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Novel paediatric formulations for the drug sodium benzoate

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Abbreviations

A	Projected pellet surface
ADI	Acceptable daily intake
AMT	Aminomethyltransferase
AR	Aspect Ratio
AUC	Area under the curve
BNF	British National Formulary
BW	Body weight
C	Compritol 888 ATO, glycerol dibehenate
CFS	Cerebrospinal fluid
CNS	Central nervous system
D	Dynasan 114, glycerol trimyristate
DD 50 %	50 % drug dissolved per labelled claim
d_{Eq}	Equivalent diameter
d_F	Mean Feret diameter
d_{F50}	Median of all mean Feret diameters
d_{Max}	Maximum Feret diameter
d_{90}	Feret diameter perpendicular to the maximum Feret diameter
DI	Discrimination index
DLD	Dihydrolipoamide dehydrogenase
DMF	Drug master file
DSC	Differential Scanning Calorimetry
EMEA	European Medicines Agency
EU	European Union
FDA	Food and Drug Administration
GCE/GCS	Glycine cleavage system
GLDC	Glycine decarboxylase
GRAS	Generally recognised as safe
H	Water content
HLB	Hydrophilic Lipophilic Balance
HPLC	High Performance Liquid Chromatography
ICH	International Conference on Harmonisation
ICU	Intensive care unit
JECFA	Joint Expert Committee on Food Additives

MCC	Microcrystalline cellulose
M	Molar
min	minutes
mM	Millimolar
MRA	Multiple regression analysis
n	Number of measurements
N	Number of observations
NCE	New chemical entity
NKH	Non-ketotic hyperglycinemia
NMDA	N-methyl D-aspartate
NMDAR	NMDA receptor
obs	observed values
OMIM	Online inheritance in man databank
P	Precirol ATO 5, glycerol distearate
PAG	Phenylacetylglutamine
PB	Sodium phenylbutyrate
PCA	Principal component analysis
Ph. Eur.	European Pharmacopeia
PLS	Projection to latent structures
pred	predicted values
Q^2	Quality of Prediction
RMSECV	Root mean square error of cross-validation
RH	relative humidity
rpm	revolutions per minute
R^2	Coefficient of determination
SD	Standard deviation
SEM	Scanning electron microscope
SLN	Solid Lipid Nanoparticles
TAG	Triacylglycerides
TRC	Taste receptor cells
UK	United Kingdom
USP	United States Pharmacopeia
W	Witocan 42/44, hard fat
WHO	World Health Organisation
x_{50}, x_{Number}	Median, Quantile
ε	Porosity
ρ_{He}	Gas pycnometric density
ρ_{M}	Mercury density

A Introduction and aim of the study

1. Introduction

A major challenge in drug development is the drug delivery for the paediatric population. Specific facts have to be taken into account with this patient group. Children are a very heterogeneous patient group ranging from newborns to adolescents with huge developmental and physical differences regarding dose, pharmacokinetics, absorption, sensitivities and compliance. The route of administration, the composition of the formulation, the dosage form and the matter of administration have to be carefully considered.
So far children are often named the orphans in drug development, but what is the matter with children suffering from orphan diseases and their treatment with therefore called orphan drugs?

An orphan disease, or rare disease, is characterized as a life-threatening or chronically debilitating disease with a low prevalence. This low prevalence is defined in the EU as less than 5 affected persons in 10,000 inhabitants. While this number seems low it translates to approximately 246,000 affected persons in the 27 member states of the EU. The origin of rare diseases is often a genetic defect, but can also be caused by rare infections, auto-immune diseases or rare poisonings. About 5,000 to 8,000 orphan diseases are known today, which means that about 6-8 % of the population in total are affected, again this translates to around 27 to 36 million people in the EU (European Commission 2008). Rare diseases are considered to have little impact on the society as a whole and have been ignored until recently. The affected persons did not benefit from health resources and services and instead suffered from a lack of information, research, diagnosis and treatment. Orphan diseases have been one of the priorities in the EU Public Health Programme of 2003 – 2008.
The term orphan drugs describes medicinal products intended for diagnosis, prevention or treatment of rare diseases. The Orphan Regulation (EC No 141/2000) in force since the year 2000 sets incentives for the pharmaceutical industry to encourage research and marketing of medicines for rare diseases. These incentives include a 10-year market exclusivity, protocol assistance and access to Centralized Procedure for Marketing Authorisation. Up to now the list of marketing authorized Orphan Drug in Europe consists of 44 products (Orphanet Report Series 2008).

This work focuses on sodium benzoate as a treatment for the orphan disease non-ketotic hyperglycinemia (NKH). It is a hereditary metabolic disease that affects the glycine metabolism. The prevalence is estimated by the European Commission as 0.02 per 10,000 EU inhabitants and the incidence is, varying by country and region in the EU, between 1 : 12,000 and 1 : 4,000,000 (Breitkreutz 2004). Sodium benzoate was granted orphan drug status for the treatment of NKH in 2002. It is used to lower the high glycine levels caused by this disease, as benzoate is able to bind glycine to form hippuric acid, which can be excreted renally. Through this therapy the severe symptoms of NKH like seizures and lethargy can be alleviated.
First attempts to develop a paediatric liquid formulation for sodium benzoate were unsuccessful, as it was impossible to mask the bitter and salty taste of the drug in a liquid formulation. In subsequent studies an alternative, a multiparticulate system, in this case saliva-resistant coated granules with sodium benzoate and hard fat as a binder for the treatment of NKH have been developed by Breitkreutz et al. (2003).

Children are a very heterogeneous patient group, the International Conference on Harmonization (ICH) divides the paediatric population by age into five categories. In the "Note for guidance on clinical investigation of medicinal products in the paediatric population" (EMEA 2000) the groups: preterm newborns, newborns, infants and toddlers, children and adolescents have been defined. The categorisation into these subpopulations has been mainly derived from physiological and pharmacokinetic differences, e.g. metabolic capacity, organ maturation and drug clearance.

In the reflection paper "Formulations of Choice for the paediatric population" by the European Committee for Medicinal Products for Human Use (CHMP 2005) the broad group of "children" of the ICH guideline ranging from 2 to 11 years is divided into two subgroups, the pre-school children between 2 and 5 years and the school children from 6 to 11 years. The division is based on the child's ability to accept and use different dosage forms. Starting approximately at the age of 6 years the majority of children receives solid dosage forms for the peroral drug administration whereas liquid formulations are predominantly used below that age (Schirm 2003). In the reflection paper the most appropriate dosage forms for each age group are evaluated.
Liquid formulations such as solutions/drops or effervescent dosage forms are rated as the dosage form of choice for infants already from their first month of life. One important consideration with liquid formulations is the dose volume, large volumes are inconvenient for patients and care takers. Usually, dose volumes below 5 ml are suitable for children under the age of 5 and volumes larger 10 ml for children of 5 years and older (EMEA 2000). However, taste is an major aspect, so the more palatable the formulation, the higher will be the tolerated dose volume.
Powders and multiparticulate formulations (e.g. granules, pellets or mini tablets) are solid dosage forms with a good acceptability, even in children below the age of 5, whereas common solid oral dosage forms such as tablets or capsules are only rated as dosage form of choice for older school children and adolescents, as the main problem is the difficulty of swallowing. The small multiparticulates can be dosed directly into the mouth of the patient or can be mixed with food or beverages prior to administration. Similar in their acceptability even for smaller children are the orodispersable dosage forms such as orodispersable tablets, lyophilised wafers or novel dosage forms such as fast dissolving films. They are easy in their administration and do not require additional liquids.
The rectal route of administration may be chosen for a number of reasons such as immediate requirement of systemic effects, unconsciousness of the patient or rejection of oral dosage forms. Suppositories are the most common rectal paediatric dosage forms and one of the dosage forms of choice for newborns and infants. However, compliance issues have to be considered as the rectal route of administration is poorly accepted in certain countries and cultures.
Parenteral drug administration such as intravenous solution or subcutaneous injections are needed for the very young and the very ill, such as preterm newborn infants and newborn infants as well as for intensive care situations.
Topical dosage forms for transdermal drug delivery are not limited in there use for certain age groups as they are easily applicable to all age groups. Instead, important penetration and permeation characteristics such as the morphological differences between the skin of adults and infants or the surface area/body weight ratio have to be considered.

But not only does a suitable dosage form need to be found and developed for paediatric purposes, additional issues have to be taken into consideration. Safe excipients are also very important for each formulation. Usually pharmaceutical excipients are labelled as "inactive ingredients" and assumed to be safe for human use, but this might not be true for a paediatric subpopulation, as children show particular differences to the "normal" adult patient. The choice of excipients may also determine the applied dosage forms, as the number of suitable excipients for manufacturing

a dosage form is often very limited. Toxicological risks are mostly associated with excipients used for liquid formulations, whereas solid drug formulations can be usually composed using non toxic excipients. So the practical advantage of liquids, including safer dose adaptation and suitability for small children, has to be weighed against easier composition of solid formulations (Breitkreutz et al.1999). Major problems with pharmaceutical excipients have been reported for benzalkonium chloride, benzyl alcohol, dyes, propylene glycol, and sulfites (Breitkreutz et al. 2007). The toxicity of excipients in newborns and infants can be explained by several factors including the insufficient metabolic capacity in the first month of life, the differences in renal clearance e.g. the much lower glomerular filtration rate in neonates or the more porous blood-brain barrier. A good example is the age-dependant toxicity of propylene glycol due to a higher bioavailability after cutaneous or peroral absorption, a higher concentration in the central nervous system and a reduced elimination due to a low-capacity metabolism. Caution should be applied to artificial colours as recent studies suggest that two dye mixtures of different composition, but both preserved with sodium benzoate, might trigger hyperactive behaviour in children (McCann et al. 2007).

As a consequence, the development of the best suitable drug products for children is a major challenge in drug development. Since January 2007 a new Paediatric Regulation [Regulation (EC) No 1901/2006 of the European Parliament and of the Council on medicinal products for paediatric use] entered into force which contains a new system of rewards, incentives and obligations for the pharmaceutical industry. This means that paediatric drug development will hopefully become an integral part of the development of medicinal products. Early strategies are needed for implementing paediatric formulations at an early stage, perhaps as early as preformulation.

2. Aim of the study

The aim of this study was the development of sodium benzoate containing dosage forms for the treatment of metabolic diseases in the paediatric population. A sodium benzoate infusion solution should be developed for the treatment of intensive care and neonatal patients. Furthermore a suitable ready-to-use formulation is needed to replace the existing practice of diluting concentrated sodium benzoate solutions with physiological saline by hospital pharmacies. The stability of this infusion and the influence of different packaging material was under investigation.

Furthermore, previously developed sodium benzoate containing granules with one hard fat as a binder (Breitkreutz et al. 2003) should be improved. Spherical pellets with narrow particle size distribution should be prepared using a cold extrusion process and following spheronisation with different lipid combinations.

The role of lipids as binders, as non-toxic alternatives to common extrusion aids, in cold extrusion/spheronisation processes were further investigated. The feasibility of using different kind of lipids as excipients in the production of these pellets and their effect on the taste were also scrutinized.

The taste of different pellet formulations should be investigated by varying means using human taste panel tests and electronic taste testing systems. The various taste testing methods were compared to each other and the use of electronic systems to replace common human taste panels evaluated.

Further insight into the taste masking of pellets through lipid binders was gained and a possible mechanism for this occurrence explored.

B General Part

1. Therapeutic applications of sodium benzoate

Sodium benzoate, commonly known as a preservative, is used in the therapy of metabolic diseases in children. The group of diseases include defects of the urea cycle and have hyperammonemia as a general symptom (Nassogne et al. 2005). One disease where sodium benzoate is used for treatment is glycine encephalopathy or non-ketotic hyperglycinemia (NKH). NKH is an autosomal recessive disorder of the glycine metabolism caused by a defect in the glycine cleavage system. In the Online Medelian Inheritance in Man (OMIM) databank it is listed under the registration number 605899 as glycine encephalopathy. The disease is caused by a defect in one of the four proteins out of the glycine cleavage system (GCS or GCE, EC 2.1.2.10). The GSC comprises four proteins, P-protein or glycine decarboxylase (GLDC, EC 1.4.4.2), T-protein or aminomethyltransferase (AMT, EC 2.1.2.10), H-protein or hydrogen carrier protein (OMIM 238330) and L-protein or dihydrolipoamide dehydrogenase (DLD, EC 1.8.1.4).

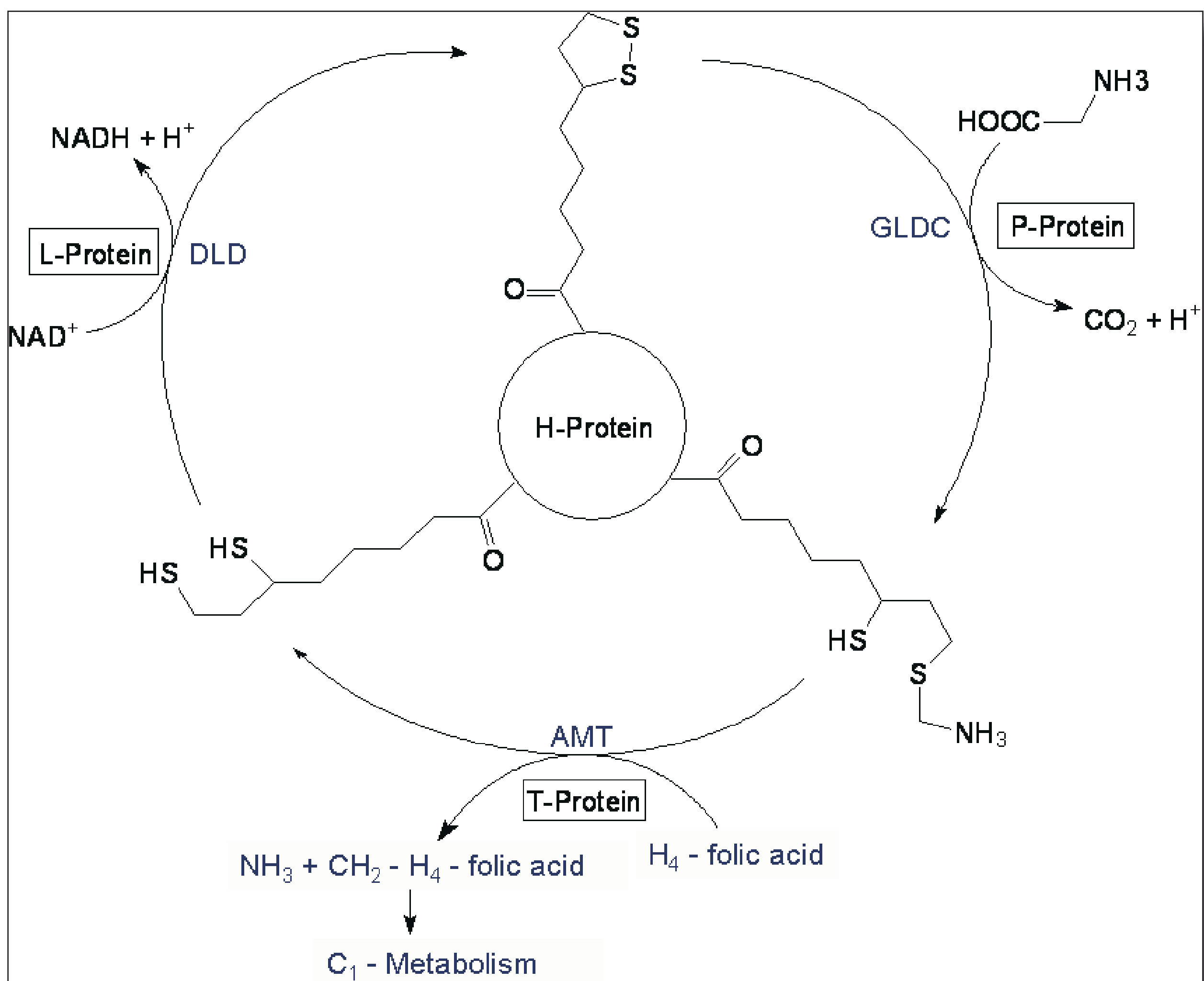

Figure 1: Schematic illustration of the glycine cleavage system and the reduction pathway of glycine through this multienzyme complex (modified from Breitkreutz 2004).

The GCS is the main catabolic pathway for glycine and it also contributes to one-carbon metabolism. It catalyses the oxidative conversion of glycine into carbon dioxide and ammonia and the remaining one-carbon unit is transferred to folate as methylenetetrahydrofolate. A deficient glycine decarboxylase activity (defect P-protein) is the most common cause of NKH (Kanno et al. 2007, Tada 1987), but also defects in T-protein (Schutgens et al. 1986, Toone et al. 2003) and one case where the H-protein was effected are described in the literature (Koyata et al. 1991). No NKH case on the base of a defect in L-protein is described so far. This might be explained by the fact that the L-protein participates also in other biotransformation pathways e.g. the pyruvate metabolism, therefore obscuring symptoms of NKH (Applegarth et al. 2001).

Most patients with NKH have the neonatal phenotype (Hennermann et al. 2002), but also cases with an atypical form (Flusser et al. 2005), a transient form (Applegarth et al. 2001) and a late-onset form appear and have been described in the literature.
Most patients suffer from lethargy, coma, seizures and apnoea in the neonatal period and about one third of the newborns with neonatal NKH die within the first 2 weeks after birth, mostly due to apnoea with fatal consequences. Apnoea improves spontaneously after 2 weeks, but most patients develop severe mental retardation and severe seizures (van Hoove et al. 2005). Congenital malformations such as agenesis or hypoplasia of the corpus callosum, and hydrocephalus have been described (Dobyns 1989, van Hoove et al. 2000).
Patients with late-onset form can exhibit differing degrees of mental retardation and seizures (Hamosh et al. 2001, Steiner et al. 2001) whereas Aiefendioğlu et al. (2003) described cases with the transient form, where patients displayed features of neonatal NKH, but whose biochemical and clinical symptoms disappeared in infancy. The outcome of these patients has been variable.
Atypical or mild NKH is phenotypically heterogeneous and non-specific, making diagnosis difficult. Described cases showed aggressiveness and developmental delay as main features (Flusser et al. 2005, Dinopoulos et al. 2005), and NKH was confirmed by genetic analysis.

Patients with a defect in the GCS have increased glycine in plasma, urine and cerebrospinal fluid (CFS) and an increased CSF:plasma glycine ratio. Important concerning the pathology is the increased glycine concentration in the CFS.
Glycine is a substrate to both inhibitoric and excitatoric amino acid receptors (Stark 2000). A strychnine-sensitive glycine receptor (Gly_A) is known, which acts inhibitoric on the CNS and the central respiration (Danysz et al. 1998).
Furthermore, glycine is a cosubstrate of the ionotropic N-methyl D-aspartate (NMDA) receptor which acts excitatoric. The NMDA receptor (NMDAR) is a membrane spanning protein complex with probably 5 subunits (NR1, NR2A-NR2D, NR3A, NR3B) per channel (Stephenson 2006). Functional channels require two NR1 and a type of NR2 subunits, where NR1 has the glycine-binding site and NR2 the glutamate-binding site. The channel is permeable for Ca^{2+}, Na^{+} and K^{+}. At resting membrane potentials, the NMDAR channel is blocked by Mg^{2+}, whereas depolarization relieves this block and allows ion flux when glycine and the actual agonist, i.e. glutamate, are present simultaneously and bind at the channel.
Due to the unphysiologically high concentrations of glycine in the CNS the NMDAR are permanently sensitized. Recent studies suggest, even though the neurotoxicity of high glycine concentrations on the brain is so far not fully understood, that the constant stimulus of NMDAR and the resulting high intracellular calcium concentrations lead to severe neuronal damage and partial apoptosis of neurons (Schmitt 2003). Especially in the maturing brains of foetuses and small children these occurrences lead to irreversible damages and severe mental and physical retardations.

Even though the pregnancies of foetuses with NKH are unobtrusive, irreversible brain damage occurs. The organism of the mother is capable of compensating the high blood glycine levels, but accumulation in other body compartments, i.e. the CSF can not be avoided. Studies with newborns suggest, that even with prenatally diagnosed NKH and conclusive immediate treatment after birth the poor long-term outcome of the disease can not be prevent, even though this regimen can favourably modify the early neonatal course (Korman et al. 2006). This further indicates glycine-induced prenatal and/or ongoing postnatal damages.

Treatment is directed toward reducing glycine levels and antagonizing the action of glycine at the NMDA receptors. The anion benzoate can, after activation through coenzyme A (CoA) in the mitochondria, bind the amino acid glycine. First benzoate is activated through CoA-ligase and then conjugated through glycine-N-acyltransferase with glycine. Hippurate, the anion of hippuric acid, is formed after the elimination of CoA. Hippurate can be eliminated renally in a fast and also quantitive manner (Kubota et al. 1991). It is therefore possible to lower the high blood glycine levels and consequently, to a lower extent also the CSF levels, with sodium benzoate. The salt form is used due to its superior water solubility in comparison to benzoic acid, which would have the same physiological effect. For the treatment of NKH doses of 250 – 750 mg per kg bodyweight/d are common (Hoover-Fong et al. 2004, van Hove et al. 2005), doses exceeding 750 mg/kg per day are associated with toxicity (van Hove et al. 1995).
The glycine plasma concentration should be below 500 µmol/L, ideally between 150 and 250 µmol/L. The CFS:plasma ratio should be less than 0.08. The physiological level of glycine in healthy subjects is 200 ± 40 µmol/L for plasma and 7 ± 3 µmol/L for CSF (Viola et al. 2002). Children with NKH displayed levels of glycine of up to 4090 µmol/L for plasma and 646 µmol/L for CSF values (Hennermann et al. 2002).
With correct doses of sodium benzoate it is possible to lower the blood plasma values into the normal range (van Hove et al. 2005), but this is not always the case for the CSF values. Side effects of the sodium benzoate therapy are gastritis and haematuria.
In combination with sodium benzoate children are often treated with dextromethorphan, a non competitive NMDAR antagonist (Randak et al. 2000, Hamosh et al.1998) or sometimes with other NMDA antagonists such as ketamine (Ohya et al. 1991, Boneh et al. 1996). To prevent or alleviate the seizure activity also anticonvulsants are given such as phenobarbital, valproic acid, diazepam or vigabatrine.
The binding of glycine by sodium benzoate is also an therapeutic option in the treatment of urea cycle disorders. Patients with lysinuric protein intolerance, ornithine transcarbamylase or carbamyl phosphate synthetase deficiency may use sodium benzoate for lowering their blood ammonia levels (Batshaw et al. 2001). Sodium benzoate therapy of urea cycle defects requires the combination with sodium phenylbutyrate, which is a licensed drug for the mono theraphy of urea cycle defects in the EU. Another indication for sodium benzoate is the treatment of portal-systemic encephalopathy in patients with chronic liver diseases, as high blood ammonia levels are an important pathogenesis factor and therapy is aimed at the prevention of the ammonia intoxication. Parenteral high-dose therapy with sodium benzoate is common as the ultima ratio (Sushma et al. 1992).
Sodium benzoate received the status as an orphan drug for the treatment of NKH from the EMEA in 2002. As NKH is a rare (orphan) disease with an estimated prevalence of about 0.02 per 10,000 EU inhabitants and is furthermore life-threatening and debilitating.
So far, there is no licensed drug product available throughout the world with sodium benzoate as an active ingredient, even though it is a standard treatment for patients with NKH. No primary reference standard, neither Ph. Eur., USP nor WHO grade is available. Sodium benzoate was rated a “new active substance” for therapeutic use as it was so far only known as a chemical substance.

A drug master file (DMF) was prepared by the Ethicare GmbH (2004). The development of sodium benzoate containing granules with a saliva-resistant coating is described by Breitkreutz et al. (2003). This novel formulation was prepared under GMP conditions and has been tested successfully in individual trials. Application as a licensed medicinal product in the EU is under preparation.

2. Lipids in oral solid formulations

Lipids (greek: lipos) is a collective term used for fats, oils, waxes and fatty substances which can be extracted from plant or animal products with lipophilic solvents and are poorly soluble in water. Large structural differences occur as not only neutral lipids such as fatty acids, acylglycerides or waxes are content of this group, but also polar or amphiphilic lipids such as phospholipids, sphingolipids and glycolipids. Classical areas of application for lipids include the use of oils and semisolid preparations for external use such as ointments, oily eye drops or lipid based injections of lipophilic drugs or suppositories.

The interest in lipid based oral drug delivery is relatively recent and is related to the growing need for novel drug delivery systems, but also for the improvement of delivery of existing drugs and for line extensions. A major part of the new chemical entities (NCE) are poorly water-soluble which is resulting in a poor bioavailability. Primarily lipid based excipients are therefore nowadays used for bioavailability enhancement with aims such as increased drug solubility, targeting lymphatic drug transport and/or modulation of enterocytes-based drug transport and disposition. Furthermore lipids can be used for taste masking or protection of the active against gastrointestinal fluids and in sustained release dosage forms.
The main lipid excipients that are used in the pharmaceutical industry have in common that they are mostly vegetable oil derivatives such as hydrogenated vegetable oils, partial glycerides, polyoxylglycerides, ethoxylated glycerides and esters of edible fatty acids and various alcohols (waxes). The common components are the fatty acids (see also Table 1).

Table 1: Nomenclature and melting temperatures of fatty acids.

Common name	Short formula number of carbons : unsaturated bonds (position of unsaturated bond)	Melting temperature (°C)
Caprylic acid	8 : 0	16.5
Capric acid	10 : 0	31.6
Lauric acid	12 : 0	44.8
Myristic acid	14 : 0	54.4
Palmitic acid	16 : 0	62.9
Stearic acid	18 : 0	70.1
Arachidic acid	20 : 0	76.1
Behenic acid	22 : 0	80.0
Oleic acid	18 : 1 (9)	16.0
Linoleic acid	18 : 2 (9,12)	-5.0
γ-linolenic acid	18 : 3 (6,9,12)	-11.0

Hydrogenated vegetable oils such as hydrogenated cottonseed oil (Lubritab™) or hydrogenated palm oil (Dynasan™ P60, Softisan™) are obtained through a catalytic hydrogenation of the unsaturated bonds with nickel, resulting in hydrophobic solids with a “waxy” appearance.

Partial glycerides can be obtained by glycerolysis, interestification or direct esterification of glycerol. Glycerolysis is a transesterification reaction of triglycerides with glycerol under heating with an alkaline catalyst. Interesterification is used to statistically redistribute the positioning of the fatty acids on the hydroxyl groups of glycerol to achieve homogeneity of the triglyceride molecules in terms of fatty acid composition. Direct esterification is the synthesis of specific mixtures of glycerides with selected fatty acids. The characteristics of the partial glycerides, e.g. physical aspects, melt characteristics or HLB, depends on the nature of the present fatty acid(s) and the degree of esterification (mono- or diglycerides). Their application can therefore vary depending on the mentioned properties. Known excipients from this group include glyceryl monostearate or GMS (Imwitor® 191, Cutina™ GMS or Tegin™), glyceryl distearate (Precirol® ATO 5) or glyceryl dibehenate (Compritol® 888 ATO).

Triacylglycerides or triglycerides (TAG) are ester of glycerol where all three hydroxyl groups are esterified by fatty acids. They are the main component of natural fats and oils. If triglycerides contain different fatty acids the amount of different triglycerides are versatile, e.g. in a fat that contains only two different fatty acids, these can stem from 6 different triglycerides, with 3 fatty acids 18 different theoretical glycerides are possible. As there are about 50 known fatty acids there are a nominal number of over 60,000 triacylglycerides possible, but only few occur naturally.
An important characteristic of triglycerides is their crystallization behaviour into different polymorphs. For monoacid TAG three polymorphs are known, called the α-, β´- and β-modification (Larsson 1966). They can be distinguished by their melting points, the α-modification being a meta-stable form having the lowest melting point, followed by the meta-stable β´-modification and the stable β-modification with the highest melting point. This can also be explained by looking at the molecular structures and the corresponding Gibbs free energy (G) values of the three forms, as α has a disordered aliphatic chain conformation and a high G value, an intermediate packing occurs in β´ and the most dense packing and lowest G can be found with β. Thermodynamic and kinetic factors determine the polymorphic crystallization. Phase changes occur step-by-step, where metastable forms nucleate first, before the most stable forms when large kinetic factors, e.g. supercooling or supersaturation, are applied. Other applied external influences such as pressure, temperature fluctuation, seeding or minimized kinetic suspend this rule and more stable forms are nucleated at reduced kinetic factors (Sato 2001). Amongst others the Dynasan® group belongs to this category, e.g. glycerol trimyristate (Dynasan® 114), which was used in this study. More complex is the situation in mixed-acid TGA with fatty acids of different chain lengths or with saturated and unsaturated fatty acids. Here more polymorphs might occur or there is no β form present and therefore β´ becomes the most stable, or two β forms are present (Sato et al. 1999), e.g. for cocoa butter there are 6 known crystalline modifications. Other effects that influence the crystallization of fats and formation of polymorphs are the melt-mediated crystallization or tempering and shear stress (Sato 2001).
In general the characteristics of the TGA are essentially determined by their fatty acids. Unsaturated fatty acids have a lower melting point than the corresponding saturated one, fats with higher contents of glycerides of the unsaturated fatty acids are therefore liquid at room temperature. Glycerides with unsaturated fatty acids change depending on the amount of unsaturated bonds due to oxidation whereas glycerides with saturated fatty acids remain relatively stable.

Polyoxyglycerides or macrogolglycerides can be produced through polyglycolysis of vegetable oils with polyoxyethyleneglycols (PEG) of certain molecular weight under heating and catalysation. They are water-soluble and their physical appearance range from viscous lipids to solids at room temperature depending on the composition of defined mixtures of mono-, di- and triglycerides and mono- and diesters of PEG. Among others, they may be composed of saturated medium chain fatty acid esters like lauroyl polyoxylglycerides (Gelucire® 44/14) or saturated long chain fatty acids like stearoyl polyoxylglycerides (Gelucire® 50/13).
Ethoxylated lipids are widely used as surfactants and to increase bioavailability of poorly-soluble drugs, one of the main products representing this group is ethoxylated castor oil (Cremophor® EL).

Polyalcohol esters of edible fatty acids are amphiphilic compounds with high to medium HLB depending on their degree of esterification and the type of alcohol used. The alcohols may be amongst others polyoxyethylene-glycols (PEG-8 stearate: Mirj® 45) or sorbitan (sorbitan mononooleate: Span® 80). They are used as solubilizers or as components of self-emulsifing systems in bioavailability enhancement (Jannin et al. 2008).

Next to high sophisticated analytics of fats and lipids such as HPLC and GC methods are the classical methods which are still part of pharmacopeial specifications. The acid value is used for the quantification of free (unesterified) fatty acids, the hydroxyl value determines free hydroxyl groups from hydroxyl fatty acids, fatty alcohols, mono- and diglycerides as well as free glycerol combined, the peroxide value detects oxidative deterioration and the iodine value measures the saturation of hydrocarbon chains. It is to note that not all unsaturated bond react as trans-fatty acids add halogen only slowly and some not at all, e.g. unsaturated bonds in phytosteroles. The saponification value is an indication for the average molecular weight of the fatty acids from a triacylglyceride mixture.

There are different techniques to produce lipid based formulations. One of the simplest and so far most common technology has been the capsule filling, where liquid or semi-solid have been encapsuled by soft or hard gelatine capsules (Jannin et al. 2006). Several considerations have to be taken into account with this technology e.g. the compatibility of the excipients with the capsule shell (Cole 1989) or the filling temperature and viscosity of the formulation. So far a range of drug products are manufactured according to this technique as there are e.g. amprenavir, cyclosporin, lopinavir or progesterone soft capsules and fenofibrate, ibuprofen or indomethacin hard capsules (Strickley 2007).

Spray cooling or spray congealing, a technique where a molten formula is sprayed into a cooling chamber, can be used to produce small solid lipid particles (Erni et al. 1980). These may then be used for tabletting to produce controlled release dosage forms (Savolainen et al. 2003). One key parameter is the drug load of the formulations, as this influences the viscosity, as dispersions generally tend to be more viscous than solutions. So far a maximum of 30 % drug load has been reported (Passerini et al. 2006). Similar to this method bigger spherical pellets can be obtained through a process where a melted suspensions is dropped onto a cooled surface (Pallagi et al. 2004).
The same equipment used for spray cooling can be used for spray drying where a liquid is sprayed into a hot air chamber to evaporate the volatile fraction, e.g. organic solvents or water (Cao et al. 2007). The process yields microparticles which may be compressed into tablets or used for other manufacturing steps, similar to particles obtained through spray cooling.

A simple, one-step procedure to produce granules based on lipids is the melt granulation or pelletisation (Everard et al 1999). In a high shear mixer the lipid excipient is either molten and sprayed onto the powder mix or the excipient is blended with the powder mix and allowed to melt through frictional forces during mixing. The key parameters to this process are mixing time, impeller speed, binder particle size and the viscosity of the binder during melt granulation (Seo et al 2001). Lipids that are used in this process amongst others are waxes (Zhou et al 1996) and acylglycerides (Hamdani et al. 2002). Typically, lipid binders are used in concentrations between 15 and 25 % depending on the fineness of the powder mixture. Further formulation parameters that should be considered during melt granulation are the drug particle size and shape, the melting point and thermoplastic behaviour of the binder and solubility of the binder (Jannin et al. 2008). Again the obtained granules or particles may be used for further processing such as the preparation of tablets (Saraiya et al. 1990). A variation of this technique is the preparation of lipid spheres in a rotary fluidized bed process (Gauthier et al. 2003).

Another technique which offers the advantage of being solvent free and which allows high drug loading as well as content uniformity for high potency low dose actives is the extrusion or extrusion/spheronisation process. In the past lipid based excipients have been included as additives to classic extrusion formulations, mainly with MCC as a binder (Liu et al. 2001, Singh et al. 2007). This was used to modify the release to obtain either enhanced dissolution or bioavailability of poorly soluble drugs (Hulsmann et al 2000) or a controlled release (Miyagawa et al. 1996).
The fraction of lipid binders were around 4 to 40 % and the drug load varied from 10 % to around 50 %. In some cases a thermal treatment of the resulting pellets or granules were performed after extrusion/spheronisation to achieve the desired release properties (Singh et al. 2007).
The cases in which lipids were used as an exclusive binder without the use of polymers or other common excipients are rare. In all cases, a melt-extrusion process is described where the formulation is extruded at temperatures above or around the melting point of the used lipid excipient (Prapaitrakul et al. 1991). Extrusion of triacylglycerides and acylglycerides at temperatures about 10 °C below the melting point of the lipids resulted in prolonged release profiles (Reitz 2007).
For polyoxylglycerides (Pinto et al. 2001) and acylglycerides (Breitkreutz et al. 2003) extrudability at room temperature in a cold extrusion was shown to be possible. The extrusion of Witocan® 42/44 as the binder with high amount of sodium benzoate resulted in immediate release granules with cylindrical shape which were coated with a saliva-resistant coating to achieve delayed drug release (Breitkreutz et al. 2003).
Another technique for lipid-based oral drug delivery are solid lipid nanoparticles (SLN), first described by Speiser (1990) as the manufacturing of lipid microparticles by spray-congealing. Classic components of SLN are glyceryl dibehenate as the solid matrix and poloxamers or polysorbates as surfactants. SLN can be produced through hot or cold high pressure homogenization. For both techniques the drug is dissolved or solubilized in the molten lipid. Afterwards it is either dispersed in a hot surfactant solution and homogenized or it is cooled, grounded and then dispersed in cold surfactant solution and then homogenized (Müller et al. 2000). Further manufacturing techniques include production through microemulsions which have the disadvantage of resulting in a relatively low concentration and yield of SLN and the use of solvents, or through a precipitation method (Siekmann et al. 1996) similar to the preparation of polymeric nanoparticles by solvent evaporation, with the disadvantage of also needing the use of solvents. They have been mainly used for controlled release in the topical administration (Souto et al. 2005), but can also be used to increase the oral bioavailability of drugs in addition to the control of their release (Hu et al. 2004).

3. Taste testing

Our sense of taste enables us to evaluate food and drinks. This evaluation promotes the ingestion of nutritious substances and should prevent the consumption of potential poisons or toxins.
The sensation of taste is mediated by taste buds located in the epithelium of the tongue. They are composed of groups of 50 to 150 columnar taste receptor cells (TRC) which are arranged to form a small taste pore through which microvilli from the taste cells extend. These microvilli of the taste cells bear taste receptors. In between the TRC are a network of dendrites of sensory nerves which are called "taste nerves". The TRC are stimulated by binding chemicals which results in a depolarization that is transmitted to the nerve fibres. The resulting action potential is transmitted to the brain. It is interesting to note that this nerve transition is possible to adapt to a certain stimulus, e.g. the initial stimulus results in a strong discharge in the nerve fibre which diminishes over time to a steady-state level.

Five different types of taste are commonly recognized: sweet, indicating energy rich nutrients (sugars); salty, indicating salts like NaCl and important for the electrolyte balance; sour, indicating acids; umami (the Japanese word for deliciousness) resulting from amino acids and bitter, indicating natural toxins like alkaloids. Each of the tastes has unique biochemical signalling pathways and some e.g. bitter taste recognition use multiple pathways.

A large number of molecules evoke taste sensations through a rather small amount of taste receptors. There are diverse mechanisms which are related and based on the structural and chemical diversity of substances for taste transduction, but all taste pathways are proposed to converge on common elements that mediate a rise in intracellular Ca^{2+} (Gilbertson et al. 2000). Some taste transduction pathways convert chemical information into cellular second messenger code (e.g. inositol triphosphate IP_3) and these messengers are typically part of a signalling cascade which leads to TRC depolarization and Ca^{2+} release. Other substances themself may constitute all or part of the initial cellular signal (e.g. Na^+, K^+, H^+).
Bitter taste is transduced through a range of different structures and compounds which bind to a family of about 30 T2R receptors. This binding leads to a transduction process such as receptor processes that use stimulus-gated ion channels, receptor mediated production of second messengers and even direct entry of some non-polar stimuli into the receptor cell.
Salty and sour taste is transduced through specific ion channels whereas umami binds to several types of glutamate receptors. So far the molecular mechanism behind the salty taste is only poorly characterised.
Recently, a new receptor type (CD36) has been identified with a high affinity for fat, or rather fatty acids which may indicate a molecular basis for fat preferences in our dietary uptake. It is an integral membrane glycoprotein and facilitator of fatty acid uptake (Laugerette et al. 2005).
Thresholds for detection varies a lot even among substances that taste the same, but usually bitter substances have very low thresholds e.g. quinine, a very bitter tasting substance and used by the Eur. Pharm. for the establishment of bitterness values, has a threshold of about 0.000008M whereas the threshold for NaCl is about 0.01M.
There is also a genetically determined difference in taste sensitivity among humans which is further increased through cultural adopted taste preferences.

The bitter taste of a large group of drug substances accounts for the majority of taste issues and problems in the development of paediatric drug formulations. Palatability is an important factor in patient compliance. Although adults might think that bitter tasting medicine work better, this is not the case for children. Taste might not be such a big issue with single dose medication schemes, but it is a major issues in multiple dose regimens for children with chronic diseases

where regimen adherence and compliance is important. Children might refuse to take their medication completely due to taste issues. Better tasting medicines make it easier for parents or caretakers to administer the needed medication to children. After efficacy and safety, one of the most important features in antibiotic formulations for parents was the taste or palatability of the formulation (Toscani et al. 2000).

It is known that children are living in a different sensory world than adults. They have a preference for sweet substances which decreases during childhood to resemble that of adults during late adolescents (Liem et al. 2002) whereas for bitterness a higher aversion is existing, especially in the very young children. However, palatability studies are often conducted with adult volunteers, even though a comparison might be difficult, as studies showed differences when taste ratings of various antibiotics by children and adults were compared (Matsui et al. 1997).

Ideally, taste testing should be performed with the designated target group of the formulation. In case of paediatric formulations it should therefore be tested in children. There are a number of regulatory concerns regarding taste test in children. This is further complicated through different legislation in different countries. A palatability study in Germany is regarded as a clinical study, which implies considerable more effort, work and difficulties until approval than a palatability study in the UK, where it is not regarded as a clinical study. In contrast to the US under EU regulation no taste studies with healthy volunteers are allowed in children, as there are ethical concerns about the lack of potential benefit to the participants as well as possible risks of adverse effects from the medication.
In general the method and design have to be carefully chosen and the age of the participating children has to be taken into account. The number of tested products is usually limited to a maximum of four to avoid taste fatigue and confusion of the children (EMEA 2003). The judgment methods can include spontaneous verbal judgment or hedonic scales, with the hedonic scales being a more standardized procedure. Both methods have been applied in studies including children from 4 years of age even though the same study suggested that correlation between verbal and hedonic assessment varies, especially in younger children (Sjövall et al. 1984).

One way to overcome the problems associated with human taste panel test, especially with children, is the taste assessment with electronic systems. Several systems for electronic taste measurements, called electronic tongues, have been developed in the last 10 years (Toko 1998, Legin et al. 2004). Currently available on the market are two systems, the αAstree E-tongue (Alpha-MOS, Toulouse, France) and the Insent Taste Sensing System SA402B (Intelligent Sensor Technology, Atsugi, Japan). Both are based on the principal of potentiometry with Ag/AgCl reference electrodes. They are used to evaluate bitterness in paediatric formulations (Ishizaka et al. 2004, Kayumba et al. 2007) or the taste masking ability of different formulations (Hashimoto et al. 2007, Li et al. 2007). So far studies suggest that e-tongues can be used as a valuable tool in formulation screening and taste assessment (Sadrieh et al. 2005) and it is likely to reduce the number of palatability studies and/or number of needed participants. Researchers have found that especially in early phases of drug development e-tongues offer valuable insight into general palatability aspects and result in important information about taste masking abilities of certain formulations. The researchers claim that much more investigation has to be done before electronic systems are able to replace human taste panel entirely, but they might lessen the need for them in some cases.

4. Taste masking

Taste masking can be defined as a perceived reduction of an undesirable taste that would otherwise exist (Sohi et al. 2004). Various techniques can be used to achieve this reduction with differing success. There are numerous papers and patents in the literature on taste masking of pharmaceuticals. The main techniques are briefly discussed in the present chapter.

Taste masking in liquid or chewable formulations often involves the use of flavouring agents and/or artificial sweeteners. It is the foremost and the simplest approach for taste masking. Children usually prefer sweet preparations with a fruity flavour, though regional differences and preferences, as always with taste, exist. Adding sugars may increase the palatability, but chronic use of such medication is problematic due to its association with excessive dental disease (Pawar et al. 2002). Citrus and red berry flavours are popular in central Europe, whereas bubble gum is a favourite in the US and liquorice is very popular in Scandinavia. An important aspect to take into consideration is the flavour stability and compatibility with the pharmaceutical formulation. The addition of phospholipids like lecithin has also been described in literature as a way to mask unpleasant bitter taste (Katsuragi et al. 1997, Suzuki et al. 2003).

Other taste masking techniques for liquid formulations make the drug insoluble in the liquid matrix and by this effectively removing the drug from the ready access to taste receptors in the mouth (McNally et al. 2006). This can be achieved through pH adjusting, increased particle sizes, increased viscosity or the chemical modification of the drug e.g. the use of insoluble prodrugs or salts.
Complexation is another technical approach for taste improvement, even though most suitable only for low dose drugs. Like solubility, modification factors such as pH and ionic strength of the solution affect the equilibrium between bound and free drug in solution. β-cyclodextrin, a sweet, cyclic oligosaccharide obtained from starch, is the most widely used complexing agent. Bitter taste reductions of about 50 % could be obtained through the preparation of 1:1 complex with cyclodextrin (Kurasumi et al. 1991).

Ion exchange resins are another approach to complexation where charged drug molecules can attach to the oppositely charged resin substrate. Insoluble adsorbates or resinates are formed through weak ion bonding thus ensuring no dissociation under saliva pH conditions. Drug molecules are released by exchanging with suitable charged ions in the gastrointestinal tract. The most frequently used polymeric network is a copolymer of styrene and divinlybenzene (Sohi et al. 2004). The unpleasant taste of various bitter tasting drugs can be masked through the use of polystyrene matrix cation-exchange resins (Indion CRP-244, Indion CRP-254) as described in the literature (Manek et al. 1981). Other used polymers are sodium polystyrene sulfonate (Honeysett 1992) or polyacrylic acid (Blase 1993).

Another feasible option for the taste masking of solid formulations is the coating with polymers to achieve a physical barrier and thus minimizing the contact between drug and taste buds. One downfall is the additional process step, as well as the use of excipients with sometimes low acceptable daily intake values (ADI) or critical toxicological evaluation, especially in case of the chronic use of paediatric medication, and the need for solvents in this process step. Various coating agents such as cellulose derivates (HPC, HPMC, CMC, EC), methacrylic acid copolymers (Eudragit) or shellac are used for taste suppression of bitter tasting drug and are described in the literature (Sohi et al. 2004).

A similar approach is the taste masking through a lipid coating. Faham et al. (2000) coated granules with molten glycerol dibehenate and Sugao et al. (1998) coated microparticles with a hydrogenated oil and surfactant mixture in a fluidized bed.

Microencapsulation is another technique to achieve small-sized taste masked particles. Microparticles can be prepared through either spray drying, phase separation (coacervation) or through solvent evaporation. For spray drying, the polymer is dissolved in a suitable solvent and drug added to form a solution or suspension and then the solvent is evaporated through spray drying. Both other methods are based on an emulsion of an aqueous drug solution and a polymeric organic solution. This W/O-emulsion is then either dispersed in a large volume of a polyvinyl alcohol containing aqueous phase, which leads to coacervation, or a phase separator like silicon oil is added, which also leads to polymer coacervation on the drug particles. Usually organic solvents have to be used and suitable techniques have to be applied to remove these from the formulations. In literature, the preparation of taste masked diclofenac sodium microcapsules with sufficient rate of drug release has been described by Al-Omran et al. (2002). The taste of salts of basic drugs was masked using a W/O/W emulsion solvent evaporation method to produce microspheres and was described by Hashimoto et al. (2002). Fast-disintegrating tablets compressed out of microparticles with taste masking properties have been described by Dobetti (2003). The microparticles were prepared by a phase separation method and contained ibuprofen as a model drug.

Incorporation of drug in lipophilic matrices can also lead to improvement of taste, as Suzuki et al. (2004) showed by preparing chewable tablets with acetaminophen and hard fat (Witocan®) as the matrix base, resulting in suppressed bitterness of the tablets. Several sweetening agents, such as xylitol, aspartame or sucrose and lecithin were added to the formulations to further improve taste and oral feeling of the tablets. The unpleasant taste of clarithromycin could be masked through an incorporation into a wax matrix consisting of 60 % glycerol monostearate and 10 % aminoalkyl methacrylate copolymer A (Yajima et al. 1999).

It can be concluded that there are numerous options for taste masking strategies. However, only few of them have been developed for paediatric purposes. Often the employed excipients are critical for paediatric use. There is an urgent need for novel taste masking strategies for the paediatric drug development.

C Results and Discussion

1. Sodium benzoate infusion

1.1 Introduction

The need for a sodium benzoate infusion results from the aetiopathology of NKH. As also newborns are effected by the disease, a suitable dosage form should be available for this age group as well. In the EMEA reflection paper "Dosage forms of choice for the paediatric population" (EMEA 2005) a suitable dosage form for preterm and term newborn infants is an i.v. solution, as seen in Table 2. Furthermore, it is the standard route of administration on intensive care units (ICU). As most children with NKH are severely affected and hospitalised in their first weeks of life a sodium benzoate infusion is a necessary and potentially life-saving treatment. So far, in clinical practice the respective hospital pharmacies are the providers of these i.v. solutions. As there is no recommended or fixed concentration for the strength of the infusions, contents may differ. Most likely, 10 % sodium benzoate solutions are prepared which have to be diluted prior to administration. The BNF for children 2006 recommends an injection with 200 mg/ml in a 5 ml ampoule (20 % solution) which, for intravenous infusion, should be diluted with 0.9 % NaCl (or 5/10 % Glucose) solution to 20 mg/ml (2 %). As recent studies about intravenous medication errors suggest (Wirtz 2003, Taxis 2003), typical preparation error rates are around 22 %. This includes incorrect prepa-

Table 2: Dosage forms of choice (EMEA); 1 = not applicable 2 = applicable with problems 3 = probably applicable 4 = good applicability 5 = best and preferred applicability.

Route / Dosage Form	Preterm newborn infants	Term newborn infants (0d–28d)	Infants and Toddlers (1m – 2y)	Children (pre school) (2 – 5y)	Children (school) (6 – 11y)	Adolescents (12 – 16/18y)
Peroral						
Solution/Drops	2	4	5	5	4	4
Emulsion/ Suspension	2	3	4	5	4	4
Effervescent DF*	2	4	5	5	4	4
Powders/ Multiparticulates	1	2	2	4	4	5
Tablets	1	1	1	3	4	5
Capsules	1	1	1	2	4	5
Orodispersable DF	1	2	3	4	5	5
Chewable tablets	1	1	1	3	5	5
Parenteral						
i.v. Solution	5	4	4	4	4	3
i.m.	3	3	3	4	4	3
s.c.	4	4	4	4	4	3

ration or miscalculation, which lead to dosing errors, which can be partly avoided by using ready-to-use formulations. So far, there is only one product available on the market containing sodium benzoate: Ammonul® Injection 10 %/10 % (Ucyclyd Pharma, Inc.) contains 10 % sodium phenylacetate and 10 % sodium benzoate in solution. It is used for the treatment of acute hyperammonemia and associated encephalopathy in infants, children and adults with urea cycle enzyme deficiencies. In 2005 Ammonul® got the FDA approval as orphan drug for urea cycle disorders. Before administration the injection has to be diluted with 10 % dextrose solution. The dosage is determined by weight for neonates, infants and young children and by body surface area for elder patients (adolescents and adults). Both drug substances contained in the solution remove nitrogen via alternate non-urea cycle enzymatic pathways (see Figure 2). As explained above, sodium benzoate conjugates with glycine to form hippuric acid and binds one mole of nitrogen for every mole sodium benzoate. Phenylacetate conjugates with glutamine in liver and kidneys to form phenylacetylglutamine (PAG) via acetylation. PAG binds two moles of nitrogen for every mole phenylacetate and is therefore more potent in the reduction of waste nitrogen than sodium benzoate. One concern regarding Ammonul® is the high concentration of sodium in the undiluted product with 30.5 mg/ml or 1.3 mol/L.

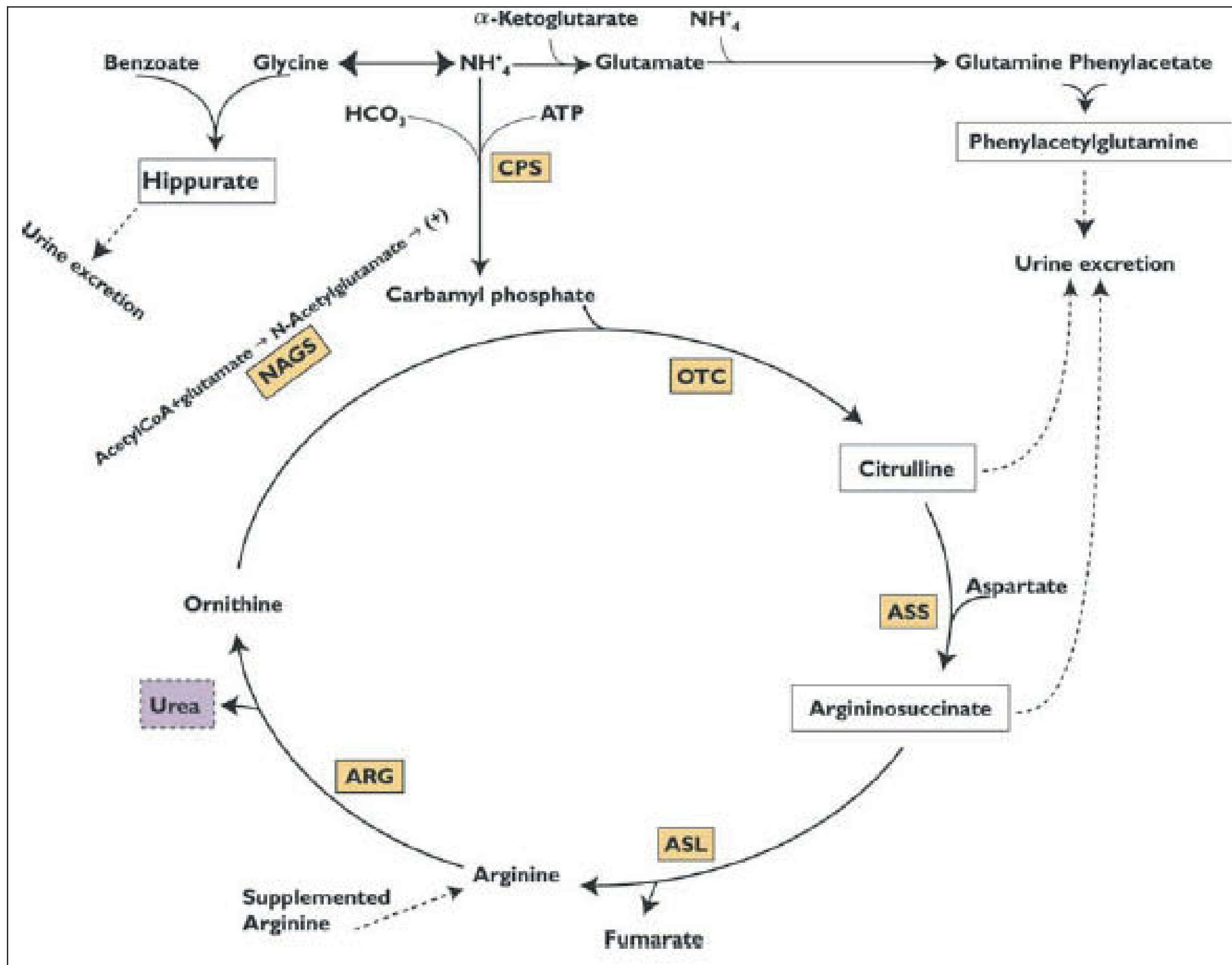

Figure 2: Schematic illustration of the alternative pathway for nitrogen disposal through benzoate and phenylacetate (FDA).
CPS = carbamyl phosphate synthetase; OTC = ornithine transcarbamylase
ASS = argininosuccinate synthetase; ASL = argininosuccinate lyase
ARG = arginase; NAGS = N-acetylglutamate synthetase

Therefore a sodium benzoate solution would not only benefit children affected by NKH, but would also be an alternative treatment for other patients, e.g. urea cycle disorders or liver cirrhosis (Oyanagi et al. 1987, Nassonge et al. 2005 and Sushma et al. 1992). Sodium benzoate may substitute substances like sodium phenylacetate and sodium phenylbutyrate (PB). Sodium phenylbutyrate is the active substance in Ammonaps® (Swedish Orphan International AB) an approved orphan drug by the EMEA and FDA (marketed in the US as Buphenly® by Ucyclyd Pharma). It is indicated as adjunctive therapy in the chronic management of urea cycle disorders and available as 500 mg tablets as well as granules. PB is a pro-drug and is rapidly converted to phenylacetate by β-oxidation.

1.2 Formulation development

Several aspects had to be taken into consideration for the development of a sodium benzoate infusion. The concentration should be suitable for direct infusion without dilution to avoid unnecessary dosing errors through calculation mistakes or preparation errors. The risk of hypernatremia has to be taken into account (Praphanphoj et al. 2000) as the concentration of sodium is high due to the salt form of the drug. The sodium concentration increases through adding sodium chloride solution as a diluent. The sodium requirement is about 3-5 mmol/kg bodyweight per day (Deutsche AG für künstl. Ernährung 1986, CHMP 2005). Furthermore, osmolality and volume of the solution have to be considered. Hyperosmolar injections may irritate small veins and produce thrombophlebitis and extravasation, hypoosmolar injections may induce haemolysis. The solution should have a suitable, i.e. physiological osmolality as well as a suitable infusion volume. As seen in Table 3, infusion volumes for children are limited, starting as low as 40-60 ml/kg in 24 h for a newborn (CMPH 2005).

Table 3: Approximate daily fluid requirements.

Infusion volume for children				
Weight [kg]	< 3	3 - 10	11 - 20	> 20 kg
Volume/24 h	150 [ml/kg]	100 [ml/kg]	1000 ml + 50 ml per kg from 11-20kg	1500 ml + 20 ml per kg > 20kg

In the present investigations a 2 % sodium benzoate solution for infusion was developed that meets the afore mentioned requirements. A 2 % solution of sodium benzoate is suitable in terms of volume per dose and through the addition of potassium chloride and calcium chloride, an adequate osmolality range is reached. This formulation followed the composition of a Ringer-solution in which the sodium chloride was replaced by sodium benzoate. The solution consists of 20 g sodium benzoate, 0.3 g potassium chloride and 0.33 g calcium chloride – dihydrate in 1L distilled water. A 3 kg infant with a daily dose of 500 mg sodium benzoate/kg bodyweight per day would receive 75 ml of this infusion, which would leave 225 ml out of the 300 ml daily fluid requirements for other infusion, as either medication or dietary supply. Regarding sodium intake, 75 ml of the solution comprise 10 mmol Na^+, which is half of the amount in a diluted solution (Table 4), and only a small part of the daily requirement of 27-45 mmol sodium for a 3 kg infant. The theoretical osmolarity was calculated as 263 mosmol/L.

Table 4: Ion concentrations [mmol/L] for different formulations.

	Diluted 10%-solution	2% Ringer-Benzoat-solution
	2 g sodium benzoate 0.72 g sodium chloride	2 g sodium benzoate
Sodium	262	139
Benzoate	139	139
Chloride	123	9
Potassium	–	4
Calcium	–	2.2

Through the novel formulation the sodium burden for the children is decreased by half. The formulation is isotonic and the electrolyte composition is well balanced.

1.3 Packaging, stoppers and compatibility

For packaging type I (neutral glass with high hydrolytic resistance) 100 ml glass containers were used, which are suitable for parenteral use. As closures halogenated butyl rubber stoppers as well as silicon stoppers with a fluorocarbon coating ("Teflon stoppers"), were used. Bromobutyl and chlorobutyl rubber is produced through the reaction of bromine or chlorine with butyl rubber (polyisobutylene). Through this process an improvement of resistance to flex fatigue, compression as well as chemical resistance is achieved. The additional coating of stoppers reduces drug and excipient migration into the stopper material and can therefore benefit the shelf life or stability of drug products. It is known that different qualities of halogenated butyl rubber closures can display physical behaviour, e.g. moisture absorption and desorption (Corveleyn et al. 1997). To investigate the possible influence of the chosen closure material on drug product stability, all of the above mentioned stoppers where used as closures and a suitable amount of vials with each stopper variety were stored for stability testing.

1.4 Stability testing

Stability testing was conducted using the three different closure varieties in the same glass type I 100 ml vials. The conditions were chosen based on the ICH guidelines for stability testing CPMP/ICH/2736/99 (CPMP 2003). 9 bottles each for bromo- and chlorobutyl rubber closures, as well as 6 for the teflon coated stoppers were stored at 40 °C and 3, respectively 2 bottles, each were analysed after 3, 6 and 12 months. The remaining vials were stored at room temperature and analysed every 3 months in a period of 24 months, again at each time point 3 bottles of each halogenated butyl rubber type closure and two bottles of the coated stopper were investigated. The tested parameters were sodium benzoate content, measured by a validated HPLC method, and ion content (sodium, potassium and calcium), measured by a validated AAS method. At the beginning the osmolality was checked, using a freezing point osmometer and the pH was recorded at the beginning and after 24 months. The measured osmolality was 270 mosmol, which is close to the calculated value of 263 mosmol. The pH of the solution remained unchanged in vials with chlorobutyl closures and changed only slightly in the coated stoppers, i.e. dropped about 0.05 from 7.65 to 7.6. The biggest change was observed in solutions with contact to bromobutyl rubber closures, here the pH was raised to 8.0 within the observed time period and would therefore be unsuitable for infusion volumes (see Table 5).

Table 5: pH values over time in sodium benzoate infusions under stability testing (room temperature, storage for 24 months).

	Bromobutyl stoppers	Chlorobutyl stoppers	Teflon stoppers
0 Months	7.69	7.69	7.65
24 months	8.00	7.70	7.60

The sodium benzoate content was stable over 24 months for all tested vials stored at room temperature, regardless of the used closure variety. As displayed in Figure 3 no significant differences could be detected over the investigated time period. All contents are in the range of 95 to 105 % of the nominal value. The same applied to the vials stored under accelerated conditions at 40 °C. Within the first six months, a slight drop could be seen for all stopper varieties, but the content after 12 months remained stable at approximately 100 % of the nominal value. No decomposition peaks were observed in the chromatograms neither under long term nor under accelerated conditions.
For the evaluation of the respective ion contents, measurement was performed by AAS. As ionisation interferences and physical interferences are described as common problems in the analysis of alkali (Na^+, K^+) and alkaline earth metals (Ca^{2+}) (Technical Documentation, Perkin Elmer) the matrix influence of the samples were analysed before actual sample measurements took place. Calcium, potassium and sodium concentrations were analysed for all vials with different stopper varieties separately over 24 months for the vials stored at room temperature and 12 months for the ones stored under accelerated conditions.

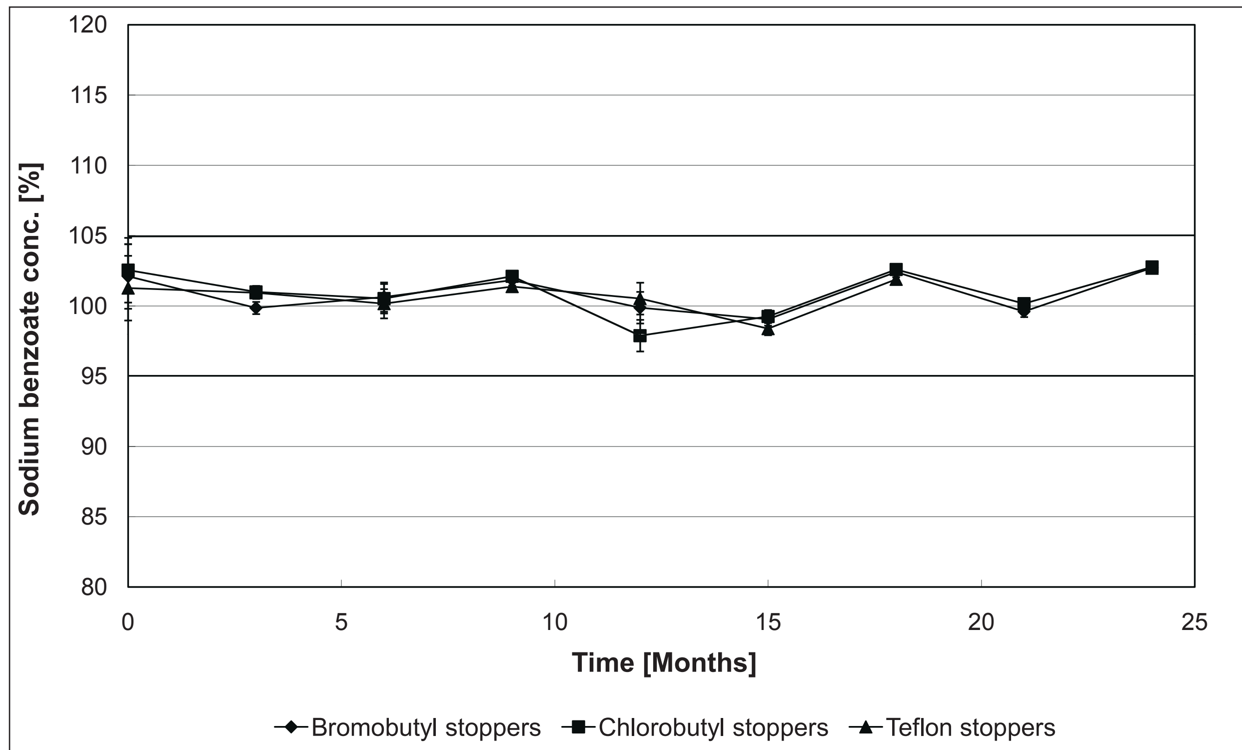

Figure 3: Sodium benzoate concentrations during storage under room conditions determined by HPLC (n = 6; mean ± SD).

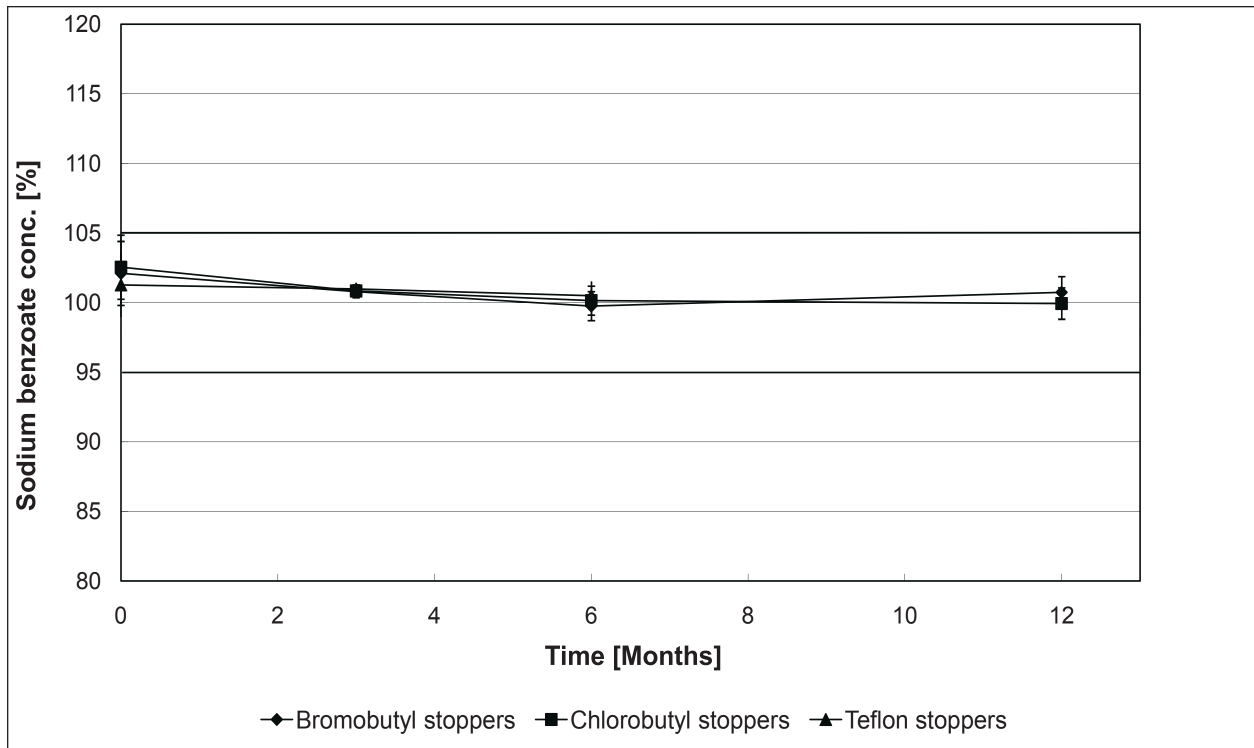

Figure 4: Sodium benzoate concentrations, determined by HPLC, during storage under accelerated conditions at 40 °C (n = 6; mean ± SD).

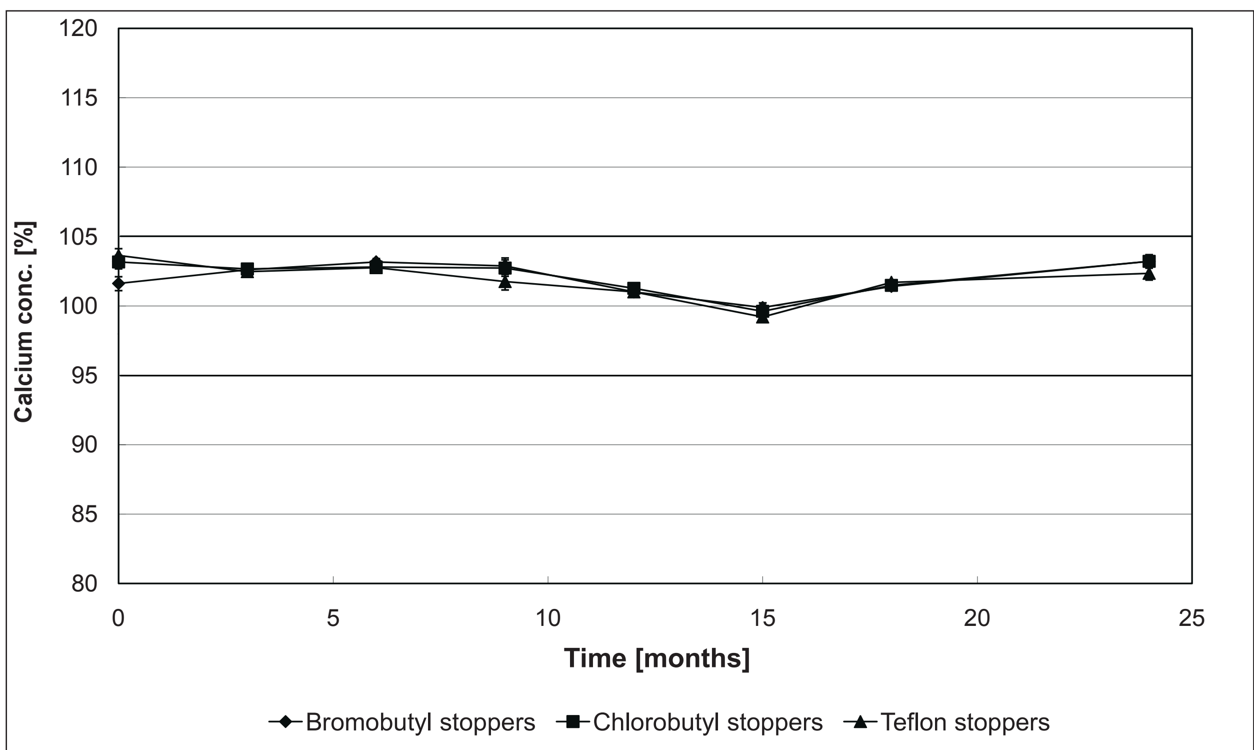

Figure 5: Calcium concentrations during storage under room conditions determined by AAS (n = 6; mean ± SD).

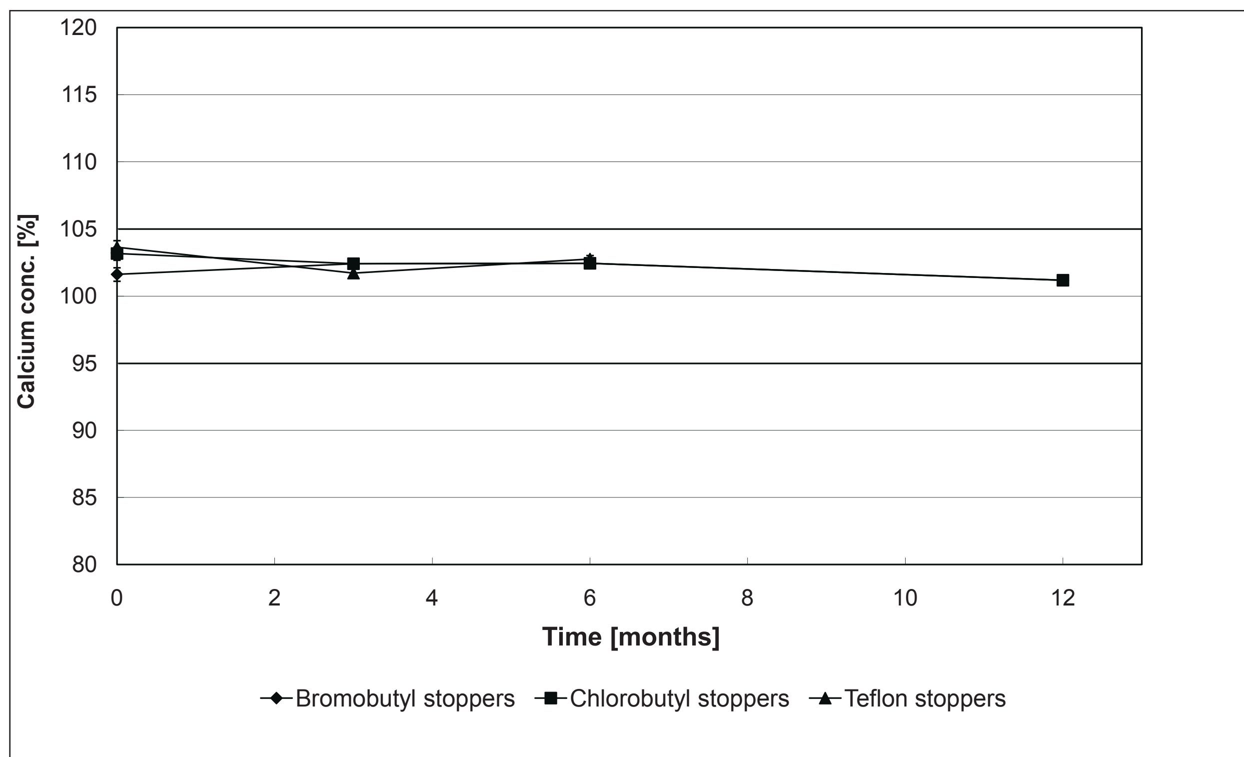

Figure 6: Calcium concentrations, determined by AAS, during storage under accelerated conditions at 40 °C (n = 6; mean ± SD).

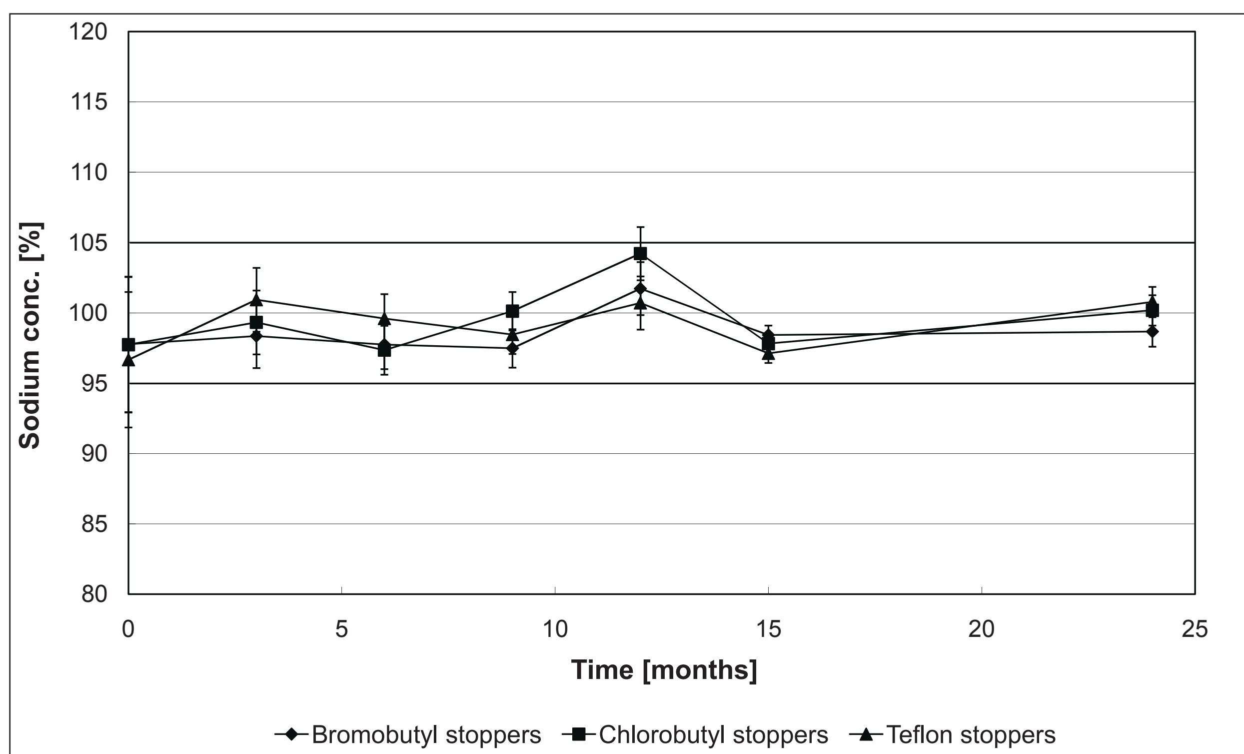

Figure 7: Sodium concentrations during storage under room conditions determined by AAS (n = 6; mean ± SD).

In Figure 5 and Figure 6 the calcium concentrations of all sample varieties under normal and accelerated conditions, respectively, are displayed. There were no differences concerning closure variety at both temperature levels and no differences in content between these levels. The same observations are applicable to the sodium content as can be seen from Figure 7 and Figure 8, even though the standard variations were higher. The determined content varied over time, but sample contents stayed within the limit of ± 5 % of the nominal content and no clear tendency of progressive lower or higher values could be detected. Concerning the potassium content, (see Figure 9 and Figure 10) no difference between storage conditions or closure variety was detectable.
Therefore, the variations were attributed to dose variations within the vials under investigation and scattering in the analytical methods.
The conclusions in respect of the developed formulation are as follows: the used closure varieties did not influence the stability of the formulation or the content of any of the used ingredients. There was no detectable temperature influence, as storage at 40 °C over a year did not lead to any detectable degradation or variation in content.
All analysed concentrations stayed within the set limits of ± 5 % of the declared content. Resulting from the above data, it is recommended to use chlorobutyl rubber closures as they have the same qualities as the teflon coated closures and are economically more viable. The bromobutyl rubber closures should not be used, as the solutions in contact displayed an elevated pH after storage. This should be avoided, as larger volumes of solutions with deranged pH values may cause irritation and pain for the patient.

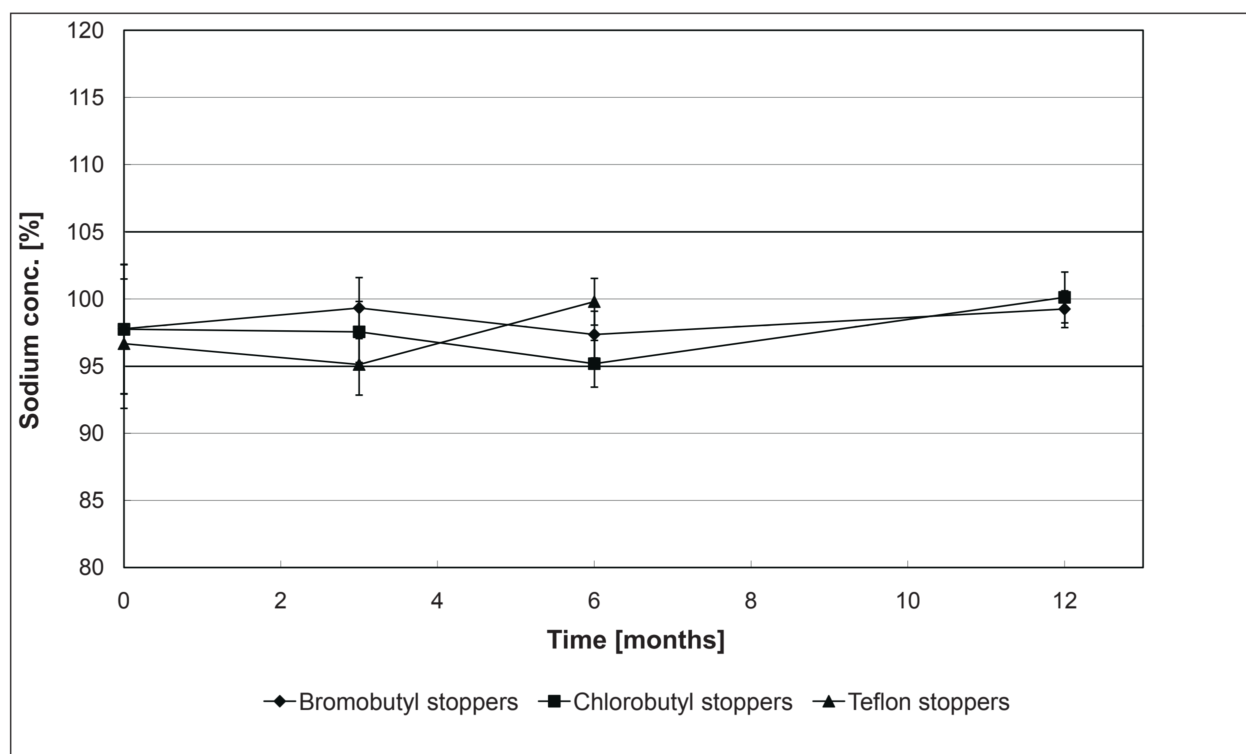

Figure 8: Sodium concentrations, determined by AAS, during storage under accelerated conditions (n = 6; mean ± SD).

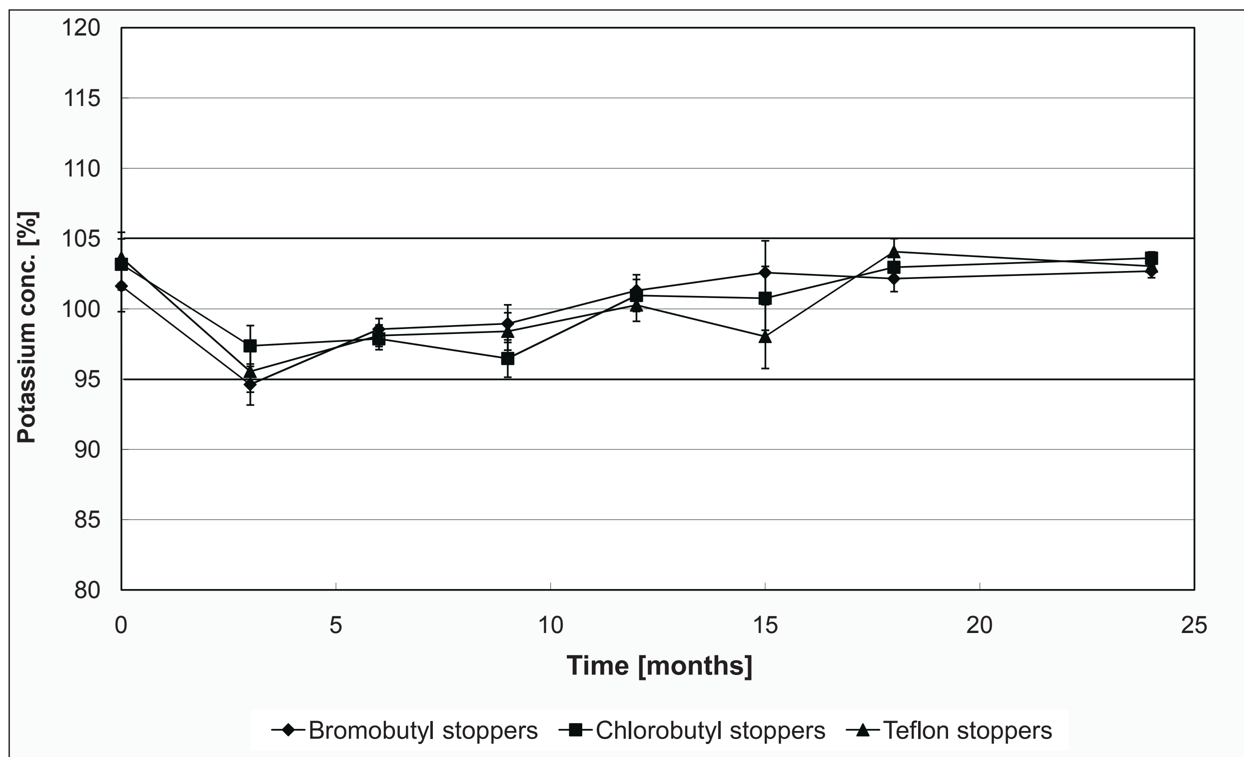

Figure 9: Potassium concentrations during storage under room conditions determined by AAS (n = 6; mean ± SD).

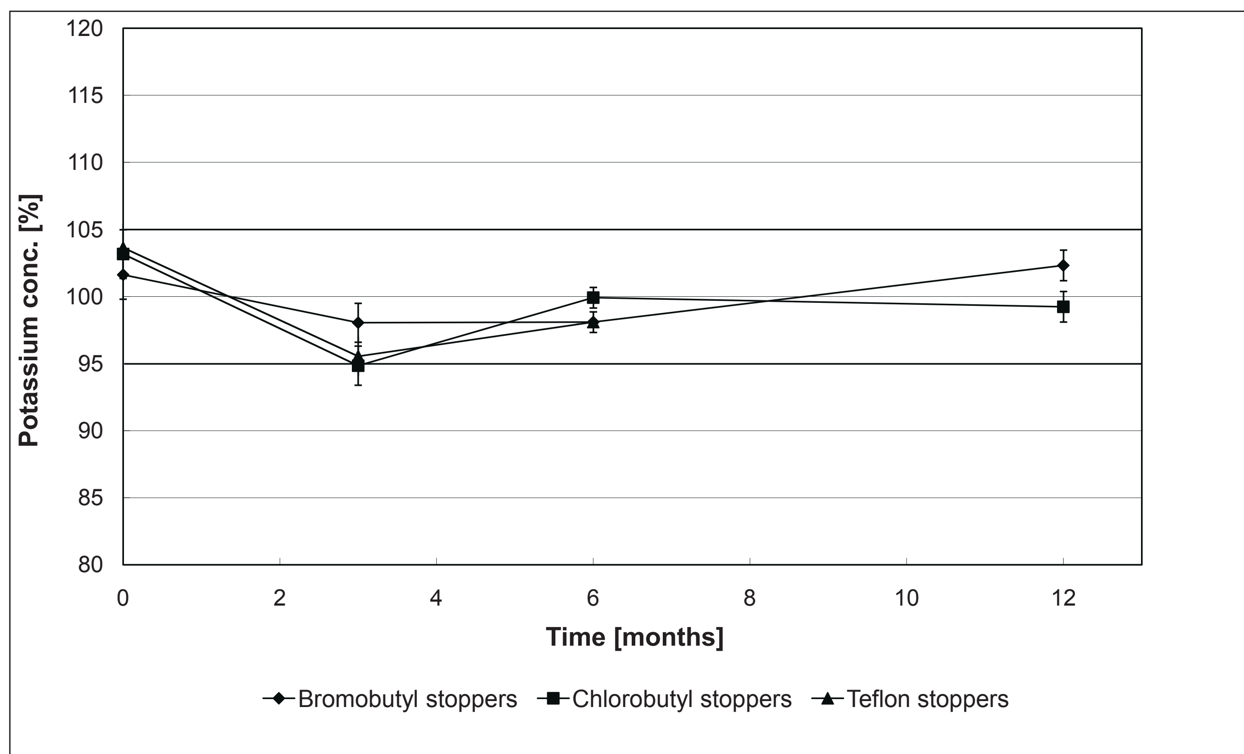

Figure 10: Potassium concentration, determined by AAS, during storage under accelerated conditions (n = 6; mean ± SD).

2. Multiparticulate formulations

2.1 Extrusion/spheronisation

2.1.1 Introduction

Pellets are increasingly used, multiparticulate drug dosage forms. The term pellet is used by a number of industries to describe a variety of agglomerates. Pellets in the pharmaceutical context are defined as isometric agglomerates with a smooth surface, a narrow size distribution and typical diameters of 0.2 to 2 mm (Kleinebudde 1997). They have several advantages in comparison to conventional monolithic dosage forms. The gastrointestinal transit time is more uniform and less sensitive to food influences. The inter- and intraindividual variability is therefore reduced. Due to the spherical shape, they are ideal particles for the application of coatings. The danger of intoxications due to dose-dumping through leakage of applied functional coatings is minimized. As the drug is released over a larger area within the gastrointestinal tract, local irritations and side effects are reduced.

In the case of paediatric drug delivery pellets have several additional benefits. If a liquid formulation, which is the dosage form of choice for under 2-year-olds (see Table 2), is not applicable as in the case of sodium benzoate due to taste issues, small pellets can be sprinkled on food and be given even to small children. Furthermore, they are also the dosage form of choice for peroral administration to over 2 year old children (EMEA reflection paper "Dosage forms of choice for the paediatric population" 2003). Dose adaptation for this very heterogeneous patient group is easy compared to monolithic dosage forms.

There are several preparation methods for pellets. One is the direct pelletisation, either wet pelletisation using a liquid binder or melt pelletisation using higher temperatures to melt the binding agent. This is usually performed in high-shear mixer/granulators or in fluidised bed granulators. Another method is the layering of seeds which leads to heterogeneous pellets with a core and a shell. Several techniques can be used for the production as there are (rotor) fluidized bed granulators or pelletisation discs (Kleinebudde 1997). In this work, pellets were produced by an extrusion/spheronisation process.

Extrusion is a process where plasticized material is pressed through defined openings. It is not only used in the pharmaceutical industry, but also in the food, ceramics and polymer industries. The type of extruders can be divided into several main classes: screw, sieve and basket, and roll and ram extruders (Vervaet et al. 1995). Screw extruders have one or two screws that feed the plasticized mass to an axial or radial extrusion die. The twin screw extruders can have counter- or co-rotating screws, where the co-rotating screws have the advantage of being self-cleaning. The extent of pressure built up in axial screw extruders depend on screw geometry, process parameters, mass properties and die design. The radial set up leads to a low pressure extrusion.
Extrusion can be differentiated into two process types, wet- and melt-extrusion. Producing pellets through a wet extrusion/spheronisation process includes several steps. A powdered material/formulation is wetted and blended, extruded to form cylindrical extrudates, which are then spheronised to form spherical pellets. The last step is the drying of these pellets. This is a critical process step, as the drying method influences the particle size, surface structure and porosity of the pellets (Kleinebudde 1994, Bashaiwoldu et al. 2004). The advantages are the possibility of high drug loads (> 95 %), a narrow size distribution with a defined size (0.2 to 3 mm), highly spherical pellets with a high density and a smooth surface, a small volume despite high doses, high

throughput and the suitability for compression into tablets. Pellet characteristics can be modified by variation of composition and processing parameters.

In melt-extrusion, heat is applied to achieve the needed viscosity to press material through the die plate. To plasticize the formulation the material is fully or partially molten. As a solvent-free preparation method this is advantageous as there is no residual solvent burden and it is applicable for hygroscopic drugs. One drawback is the influence of heat stress on thermal sensitive drugs. This can be overcome by a process where no thermal influence is required to press the formulation through the die plate. The recently introduced extrusion of formulations containing lipids can be performed at room temperature as the applied pressure during the extrusion process is enough to plasticize the material. A “cold” extrusion of pellets on the basis of lipids becomes possible (Breitkreutz et al. 2003). So far only prolonged release pellets based on lipid binders have been described in the literature (Reitz 2007).

The point of origin for this part of the work has been a previous study about paediatric drug formulations of sodium benzoate (Breitkreutz et al. 2003). In this work, granules with a lipophilic binder, i.e. the hard fat Witocan 42/44, containing 80 % sodium benzoate have been prepared. However, the prepared granules were not spherical but more cylindrical shaped. These were now used as a comparison, as one aim of this study was to improve the previously developed formulation to achieve spherical pellets with the same high drug load with preferably low toxicity excipients in a simple extrusion/spheronisation process. Wet extrusion pellets prepared with MCC and κ-carrageenan were compared to lipid pellets prepared by cold extrusion. All pellet batches were characterised through image analysis and compared in their roundness, size and size distribution. Furthermore dissolution studies were performed to analyse differences in release behaviour, as suitable immediate-release pellets should be obtained.

2.2 Wet Extrusion

2.2.1 MCC and κ-carrageenan

Microcrystalline cellulose (MCC) has been used as a standard extrusion/spheronisation aid for decades as it can be added in small quantities to various formulations to achieve the desired pelletisation properties (Conine et al. 1970, Reynolds 1970). It has been widely investigated to understand its mechanism in the wet extrusion/spheronisation process (Kleinebudde 1997b). However, there are some disadvantages associated with MCC formulations, as some drugs may adsorb to MCC which will alter the dissolution time (Okada et al. 1987) or cause non-disintegration of the pellets (Kleinebudde 1994). Therefore a search for alternative extrusion aids, which are able to overcome the problems associated with MCC while maintaining the positive properties of MCC, is going on. One suitable alternative is the relatively novel excipient carrageenan (Bornhöft et al. 2005). Carrageenans are acid polysaccharides which are produced from red seaweeds. They are often used in the food industry as thickener, binder and stabiliser. It was shown that κ-carrageenan can be used as a possible substitute for MCC, as spherical pellets with short disintegration times and fast drug release, independent from drug solubility, could be obtained (Thommes et al. 2006).

To compare wet extruded formulations to those obtained through cold lipid extrusion, 20 % of binder and 80 % of sodium benzoate was always used. Furthermore in these preliminary experiments, the option of exchanging the therapeutically administered sodium benzoate through benzoic acid was explored. Benzoic acid is physiologically equivalent to its sodium salt form and offers the advantage of reducing the sodium burden for affected children, who often suffer from

hypernatremia. The aspect ratio (length-width ratio) was used to describe the sphericity of the pellets. As seen in Figure 11 appropriate spherical pellets with AR < 1.2 could be obtained with MCC, and by changing the active ingredient from salt to the acid form for κ-carrageenan. Especially in contrast to the lipid formulation with Witocan as a binder it is obvious that it is much easier to produce spherical pellets with the classical wet extrusion techniques. The differences between the κ-carrageenan batches with sodium benzoate and with benzoic acid as active components can be explained by the fact that κ-carrageenan strongly interferes with sodium ions which affects the gel forming capacity (Wittgren et al. 1998). Even though benzoic acid proved to be superior to sodium benzoate in terms of the roundness of the pellets and the narrow size and shape distributions it proved to be inappropriate in the subsuqent drying process due to its high vapour pressure. During the drying step, which is needed to evaporate the residual water, benzoic acid partly vaporises and the resulting pellets display sponge-like structures, which also can be found in dried extrudates (Figure 12), whereas sodium benzoate containing pellets and extrudates display smooth surfaces (Figure 13). Content uniformity referred to the labelled claim could not be achieved. Therefore benzoic acid was rejected as an active component for the subsequent studies. The pellet and batch properties of the formulations obtained through wet extrusion are displayed in Table 6.

Table 6: Pellet and batch properties of different formulations.

Formulation	Carrageenan	MCC	Carrageenan BA	MCC BA
Binder (20%)	κ-carrageenan	MCC	κ-carrageenan	MCC
Active (80%)	Sodium benzoate	Sodium benzoate	Benzoic acid	Benzoic acid
Equivalent dia. (mm)	1.47 ± 0.24	1.42 ± 0.15	1.25 ± 0.19	1.10 ± 0.27
Loss on drying (%)	41.7 ± 0.5	37.9 ± 0.4	92.2 ± 2.8	54.9 ± 2.2
Yield 0.63-2.0 mm (%)	96	98	99	99
10%-interval (%)	60	80	59	42
Aspect ratio	1.3	1.11	1.07	1.08

Pellets with sodium benzoate have slightly bigger equivalent diameters than those containing benzoic acid. The higher loss on drying in pellets with benzoic acid can be explained by the aforementioned fact, that benzoic acid vaporised during the drying step. Therefore, not only the loss on drying, but also the loss on active substance was determined. Next to size and shape of pellets another important property for the pellet quality is a narrow particle size distribution. The 10% interval is used to describe the particle size distribution, which is based on the dimensionless diameter. It contains the fraction of pellets within the 0.9 – 1.1 interval of the dimensionless diameter. This fraction was calculated for all formulations. It is independent from the production method thus allowing a batch-to-batch comparison. The size distribution was characterised as "good" if the fraction of the 10% interval exceeds 50% (Thommes et al. 2006). All batches except the one with benzoic acid and MCC as a binder passed this criterion and therefore were regarded to have a sufficient narrow size distribution.

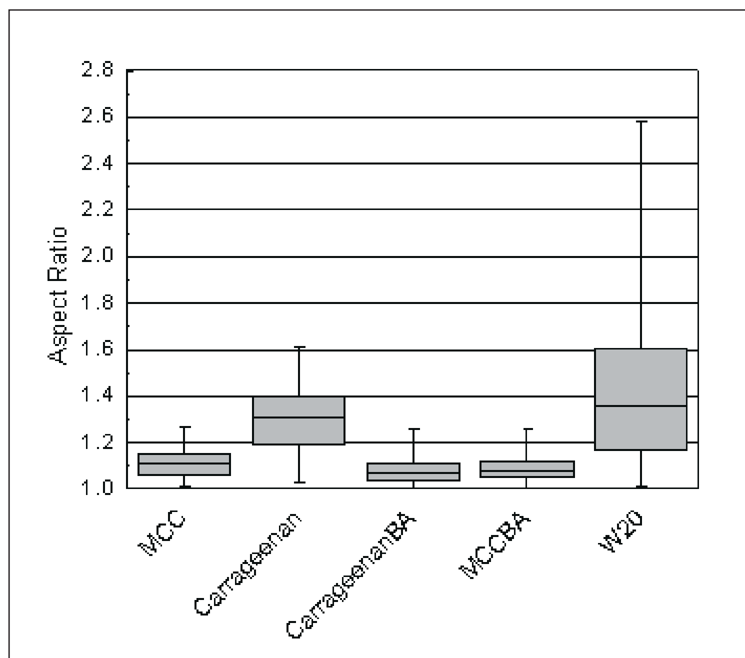

Figure 11: Aspect ratios of different formulations (x_1, x_{25}, x_{50}, x_{75}, x_{99}, n>500) for pellets made by conventional wet extrusion and comparison to formerly produced lipid pellets with 20 % Witocan 42/44 as a binder (W20). For formulation composition see Table 6.

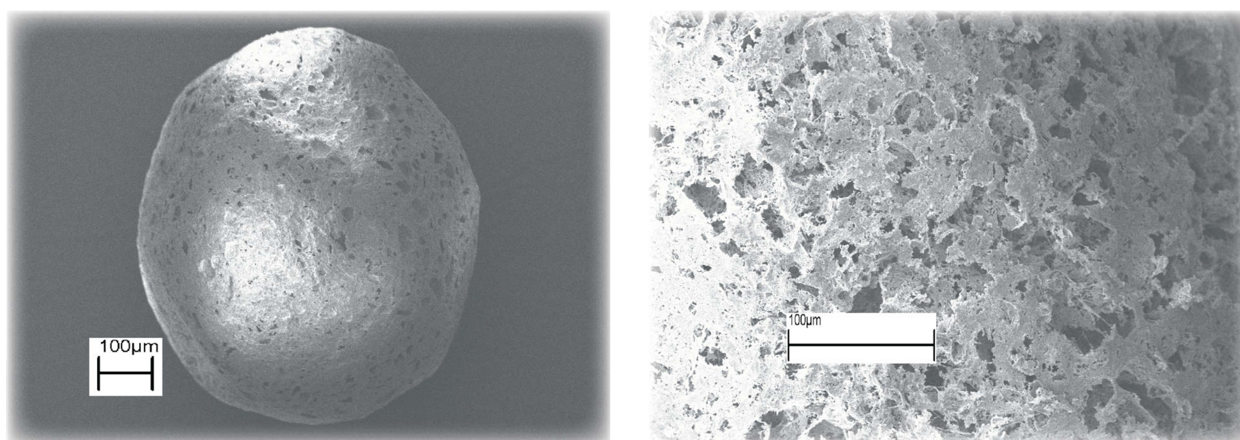

Figure 12: Scanning electron micrographs of a dried pellet (left) and dried extrudate (right) with 80 % benzoic acid and 20 % MCC. Scale bar represents 100 µm.

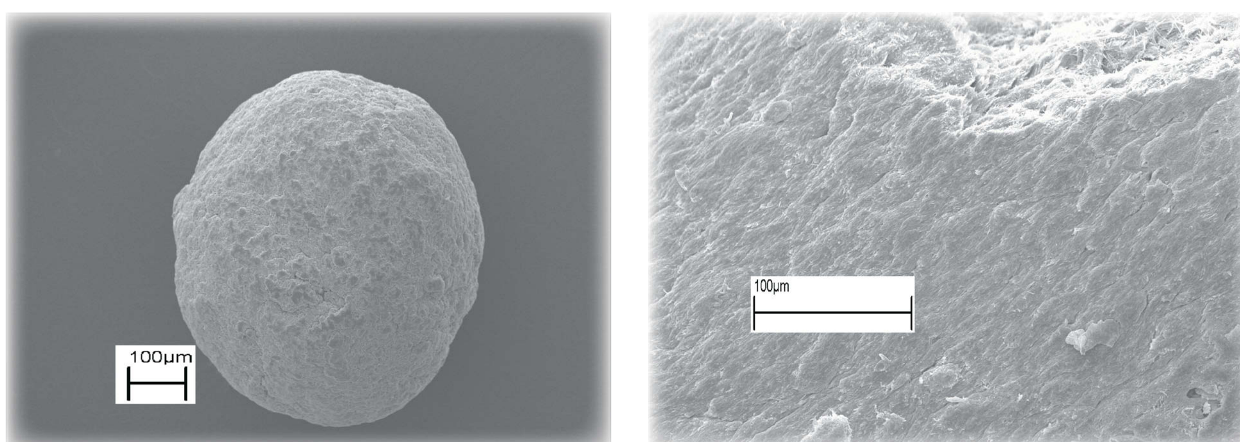

Figure 13: Scanning electron micrograph of a dried pellet (left) and dried extrudate (right) with 80 % sodium benzoate and 20 % MCC. Scale bar represents 100 µm.

As a conclusion the batches of sodium benzoate with MCC and κ-carrageenan were included in the dissolution studies as a reference, but were not regarded as a therapeutic alternative. As mentioned before, benzoic acid is highly volatile and the use of κ-carrageenan is limited due to safety reasons. As the prescribed doses of sodium benzoate are extremely high – 250-750 mg/kg BW per day – only those substances can be used as excipients, which are recognised as safe in almost all doses (Breitkreutz et al. 2003). The database of food additives (JECFA) evaluated by the World Health Organization (WHO) limits the acceptable daily intake (ADI) for κ-carrageenan as 75 mg/kg BW. This means that even without calculating special paediatric safety margins, the highest possible dose of sodium benzoate would be 300 mg/kg body weight per day, which in some cases is less than half of the required daily dose. Furthermore the UK Food Advisory Committee recommended the removal of carrageenan as an additive in infant food formulas, because of its ability to induce inflammatory responses in animals (MAFF 1992). In contrast, MCC has no limitations for ADI, but can be absorbed in the intestinal tract through persorption (Pahlke et al. 1974, Kotkoskie et al. 1996). Due to physiological characteristics in the paediatric population the risk is expected to be higher in children. The lipid binders appear as a valuable alternative to the commonly used extrusion aids in the special case of high dose sodium benzoate formulations.

2.3 Solid Lipid Extrusion

2.3.1 Formulation variation

Four lipids with different nature were chosen as binders for lipid pellet formulations. All formulations included Witocan 42/44, a powdered hard fat, previously used for the production of salvia-resistant coated sodium benzoate granules. It has the lowest melting range of 42-44 °C. The basic formulation was therefore, analogue to the work from Breitkreutz et al. (2003), 80 % sodium benzoate and 20 % hard fat. The 20 % hard fat fraction was then varied to binary mixtures of lipids by exchanging 5 % through either glycerol dibehenate (Compritol 888 ATO), glycerol trimyristate (Dynasan 114) or glycerol distearate (Precirol ATO 5). Furthermore, ternary mixtures were prepared by exchanging the 5 % through 2.5 % of two of these lipids. The compositions of all 7 formulations are displayed in Table 7.

Table 7: Composition of formulations based on lipids as binders.

Formulation	Sodium benzoate [%]	Witocan 42/44 [%]	Compritol 888 ATO [%]	Dynasan 114 [%]	Precirol ATO 5 [%]
W20	80	20			
W15 C 5	80	15	5		
W15 D 5	80	15		5	
W15 P 5	80	15			5
W15 C 2.5 D 2.5	80	15	2.5	2.5	
W15 P 2.5 C 2.5	80	15	2.5		2.5
W15 P 2.5 D 2.5	80	15		2.5	2.5

W Witocan 42/44 P Precirol ATO 5 D Dynasan 114 C Compritol 888 ATO

Numbers indicate the percental fraction of the lipid in the formulation

2.3.2 Process parameters

The same process parameters were used for all formulations to allow batch to batch comparison. The powder feed rate was always 40 g/min extruded with 50 rpm through a 2.5 mm die plate with 23 holes of 1 mm in diameter. The extruder was cooled to 25 °C at all times. The simple screw configuration with only conveying elements was chosen to allow an easier adaptation to other extruder types. The conveying elements were inserted in order of decreasing pitch length (from 40 mm to 20 mm). Where the high pitch values provided a homogeneous feeding and filling of the extruder barrel and the decreasing pitch values enabled a proper pressurisation of the material towards the die. The extrusion process was found to be very robust. None of the tested formulations showed any problems during extrusion and suitable smooth extrudates were produced. Under the process conditions the extrusion aids were not thoroughly melted. The different melting points of the chosen lipids are shown in Table 17 and typical DSC curves with the melting peaks of the powdered lipids are displayed in Figure 14. Hence, binding of the drug substance is achieved by softening or melting of small parts of the binder, but not by thoroughly molten excipients. The critical production step in the preparation of lipid pellets proved not to be the extrusion, as all lipids, irrespective of their nature and individual melting points, were plasticized through the pressure at the die plate and produced smooth extrudates.

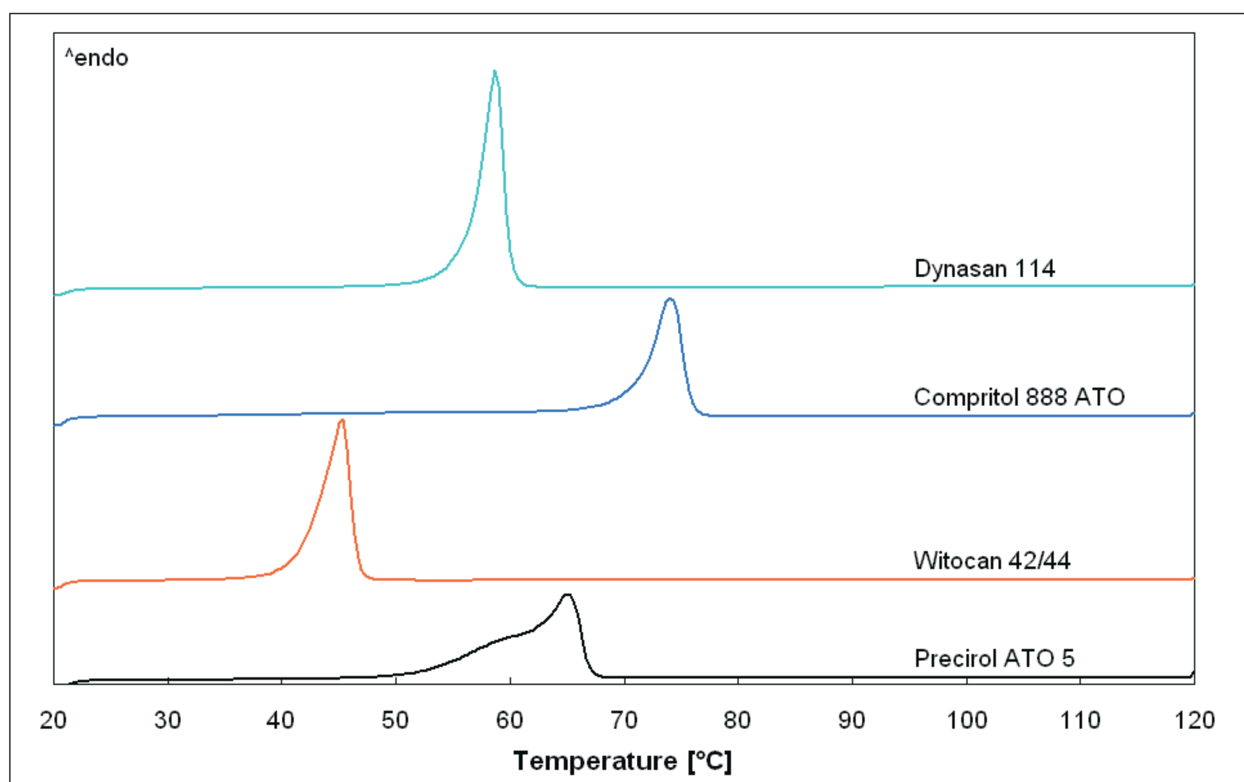

Figure 14: DSC tracks of untreated lipid powders, recorded at a heating rate of 10 K/min. All signals are scaled to sample weight.

2.3.3 Spheronisation

The critical process step with these lipid formulations proved to be the spheronisation process. To find the optimal spheronisation parameters to obtain spherical pellets, the basic formulation consisting of 80 % SB and 20 % hard fat was rounded after extrusion at different spheroniser temperatures, speeds and for different time intervals. Three temperatures (25 °C, 33 °C and 40 °C) and three spheronisation plate speeds (750 rpm, 1000 rpm and 1500 rpm) were investigated. 300 g samples were spheronised for up to 15 min. Representative samples were analysed by image analysis.
Various parameters are used to characterise the shape of pellets as a quality property (Hellen 1993, Podczeck et al. 1999). The parameter most commonly used and well suited for pellets produced through extrusion/spheronisation is the aspect ratio (AR), a length-width ratio (Bouwman et al. 2004). A mean aspect ratio lower or equal to 1.2 was considered as sufficient for pharmaceutical pellets. Aspect ratios above that value were regarded as insufficient (Kleinebudde 1995) as an indication of cylindrical granules, but not pharmaceutical pellets. The influence of spheronisation time and friction plate speed at a temperature of 33 °C is displayed in Figure 15. Temperatures far below the melting point of the binder proved to be insufficient as well as temperatures too close to it. Aspect ratios resulting from those experiments are not displayed. Either no pellets were obtained, but rather extrudate fragments due to insufficient energy introduction, or the extrudate fragments/cylinders started to agglomerate quickly due to melting of the lipid binder. In this study a spheroniser temperature of 10 °C below the melting point of the binder, i.e. 33 °C, revealed the best results. An increase in spheronisation time and a higher elevated speed of the friction plate resulted in lower aspect ratios.

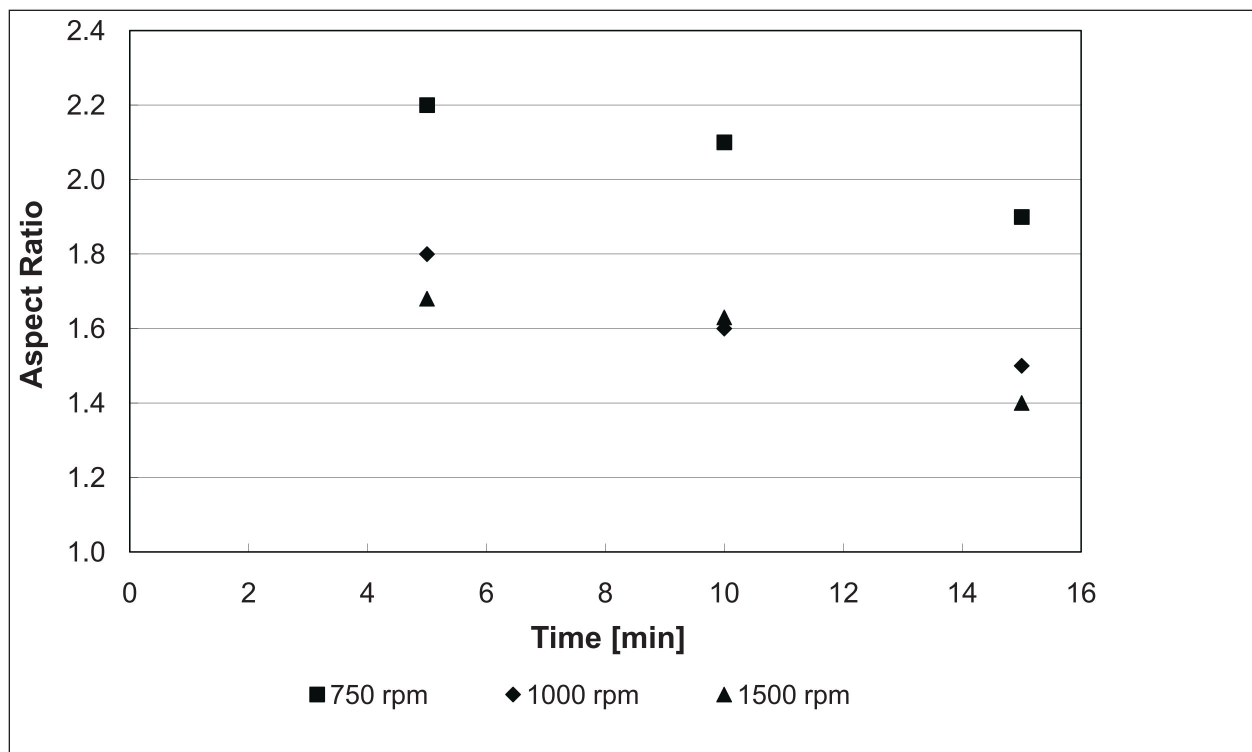

Figure 15: Influence of spheronisation time and speed on the median aspect ratio of a pellet formulation with 80 % sodium benzoate and 20 % Witocan 42/44 at a spheronisation temperature of 33°C.

As a result of these findings further spheronisation for all batches was set to 1500 rpm, the highest possible rotating speed of the spheroniser plate, for 15 minutes at 33 °C.

2.3.4 Pellet form, size and size distribution

The pellet and batch characteristics for all produced formulations are given in Table 8. The equivalent diameter for lipid pellets ranges from 0.89 to 1.65 mm, depending on the used lipid mixture whereas the formulations with MCC and κ-carrageenan ranged from 1.1 to 1.47 mm.
Three formulations exceeded the 50 % limit for the 10 % interval and were therefore rated as "good". This coincided with the three lowest aspect ratios, which all were below 1.3. Only one batch displayed an AR <1.2 and therefore the combination of 15 % Witocan 42/44, 2.5 % Dynasan 114 and 2.5 % Precirol ATO 5 is assumed to be the best formulation regarding size distribution and shape. Concerning the characterised properties it is close to the pellets made from MCC or κ-carrageenan by wet extrusion. In Figure 16 box plots with the aspect ratios of pellets made from binary and ternary mixtures of different lipids are displayed. It is obvious that the adding of Compritol, which has the highest melting range of the investigated lipid, has a negative effect on the sphericity of the pellets. Four of the batches (two ternary and two binary mixtures) show better AR and smaller size distributions than the reference batch.
The comparison of the general methods, cold solvent-free solid lipid extrusion and traditional wet extrusion, shows that it is much easier to produce spherical pellets with the classical wet extrusion techniques. However, it is possible to achieve spherical pellets with a sufficient narrow size distribution also with lipid binders. Process parameters have to be closely adapted to the specific formulation, here especially in the spheronisation process. For instance the spheronisation temperature has to be precisely adjusted to obtain suitable pellet properties.

Table 8: Pellet and batch properties of different formulations (Batch coding as in Table 7).

Formulation	Equivalent dia. (mm)	Loss on drying (%)	Yield 0.63 - 2.0 mm (%)	10% - interval (%)	Aspect Ratio
W15 C5	0.94 ± 0.22	–	98	43	1.46
W15 P5	1.42 ± 0.33	–	97	43	1.31
W15 D5	1.65 ± 0.21	–	99	72	1.28
W15 C2.5 D2.5	0.89 ± 0.16	–	98	58	1.29
W15 P2.5 C2.5	1.45 ± 0.33	–	97	45	1.46
W15 P2.5 D2.5	1.61 ± 0.32	–	98	66	1.16
W20	0.96 ± 0.21	–	98	44	1.31

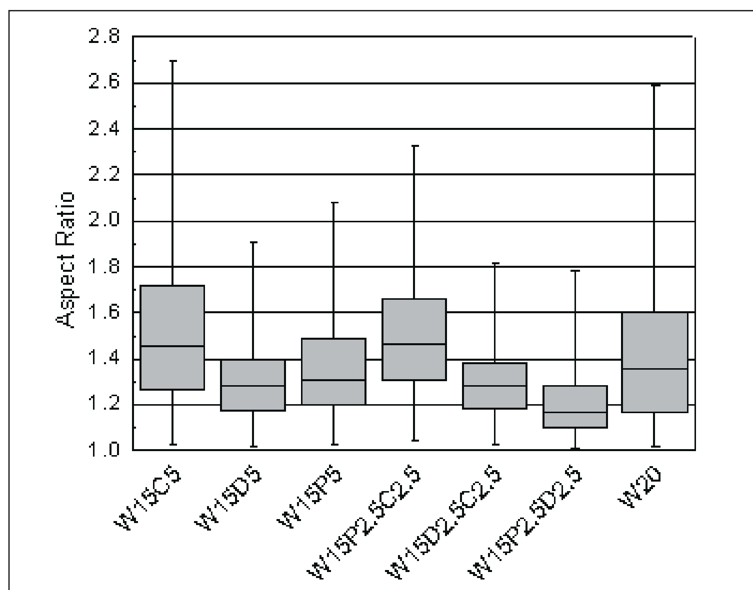

Figure 16: Aspect ratios of pellets (x_1, x_{25}, x_{50}, x_{75}, x_{99}, n>500) with different lipid mixtures made by solvent-free solid lipid extrusion.

2.4 Sodium benzoate dissolution

2.4.1 Dissolution media and process parameter

Dissolution tests were performed for all batches. The influence of different dissolution media, the temperature and the influence of storage conditions on the release profile of the lipid pellets were investigated. The basket method was employed to prevent the flotation of the pellets and to ensure complete wetting during dissolution. Preliminary investigations showed that the rotating speed of the baskets had no influence on the release profile and was therefore kept constant at 150 rpm in all tests. It is known that dosage forms based on lipids can change their release profiles over time (Sutananta et al. 1995) as for example thermal influence during extrusion/spheronisation processes can lead to polymorphic changes. As the potential to form immediate-release dosage forms was studied all pellet batches were stored for 12 months at room temperature before dissolution testing. This was done to ensure the transformation into stable forms. The focus was the investigation of steady release profiles and not intermediate forms that might still change to a varying dissolution profile.
The release of SB from MCC and κ-carrageenan pellets can be seen in Figure 18. As expected a fast release of more than 90 % of the drug within the first 10 min is achieved.
In Figure 18, the release from different batches with various lipid binders is presented. It is obvious that due to the lipid nature of the extrusion aids the release is modified compared to the pellets made by wet extrusion. Even so, all batches released more than 90 % in less than 2 hours. There is neither a clear definition for immediate-release dosage forms in the Ph. Eur. nor in the USP. A suitable range would be the release of at least 80 % in 60 min. If this is applied to the release from the presented lipid pellets, all formulations except one, the combination of hard fat, glycerol distearate and glycerol dibehenate comply and can therefore be described as immediate release dosage forms. Noticeable is furthermore the influence of glycerol distearate on the dissolution profile, as all batches containing this lipid have a release of more than 90 % in 40 min. Whereas the addition of glycerol trimyristate or glycerol dibehenate decelerates the release of the drug. The reasons for the general rapid drug release, compared to the recent reports on solid lipid extrusion (Michalk 2007, Reitz 2007), are the high drug load of 80 % and the very good water solubility of sodium benzoate.

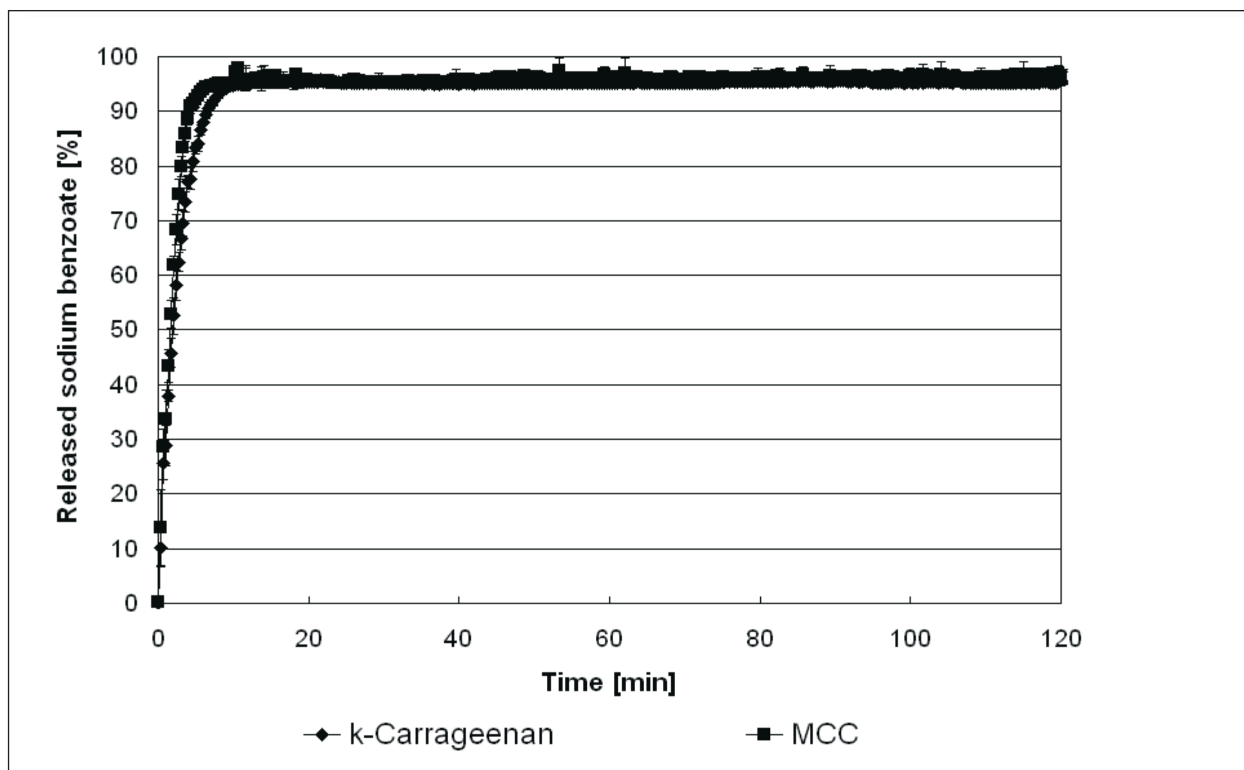

Figure 17: Sodium benzoate release from pellets made by wet extrusion.
Dissolution media: purified water (mean ± SD, n=6), Temperature: 37 ± 0.5 °C, 150 rpm, Basket method.

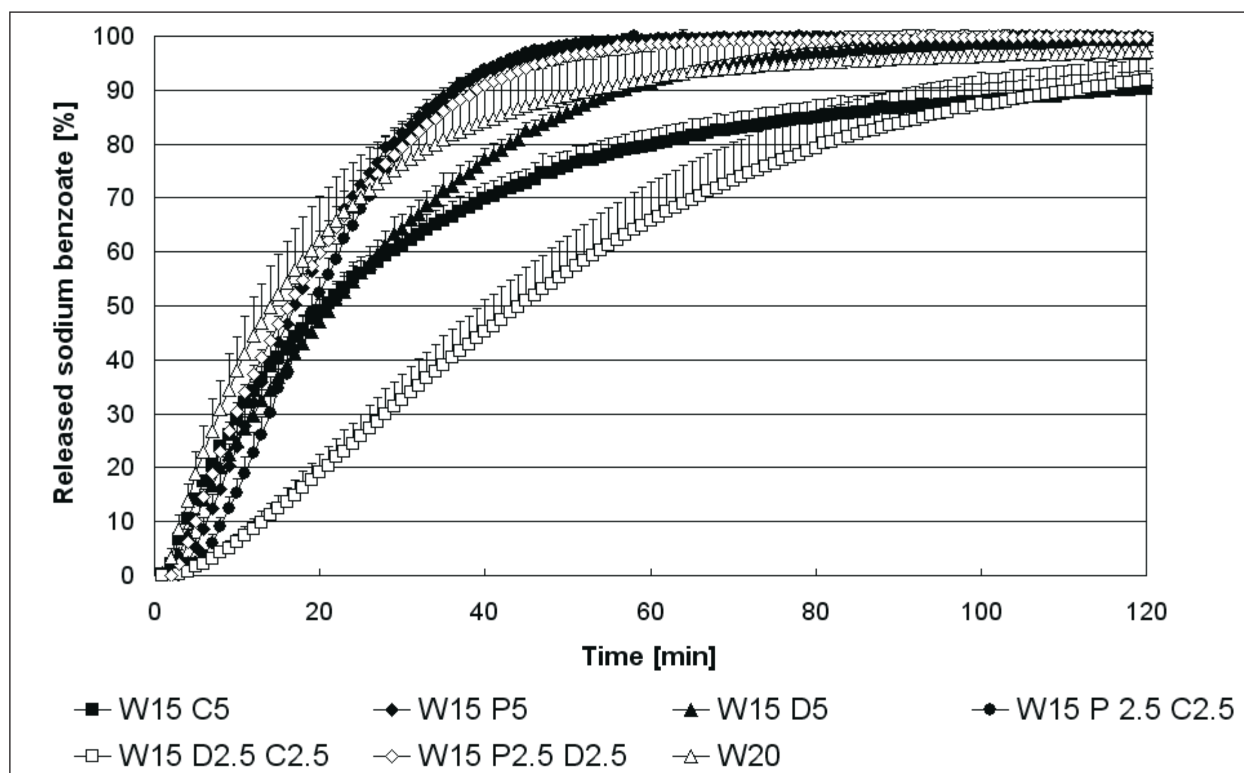

Figure 18: Sodium benzoate release from lipid pellet formulations.
Dissolution media: purified water (mean + SD , n=6), Temperature: 37± 0.5 °C, 150 rpm, Basket method.

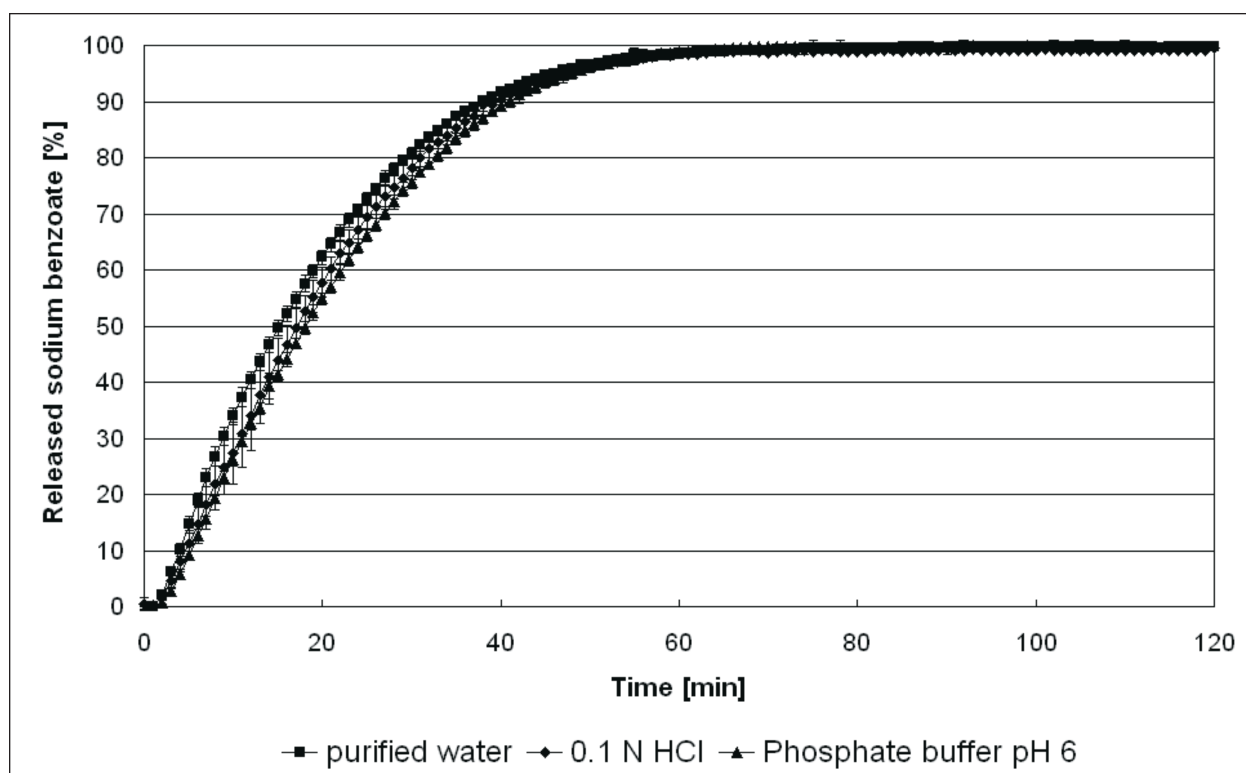

Figure 19: Influence of dissolution media type on release characteristics of lipid sodium benzoate pellets.
Formulation W15 P2.5 D2.5 (mean ± SD, n = 6) Temperature: 37± 0.5 °C, 150 rpm,
Basket method.

For the investigation of the pH influence on the release characteristics, 0.1 N HCl-solution with a pH of 1 and a phosphate buffer solution with a pH of 6 were also used as dissolution media. The phosphate buffer was chosen from the Ph. Eur. monography 2.9.25.: "Dissolution test for medicated chewing gum" as the release in the oral cavity should be simulated.
As displayed in Figure 19, exemplified by one batch, no differences between the drug release into different dissolution media could be detected at 37 °C. This applied to all batches regardless of the used lipid composition.
In contrast to the pH of the media, another parameter influenced significantly the release of lipid pellets: the temperature of the media.

2.4.2 Temperature effect

So far, the test performed with pellets consisting of SB and Witocan 42/44 revealed no influence of the temperature, as can be seen in Figure 20. During the studies on taste perception of different formulations (see Chapter 3) it was noted that lipid pellets stirred at room temperature showed poor release of sodium benzoate. To verify this observation, dissolution tests of lipid pellets at room temperature were performed. The preliminary test was performed with the optimal pellet batch containing the lipid mixture of hard fat, glycerol distearate and glycerol trimyristate. As can be seen in Figure 21, less than 5 % of drug was released under these conditions, whereas a temperature of 37 °C led to complete drug release within 60 min. To obtain further insight into the mechanism behind this phenomenon dissolution profiles between the basic data at 24 °C and 37 °C were investigated.

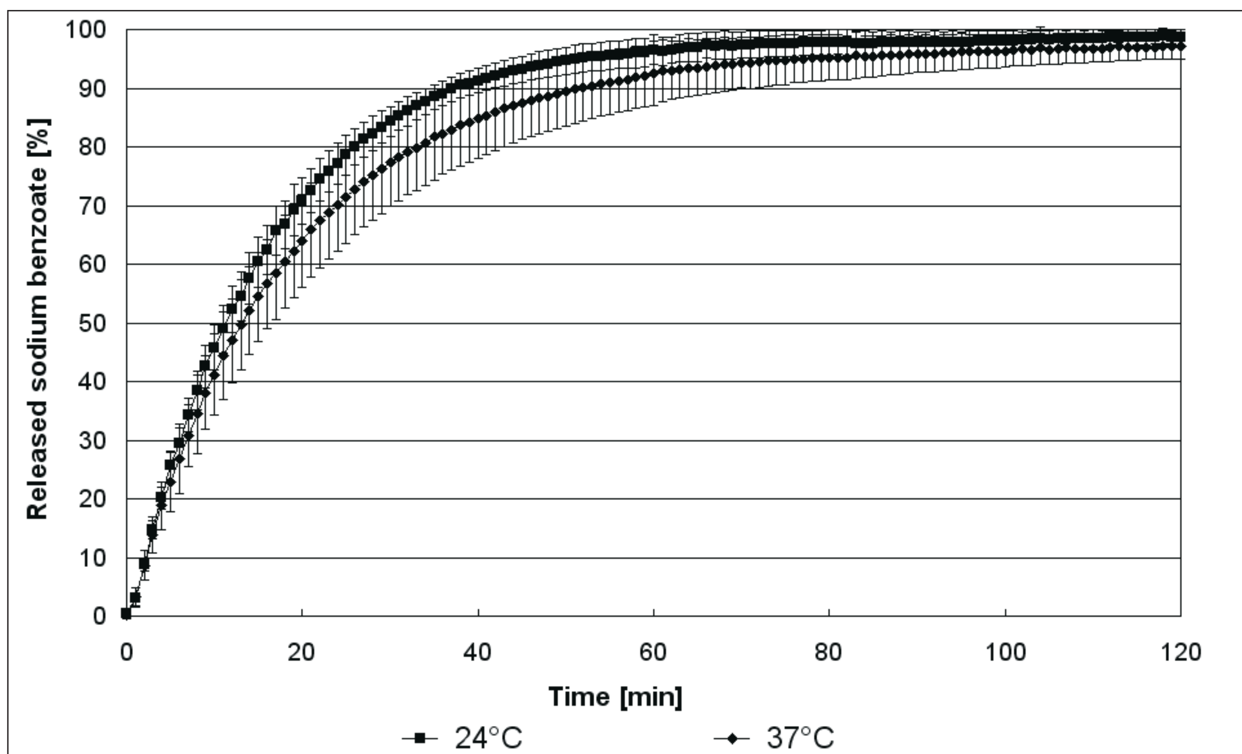

Figure 20: Influence of dissolution media temperature on release characteristics of lipid sodium benzoate pellets.
Formulation W20 (mean ± SD , n=6), Dissolution media: purified water, 150 rpm, Basket method.

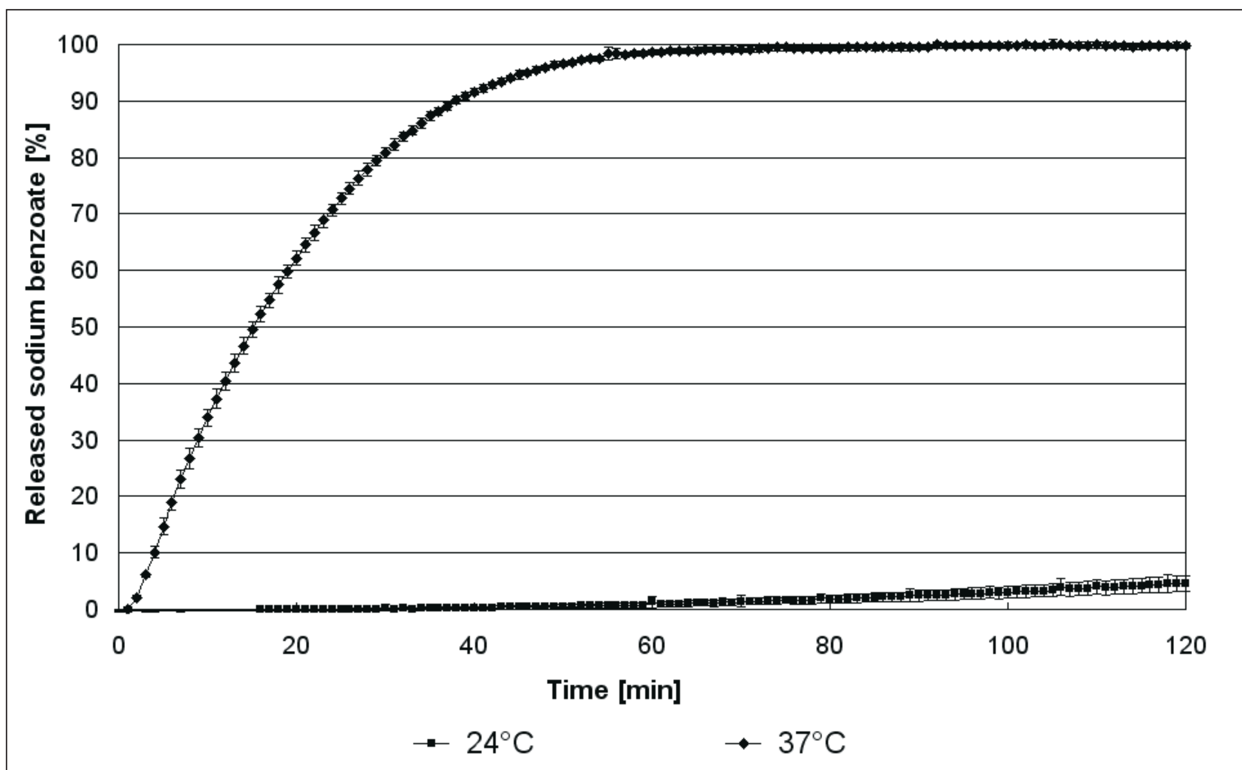

Figure 21: Influence of dissolution media temperature on release characteristics of lipid sodium benzoate pellets.
Formulation W15 P2.5 D2.5 (mean ± SD , n=6), Dissolution media: purified water, 150 rpm, Basket method.

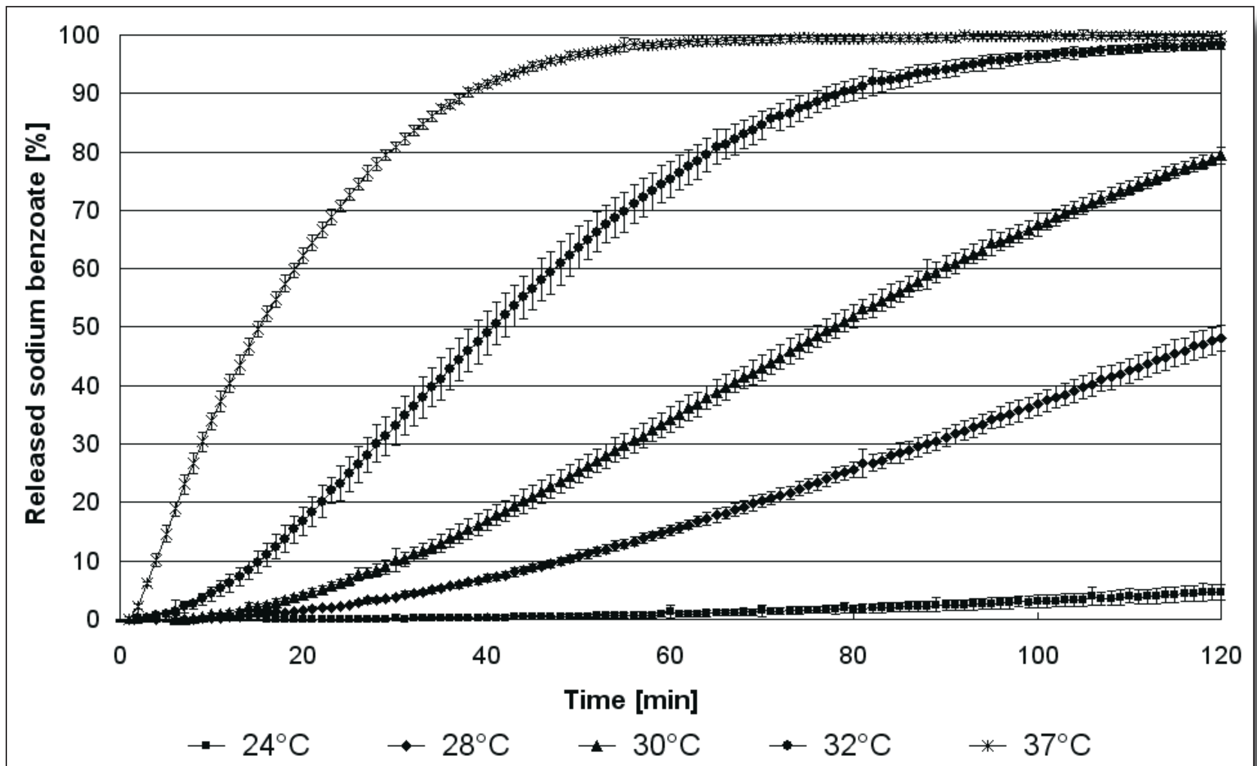

Figure 22: Influence of dissolution media temperature on release characteristics of lipid sodium benzoate pellets. Formulation W15 P2.5 D2.5 (mean ± SD , n=6), Dissolution media: purified water, 150 rpm, Basket method.

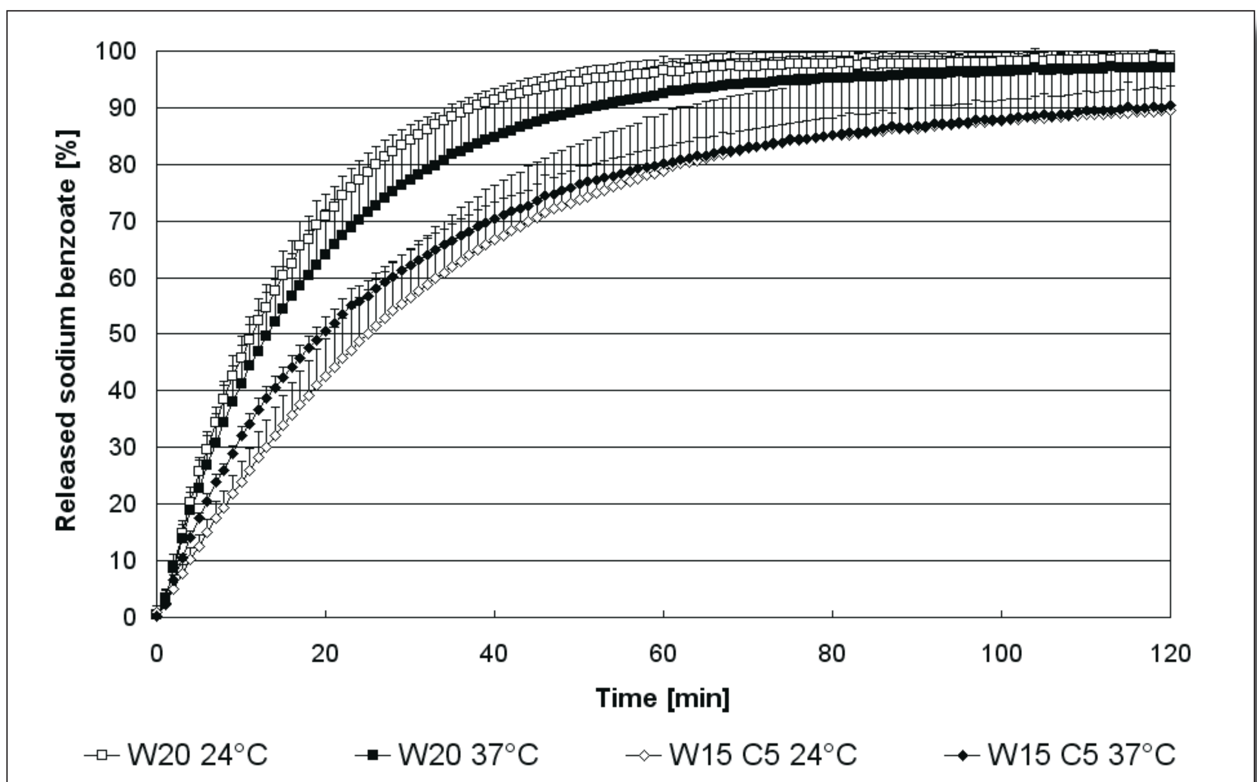

Figure 23: Influence of dissolution media temperature on release characteristics. Dissolution media: purified water (mean + SD, n=6), 150 rpm, Basket method.

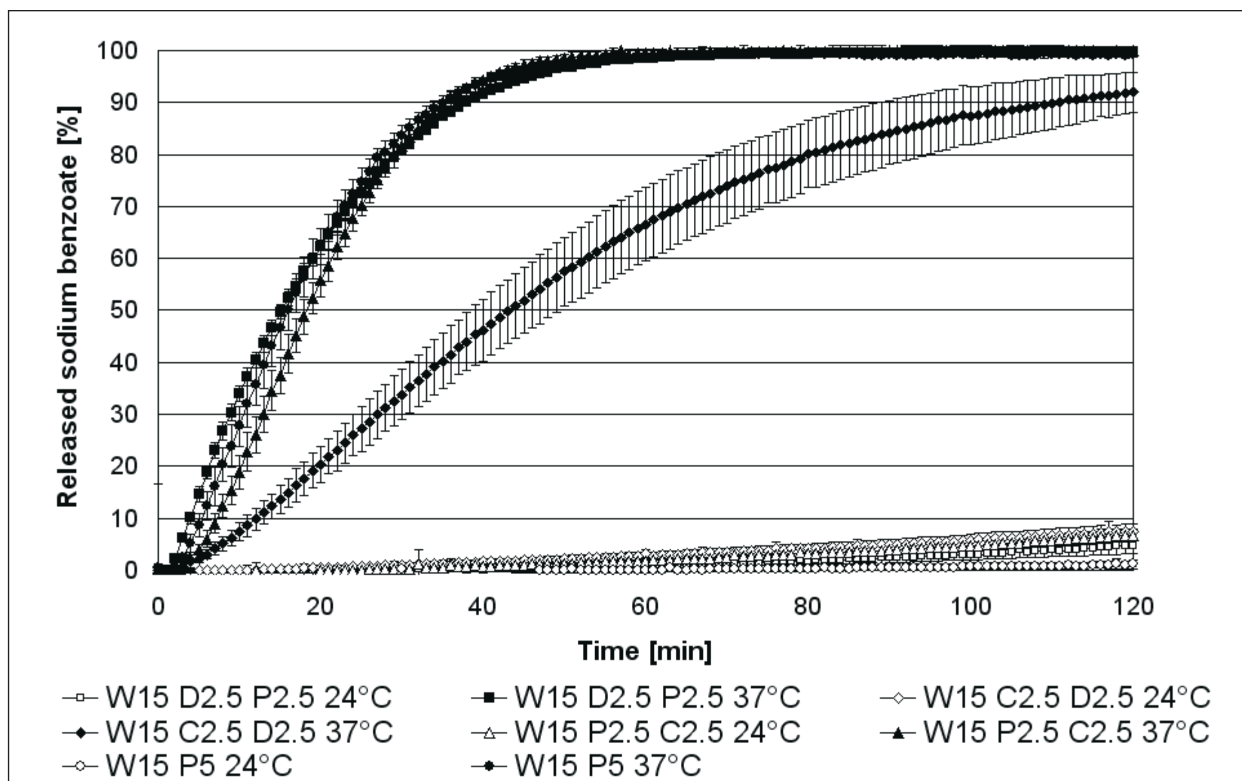

Figure 24: Influence of dissolution media temperature on release characteristics. Dissolution media: purified water (mean ± SD , n=6), 150 rpm, Basket method.

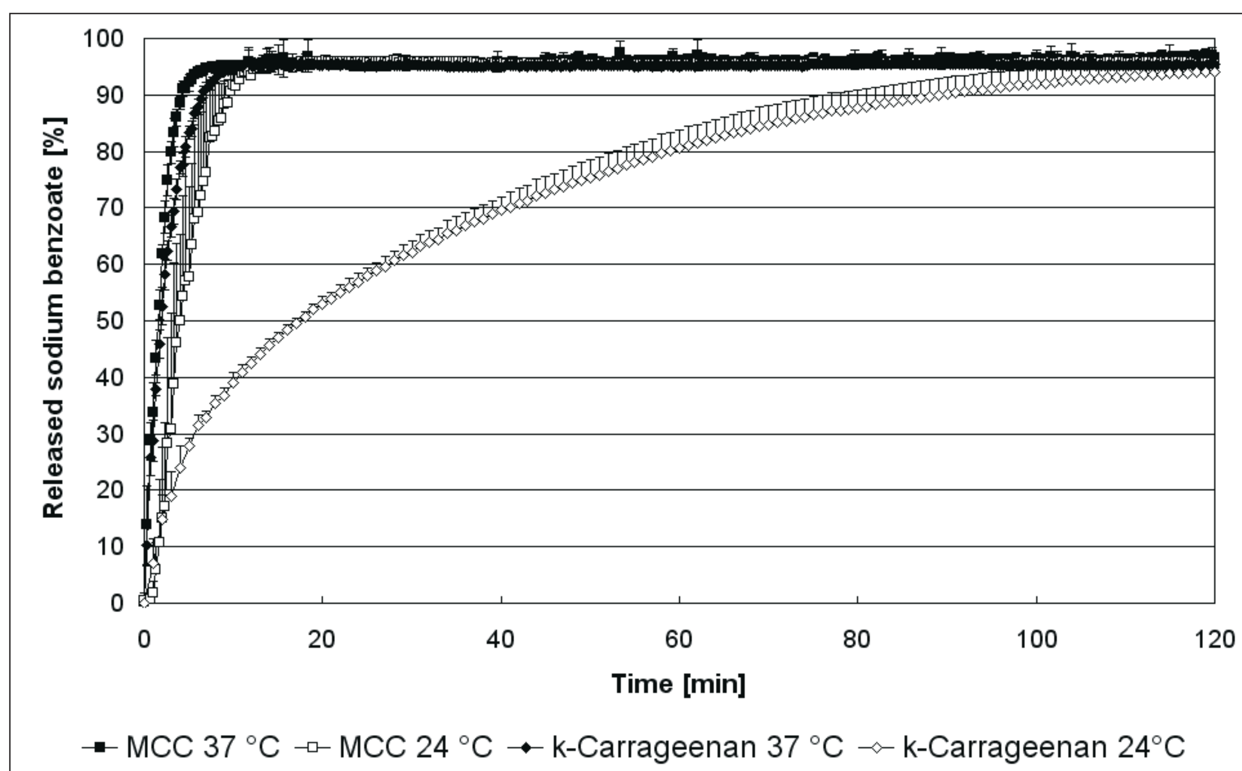

Figure 25: Influence of dissolution media temperature on release characteristics of sodium benzoate pellets. Dissolution media: purified water (mean + SD , n=6), 150 rpm, Basket method.

As Figure 22 shows, with an increase in temperature of the dissolution media, the drug release increases. There is no threshold temperature to reach maximum release, rather a gradual increase. Consequently all other lipid batches were also tested under these conditions and formulations could be divided in two groups. The pellet batches containing hard fat or a mixture of hard fat and glycerol dibehenate displayed no temperature sensitive release profiles.
As displayed in Figure 23 release profiles remained similar and did not vary by temperature change for these two batches. In contrast, as can be seen in Figure 24, the other two binary and three ternary mixtures exhibit a significant influence of media temperature on the dissolution characteristics. Less than 10 % drug release over two hours could be observed in any of these pellet batches.
As a comparison, also the pellets containing MCC and κ-carrageenan were investigated to evaluate the temperature influence. For MCC no effect of dissolution media temperature could be found in contrast to κ-carrageenan were the cold dissolution media led to a slower, but still complete release of the drug (see Figure 25).
Furthermore it became obvious that in contrast to the previously described missing influence of the dissolution media type on the drug release from lipid pellets, the release under room temperature conditions varies with the media type, depending on the lipid mixture. Three batches, two binary mixtures with hard fat and either glycerol trimyristate or glycerol dibehenate and the reference batch with 20 % hard fat, showed a delayed drug release in the acidic media of 0.1 N HCl, as can be seen in Figure 26. Sodium benzoate can form slightly soluble benzoic acid in acid environment. If benzoic acid is formed during dissolution, the pellet surfaces will be coated by the crystals and the release might be delayed through this effect. For all three ternary mixtures as well as the binary mixture of hard fat and glycerol distearate this effect was not observed.

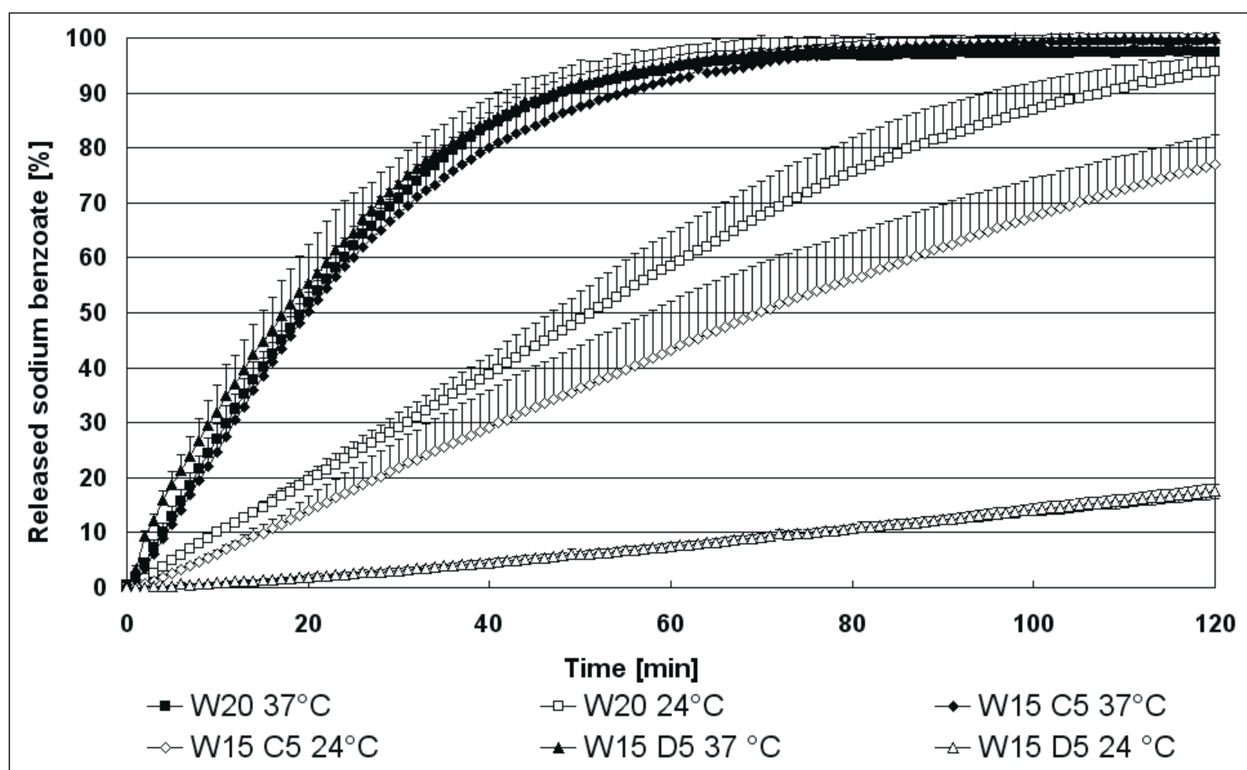

Figure 26: Influence of dissolution media temperature on release characteristics of lipid pellets. Dissolution media: 0.1 N HCl-solution pH 1 (mean + SD , n=6), 150 rpm, Basket method.

To further investigate the discovered effects, pellets were observed by scanning electron microscopy. The micrographs of lipid pellets, as can be seen in Figure 27, display several different surface features independently from the used lipid mixture. The lipid pellets have mostly a smooth surface, indicating molten lipids, which is repeatedly broken by pore-like structures. All pellet batches displayed these features irrespective of their temperature dependent dissolution. To further analyse differences in surface structure the pellets were also investigated after dissolution.

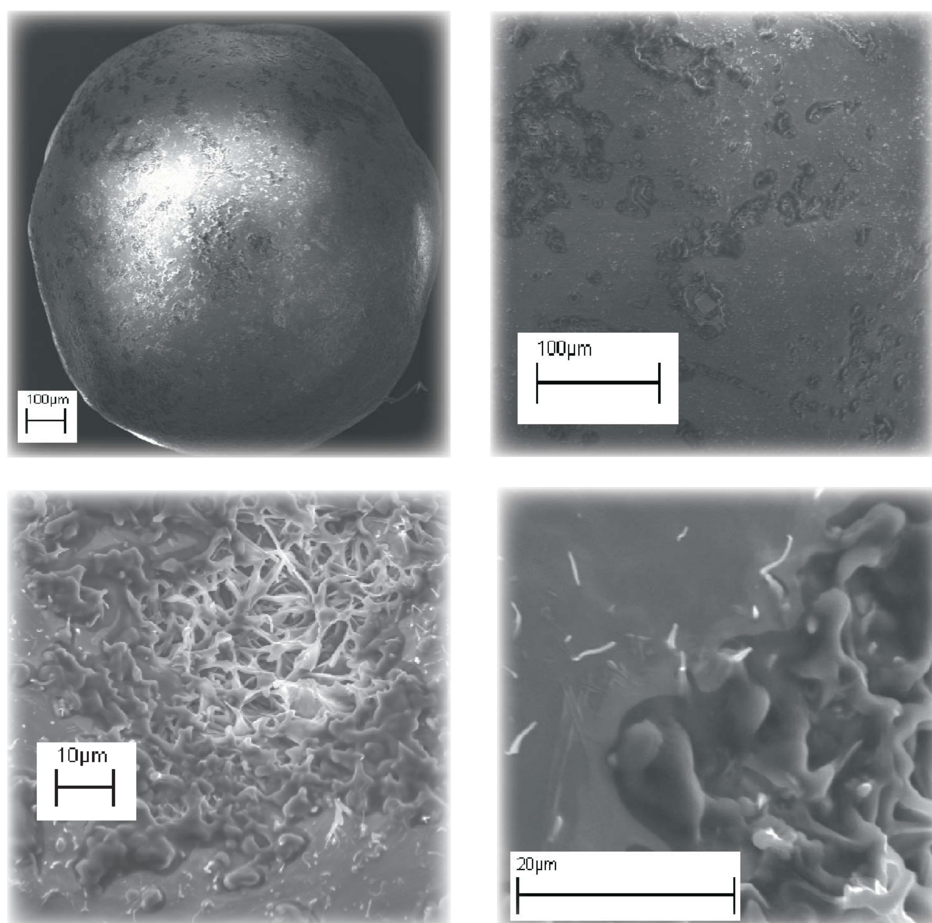

Figure 27: Scanning electron micrographs of the pellet formulation W15 P2.5 D2.5.

Scanning electron microscope analysis of lipid pellets before and after dissolution at different temperatures revealed interesting differences in the surface appearances.
Pellets released at a temperature of 24 °C show a similar surface structure as untreated pellets of the same batch (Figure 28). In smaller magnifications (40x, 100x; as adjusted at the microscope) no differences are apparent. Larger magnifications (400x, 1000x) show differences, like a flaked surface with more pores and needle-like structures, which are probably recrystallised sodium benzoate. In clear contrast to this are the surfaces of pellets after release in 37 °C media. Here even at small magnifications the pellets look shrunk and deformed. At a closer look none of the previous described features are present. The surfaces have a molten-like structure with no visible pores. As 37 °C is close to the melting point of the main binder Witocan 42/44, it is possible that the lipids start melting during dissolution forming such surfaces. Again, independently from the composition of the lipid mixture, the pellet surfaces have the same characteristics and features. An implication for the dissolution profile cannot be derived from the surface appearance.

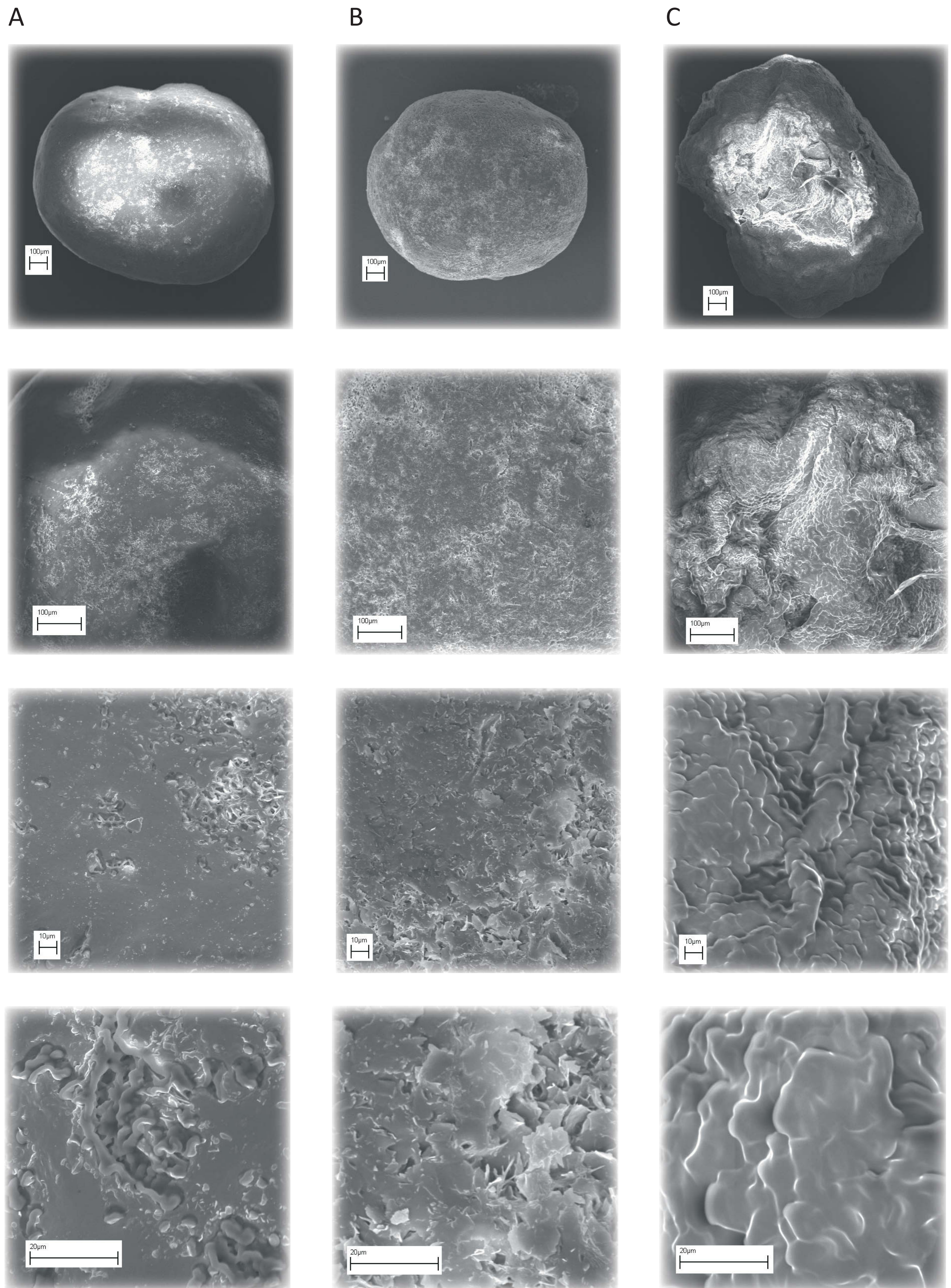

Figure 28: Scanning electron micrographs of a pellet formulation W15 D5.
A pellet after extrusion/spheronisation B pellet after dissolution at 25 °C C Pellet after dissolution at 37 °C.

2.4.3 Self-coating of the lipid pellets

To understand the mechanism behind the observed dissolution effects, especially the difference in the dissolution profiles at different temperatures, correlation with other pellet properties were investigated. The different dissolution profiles could not be correlated with the AR of the respective pellet batches. The same applied to the porosity of the pellets, which was approximately 4 %. Another potential influence, the melting point of the used lipids, did also not determine the dissolution profile, e.g. the exchange of 5 % hard fat (melting point of 42-44 °C) with glycerol dibehenate, the lipid with the highest melting point of 73 °C, resulted in approximately the same dissolution profiles which were not affected by temperature (see Figure 23).
It is noticeable that glycerol distearate has a significant impact on the dissolution profile, as even small amounts (2.5 %) of this lipid in a formulation alters the dissolution at room temperature. This becomes also apparent in DSC measurements, as all pellet batches containing Precirol, irrespective of the respective amount or lipid mixture, display the same peaks or shoulders in the DSC measurements, as can be seen in Figure 29.

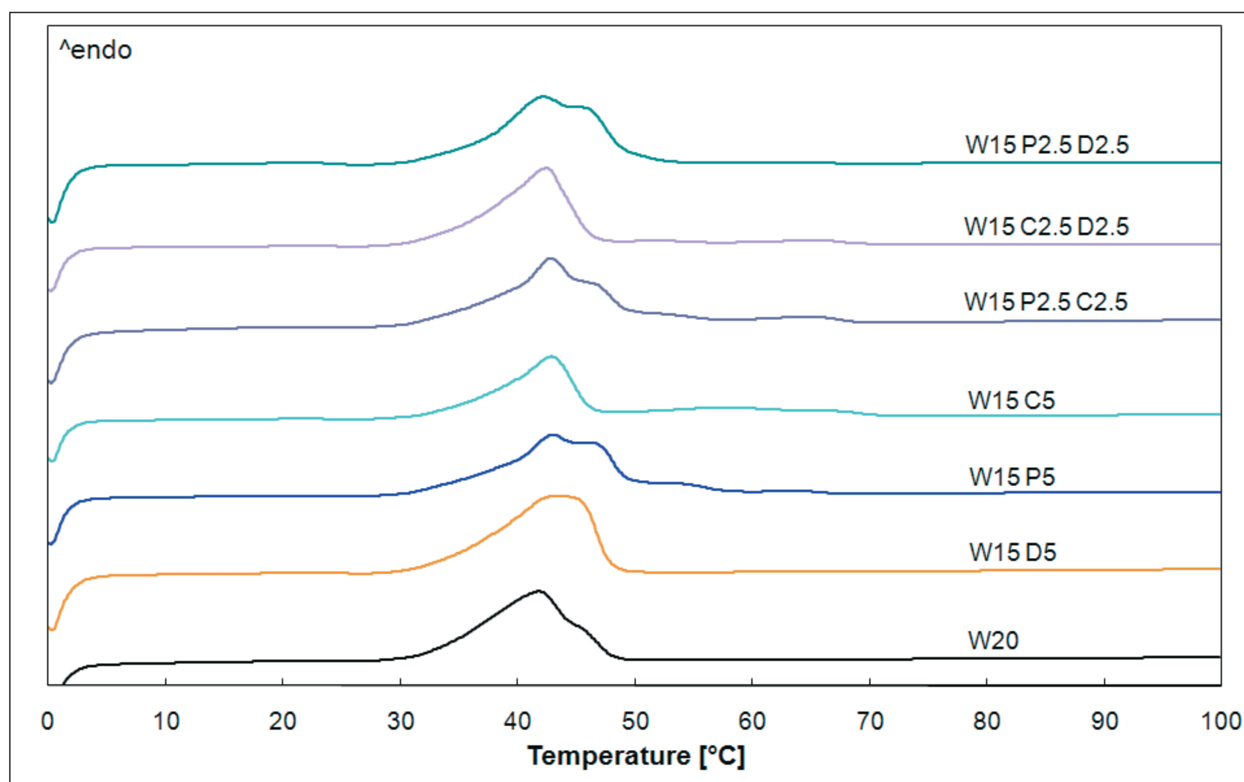

Figure 29:DSC tracks of different lipid pellets, recorded at a heating rate of 10 K/min. All signals are scaled to sample weight.

Furthermore the DSC measurements indicate that melting of the lipid binder is determined by the 15 % hard fat in all combinations. The 5 % change of hard fat to lipids with higher melting points did not lead to significant higher melting peaks in the respective pellets. The DSC curves for binary and ternary mixtures support this, as the melting curves of the lipid mixtures display peaks around or even lower than the melting point of the hard fat Witocan 42/44 both after the first and second heating of the samples. It is probable that the higher melting lipid binders dissolve in the molten or melting hard fat at temperatures around 40 °C as can be seen from Figure 30 and Figure 31. During spheronisation this may lead to a plasticization of all lipids in the outer parts of the pellets, regardless their melting points and initial composition.

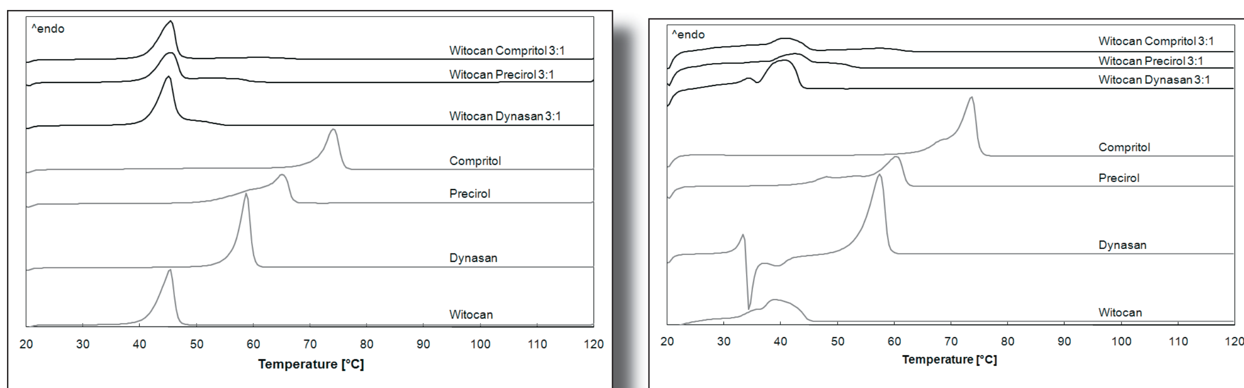

Figure 30: DSC tracks of binary lipid mixtures, first (left) and second (right) heating (10 °C/min). All signals are scaled to sample weight.

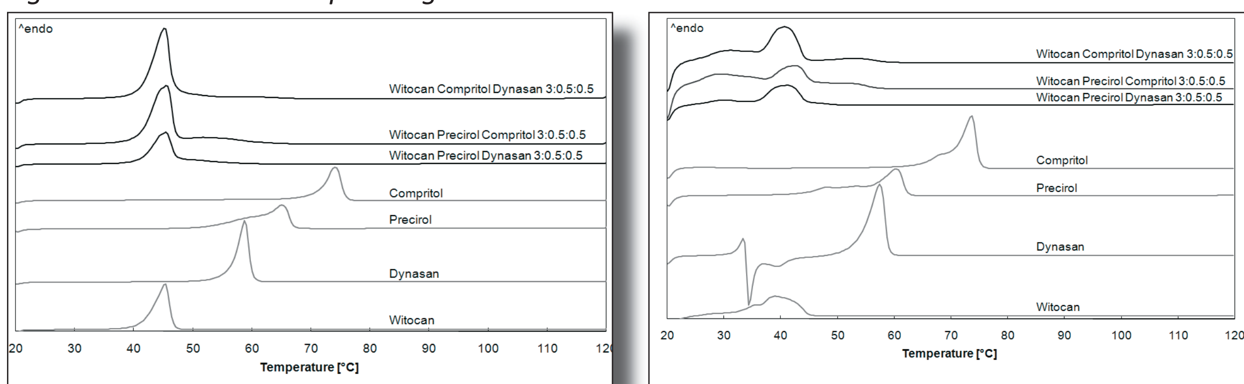

Figure 31: DSC tracks of ternary lipid mixtures, first (left) and second (right) heating (10 °C/min). All signals are scaled to sample weight.

A possible mechanism to explain the observed effects is the assumption of a lipid coating of the pellets. During the extrusion process, although no heat is used to plasticize the mixtures before the die passage, some parts of the lipid mixture melt due to the friction and pressure forces, especially on the extrudate surfaces. This effect is amplified in the spheronisation process where external heat and frictional heat of the pellet bed lead to partial melting of the binder and formation of spherical pellets.

A separation process between molten binder and the active crystalline drug takes places. The molten parts of the lipids layer in higher concentrations on the surfaces of the pellets, which leads to a coat of lipids around each pellet. The composition of this coat, i.e. the amount of higher melting lipids dissolved in hard fat, determines the release behaviour of the respective pellet batch. The layer beneath this coat is slightly depleted of lipids whereas the pellet cores have an even distribution of binder and active drug. This mechanism of self-coating through the lipid binder is supported by pictures obtained through scanning electron microscopy of split pellets. It has not been reported in the literature up to now. The term self-coating has been previously used by the working group of Ulrich to describe an effect during hot melt technology where higher concentrations of the active are found on the sides than in the core of pastilles (Römbach et al. 2007).

In Figure 32,A the plan view of a pellet half is displayed. The black arrows point to the visible layer all around the pellet which is detectable even at this small magnification (100x, adjusted at apparatus). The same structures are magnified in Figure 32,B (1000x, adjusted at apparatus), where an approximately 10 µm layer on the outer surface is clearly visible. In Figure 33 the same pellet structures are displayed at larger magnifications Again a distinct layer of approximately 10 µm

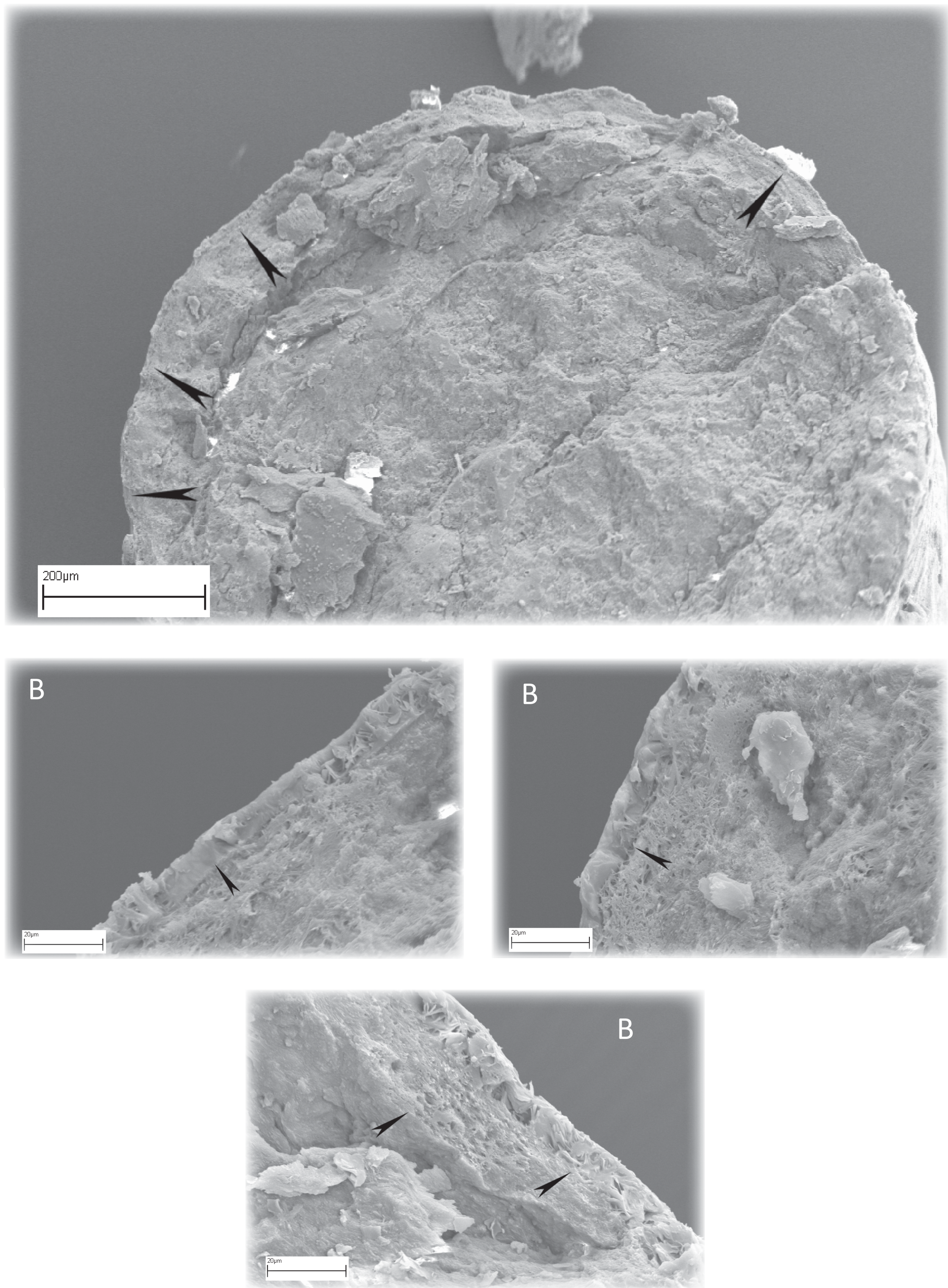

Figure 32: Scanning electron micrographs of a pellet containing 80% sodium benzoate, 15% Witocan and 5% Compritol (formulation W15 C5). A was taken at an adjusted apparatus magnification of 100 B were taken at an adjusted apparatus magnification of 1000.

can be distinguished on the surfaces of the pellet and beneath a thicker layer of different texture. The outer layer appears congealed and smooth, whereas the second layer has a rather rough and even needle-like structure. This supports the hypothesis that a layer of drug-free or nearly drug free binder forms the outer layer followed by a layer with depleted binder content and therefore increased drug concentration.

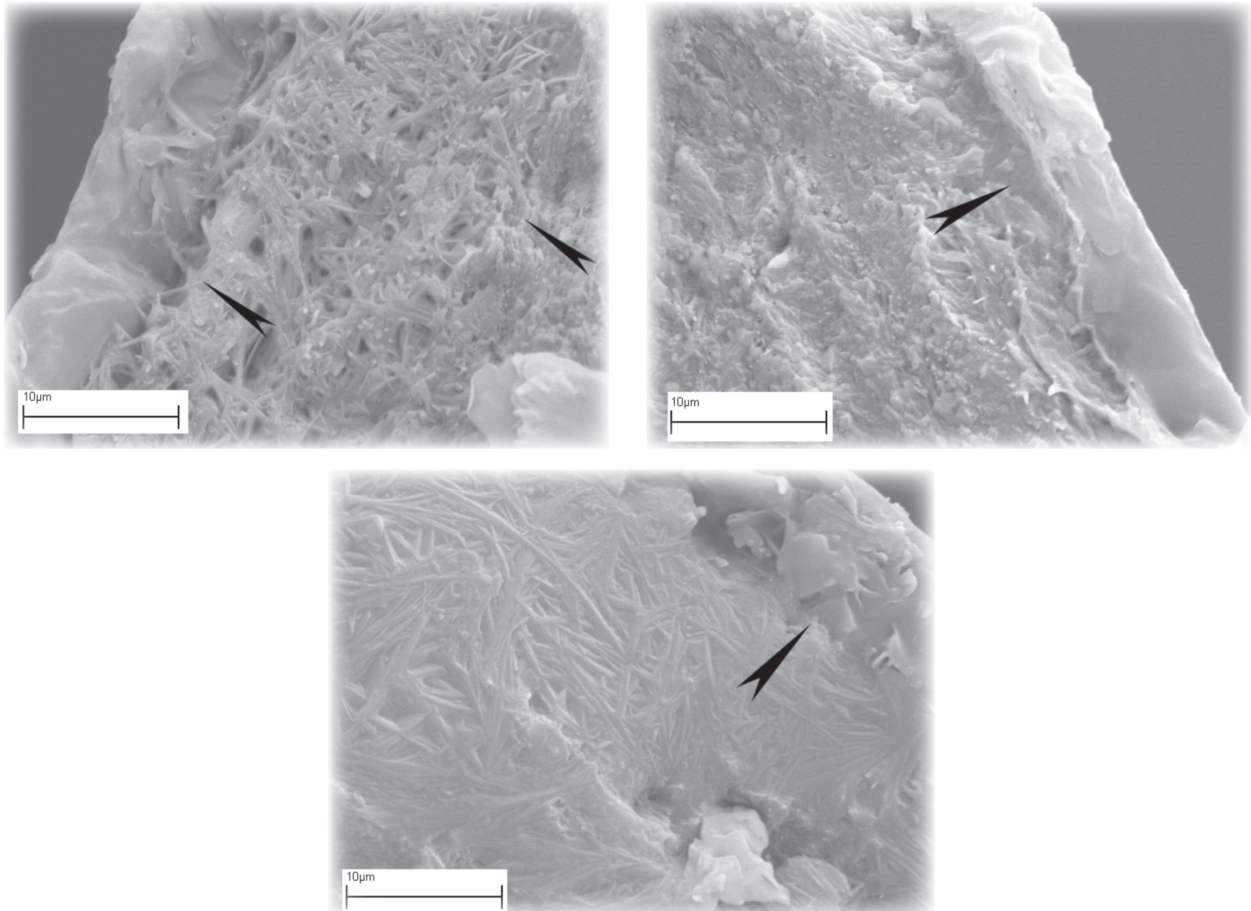

Figure 33: Scanning electron micrographs of a pellet (W15 C5) section with lipid layer and subjacent structures.

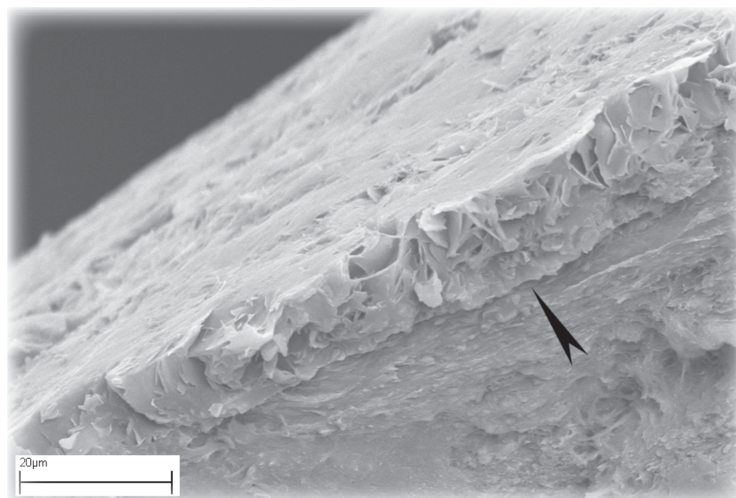

Figure 34: Scanning electron micrographs of a pellet containing 80% sodium benzoate, 15% Witocan and 5% Precirol (W15 P5).

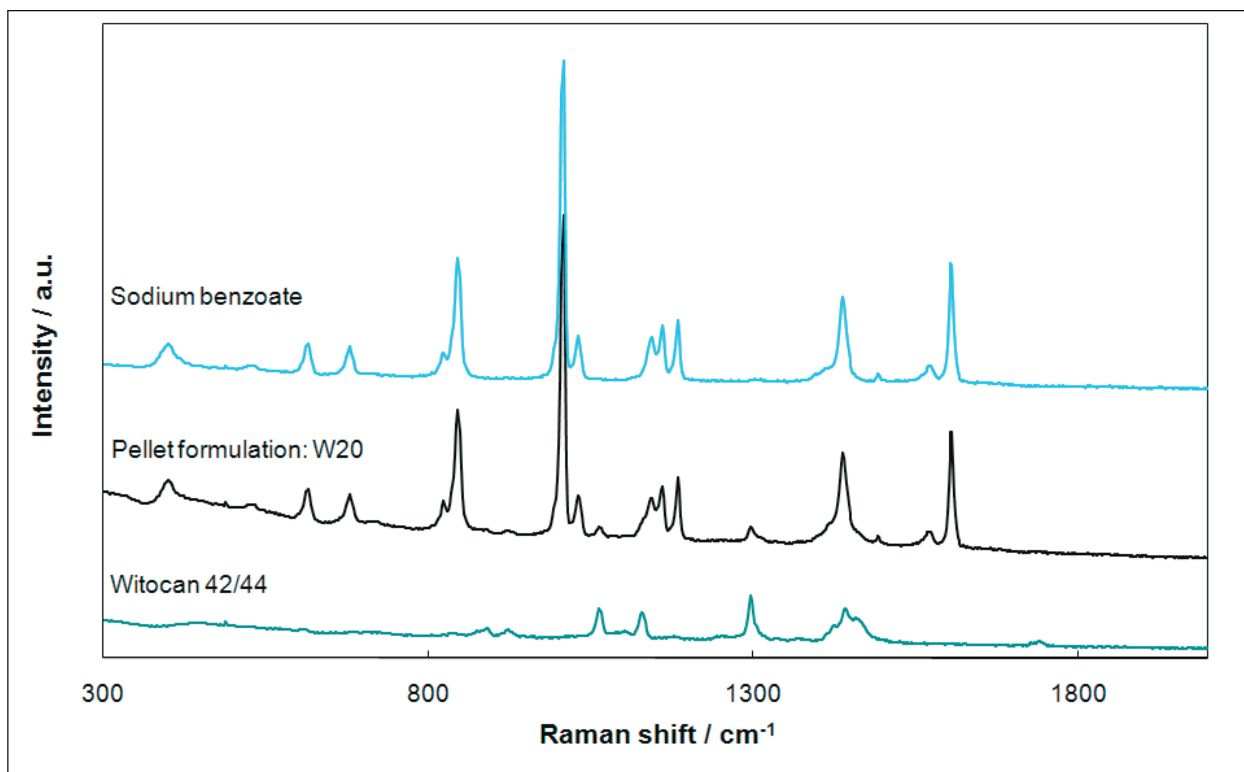

Figure 35: Raman spectra of pellet batch W20 and the respective excipients.

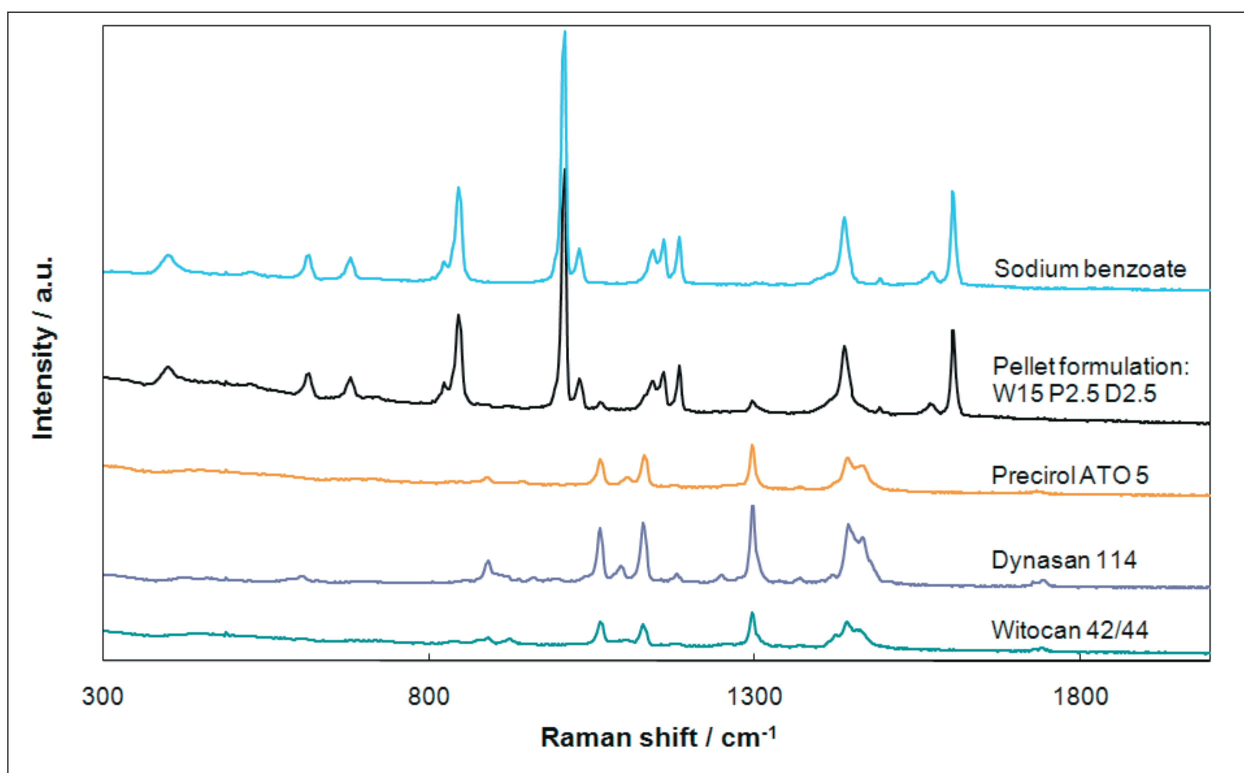

Figure 36: Raman spectra of pellet batch W15 P2.5 D2.5 and the respective excipients.

These aforementioned structures were found within all pellet batches disregarding their temperature depended release profiles. The pellet with hard fat and glycerol distearate displayed in Figure 34 reveals the same visible layer as the previously described pictures with pellets containing hard and glycerol dibehenate.
This rearranging of compounds within the pellet during extrusion/spheronisation takes place without changes in the crystalline structure as Raman analysis of the different pellet batches and their respective excipients suggest. In Figure 35 and Figure 36 the Raman spectra of two pellet batches (one with only 20 % hard fat as a binder and one with 15 % hard fat, 2.5 glycerol distearate and 2.5 glycerol trimyristate) are displayed. There are no detectable changes in their peak pattern.
Another verification of the hypothesis would be an increased drug release of milled pellets at room temperature of a batch, which showed no release at this temperature in intact conditions. The drug dissolution of pellets containing hard fat, glycerol distearate and glycerol trimyristate previously showing nearly no release at 24 °C over 2 hours, display a rapid release of sodium benzoate after milling i.e. destroying of the lipid coat (Figure 37). In this figure, unlike to before displayed release profiles, only the percentage of actual released sodium benzoate is shown, instead the percentage of labelled sodium benzoate content. Due to the milling a correct proportional initial weight of drug and binder could not be ensured and therefore no theoretical nominal content of sodium benzoate be calculated. The curves can still be compared as the kinetic of the release stays the same independent from the chosen data presentation. The temperature controlled release is therefore depending upon the presence of a lipid coat and controlled by it. The release can be modified by the composition of the used lipid mixture.

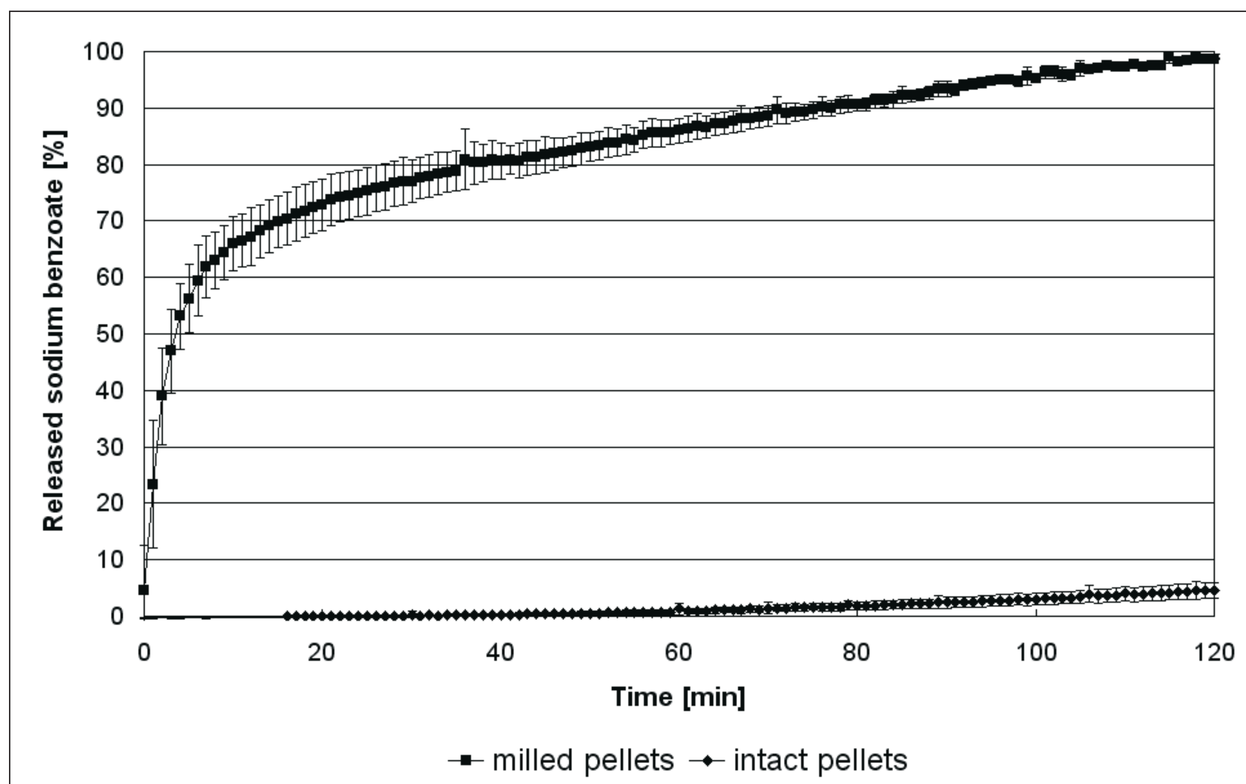

Figure 37: Dissolution profile of pellets containing 80 % sodium benzoate, 15 % Witocan, 2.5 % Precirol and 2.5 % Dynasan (W15 P2.5 D2.5). Milled pellets: Percentage of actual released sodium benzoate; Intact pellets: Percentage of labelled sodium benzoate content.
Dissolution media: purified water (mean ± SD , n=3), Temperature 24 °C , 150 rpm,
Basket method.

Further investigation of the taste masking characteristics included a prolonged dissolution test of the pellet batch containing hard fat and glycerol distearate. This batch showed the most pronounced temperature effect with less than 2 % drug release in 2 h with a dissolution media temperature of 24 °C.
Figure 38 displays the release profile of the same batch over a longer time period in a standard dissolution test with a media temperature of 24 °C. As can be seen from above, within the first 5 h about 10 % of the drug was released, whereas a complete drug release is reached after 45 h. The gaps in this dissolution profile occurred through the fact that a piston pump was used, which was switched off after several hours of continuous pumping to avoid overheating or breakdown.

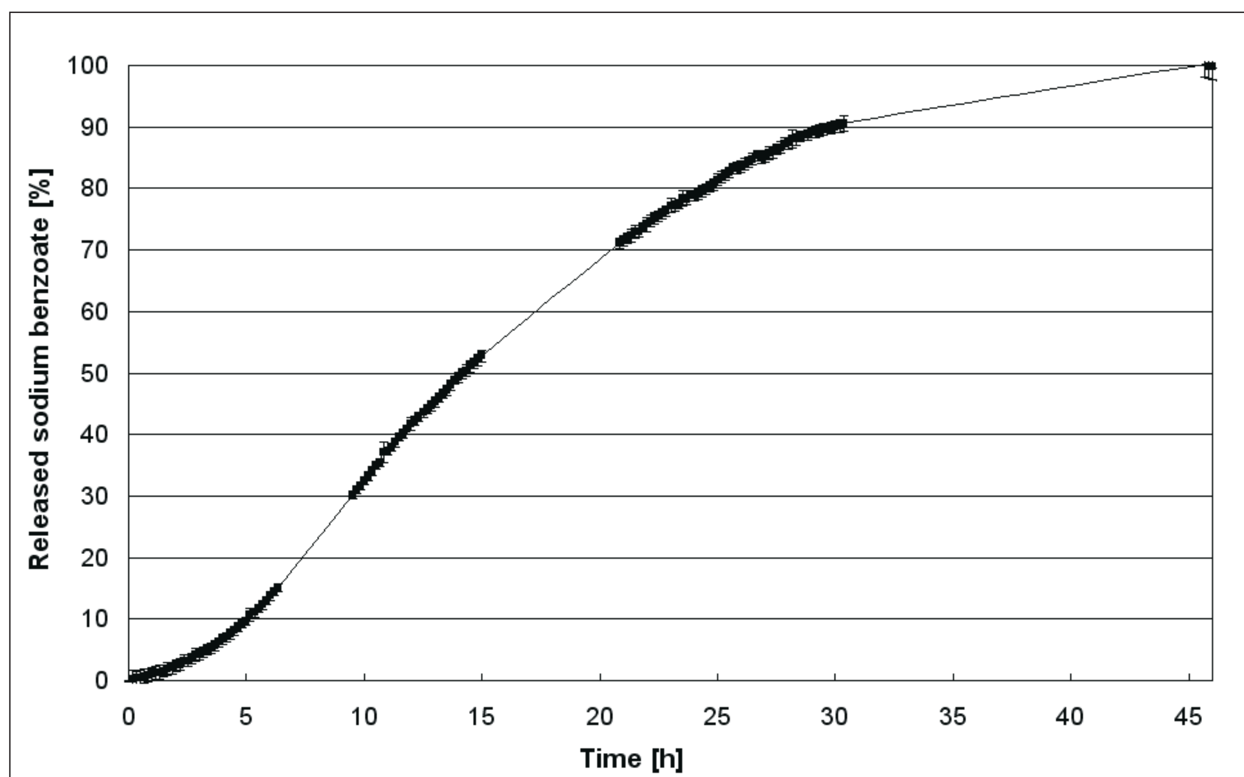

Figure 38: Dissolution profile of pellets containing 80 % sodium benzoate, 15 % Witocan, 2.5 % Precirol and 2.5 % Dynasan (formulation W15 P2.5 D2.5). Dissolution media: purified water (mean ± SD , n=6), Temperature 24 °C, 150 rpm, Basket-Method.

Therefore it can be concluded that the taste masking effect is present at least for the first 5 hours, as a drug release of less than 10 % can be regarded as nearly tasteless. Further studies would be needed to explore the potential of an extended taste masked formulation. Drug release in stationary systems such as stable suspensions which are only agitated occasionally might be different from the continuous stirring in a pharmacopeial dissolution test.
In Table 9 the minutes until 50 % of the labelled sodium benzoate content is released are displayed for all tested formulations. Through the difference in the 50 % drug release between 24 °C and 37 °C the formulations can be divided into two categories. The formulation with 20 % hard fat (W20) and the one with 15 % hard fat and 5 % glycerol dibehenate (W15 C5) have similar release profiles at room temperature and 37 °C, thus the small difference of 6, respective 5 minutes. Both present similar drug release profiles independent from the dissolution media temperature.

Table 9: Comparison of dissolution times until 50 % drug release of the labelled sodium benzoate content for all lipid pellet formulations at dissolution media temperatures of 24 °C and 37 °C.

Formulation	DD 50% / 37 °C [min]	DD 50% / 24 °C [min]	Δ DD 50%
W15 C5	20	25	5
W15 P5	16	>120	>104
W15 D5	20	>120	>100
W15 C2.5 D2.5	43	>120	>77
W15 P2.5 C2.5	18	>120	>102
W15 P2.5 D2.5	15	>120	>105
W20	13	19	6

DD 50% = 50% drug dissolved per labelled claim

In the second category are the following four formulations: W15 P5, W15 D5, W15 P2.5 C2.5 and W15 P2.5 D2.5. All display a DD 50 % value of 15-20 min at 37 °C, which is close to the 20 % hard fat formulation, whereas the DD 50 % at room temperature is exceeding 120, as none of these formulations released 50 % of the drug within the observed timeframe of 2 hours. All four formulations have therefore a Δ DD 50 % exceeding 100, which is an indication of the temperature dependent drug release of these pellets. The combination of hard fat, glycerol dibehenate and glycerol trimyristate (W15 C2.5 D2.5) may be added to the second categories. A temperature influence on the release is clearly visible with a Δ DD 50 % > 77, even though the drug release at 37 °C is delayed in comparison to the 6 other formulations with a DD 50 % of approximately doubled. Further research is necessary to develop a deeper insight into the exact influences of individual lipids on the drug release profiles, as so far it is only possible to attribute general tendencies and influences towards the lipids in the investigated formulations.

2.4.4 Drug release in food

To further investigate the taste masking ability the release of sodium benzoate from the developed lipid pellets into different foodstuff was tested. The previously described pharmacopeial dissolution tests do not give evidence for the behaviour under in-vivo conditions. As a comparison the saliva-resistant coated granules, developed by Breitkreutz et al. (2003), were used. A typical drug administration situation was simulated. 2 g pellets from each batch, equivalent to 1.6 g sodium benzoate, which is a typical single dose for a one-year-old infant, were mixed with 100 g food. Samples of the food were taken after 5, 10 and 20 minutes in between intermittent stirring. As can be seen from Figure 39 and Figure 40, the release from the saliva-resistant coated pellets was higher than for the uncoated lipid pellets, where the release within the first 10 min was hardly detectable and even after 20 min still ranged under 1 %. The release for the coated pellet batch was about 4 % after 5 min, 6 % after 10 min and around 10-11 % after 20 min for the samples stirred in applesauce and vanilla yoghurt, but about 2 % less for the samples in milk and vanilla pudding. The difference can be explained by the differing pH values of the food. Applesauce and vanilla yoghurt have acidic pH values around pH 3 and pH 4, whereas milk and vanilla pudding are neutral. The faster release is obtained because the saliva-resistant coat is soluble in acid media. The uncoated lipid pellets contained hard fat, glycerol distearate and glycerol trimyristate as binder (formulation W15 P2.5 D2.5) and were chosen due to their pronounced characteristics described in the previous chapters. The taste masking of lipid pellets can therefore be rated similar to pellets coated with amino-polymethacrylates (Eudragit E) and was even more efficient under typical drug administration conditions.

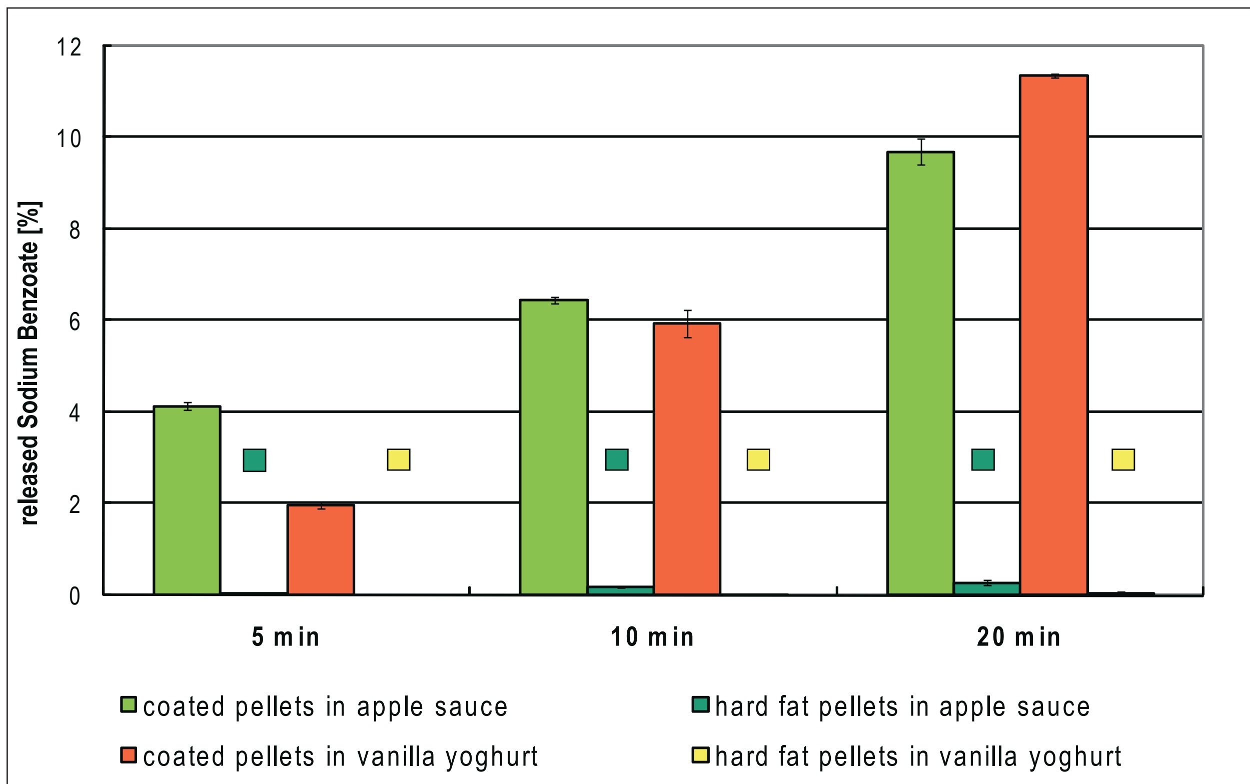

Figure 39: Release of sodium benzoate from two different pellet formulations, 2g pellets mixed with 100g food stuff (n = 3, mean ± SD).

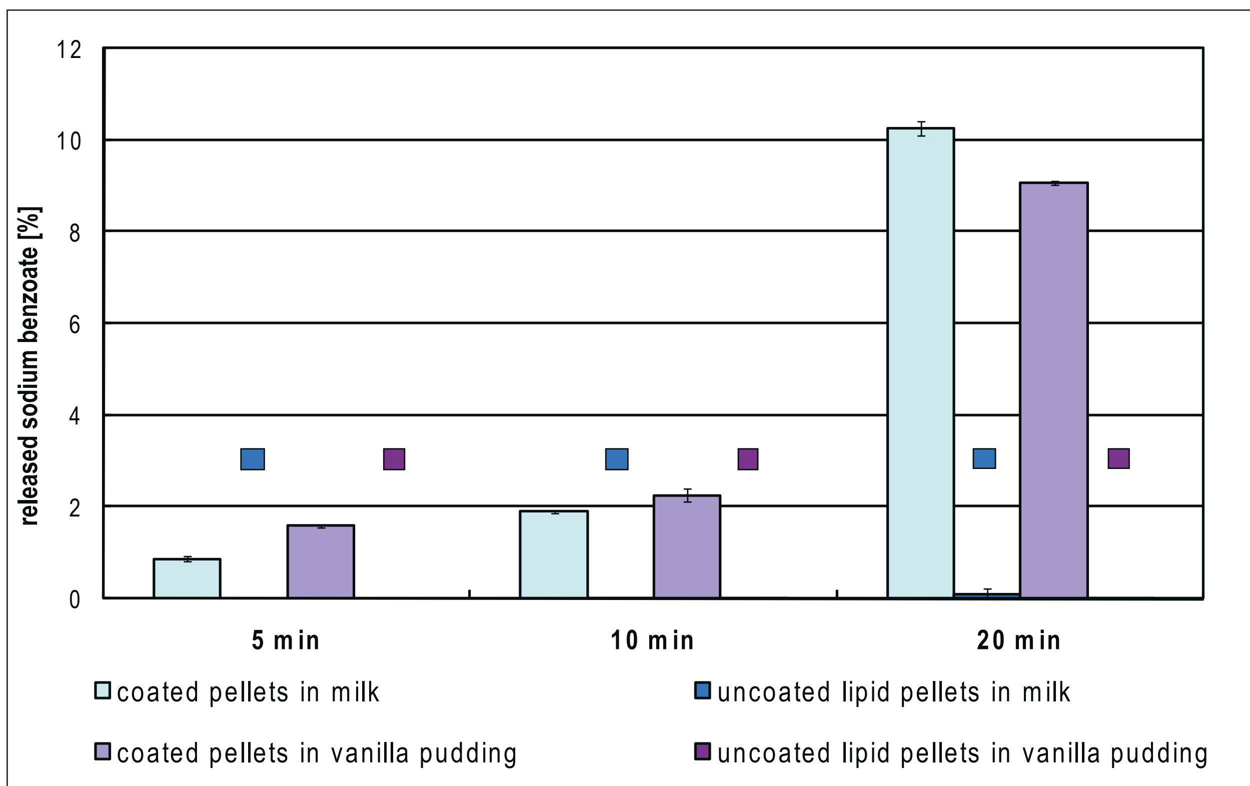

Figure 40: Release of sodium benzoate from two different pellet formulations, 2g pellets mixed with 100g food stuff (n = 3, mean ± SD).

2.4.5 Stability

As it is well known that lipids can undergo changes during thermal treatment (Sutananta et al. 1994) and under storage conditions (Laine et al. 1988), the effect of storage at elevated temperature on the dissolution profile was investigated. Therefore, all lipid pellet batches were stored under accelerated conditions at 32 °C for 4 weeks, which is 10 °C below the melting point of the hard fat Witocan 42/44, present in all formulations. This was done to examine the influence of ageing, temperature and long-time storage. Changes in the release profiles were expected after storage at the predefined conditions. The former saliva-resistant coated pellets were stable at 25 °C/60 % RH and 30 °C/65 % RH for more than one year, but displayed caking at 40 °C/75 % RH.
Interestingly, again the developed batches could be divided into two groups. In Figure 41, the same batches (with hard fat or hard fat and glycerol dibehenate as a binder) that were not influenced by the dissolution media (Figure 23), were also not influenced in their dissolution profiles by the temperature under accelerated storage conditions. It is possible that the batch containing 5 % glycerol dibehenate would belong to the other category where changes during storage occurred. The storage temperature of 32 °C is very low for a lipid with a melting point of approx. 70 °C, but due to the 15 % hard fat no higher storage temperatures could be tested.

In Figure 42 all batches with changed release profiles are displayed and it is interesting to note that this effect was seen in all batches containing glycerol distearate. The release of these batches was slower in the first hour of dissolution, but still complete within less than 2 hours. If the aforementioned definition of immediate release, as 80 % drug release in 60 min, is again applied it is interesting to note that all batches still comply with the claimed release.
In Table 10, analogue to Table 9, the DD 50 % values of all formulations before and after storage are displayed. Similarities become apparent as again the formulations W20 and W15 C5 can be combined in one category of formulations where the investigated storage conditions have no influence on the dissolution profile. In both cases the Δ DD 50 % is only 2, indicating only a minor deviation between both release profiles. As stated above, the three formulations containing glycerol distearate (W15 P5, W15 P2.5 C2.5 and W15 P2.5 D2.5) represent the next category of formulations where the storage conditions have a moderate influence on the dissolution profile. Release is slowed down within the first hour, indicated by Δ DD 50 % values between 14 and 20 (see Figure 42). Singled out are two formulations, one binary combination and one ternary (W15 D5 and W15 C2.5 D2.5), where the storage under increased temperature had a strong effect on the release profile, leading to incomplete dissolution within the investigated 2 hour period. Through the temperature influence intermediate release profiles emerge, indicating a convergence from complete release at 37 °C to an incomplete release at 37 °C similar to the one at room temperature.

It may therefore be possible to say that combinations including glycerol distearate are less susceptible to temperature and storage effects than combinations with glycerol trimyristate and/or glycerol dibehenate. Hard fat is seemingly not influenced by the tested storage conditions, as well as a combination of just hard fat and glycerol dibehenate, even though this might be due to the relatively low temperatures during storage in comparison to the melting range of glycerol dibehenate.

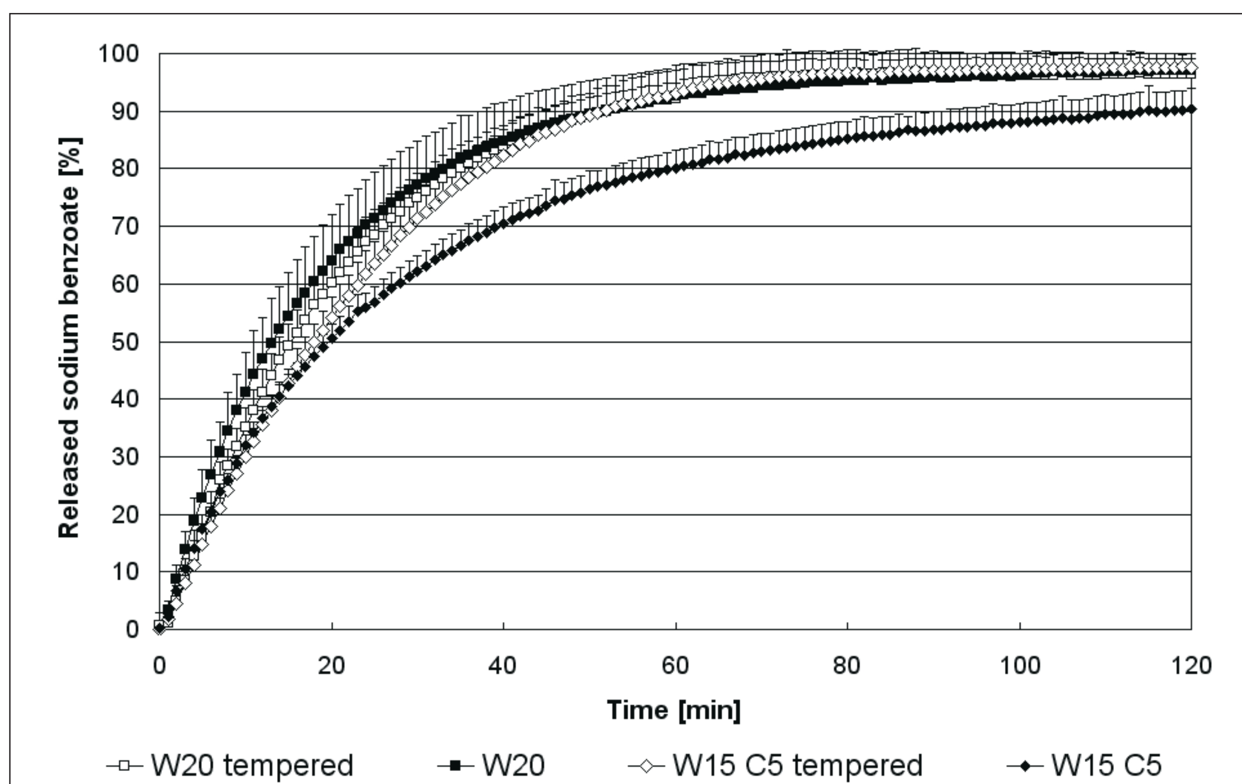

Figure 41: Influence of storage under accelerated conditions on release characteristics, Dissolution media: purified water (mean ± SD, n=6), Temperature: 37 °C ± 0.5 °C, 150 rpm, Basket-Method.

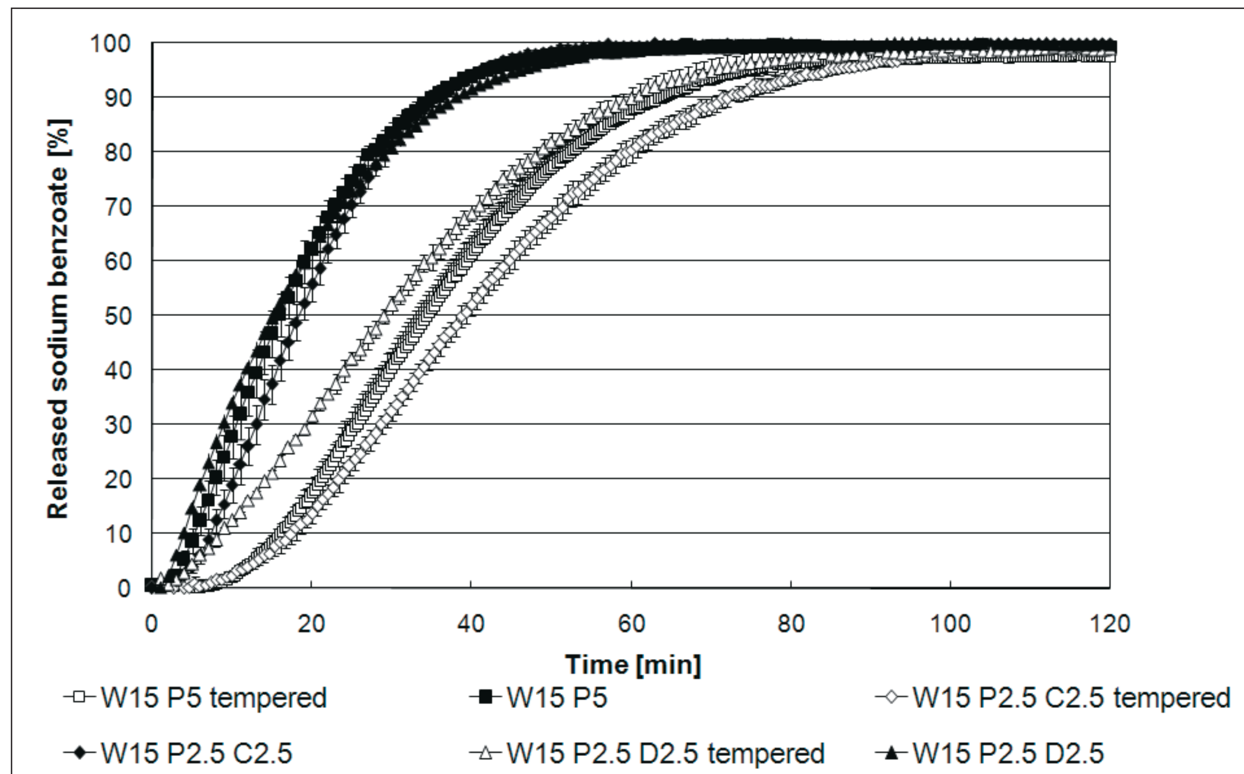

Figure 42: Influence of storage under accelerated conditions on release characteristics, Dissolution media: purified water (mean ± SD, n=6), Temperature: 37 °C ± 0.5 °C, 150 rpm, Basket-Method.

Table 10: Comparison of dissolution times until 50 % drug release of the labelled sodium benzoate content for all lipid pellet formulations before and after storage at 32 °C for 4 weeks.

Formulation	DD 50% / 37 °C [min]	DD 50% / 37 °C tempered [min]	Δ DD 50%
W15 C5	20	18	2
W15 P5	16	34	18
W15 D5	20	86	66
W15 C2.5 D2.5	43	98	55
W15 P2.5 C2.5	18	38	20
W15 P2.5 D2.5	15	29	14
W20	13	15	2

DD 50% = 50% drug dissolved per labelled claim

The X-ray diffraction of all pellet batches before and after storage showed no differences (see Figure 43 for exemplary diffractrograms of compressed pellets containing hard fat, glycerol distearate and glycerol trimyristate) and it is therefore possible to conclude that no change in crystalline structure took place under the given conditions.
Obviously, the composition of the lipids determines not only the temperature dependent drug release, but also their tendency to undergo changes by thermal treatment. It is essential in drug development that the drug dissolution profiles are kept constant over time. Therefore, it is essential to distinguish between the best-shaped pellet formulation, desired release profiles and the best maintenance of these drug release profiles.

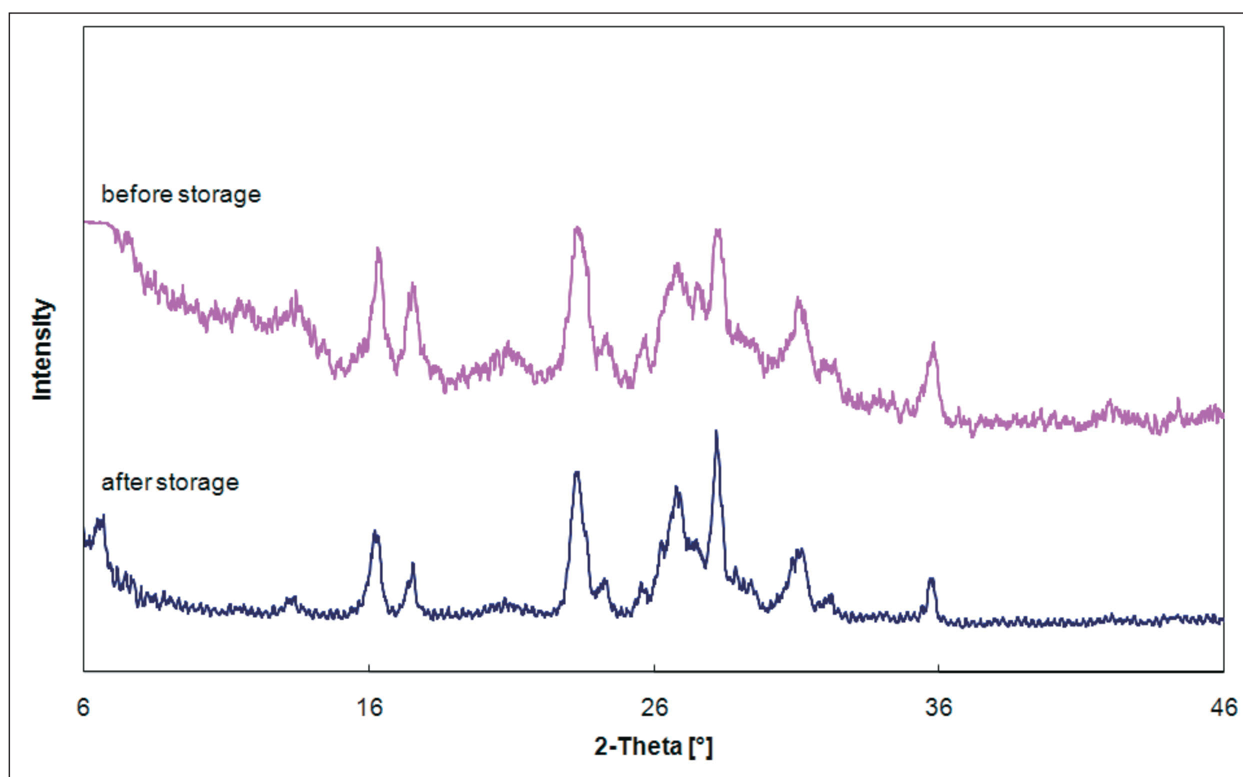

Figure 43: X-ray diffractograms of pellets, containing 80 % sodium benzoate, 15 % Witocan, 2.5 % Precirol and 2.5 % Dynasan (W15 P2.5 D2.5), before and after storage at 32 °C for 4 weeks.

2.4.6 Conclusion

The newly discovered principle of self-coating of lipid pellets, possible through the behaviour of the lipid binder during the extrusion/spheronisation process, offers interesting new aspects for the use in not only paediatric oral dosage forms. Taste masking through an additional salvia-resistant coating might be avoided in some cases and the potential to design desired release profiles through the choice of lipid composition offers an interesting field, which should be explored further. It offers a variety of interesting possibilities to develop new modified release dosage forms without the need of an additional time- and resources-consuming coating step with potentially toxic excipients. Suitable fields of application may be taste masked suspensions, or granules and pellets for reconstitution of suspensions.

Through the variation of the lipid binders different release profiles can be obtained and through this effect desired drug release profiles can be designed. Further research is necessary to gain a deeper understanding of the mechanism behind the discovered effect of certain lipid mixtures. So far it is not possible to relate defined lipid characteristics with drug release profiles. It has to be investigated which fractions of the different lipids lead to the formation of the lipid layer on the pellet surfaces and which are responsible for the temperature dependent release. However, it is clear that especially glycerol distearate has an important influence on the described temperature dependent release. This temperature effect can result in taste masked drug formulations or other modified release dosage forms while still maintaining an immediate-release profile under physiological temperatures.

3. Taste assessment

3.1 Taste analysis of different pellet formulations

It has been reported that lipids are able to function as taste masking agents (Michalk 2007, Suzuki et al. 2003) due to their lipophilic characteristics, which include reduction in wettability and solubility leading to decreased contact times with taste buds in the oral cavity. Granules (Faham et al. 2000) and powders (Sugao et al. 1997) have been coated with lipids or waxes to mask unpleasant tastes. Several hard fats, including Witocan 42/44, have been tested as bitter masking agents in chewable tablets with satisfactory results (Suzuki et al. 2004). Usually adult volunteers in human taste panels are used for the assessment of the taste of drug formulations. This is challenging for paediatric dosage forms as taste panel tests in children are difficult to perform (Sjövall et al. 1984, Visser et al. 2000, Liem et al. 2004). The method and design has to be carefully chosen and have to be adjusted to the age of the participating children. Especially young children under the age of four tend to lack the discriminatory ability and the consistency needed to obtain valid data. Procedures should be short and easy to understand to avoid fatigue and confusion of the children. The judgment methods can include spontaneous verbal judgment or hedonic scales, with the hedonic scales being a more standardized procedure. In the EU paediatric taste panels are only allowed with affected children who benefit from the tested medication, as in contrast to the US where healthy children are also allowed to be included in taste panel tests. Especially in paediatric formulations taste plays an important role as children reject medications with an unpleasant taste, but is difficult to measure and quantify. Children are more sensitive towards bitter tastes than adults as bitter works as a protection mechanism against potential poisoning. In general taste is difficult to analyse as personal preferences can differ widely. Furthermore if adults are used, as test persons for childrens´ medications it is difficult to extrapolate these results onto the paediatric target population.
The recently developed electronic taste measurement systems, also called e-tongues, are new options which offer the opportunity to give a taste prediction without the need of human test persons. Currently there are two systems available on the market both working on the principal of potentiometry. Further information on the respective system and data analysis is given in the respective chapters.
Five different pellet formulations with a drug load of 80 % sodium benzoate, but differing in the nature of the used binder, where analysed by the two e-tongues and also tested by a human taste panel. The results were compared to each other and related to the actual released sodium benzoate concentration, which was determined by UV-analysis. Furthermore, the performance of these two new systems was validated and compared to each other based on the model of sodium benzoate pellets.

Table 11: Labelling of the five pellet batches tested with electronic tongues.

Labelling	Binder
Carrageenan	20% κ-carrageenan
MCC	20% MCC
Hard fat	20% Witocan ® 42/44
coated SB pellets	20% Witocan ® 42/44, with saliva-resistant coating
Lipid Mixture	15% Witocan® 42/44, 2.5% Precirol®, 2.5% Dynasan 114®

Table 11, the respective labelling for the tested batches is displayed as used in the following figures. To analyse the taste masking differences between the different batches and to allow the testing with the e-tongues, who are only able to analyse liquid samples, an in-vitro dissolution test was developed to simulate real administration conditions. 2 g pellets, equal to 1.6 g sodium benzoate, were put in 100 ml purified water and stirred for different time intervals (see F2.4.5) at room temperature. The dissolution solution was tested by adult volunteers as well as analysed by the two e-tongues.
Furthermore the solutions were analysed by UV to detect the actual released sodium benzoate content. In Figure 44, the drug release profiles for the respective batches are displayed.

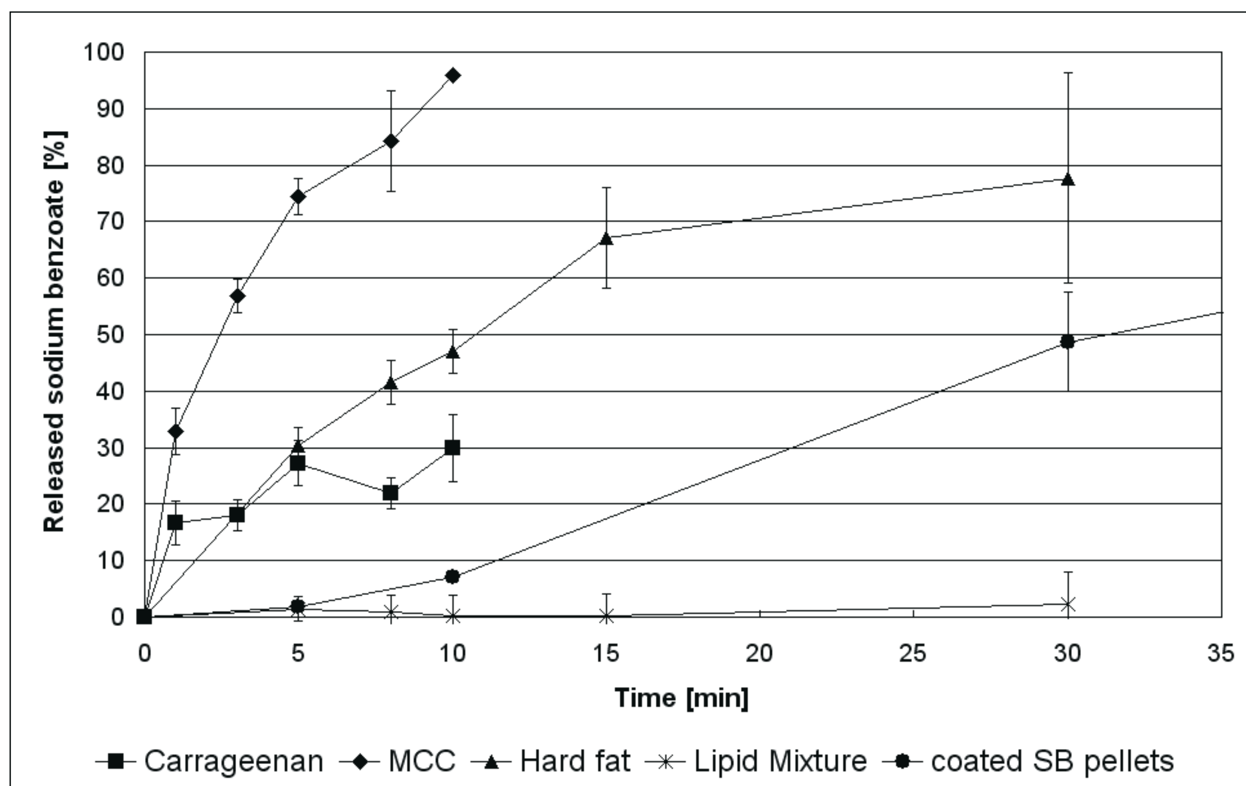

Figure 44: Release of sodium benzoate under in-vitro test conditions of the five tested pellet batches. Dissolution media: 100 ml purified water, room temperature (n = 3, mean ± SD).

Pellets with MCC as a binder show rapid and full release within the first 10 minutes of stirring. The same would be expected with κ-carrageenan as a binder, but the curve differs significantly. The difference can be explained by the fact that these pellets formed a gel under the given dissolution conditions. This gel could not be measured and the attempt to filter off a clear solution led to the loss of sodium benzoate. The saliva-resistant coated pellets showed clearly the taste masking effect of the Eudragit E-coating as less than 5 % of sodium benzoate were released in the first 5 min, followed by an increase afterwards. The influence of lipid mixtures on the release profile of sodium benzoate has been discussed in the previous chapter, but has been first discovered during these taste assessment experiments, as this effect between the previous used hard fat as a binder and the mixture of hard fat, glycerol trimyristate and glycerol distearate is only identifiable at room temperature. For the statistical evaluation of the e-tongue data with the in vivo and in vitro experiments this data set was excluded, as a correlation with values equal or close to zero was not possible, whereas all samples from all other formulations were statistically evaluated.

3.2 Taste panel

The results for the human taste sensation of the tested solutions are displayed in Figure 45. Profiles similar graphs to the dissolution curves were obtained, indicating a good detection of different sodium benzoate concentrations through the volunteers. The higher deviations can be explained by the high inter-personal deviation in taste perception of the volunteers. Again pellets with MCC as a binder showed high taste scores, indicating a bad taste or high concentrations of sodium benzoate. The taste masking of the coated pellets can be seen in the low taste scores within the first 10 min, as well as the taste masking of the lipid mixture with low taste values within the whole tested time period of 30 min. Therefore it can be concluded that the lipid pellets have the same ability to mask the unpleasant taste of sodium benzoate as the coated pellets with the additional advantage of having a longer expression of the taste masking effect.

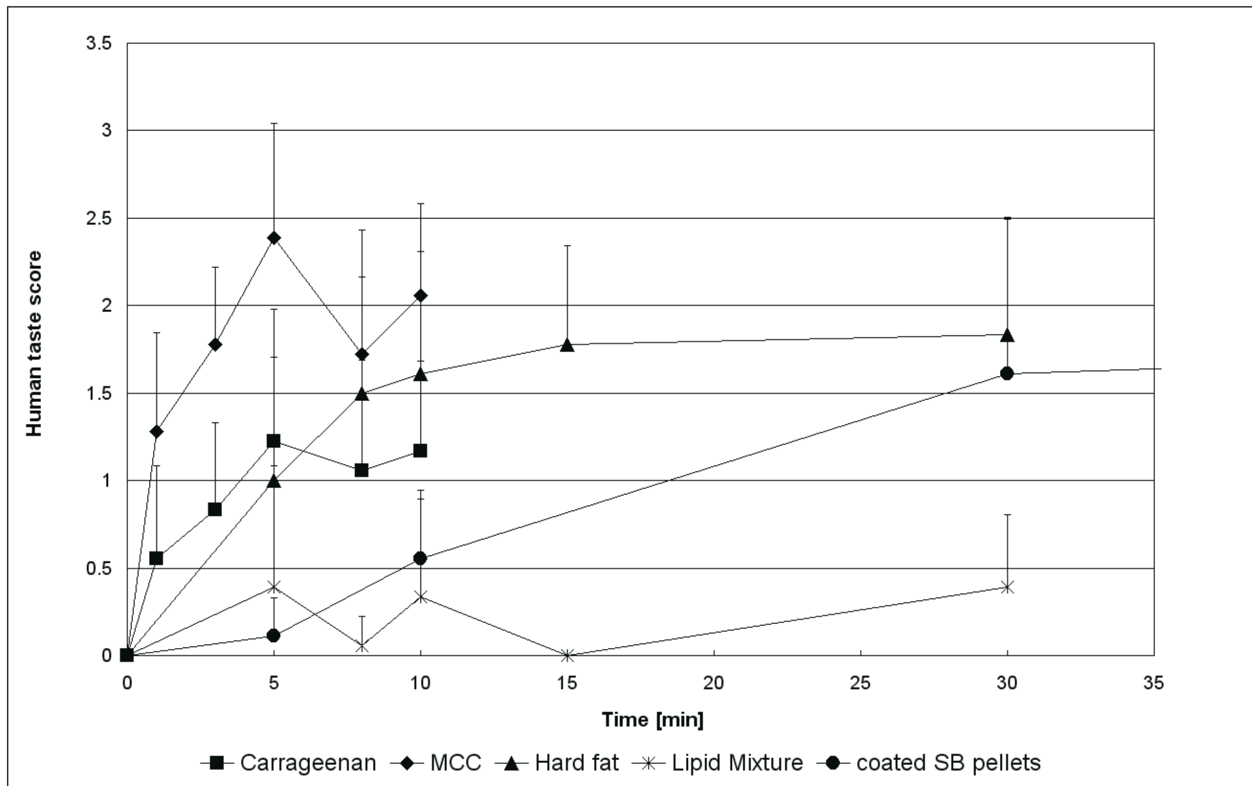

Figure 45: Taste evaluation by adult volunteers for different pellet batches on a scale from 0 to 3.5, equalling no to very bad taste (n = 9, mean + SD).

If the results from the human taste panel are correlated directly with the released sodium benzoate percentage (Figure 46), it is interesting to note that the accordance of human taste sensation to the concentration of sodium benzoate is good until a certain threshold is reached. In the case of sodium benzoate a concentration above 60 % released drug, equalling a 1 % aqueous SB solution, led to the difficulties of the study participants to rank these samples accordingly. The subjects were unable to distinguish between a “bad” tasting sample and an “even worse” tasting one. Concentrations >1 % were rated with the highest marks from 1.8 to 2.5 points on the scale. This again demonstrates the importance of a taste masked formulation as even low concentrations of sodium benzoate lead to unacceptable taste impressions.

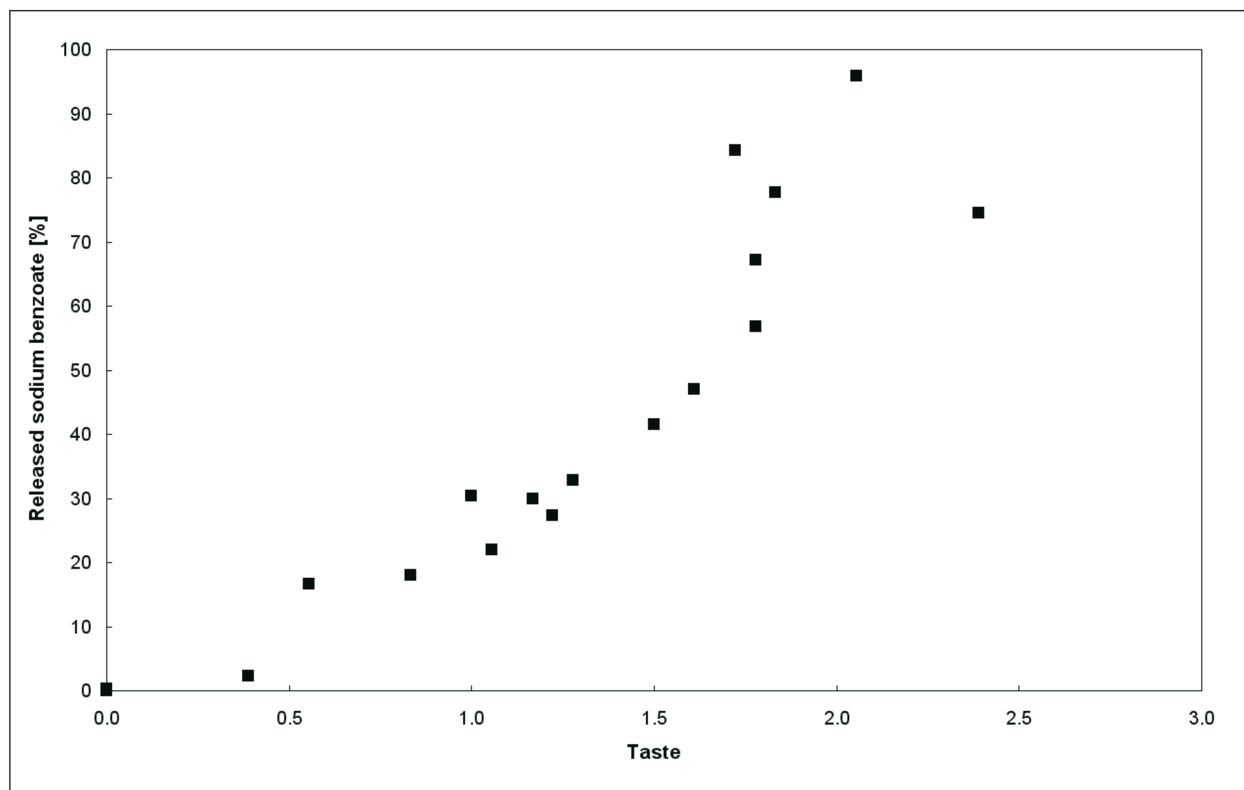

Figure 46: Direct correlation of human taste evaluation to actual sodium benzoate concentrations.

3.3 Astree e-tongue

After the conduction of the recommended system checks a calibration with 5 sodium benzoate solutions ranging from 2.5 % to 0.00025 % (equal to 173.5 mM to 0.017 mM) was performed (see also F 2.4.6). Linearity was demonstrated for concentrations as low as 0.025 % (1.7 mM). For each tested pellet formulation, a special corresponding placebo formulation (matrix without an active ingredient) was prepared. This was necessary, as the Astree e-tongue is only able to detect taste differences between formulations. This may cause, especially in the case of solid dosage forms like pellets, considerable additional efforts. It is not always easy to prepare similar placebo formulations when high drug loads have to be renounced and of course time and cost consuming to prepare a new placebo formulation for each change of excipients. Each batch with 12 samples (6 active and 6 placebo samples) was analysed separately, first the 6 placebo samples followed by the 6 samples containing sodium benzoate. This procedure was replicated 7 times. The first and seventh sample (P0 and A0) for the time point 0 min of each release, contained purified water.

Exemplary for all batches, the results from pellets with MCC as a binder will be discussed in detail to demonstrate the data handling with the Astree e-tongue. The sensor responses for all sensors to all samples were collected in one data set. Out of this set, a taste print for each sensor was established analogue to the one displayed in Figure 47, i.e. the response from this sensor to all samples throughout the 8 runs. For each batch 7 taste prints representing the 7 used sensors were built. If a sensor drift can be seen, like in Figure 47, it has to be decided to remove several runs. For the MCC batch, the first 3 replicates were not taken into account. Usually the sensors need time to stabilize their signals in new samples, therefore the first 1 to 3 replicates were removed in all batches. Parallel to the individual sensor responses all root mean square errors for all samples and sensors are analysed as can be seen in Table 12. Out of the means, unstable sensors, e.g. BB1

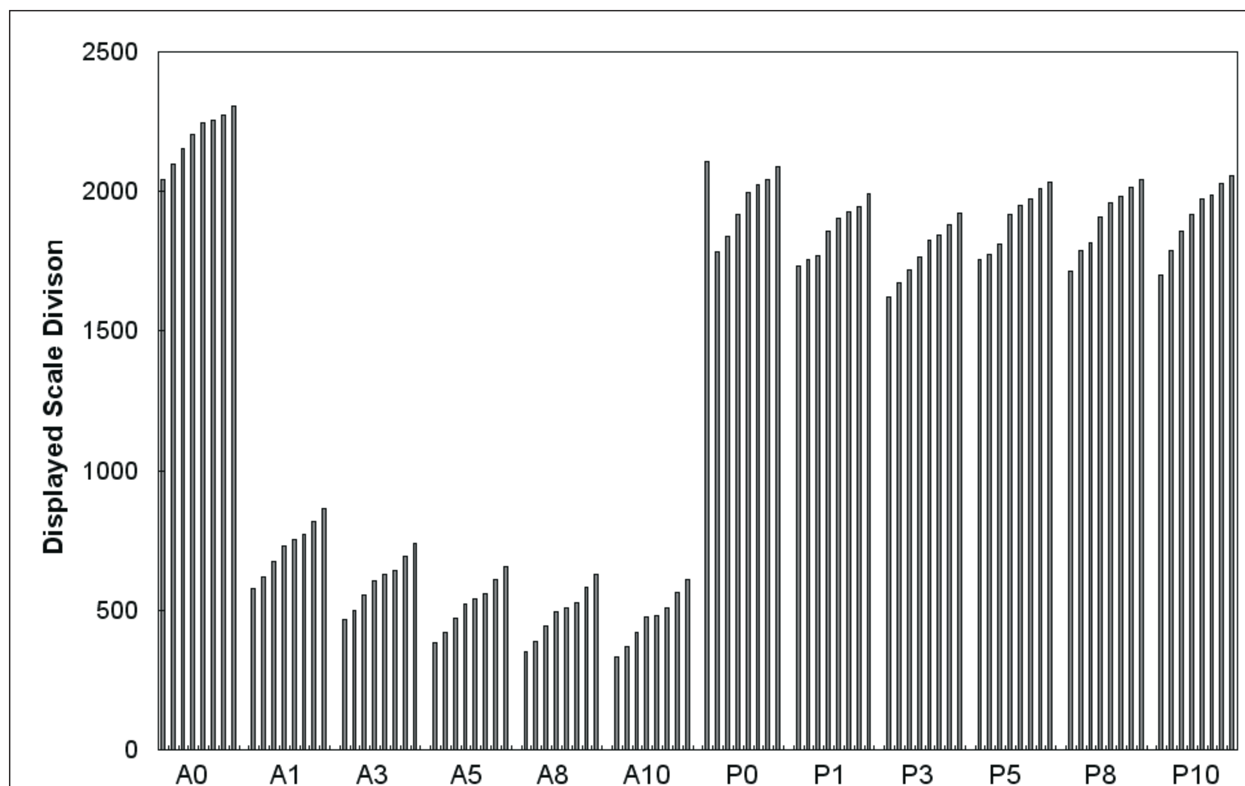

Figure 47: Sensor BA responses at different time points (numbers: minutes of drug release) for dissolution fluids from MCC pellets (n = 8). P and A indicating placebo and active samples, respectively.

with a mean RSD of 15.6, or samples difficult to analyse for all sensors, e.g. P0 with a mean RSD of 16.5, can be identified and discarded for subsequent evaluation. If sensors can not differentiate between samples, which is visible in a taste print of the respective sensor, they are removed from the data set. The removal of sensor(s) and replicate data lower the mean RSD, e.g. the mean RSD for sensor BB1 dropped from 15.6 to 0.41 after removal of the first the replicates.

The resulting new data set was then used for a multivariate data analysis and displayed in a Principal Component Analysis (PCA), a multivariate analysis tool which allows multidimensional data to be displayed in a two-dimensional way. In the 7-dimensional data space, resulting from the use of 7 different taste sensors, a two dimensional space is fitted, so that most of the data information is reduced to this plane. Now the data can be represented in a normal x-y chart, the PCA map, where most of the information lies on the x-axis.
As can be seen from Figure 48, 95.87 % of the information is displayed on the x-axis, whereas 3.63 % lies on the y-axis. The rest of the information (0.5 %) can not be displayed by this map. For each sample a data cluster set is visible on this map. Data points close together in the map represent similar taste information, data cluster further apart from each other indicate taste differences. For each sample set (placebo and active containing sample, e.g. P3 and A3) a distance in the PCA map can be calculated. A small distance means similarity in taste, i.e. neutral or close to neutral taste of an active containing sample compared to the corresponding placebo, whereas a bigger distance represents deviating taste, i.e. more released sodium benzoate. Both tested series (placebo and active samples) show a certain alignment and form a consecutive row, reversed in their directions (Figure 48), which is surprising for the placebo samples, as these are, according to human volunteer ratings, tasteless. At this point it is unknown which structures in the dif-

Table 12: Intra-analysis repeatability for all sensors and all samples (MCC) before (RSD1) and after (RSD2) elimination of one sensor (ZZ) and the first three replicates .

RSD1	ZZ1	AB1	BA1	BB1	CA1	DA1	JE1	Mean
A0	14.17	10.88	3.91	21.50	3.83	8.64	0.60	9.08
A1	0.63	9.94	12.52	8.62	7.10	6.34	1.26	6.63
A3	0.46	9.58	14.33	5.12	8.01	4.73	0.44	6.10
A5	0.25	8.81	16.54	3.08	8.26	3.13	0.71	5.83
A8	0.52	8.81	17.85	2.12	7.56	2.88	0.82	5.79
A10	0.16	8.04	18.65	1.42	6.32	2.53	0.96	5.44
P0	25.05	48.87	5.55	18.46	4.84	9.87	2.86	16.50
P1	15.69	31.92	4.90	17.65	5.59	8.28	0.91	12.13
P3	6.57	21.79	5.53	16.55	5.76	7.08	1.31	9.23
P5	4.61	16.78	5.31	16.22	5.25	6.94	1.41	8.07
P8	4.02	14.05	5.80	15.97	5.40	6.45	1.78	7.64
P10	3.50	12.62	6.07	15.60	5.45	6.26	1.98	7.35
Mean	3.50	12.62	6.07	15.60	5.45	6.26	1.98	

RSD2	AB1	BA1	BB1	CA1	DA1	JE1	Mean
A0	0.54	1.44	0.60	0.91	0.64	0.46	0.77
A1	0.87	6.06	0.29	2.19	0.91	0.41	1.79
A3	1.29	7.28	0.16	2.30	0.98	0.22	2.04
A5	1.73	8.42	0.15	2.81	0.75	0.18	2.34
A8	1.83	9.12	0.24	3.32	0.70	0.37	2.60
A10	2.20	9.82	0.14	3.45	0.70	0.33	2.77
P0	1.18	2.80	0.77	2.56	1.25	0.56	1.52
P1	0.69	2.30	0.45	2.07	1.18	0.55	1.21
P3	0.58	2.86	0.65	2.10	1.50	0.88	1.43
P5	0.74	2.07	0.52	1.51	1.05	0.40	1.05
P8	0.63	2.35	0.59	1.61	1.27	0.57	1.17
P10	0.64	2.38	0.38	1.59	1.33	0.56	1.15
Mean	1.08	4.74	0.41	2.20	1.02	0.46	

ferent binders cause to these sensor responses, as this phenomenon was not only detected with MCC, but also with other binders. Another unclear point are the data clusters for both zero samples. Both contain purified water, stand outside their respective rows and differ notable in their position. The e-tongue seems not to be able to recognise these two samples as the same, just as similar, as the A0 sample is close to the placebo samples (P0 to P10) and far away from the active containing samples (A1 to A10). An effect which might be explained by positioning effects on the sample rack which were not analysed in detail. Another explanation might be the inability of the sensors to react to purified water, as a certain conductivity is needed for proper detection of sensor signals, according to the recommendations provided for the use of the second e-tongue.

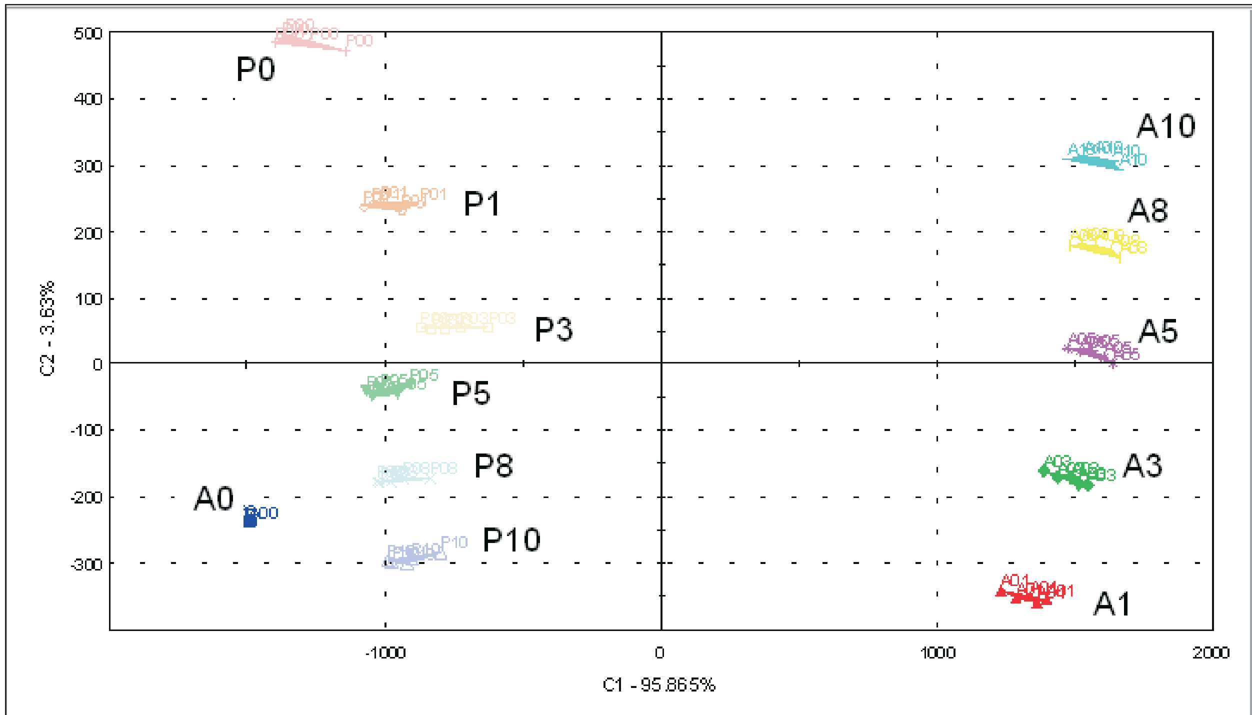

Figure 48: PCA map for all MCC samples generated by the αAstree Software. Discrimination index: 96.12, n = 7, C1 = Main component, C2 = Second Main Component, A0 to A10 = Active samples, P0 to P10 = Placebo Samples, Numbers indicating minutes of drug release.

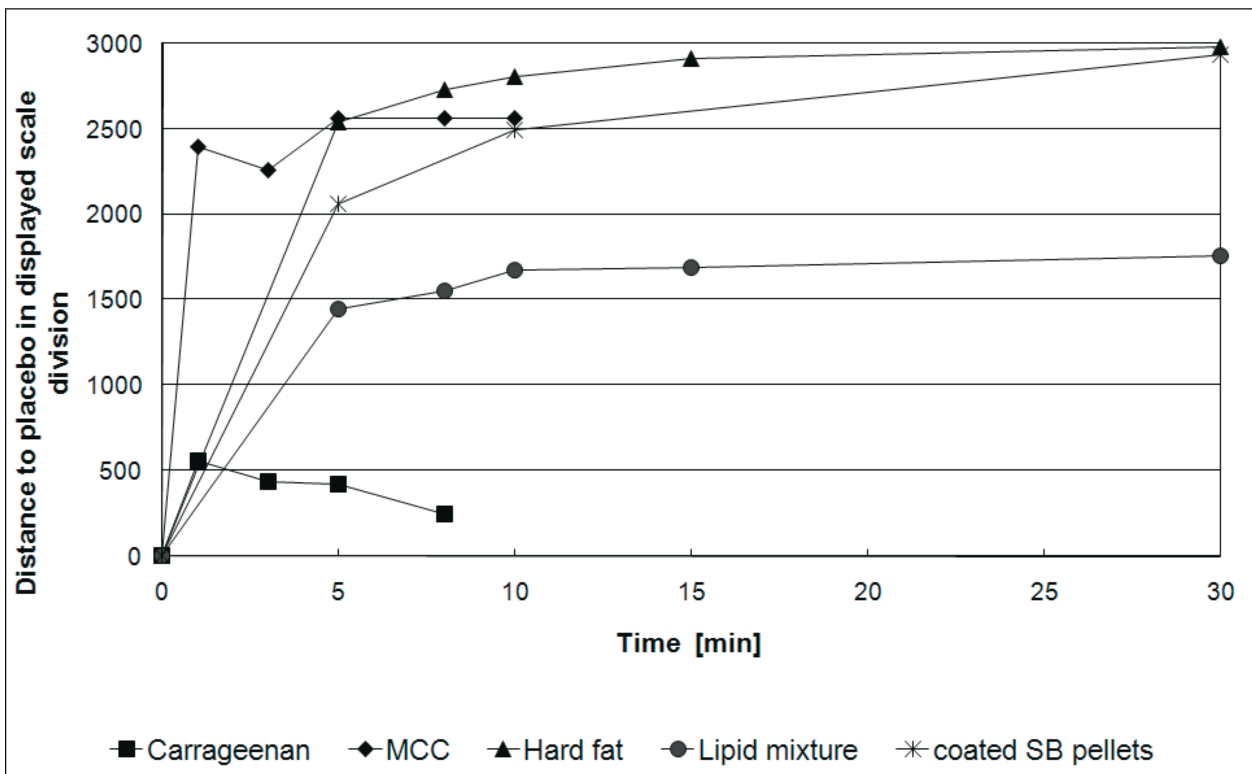

Figure 49: Placebo-to-active distances in PCA map for all pellet batches. Distances were calculated from 7 individual measurements displayed in the PCA map by the αAstree Software.

The distances between all sample sets and all batches, excluding all zero samples, are displayed in Figure 49. Again the batch with κ-carrageenan sticks out with unusual low distances, i.e. little taste information of the samples, due to the same above mentioned reasons. MCC and hard fat pellets show high distances within the first 10 min indicating big taste differences to the placebo samples, i.e. higher release of sodium benzoate than the coated pellets or the pellets containing a lipid mixture. It is important to note that the general order of the batches, MCC and hard fat pellets with a faster release than the coated pellets and surprisingly the lipid mixture pellets with a better taste than the coated pellets, could be detected by the Astree e-tongue. A relative classification and placement was possible with this electronic taste measuring system. Sensor responses correlated with sodium benzoate concentration as the increase in the placebo to active distances suggests. Unfortunately, only relative values can be established and general taste impression, e.g. closer to placebo (good/neutral taste) or further away from placebo (bad/intense taste), can be given.

3.4 Insent Taste Sensing System

Before the measurement of the different pellet batches a calibration was performed using 8 aqueous sodium benzoate solutions (concentration ranged from 0.1 to 200 mM)(see also F 2.4.6). These calibration solutions were analysed three times, on different days, to check the stability and reproducibility of the sensor responses. Furthermore the influence of the sample position on the sample rack was analysed, as the measurements were performed in ascending, descending and random order. First, the direct sensor responses [in mV] were analysed, before the data treatment was performed using the Insent software.

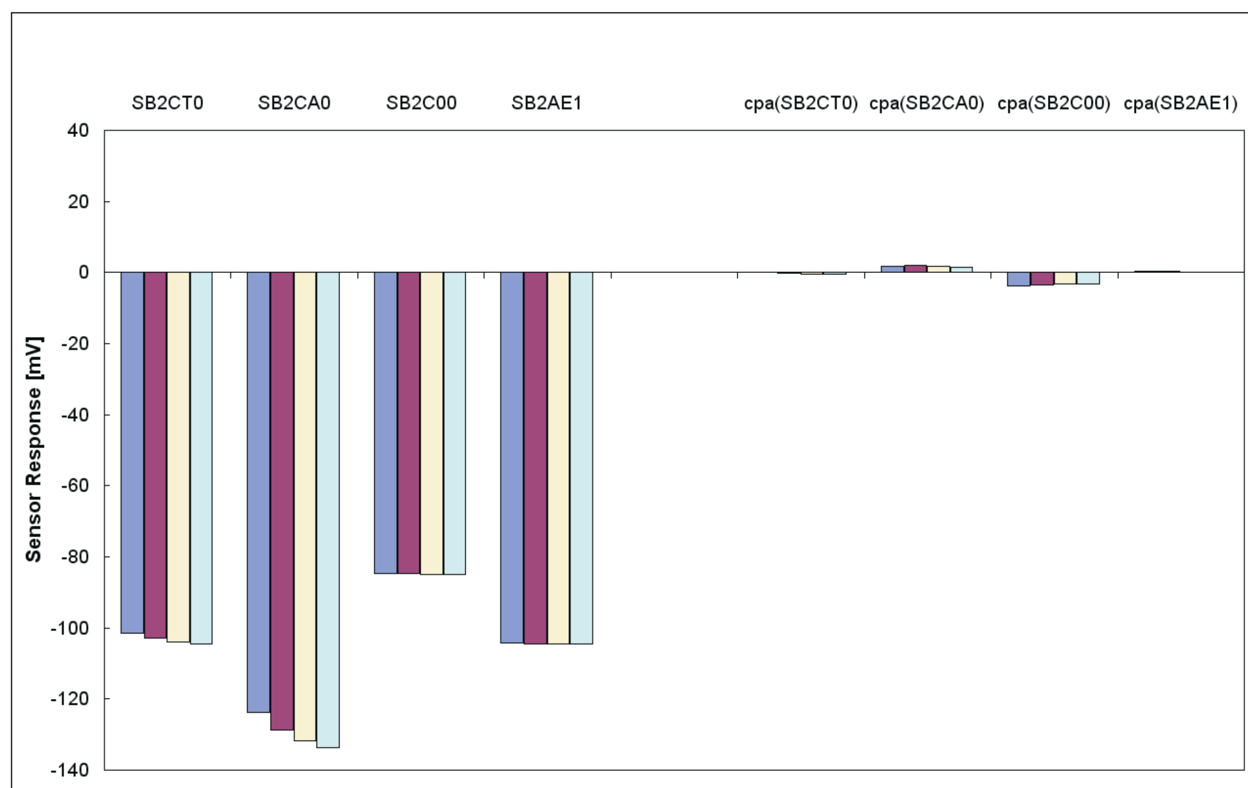

Figure 50: Sensor responses of all sensors (SB2CT0, SB2CA0, SB2C00, SB2AE1) for four runs, for one calibration sample (111 mM sodium benzoate). On the right the delayed sensor signals (CPA) are displayed.

Exemplary, in Figure 50 the sensor responses from all four sensors (saltiness: SB2CT0, sourness: SB2CA0, astringency: SB2AE1 and bitterness: SB2C00) are displayed for the calibration sample with a concentration of 111 mM sodium benzoate (which is equal to 100 % release of 2 g pellets). Each bar represents one run from the test series. Through this data application it is possible to detect drift in sensor responses, as can be seen with sensor SB2CA0. CPA values are the sensor response to the standard solution immediately after the measurement of the active sample, in between these two measurements the sensors are only cleaned very shortly. This measurement procedure is performed to detect any delayed sensor signals due to adherence of sample substance to the sensor, indicating an aftertaste of the sample. As a standard procedure, all batches were measured four times and the first run was omitted in further data handling. To analyse the response from separate sensors, sensor data from one sensor for all measured samples are displayed in a bar chart (Figure 51) for the bitterness sensor and all calibration samples. The direct sensor response turned more negative with an increase in sodium benzoate concentration, whereas the signal for the aftertaste only changed with higher concentrations. This is possible as low concentrations of sodium benzoate are likely to get washed off in the short cleaning steps between relative value measurement and CPA (aftertaste measurement), whereas higher concentrations would need a longer cleaning process and therefore are detectable in the CPA procedure.

As sodium benzoate is a salty and bitter tasting drug, the raw data of sensors SB2CT0 and SB2C00 are displayed in Figure 52. As can be seen from the graph, the saltiness sensor SB2CT0 is log-linear in its response to the sodium benzoate concentration throughout the calibration range, but the limit of detection is 0.3 mM sodium benzoate, as the sensor is not possible to distinguish between it and the 0.1 mM sample. The bitterness sensor SB2C00 shows a bigger scattering, especially with low concentrations of sodium benzoate, but is able to detect differences between all samples.

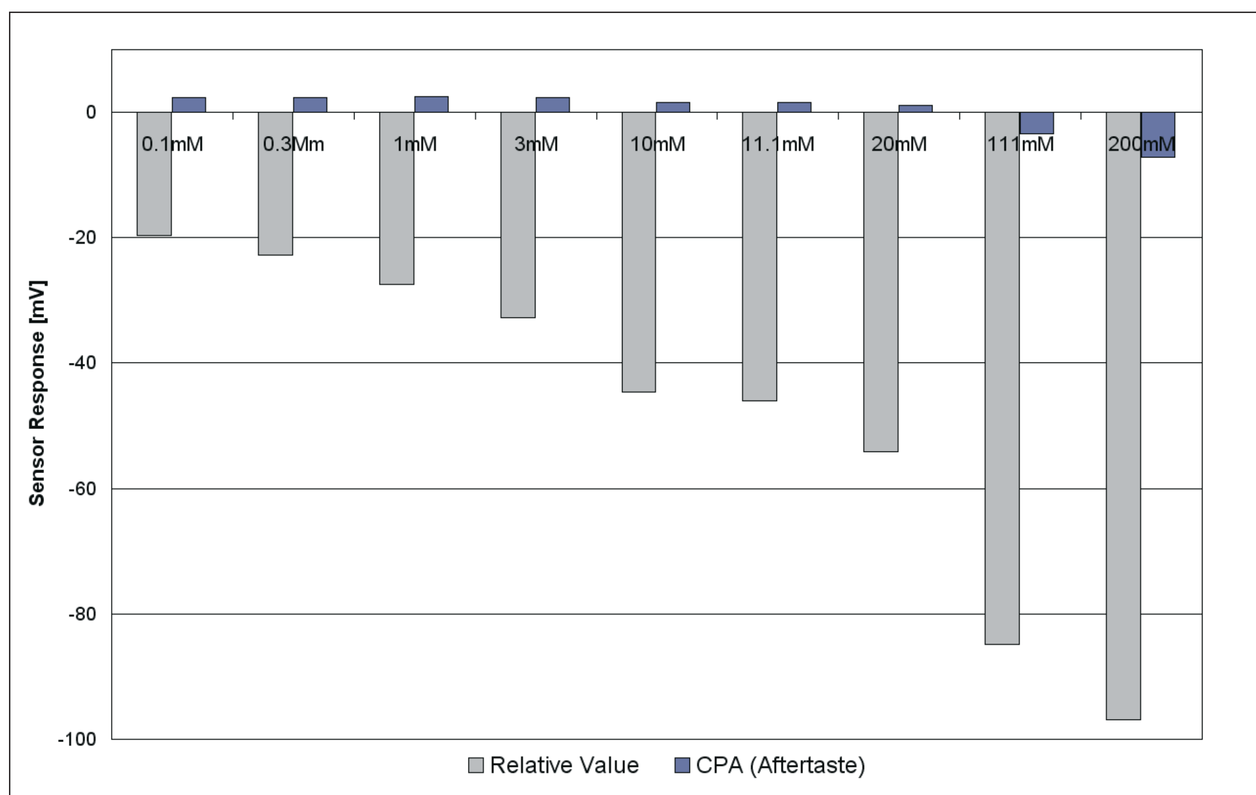

Figure 51: Sensor responses (incl. CPA values) from one sensor (SB2C00 = bitter) for all calibration samples (0.1 to 200 mMol), n = 3.

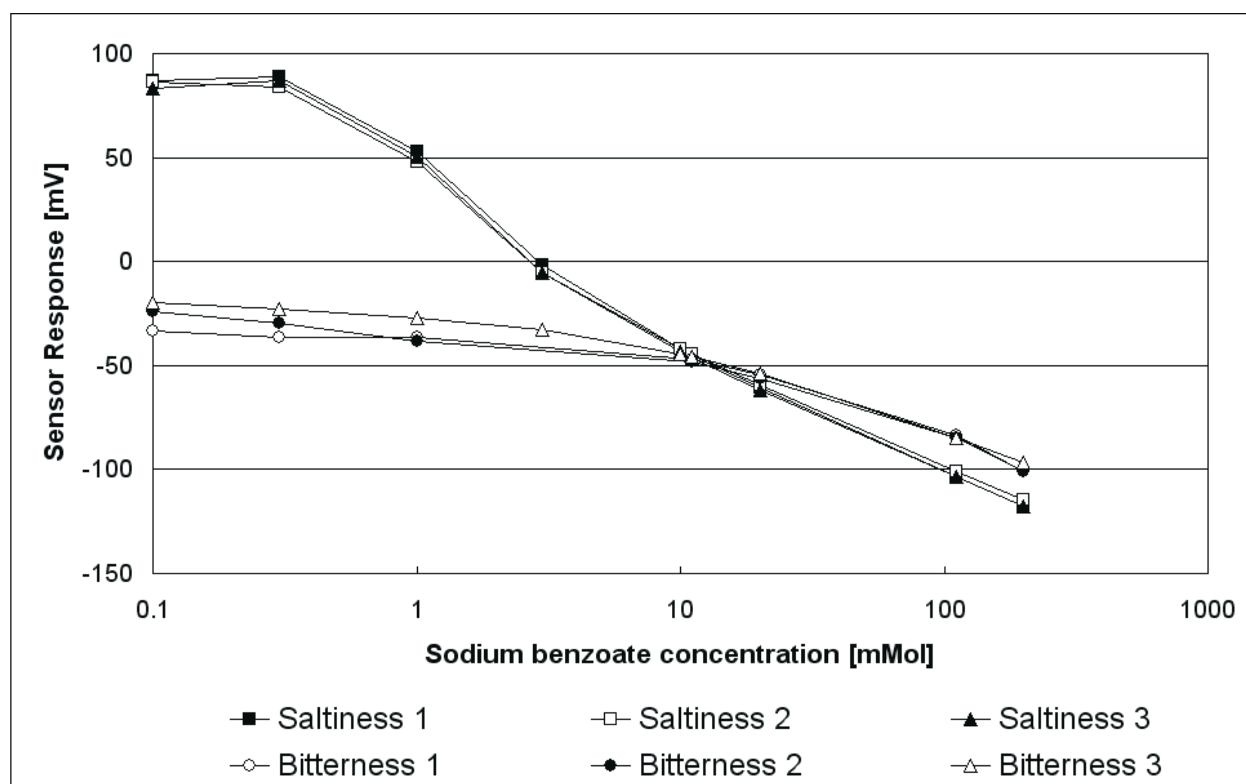

Figure 52: Sensor responses [mV] for the bitterness (SB2C00) and saltiness (SB2CT0) sensors for the three separately performed test series with calibration samples from 0.1 to 200 mMol.

After the calibration confirmed the ability of the e-tongue to measure and detect sodium benzoate in the estimated concentration range the pellet batches were investigated using the same set-up. Even though the Insent e-tongue is capable of performing measurement of samples without the respective placebo formulations they were also measured to keep the settings similar in both e-tongue experiments. As done with the Astree data, exemplary for all pellet batches the results from pellets with MCC as a binder will be discussed in detail to demonstrate the data handling with the Insent e-tongue.
As done with the calibration samples, data was presented ordered by samples (Figure 53) and sensors (Figure 54). After elimination of the first run, the standard deviations (SD) for all sensor and samples are displayed in Table 13. For the active containing samples the SD is < 1, whereas the SD for the placebo samples range between 1-2. The Insent sensors need a minimum conductivity to work properly. It is unlikely that this is achieved by placebo pellets and this might cause the higher SD values. It is also noteworthy that the sensors for bitterness (SB2C00) and saltiness (SB2CT0), the taste of sodium benzoate, display the lowest SD (< 0.5) compared to the other two sensors. In general the deviations, regarding samples or sensors, are lower compared to the Astree e-tongue. More stable and reproducible sensor responses were obtained with the Insent e-tongue.

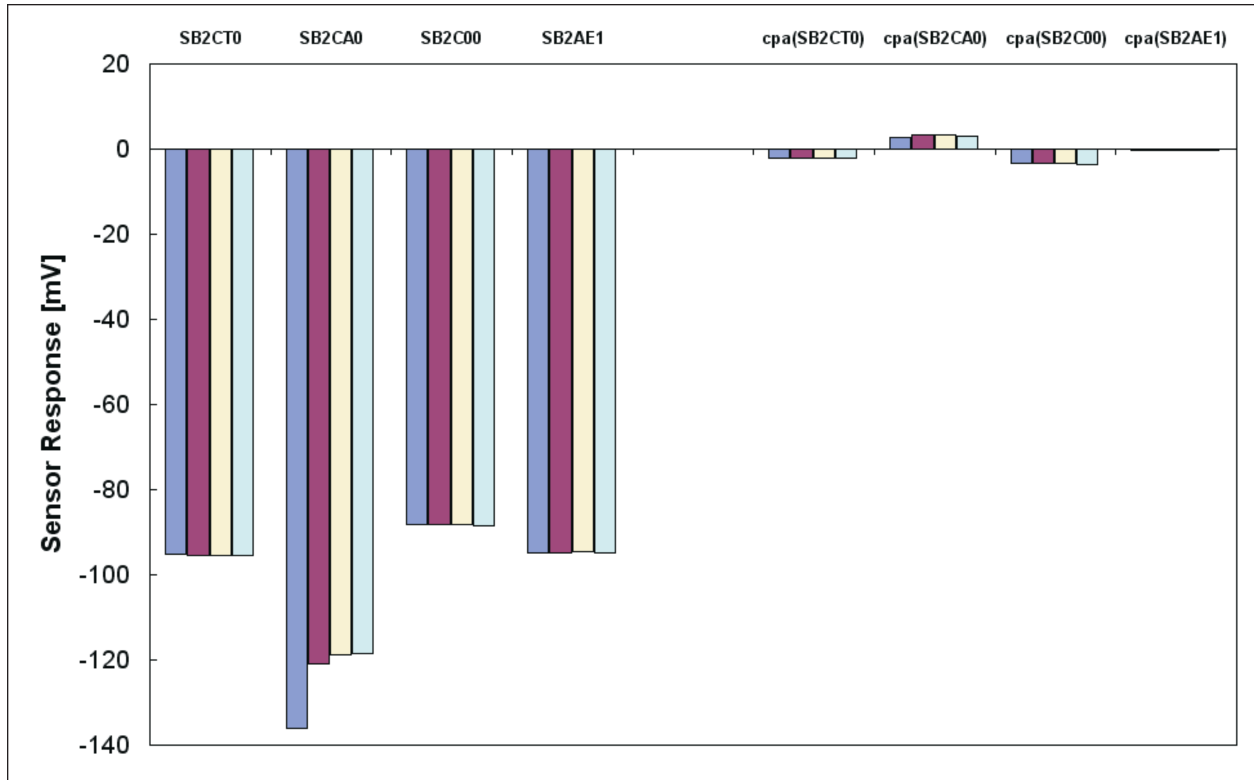

Figure 53: Sensor responses of all sensors (SB2CT0, SB2CA0, SB2C00, SB2AE1) for four runs, for one MCC sample (A5). On the right the delayed sensor signals (CPA) are displayed.

Table 13: Intra-analysis repeatability (Standard deviation of mean) for all sensors and all samples with MCC as a binder, after elimination of the first run (n = 3).

SD	SB2CT0	SB2CA0	SB2C00	SB2AE1	Mean
P1	0.24	1.13	1.58	4.27	1.81
P3	0.50	1.30	0.58	2.91	1.32
P5	0.89	0.96	0.43	2.57	1.21
P8	0.88	1.22	0.32	3.44	1.46
P10	1.00	1.07	0.41	2.85	1.33
A1	0.04	1.59	0.17	0.04	0.46
A3	0.07	1.83	0.16	0.07	0.53
A5	0.04	1.28	0.21	0.05	0.40
A8	0.03	1.04	0.05	0.01	0.28
A10	0.06	0.78	0.05	0.04	0.23
Mean	0.38	1.22	0.39	1.62	

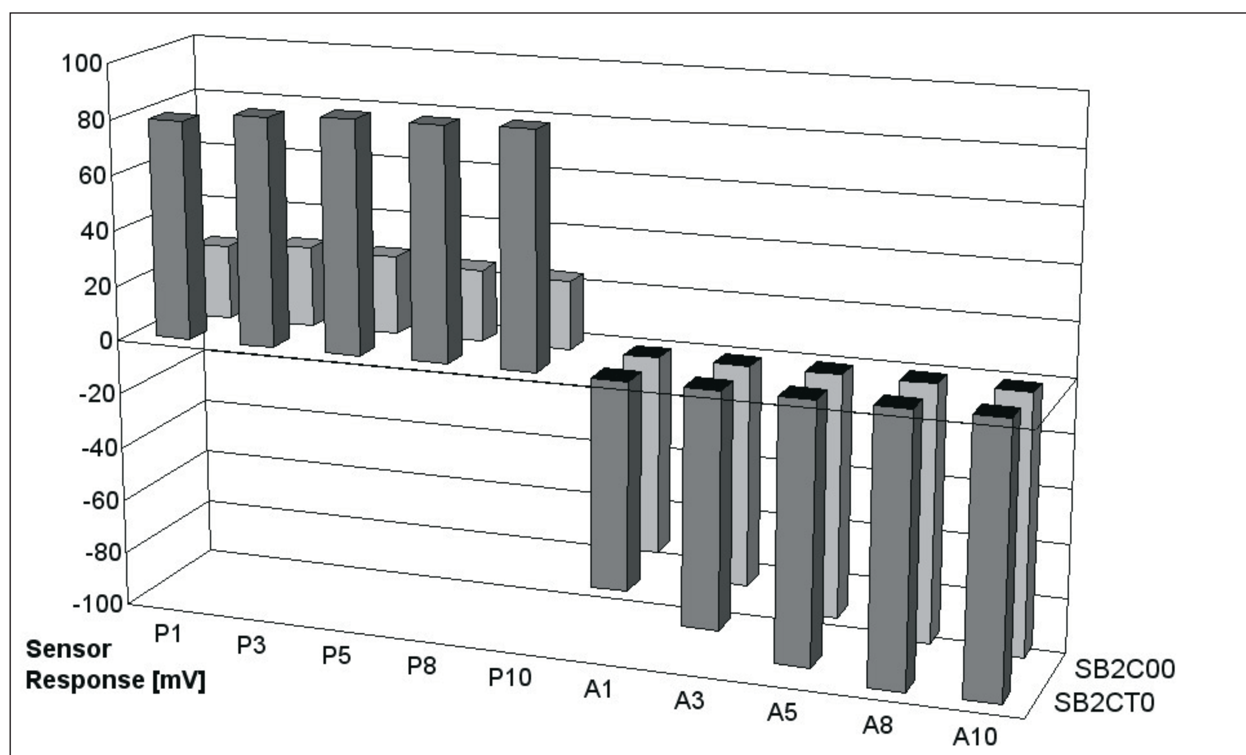

Figure 54: Sensor responses from two sensors (SB2C00 = bitterness and SB2CT0 = saltiness) for all MCC samples (incl. placebo), n = 3.

As seen in Figure 54, the responses to the placebo samples (positive) can be clearly distinguished from the results for the active containing samples (negative sensor signals). Both, the bitterness and saltiness sensor, show stable or only slightly varying values for the placebo samples, whereas the response to the active samples show an increase in negativity with an increase in released active substance.
After data evaluation with the Insent Software, including the transformation from actual sensor responses in mV into dimensionless taste information (see also F2.4.6), further data representation was possible.
A radar plot for all MCC samples can be seen in Figure 55. Again the placebo pellets are clearly distinguishable from the active samples and the sensitivity of certain sensors can be seen. The samples differ widely in bitterness and aftertaste from bitterness, as well as in aftertaste from astringency, whereas the results from the saltiness and astringency sensor are located closer by, similar to the sourness sensor response. With this plot also the direction of sensor response is possible to depict. Depending on the sensor and the active drug, sensor responses can become more positive or more negative with an increase in drug concentration. This direction change is indicated by crossing lines in the radar plot, as can be seen between bitterness and astringency.
In 2D-plots, 2 different taste information values can be displayed parallel to each other. The axes can be chosen freely, so all possible correlations can be displayed. Depicted in Figure 56 are the taste information from saltiness and bitterness for all MCC samples.

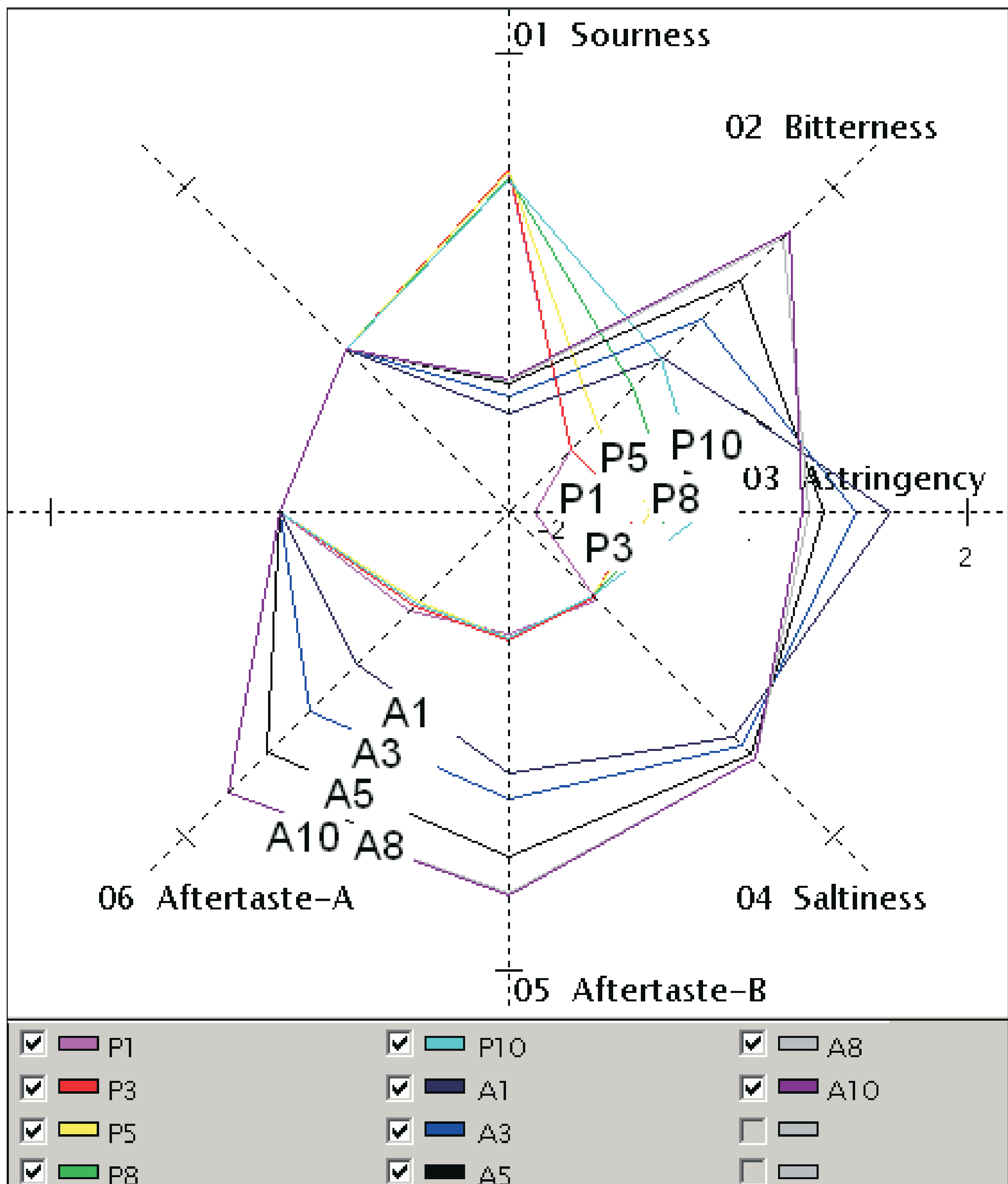

Figure 55: Radar chart for all MCC samples (n = 3).

Figure 56: 2D-Plot with taste information for saltiness and bitterness for all MCC samples (n = 3, mean ± SD).

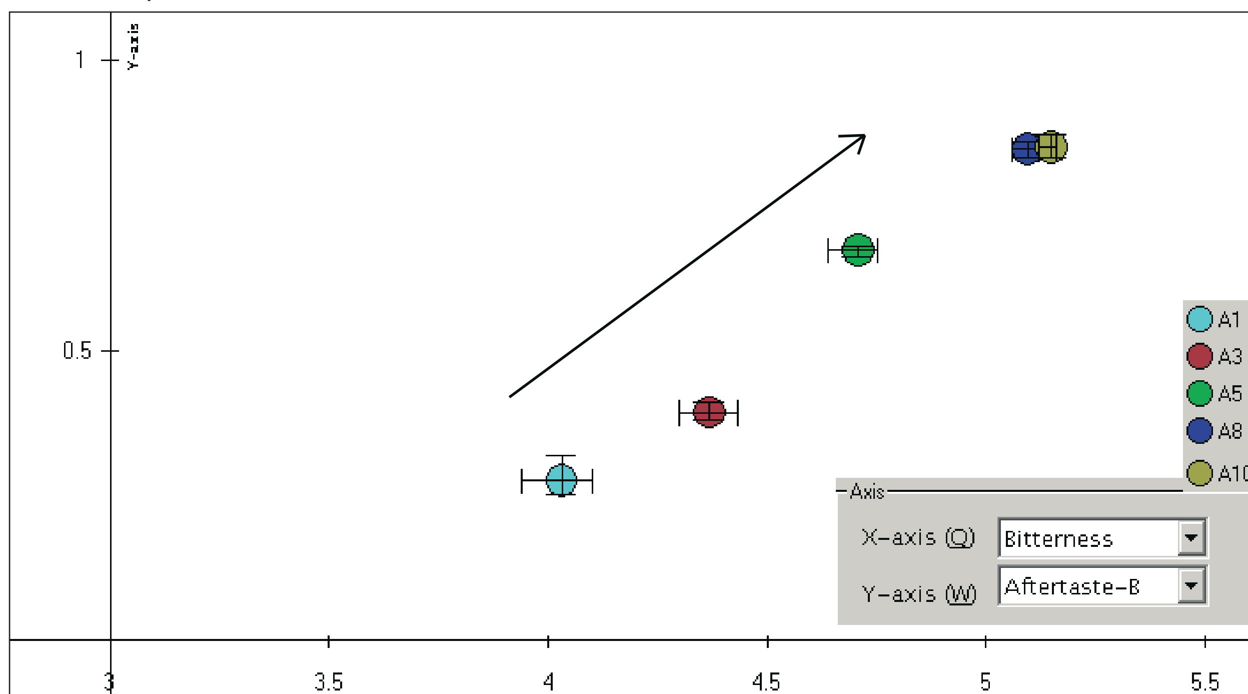

Figure 57: 2D-Plot with taste information for bitterness and aftertaste from bitterness for active MCC samples (n = 3, mean ± SD).

The placebo samples are located in a cluster on the left (negative) side of the diagram and can only be distinguished through their bitterness value, but range tighter together than the active samples. The active samples can be distinguished through both values which score increased taste values with an increase in released sodium benzoate. Another 2D-plot can be seen in Figure 57, where bitterness is plotted against aftertaste from bitterness. Again sensor responses increase with an increase of sodium benzoate concentration, even though the absolute values are in a relative small range compared to other sensor responses, e.g. saltiness. The change from negative mV data in Figure 54 to positive taste values in the 2D-plots occurs through the insertion of the mV data into a equation to receive the respective taste values (see Table 20).
This data analysis with the respective bar graphs, radar charts and 2D-plots was performed for all pellet batches, in the same manner as reported for the MCC pellets. In all cases a correlation between the bitterness and saltiness taste values, or even the raw data in mV, and an increase in sodium benzoate release could be established.
To compare all five batches with each other, the taste values for saltiness and aftertaste from bitterness for all active samples in each batch are displayed in Figure 58. As with the human taste panel and Astree e-tongue data, a certain order of taste can be established for the batches. High taste values, especially for saltiness can be seen for MCC pellets, followed by the hard fat and carrageenan containing pellets. Low and in this case even negative values are given for the pellets with the lipid mixture as a binder and the coated SB pellets reach values in between and are again higher. The same applies to the taste values for aftertaste from bitterness, just in a smaller range.

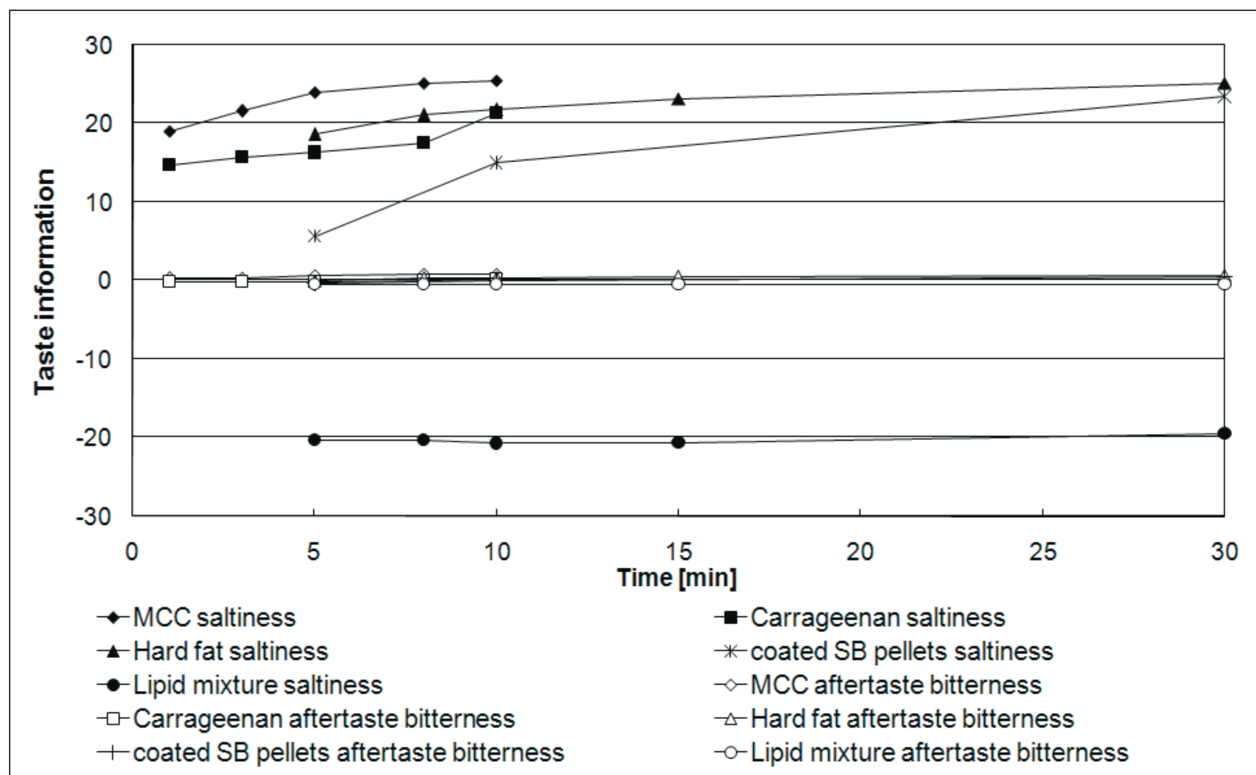

Figure 58: Taste information saltiness and aftertaste bitterness from two sensors (SB2CT0 and SB2C00) for pellet batches releasing sodium benzoate over time.

Concluding it is possible to say that the Insent e-tongue is able to detect sodium benzoate in an aqueous solution as well as released from different pellet formulations. A correlation between the increase in sodium benzoate concentration and sensor responses is clearly established. Furthermore six different taste qualities (saltiness, sourness, bitterness, astringency, aftertaste bitterness and aftertaste astringency) can be differentiated and data can be displayed accordingly. Correlations between taste qualities can be made visible.

3.5 Correlation Taste panel/UV data vs. E-tongue

With the provided software of the Insent taste sensing system comes a feature to correlate the obtained taste values with own data sets. In this case, the data obtained through the human taste panel test and released sodium benzoate concentration was used. Through multivariate data analysis (PLS) the six taste information values were correlated to the given data set and used to calculate a predictive value, either as a human taste value (rated from 0 to 3) or a released sodium benzoate percentage (0 to 100). This was performed for all pellet batches so that two values (human taste value and released SB %) was obtained for each tested active sample, calculated by the e-tongue. As can be seen in Figure 59 and Figure 60, linear relationships could be established for both correlations. The higher deviation in the correlation with the human taste can be explained by the high intra-individual variation within the human test subjects.
Therefore it can be concluded that it is possible to get taste informations with the Insent taste sensing system for sodium benzoate which are comparable and similar to the actual perceived human taste and correlate with sodium benzoate concentration in the tested solution. Further it is possible to use the obtained data to analyse unknown samples and get taste evaluations without the need of taste assessment panels.

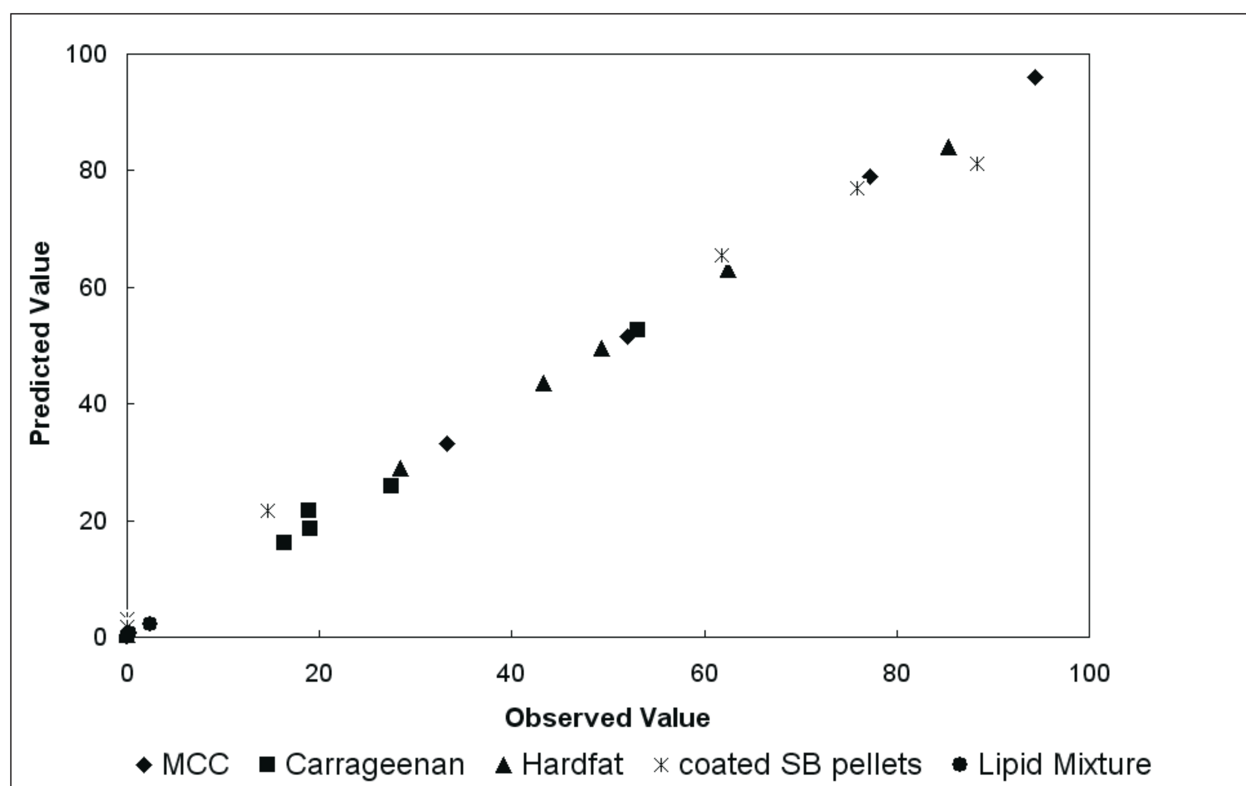

Figure 59: E-tongue prediction (Insent) vs. measured UV-data for sodium benzoate release.

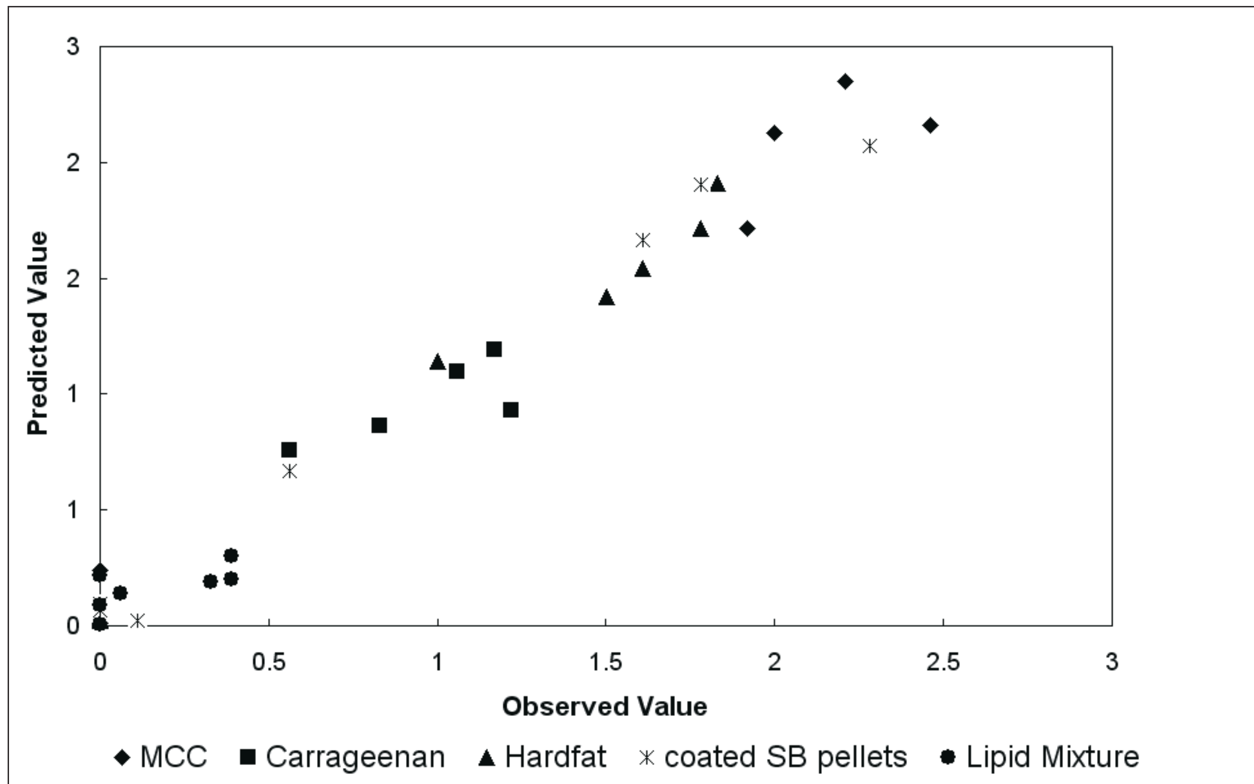

Figure 60: E-tongue prediction (Insent) vs. human taste scores from taste panel tests.

3.6 Comparison of the two e-tongues

The two used electronic taste measurement systems discussed in the previous chapters were compared to each other regarding their data analysis, measurement procedure and ease of handling. This is the first report on comparative investigations with the two e-tongues that are commercially available.

Measuring principle
Both sensor systems are based on the principle of potentiometry. With the Astree e-tongue silicon transistors are used which are sensitive towards electrostatic charging. Sensor handling is delicate and the detection of a broken, or out of range acting, sensor is difficult. A sensor array of 7 sensors and one reference electrode is used, where all sensors are able to detect various substances in solution, but differ in their sensitivity. This means a unique pattern out of the 7 sensor answers is obtained for each substance, but no individual taste qualities can be distinguished.
The Insent e-tongue can be equipped with up to 8 sensors which are able to detect 11 taste qualities (including the detection of 3 aftertastes). In this study four sensors with 6 taste qualities were used and evaluated. The sensors have to be filled with a Ag/AgCl-solution (inner solution) before measurement and can be stored under wet conditions in this filled state or can be cleaned and stored dry by the respective user. A sensor check should be performed before each measurement to assure intact sensors. Each sensor has a certain voltage range for the sensor answer during sensor check, the sensor should not be used if the sensor response is off this range.

System preparation
The preparation phase with the Astree e-tongue includes a conditioning and calibration phase. If the sensor array fails the given thresholds on stability, repeatability, drift and shift of the sensor response, tests can be repeated or thresholds lowered.
For the Insent e-tongue the above mentioned sensor check is performed to check data drift and stability of each sensor. Furthermore individual solutions for each sensor can be prepared and used as a taste sample for the corresponding sensor (bitterness, sourness, etc.).

Sample measurement
As mentioned before, placebo formulations for all tested samples are needed when the taste testing is performed with the Astree e-tongue. This, in combination with the cross-sensitive sensors, allows only relative evaluations of the taste sensation. Different formulations are difficult to compare to each other and no definite taste quality (bitter, sour, etc.) can be assigned to the samples.
With the Insent e-tongue placebo formulations are not needed. Furthermore as a reference or zero point either the standard solution or a solution selected by the user can be chosen. This allows comparison of absolute values (mV or taste information values) between samples, even of different origin.

Data analysis
Based on raw data analysis, the first 1-3 replicates of the 8 runs from the Astree e-tongue are deleted, as well as sensors lacking discrimination between samples. These revised data sets are then used for multivariate data analysis. Sample data is displayed in PCA maps and distances of active sample to placebo are derived from these maps. Correlation with additional data (either actual sample concentrations or human taste data) is possible through PLS, but has not been performed in this study using the Alpha-Mos Astree software.
The standard Insent procedure suggests 4 runs with deletion of the first replicate for further data analysis. The sensor data is then centred to the chosen zero point (standard solution or chosen reference solution) and transformed into taste information values. This taste data can be displayed in 2-D plots, PCA maps or radar plots. Furthermore the correlation with additional data through PLS can be performed, here it is possible to chose the number of used variables which also can be ranked according their importance for the overall model. Even an interpretation of the raw data in mV is possible. Raw data can be exported to other software programmes, e.g. to obtain direct correlations with concentrations.

Table 14: PLS data for four pellet batches. Correlation of Astree Taste data with human taste evaluation data and sodium benzoate concentrations (determined by UV).

Correlation Astree e-tongue vs. Taste Panel:				Corelation Astree e-tongue vs. Concentration:			
	R2Y	Q2	RMSECV [%]		R2Y	Q2	RMSECV [%]
MCC	0.85	0.736	22.99	MCC	0.98	0.973	6.03
Carrageenan	0.994	0.992	1.46	Carrageenan	0.98	0.97	0.73
Hard fat	0.94	0.932	7.58	Hard fat	0.93	0.92	12.17
coated SB pellets	0.98	0.98	8.15	coated SB pellets	0.98	0.978	15.73

Table 15: PLS data for four pellet batches. Correlation of Insent Taste data with human taste evaluation data and sodium benzoate concentrations (determined by UV).

Correlation Insent e-tongue vs. Taste Panel:				Correlation Insent e-tongue vs. Concentration:			
	R2Y	Q2	RMSECV [%]		R2Y	Q2	RMSECV [%]
MCC	0.95	0.819	7.98	MCC	0.991	0.988	5.57
Carrageenan	0.94	0.88	36.90	Carrageenan	0.998	0.995	1.93
Hard fat	0.73	0.594	5.43	Hard fat	0.996	0.989	7.38
coated SB pellets	0.87	0.815	12.44	coated SB pellets	0.986	0.978	10.77

For further comparison of both e-tongues, multivariate data analysis for four of the five pellet batches was performed with an independent software (SIMCA-P, Umetrics AB, Umeå, Sweden). The predictivity of the models were compared with each other. The taste data from both e-tongues was correlated with either the actual sodium benzoate concentration determined by UV-spectroscopy or the taste score obtained through human taste panel test through PLS as implemented in Simca-P version 10.0.4. All PLS models were validated using the leave-one-out cross validation (Q^2) test. For all pellet batches the RMSECV was compared within the two equipments. With MCC and hard fat pellets the Insent e-tongue appears to have a better correlation for both, the human taste and concentration data, indicated by lower RMSECV values. The same applies to the Astree data and carrageenan pellets, even though this data should be treated critically as the gel formation and the interaction of the carrageenan with the sodium impaired the results as no proper solution, which was needed for e-tongue measurement, was obtained. The coated sodium benzoate pellets give a split result, overall it is the batch with the highest RMSECV, therefore having the models with the worst predictivity. Concerning the correlation with human taste data, the Astree e-tongue is better, concerning concentration, the Insent e-tongue obtained better values.
If all the above compared points are comprised, the Insent e-tongue is favourable to the Astree e-tongue in the prediction of taste for sodium benzoate releasing pellets. The main points are the better data handling and analysis abilities, ease of sensor handling and slightly better predictivity by PLS models.

3.7 Conclusion

Electronic taste measurement systems offer a new and valuable technique for formulation development. They are capable of detecting taste differences in different formulations and these findings correlate with actual drug concentration and perceived human taste. This makes it possible to test and analyse new and unknown samples without further need of conducting human taste panel tests at least in the early phases of formulation development. These cumbersome, time-consuming and sometimes even difficult to conduct tests may be replaced by analytical means while still achieving similar results. So far, only sodium benzoate has been tested in detail for this study, but further investigational studies in our work group revealed the usefulness of an e-tongue also for a range of other bitter tasting drugs. Therefore a broader field of application is possible. One question that remains is the transferability from these findings to the paediatric population. Children have a different taste perception than adults, and are more sensitive towards bitter taste and prefer sweeter tastes. If a model is built up for taste prediction using adult volunteers, the outcome might be different if children would have been used as test subjects.
Nevertheless, this study proves the general usefulness and usability of these new analytical tools.

D Summary

Novel drug formulations with sodium benzoate as an active ingredient were developed in the present work: an infusion solution and taste masked pellets with lipid binders.

The infusion is intended for the use in neonates with non-ketotic hyperglycinemia or urea cycle defects and for emergency cases. In contrast to common infusions prepared in hospital pharmacies it lowers the risk of hypernatremia in newborns.
The taste masked pellets are intended for infants and elder patients who are able to swallow small-sized particles together with food. In contrast to already existing pellets with sodium benzoate the newly developed pellets do not need a polymer coating for an efficient taste masking as demonstrated by different in vitro and in vivo taste assessment methods. The pellets show a self-coating mechanism which was discovered and described in the present work for the first time.

The particular results are: For the infusion solution a 2 %, isotonic formulation was obtained with reduced sodium levels. The solution underwent stability testing for 2 years meanwhile the testing of different stopper material as vial closures was included. The formulation was stable during the tested time period, since the content of the tested ingredient was in between 95 % and 105 % of the nominal content for all batches. Only one stopper variety, the bromobutyl rubber stoppers, were evaluated as insufficient, as the pH value changed significantly over the tested period.

Sodium benzoate containing pellets with MCC or κ-carrageenan prepared through and wet extrusion process, also pellets with four different lipids as binders were investigated as suitable dosage forms in 7 different mixtures and prepared by cold solid lipid extrusion.
All developed pellet batches were characterised and compared to each other as well as to previously prepared salvia-resistant coated granules with sodium benzoate.
It is possible to obtain pellets with suitable batch characteristics such as spherical shape indicated by AR < 1.2, narrow particle size distribution, a high drug load of 80 % and immediate release profiles through an extrusion/spheronisation process and appropriate mixture of different lipids.

Within the investigations it became evident that it is possible to mask the unpleasant taste in this lipid pellets without the need of a coating. We called this principle "self-coating" of lipid pellets. This mechanism first described in this work, is a new technique in taste masking strategies. The mechanism can be explained by the production process. To achieve spherical pellets, the respective extrudates are rounded in a spheroniser, which is heated to temperatures of approximately 10 °C below the melting range of the lipid with the highest fraction in the mixture and the lowest melting range. During this process, some parts of the lipid binder melt due to the heat of the spheroniser and frictional forces in the pellet bed. A separation of the molten lipid binder and the solid crystalline sodium benzoate takes place. This leads to a layer of lipids of approximately 10 µm around the surface of the pellets which acts as a diffusion barrier and masks the unpleasant bitter and salty taste. This taste masking effect is based on a temperature dependent release of the drug through this layer. Selection of appropriate lipid formulations influence the diffusion through this layer and release profiles can therefore be fitted to desired kinetics. It is possible to obtain pellets which display hardly any drug release at lower temperatures and are therefore able to mask an unpleasant taste at these temperatures whereas a higher temperature (37 °C) leads to a complete drug release. Further insight has to be gained into the specific nature of the components in the respective lipids that lead to the differences in the temperature dependent drug release effects through this layer.

In taste panel tests with adult volunteers and comparison of sodium benzoate release in different food, it could be demonstrated that these lipid pellets have a better taste masking ability than saliva-resistant coated granules.
The taste assessment was also performed in vitro using novel chemometric sensor systems called "electronic tongues". Two of these e-tongues were used for the determination of taste of 5 different pellet formulations all containing sodium benzoate. The two e-tongues were evaluated and compared in their handling and measurement routine, data analysis and ability for the prediction of taste. The Insent e-tongue gave a slightly better impression due to easier handling and measurement routine and more abilities in data analysis. Both tested systems were able to detect differences in between the tested formulations. Correlations between taste values from the human taste panel as well as concentration of released sodium benzoate and data from the e-tongues were possible. E-tongues offer valuable alternatives to the common method of human taste panel tests for the evaluation of taste, especially in cases where taste assessment studies are difficult, e.g. due to regulatory or organisation issues, as in the formulation development for the paediatric population.

E Zusammenfassung

In der Arbeit wurden neue Arzneiformen, eine Infusionslösung und geschmacksmaskierte Pellets, mit Natriumbenzoat als Arzneistoff entwickelt.

Die Infusionslösung ist für die Notfallversorgung von Neugeborenen mit non-ketotischer Hyperglycinämie oder Harnstoffzyklusdefekten bestimmt. Im Gegensatz zu den bisher in Krankenhausapotheken hergestellten Infusionslösungen bietet die neu entwickelte Infusionslösung den Vorteil, das Risiko von Hypernatriämien zu verringern.
Die geschmacksmaskierten Pellets sind für Kleinkinder und ältere Patienten gedacht, die in der Lage sind, diese kleinen Partikel zusammen mit der Nahrung einzunehmen. Im Gegensatz zu den bisher existierenden Natriumbenzoat-Granulaten kann bei den neu entwickelten Pellets auf eine Geschmacksmaskierung, die auf einem separaten Prozess beruht, verzichtet werden. Dies wurde durch verschiedene in-vivo und in-vitro Untersuchungsmethoden gezeigt. Der Mechanismus der Geschmacksmaskierung, welcher erstmals in dieser Arbeit entdeckt und beschrieben wurde, beruht auf einer in-situ Bildung von Lipidschichten.

Für die Infusionslösung wurde eine 2 % isotone Formulierung mit reduziertem Natriumgehalt entwickelt. Der Einfluss verschiedener Stopfenmaterialien auf die Stabilität der Infusionslösung wurde während einer zweijährigen Stabilitätsstudie untersucht. Die Formulierung erwies sich als stabil über den getesteten Zeitraum, da alle untersuchten Inhaltsstoffe für die getesteten Chargen im Bereich von 95 % bis 105 % des angegebenen Gehaltes lagen. Nur die Brombutylstopfen wurden aufgrund Ihres negativen Einflusses auf den pH-Wert der Infusion als ungeeignet bewertet.

Mittels Feuchtextrusion wurden Natriumbenzoat-haltige Pellets mit MCC und κ-Carrageenan als Bindemittel hergestellt. Weiterhin wurden vier Lipide und Ihre Mischungen als Bindemittel zur Herstellung von Lipidpellets durch kalte Festfettextrusion untersucht.
Die hergestellten Pelletchargen wurden charakterisiert und miteinander sowie gegen die bisher bekannten speichelresistent-befilmten Natriumbenzoatgranulate verglichen.
Pellets mit geeigneten Eigenschaften, wie einem Seitenverhältnis < 1,2, enge Partikelgrößenverteilung, einer hohen Arzneistoffbeladung von 80% und schneller Arzneistoff-Freisetzung können durch einen Extrusions/Sphäronisationsprozess und unter Verwendung einer geeigneten Lipidmischung erhalten werden.

Es stellte sich während der Untersuchungen heraus, dass es möglich ist, den schlechten Geschmack des Natriumbenzoates in den Lipidpellets ohne zusätzliches Überziehen der Pellets durch einen funktionellen Film zu erreichen. Der Mechanismus der in-situ Bildung von Lipidschichten wurde erstmals in dieser Studie beschrieben und stellt eine neuartige Form der Geschmacksmaskierung da. Der Mechanismus beruht auf der Prozessführung bei der Herstellung der Pellets. Zur Herstellung sphärischer Pellets werden die entsprechenden Extrudate in einem erwärmten Sphäroniser gerundet. Die Temperatureinstellung liegt bei 10 °C unter dem Schmelzbereich des Fettes mit dem niedrigsten Schmelzbereich. Während des Sphäronisationsprozesses werden durch die Erwärmung des Sphäronisers und die entstehende Reibungswärme im sich bewegenden Pelletbett Teile der Fette angeschmolzen. Es kommt es zu einer Trennung von geschmolzenem Fett und kristallinem Natriumbenzoat, welches dazu führt, dass sich an der Oberfläche der Pellets eine ca. 10 µm dicke Lipidschicht bildet. Diese funktioniert als Diffusionsbarriere und maskiert den schlechten Geschmack des Natriumbenzoats. Dieser Mechanismus der Geschmacksmaskierung beruht auf einer temperaturabhängigen Freisetzung des Wirkstoffes aus den Pellets. Durch die Auswahl von geeigneten Lipidmischungen ist es möglich, die Diffusion durch diese Schicht

zu beeinflussen, um so erwünschte Freisetzungsprofile zu erhalten, sodass bei niedrigen Temperaturen fast keine Arzneistofffreisetzung stattfindet und damit der Geschmack maskiert ist, jedoch bei höheren Temperaturen (37 °C) eine vollständige Freisetzung vorliegt. Weiterführende Untersuchungen sind nötig, um tiefere Einblicke in die genaue Zusammensetzung dieser Lipidschichten zu erlangen, damit der Einfluss einzelner Lipide auf die temperaturabhängigen Freisetzungseigenschaften dieser Lipidschichten charakterisiert werden kann.

In Geschmacksuntersuchungen mit freiwilligen erwachsenen Testpersonen und durch vergleichende Freisetzungsuntersuchungen in verschiedener Nahrung konnte gezeigt werden, dass die hergestellten Lipidpellets eine bessere Geschmacksmaskierung aufweisen als die speichelresistent überzogenen Granulate.

Zur in-vitro Geschmacksuntersuchung wurden neuartige chemometrische Sensorsysteme, sogenannte elektronische Zungen (e-tongues), eingesetzt. Die beiden auf dem Markt befindlichen Systeme wurden genutzt, um den Geschmack von fünf verschiedenen Pelletchargen zu bestimmen. Die beiden E-tongues wurden anhand Ihrer Handhabung, Versuchsdurchführung, Datenauswertung und der Qualität der Geschmacksvorhersage beurteilt und verglichen. Die Insent E-tongue wurde aufgrund Ihrer einfachen Sensorhandhabung und Versuchsdurchführung sowie der größeren Möglichkeiten in der Datenauswertung positiver bewertet. Beide Systeme waren in der Lage, Unterschiede zwischen den einzelnen Formulierungen zu detektieren. Weiterhin war es möglich, eine Korrelation zwischen den Probandenbewertungen sowie dem freigesetzten Natriumbenzoat-Anteil in der Lösung und den Werten der E-tongues zu erstellen.
Der Einsatz von E-tongues ist eine mögliche Alternative zu den üblicherweise eingesetzten Geschmacksuntersuchungen mit freiwilligen Testkollektiven. Besonders in kritischen Fällen wie der Entwicklung neuer Arzneistoffe mit unbekannter Toxizität oder im Bereich neuer Kinderarzneimittel, in dem Geschmacksstudien nur sehr schwer und unter hohem organisatorischen Aufwand durchzuführen sind, wären E-tongue-Messungen von Nutzen.

F Experimental Part

1. Materials

1.1 Sodium benzoate

Sodium benzoate (CAS No 532-32-1) is the sodium salt of benzoic acid and has the chemical formula C_6H_5COONa. It has a molar mass of 144.1 g/mol, a density of 1.44 g/cm^3 and a melting point around 420 °C. It is very soluble in water (550-630g/L at 20 °C) and hygroscopic at a relative humidity above 50 %. Its pH is about 7.5 at a concentration of 10 g/L in water and the pK_a is 9.8. Sodium benzoate is produced through liquid-phase oxidation, in the presence of a catalyser, of toluene to benzoic acid, which is then neutralised with sodium hydroxide to form sodium benzoate. It is used as a preservative in foodstuff (E211), beverages, toothpastes, mouthwashes, cosmetics and pharmaceuticals due to its bacteriostatic and fungi static quality and is most suitable for foods, fruit juices and soft drinks that are naturally in an acidic pH range, preferably in a pH range below 4.5. Benzoic acid permeates endothelial cells and lowers the intracellular pH. This leads to an inhibition of glycolysis, which causes a fall in ATP and thus restricts cell growth (Krebs 1983). The European Commission limits the use of benzoic acid and sodium benzoate in food at 0.5 % (EC 1995). About 0.1 % is usually sufficient to preserve an aqueous product. In humans, the acute toxicity is low; however, sodium benzoate is known to cause non-immunological contact reactions (pseudo-allergies). It has a salty and bitter taste, which is not detected by 25 % of the population. In the acidic conditions of the stomach, benzoate will change from the ionized form to the undissociated benzoic acid. As a result, the metabolism and systemic effects of benzoic acid and sodium benzoate are the same. Benzoic acid can be found naturally in many plants, as it is produced as an intermediate in the formation of other compounds. It may be formed from cinnamic acid through a β-oxidation-like process or through a direct way, without the formation of cinnamic acids, branching from the shikimic acid (Hänsel 1999). High concentrations can be found in gum benzoin, i.e. up to 20 % (Hänsel 1999) or certain berries like the Vaccinum-species, e.g. cranberries which can contain as much as 300-1300 mg free benzoic acid per kg fruit (Hegnauer 1966). The role and effect of sodium benzoate as a therapeutic agent has been discussed in chapter B1. The used sodium benzoate qualtities, as well as the saliva-resistant coated sodium benzoate pellets, used as a reference for pellet characterisation, are displayed in Table 16.

Table 16: Sodium benzoate.

	Quality	Batch number	Supplier
Sodium benzoate	Ph. Eur. Ph. Eur.	200620609 01/2003	Ethicare GmbH, Haltern, Germany
Sodium benzoate	Ph. Eur.	2720	Riedel-de Haën, Seelze, Germany
Saliva-resistant coated sodium benzoate granules	Clinical batch	260402	Ethicare GmbH, Haltern, Germany

1.2 Lipid grades

Different lipids and binary or ternary mixtures of these lipids were used in the preparation of pellets through an extrusion/spheronisation process.

Table 17: Used lipid grades.

	Witocan® 42/44 „Hard fat“	Dynasan® 114 „Glycerol trimyristate“	Precirol® ATO 5 „Glycerol distearate“	Compritol® 888 ATO „Glycerol dibehenate“
Quality	Ph. Eur.	Food grade	Ph. Eur.	Ph. Eur.
Composition	90 % < Triglycerides	95 % < Triglycerides	25-35 % Triglycerides	21-35 % Triglycerides
			40-60 % Diglycerides	40-60 % Diglycerides
			8-22 % Monoglycerides	13-21 % Monoglycerides
Melting Point	42-44 °C	55-58 °C	53-57 °C	69-74 °C
HLB	2	2	2	2
Hydroxyl number	15	10	100	100
Batch number	106016	512158 402156	34196	106052
Supplier	Sasol, Witten, Germany	Sasol, Witten, Germany	Gattefossé, Weil am Rhein, Germany	Gattefossé, Weil am Rhein, Germany

1.3 Other substances

The following other chemical substances (Table 18) were used in this work.

Table 18: Other used substances.

	Quality	Batch number	Supplier
Acetic acid	p.a.	41151235	Carl Roth GmbH, Karlsruhe, Germany
Acetonitrile	HPLC		Fisher Scientific, Loughborough, UK
Ammonium acetate	Ph. Eur.	10150	Riedel-de Haën, Seelze, Germany
Benzoic acid	Ph. Eur.	40750	Riedel-de Haën, Seelze, Germany
Calcium chloride	Ph. Eur.	4J008226	KMF, Lohmar, Germany
Destilled water	Ph. Eur.		in- lab destillation
Hydrochloric acid 1 mol/l (1N)	p.a.		Merck, Darmstadt, Germany
κ-carrageenan (Gelcarin GP 911 NF)		40701170	FMC, Philadelphia, PA, USA
Microcrystalline Cellulose (Sanaq 102)		216150	Pharmatrans Sanaq, Basel, Switzerland
Potassium chloride	Ph. Eur.	4E001643	KMF, Lohmar, Germany
Potassium hydroxide 0.1 mol/l (0.1N)	p.a.		Merck, Darmstadt, Germany
Purified water	Ph. Eur.		Reverse Osmosis
Silver chloride	purum	50770	Riedel-de Haën, Seelze, Germany
Sodium hydroxide 1 mol/l (1N)	p.a.		Merck, Darmstadt, Germany
Tartaric acid	Ph. Eur.	50610	Riedel-de Haën, Seelze, Germany

2. Methods

2.1 Infusion preparation

2.1.1 Manufacturing

All used glass and other equipment, including the later used stoppers and glass containers (Type I, Eur. Pharm.), were sterilized at 160 °C for two hours. The ingredients for the solution were weighed out and put in a volumetric flask. The powders were then dissolved in distilled water.

2.1.2 Filtering and sterilization

Before sterilisation, the solution was filtered through a 0.2 µm nylon bottle-top filter (ZAPCAP®-CR, Schleicher & Schuell MicroScience, Riviera Beach, FL, USA) directly into the vials. Preliminary tests were performed to ensure that sorption of active ingredients on filter material do not takes place. These were then closed with bromobutyl (Type V9003/FM257/1, Lot 0305340, Zscheile & Klinger, Hamburg, Germany) and chlorobutyl (Type 4443/4106/50 , Lot 2208057, Zscheile & Klinger, Hamburg, Germany) rubber stoppers as well as teflon-coated FluroTec stoppers (Type PH 4104/40, West Pharmaceutical Services, Eschweiler, Germany) which were retained by an aluminium crimped seal (Type ZK 3214, Lot 900369, Zscheile & Klinger, Hamburg, Germany) and autoclaved (tuttnauer 3850 EL, Tuttnauer, Breda, The Netherlands) for 15 minutes at 121 °C and 2 bar.

2.1.3 Storage

Infusion vials with each stopper variety were then stored at room temperature to allow stability testing over 24 months with a three month sample interval (n=3). Six vials for chloro-, bromobutyl stoppers and Teflon stoppers, were stored under accelerated conditions at 40 °C (ET 6130, Heraeus, Hanau, Germany) for six months according to the ICH Note for Guidance on Stability Testing (CPMP/ICH 2003).

2.2 Infusion characterisation

2.2.1 HPLC-UV (DAD)

Sodium benzoate content of infusion solutions was determined by a validated high-performance liquid chromatography (HPLC) method, developed by Breitkreutz et al. (2003). A HPLC apparatus Hewlett-Packard 1090 Series II (Agilent, Böblingen, Germany) with an UV-diode array detector and a Rheodyne injection loop 7125 (Alsbach, Germany) with a volume of 20 µl was used to obtain chromatograms. A Microliter #702 injection tip (Hamilton, Reno, USA) with 25 µl volume was used to inject the analysed solutions. Analysis of the data was performed with the Hewlett-Packard ChemStation Rev. A.06.03 (Agilent, Böblingen, Germany). The method was validated based on the ICH Guideline Q2 (R1) "Validation of Analytical Procedures" (2003). An identification of the peaks was based on the retention time, referred to benzoic acid (p.a. as laboratory standard) as internal standard. The laboratory standard was calibrated with Benzoic acid USP Reference Standard 05500 (Lot F-5). The resulting calibration factor was 1.0015. Due to the acidic pH the resulting peaks could be attributed to benzoic acid, and therefore benzoic acid could be used as the reference standard substance.
The eluent was a mixture of 30 % acetonitrile and 70 % 0.01 M aqueous ammonium acetate solution adjusted to pH 4.5 by acetic acid with a flow rate of 1 ml/min. A reverse phase prepacked column Agilent Eclipse XDB-C18 (dimensions: 4.6 x 150 mm, size of packaging material: 5 µm,

Agilent) was used with a column temperature of 40 °C. The wavelength for peak evaluation was 225 nm.
A stock solution was used to prepare solutions with 50, 75, 100, 125 and 150 % content of the labelled claim which were then analysed in random order (n = 2). The detected value was calculated according to following equation:

$$Content[\%] = \frac{A_{Sample} \times 144.1 \times e_{BA} \times 10000}{A_{BA} \times nc \times 122.1 \times e_{Sample} \times 2} \quad \textbf{(Eq. 1)}$$

with A_{Sample} and A_{BA} as AUC of the sample and the standard, respectively. e_{BA} and e_{Sample} are the initial weight and *nc* is the nominal content of each sample. The recovery rate was 99.81 % (n = 10). The linearity was calculated from these samples, resulting in a linearity with the calibration equation y mAU*s = 14.727 x mg/100 ml. The coefficient of determination was 0.9994 at 225 nm from 1.5-4.5 mg/100 ml (50-150 % content). The limit of quantification and qualification was determined with eight solutions ranging from 1.5 to 0.0156 % content resulting in a limit of quantification of 0.0019 mg/ 100 ml and limit of qualification 0.000059 mg/100 ml. The precision, determined by the relative standard deviation of six separately weighed samples (n = 2), was 1.45 %.
50.0 mg benzoic acid was completely dissolved in 100 ml distilled water in a volumetric flask; 5.0 ml of the solution was diluted to 100.0 ml with eluent and the resulting solution marked as laboratory standard solution. During sample measurements, the standard solution was injected two times before sample measurement started and after every fifth sample. Sample data was only analysed if the relative standard deviation of the standard solution was < 2 %.
To prepare samples from infusion solutions, 3.0 ml infusion solution was diluted to 100 ml with distilled water in a volumetric flask and 5.0 ml from this solution was then mixed with eluent in a 100 ml volumetric flask. Each sample was measured twice.
The content was calculated according to following equation:

$$Content[\%] = \frac{A_{Sample} \times 144.1 \times e_{BA} \times 100}{A_{BA} \times 122.1 \times e_{Sample}} \quad \textbf{(Eq. 2)}$$

With A_{Sample} and A_{BA} as AUC of the sample and the standard, respectively, and e_{BA} and e_{Sample} as the initial weight.

2.2.2 Atomic absorption spectroscopy

The content of sodium, potassium and calcium in the infusion solutions were analysed by atomic absorption spectroscopy (AAS-1100-B, Perkin-Elmer, Überlingen, Germany). An acetylene/air flame with 10 cm width was used to atomize the samples. As a light source, hollow cathode lamps of the respective elements were used. The calibration was done with two reference solutions (higher and lower concentration around the nominal value) for each element and each analysis time point. Due to ionisation and physical interferences the reference solutions contained the same matrix as the samples, e.g. the reference solutions for sodium contained, next to a set amount of sodium also calcium and potassium in the same concentration as it would be in the sample solution. For the detection of sodium a Perkin Elmer Intensitron™ hollow cathode lamp (Part #3036065, Serial #402606, Perkin Elmer, Überlingen, Germany) was used with 8 mA current. The spectral line was 589.0 nm and the gap length of the monochromator 0.4 nm. Reference solutions with 0.8 and 1.2 mg sodium/l were used for calibration. For sample preparation, 0.3 ml of the infusion solution was diluted to 1 l in a volummetric flask to obtain a nominal concentration of approx. 1 mg/l. For potassium, a Perkin Elmer Photron™ hollow cathode lamp (Part No P941,

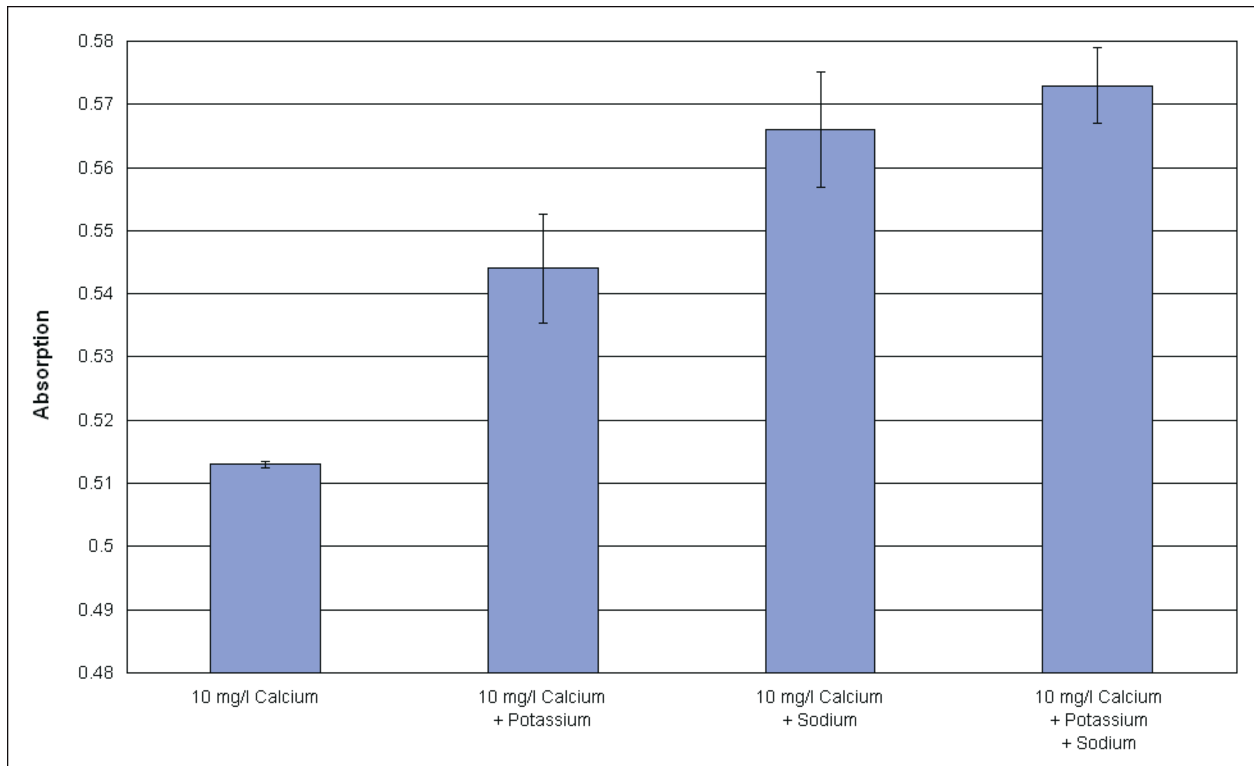

Figure 61: Matrix effect for calcium; Influence of potassium and sodium (n = 3, mean ± SD).

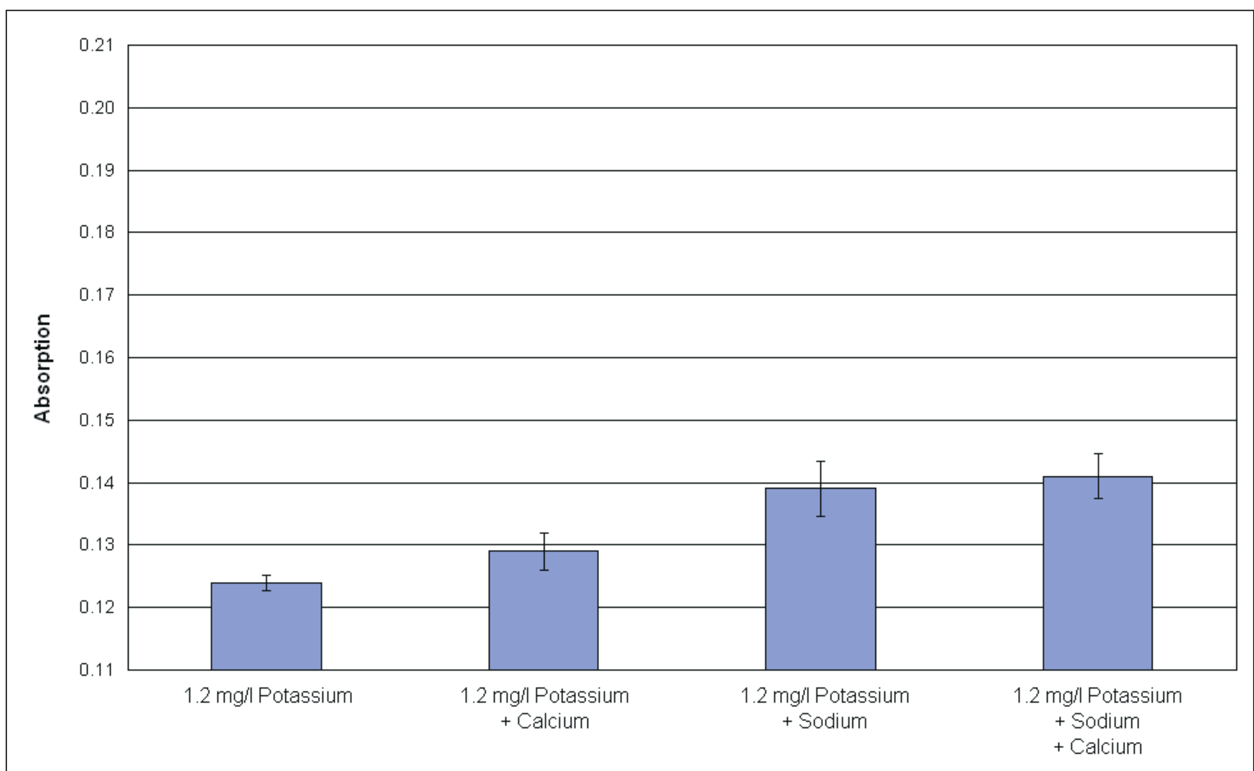

Figure 62: Matrix effect for potassium; Influence of calcium and sodium (n = 3, mean ± SD).

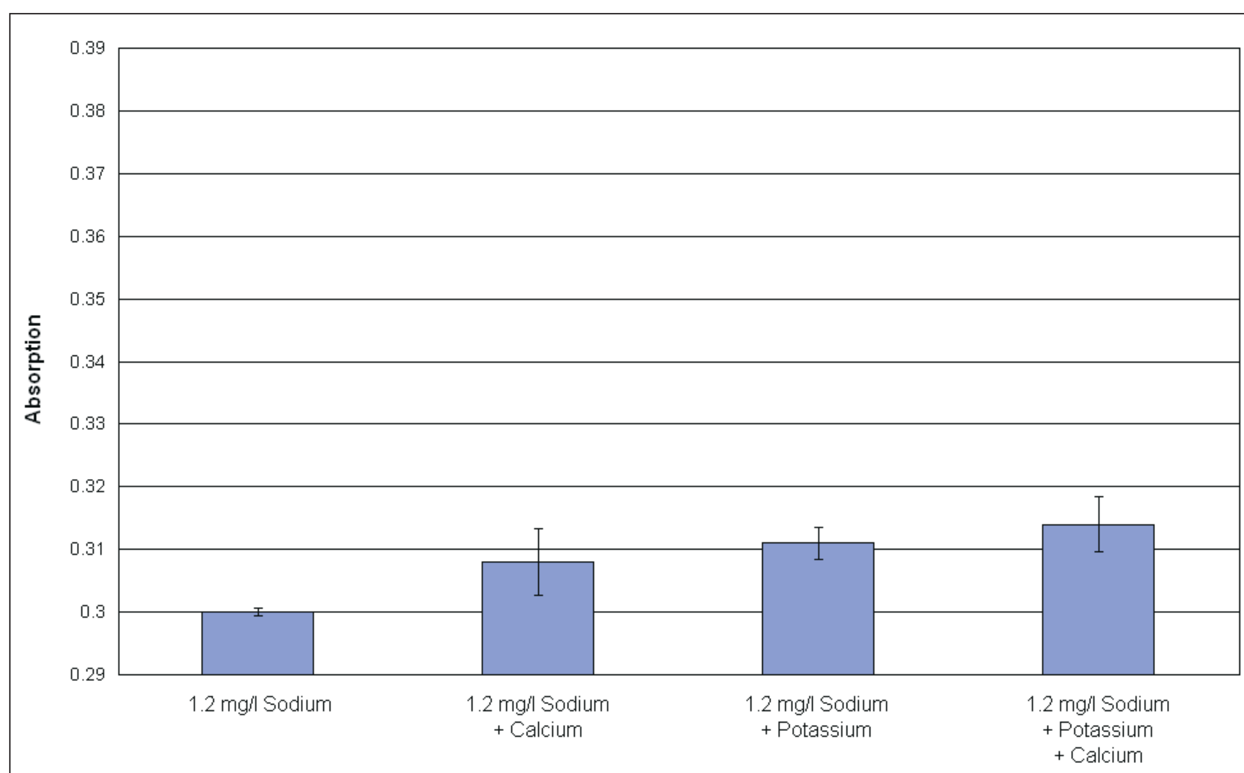

Figure 63: Matrix effect for sodium; Influence of calcium and potassium (n = 3, mean ± SD).

Serial No HBE 0081, Überlingen, Germany) was used with 15 mA current. The analysed spectral line was 766.5 nm and the monochromator gap length 0.4 nm. The reference solutions contained 0.8 and 1.2 mg potassium/l. 6.0 ml of the infusion solution was diluted to 1 l to obtain samples with a nominal content of approximately 1 mg/l. For calcium, again an Intensitron™ lamp (Part #3036017, Serial #206597, Perkin Elmer, Überlingen, Germany) was used with 10 mA current. The spectral line was 422.7 nm and a monochromator gap length of 0.7 nm was set. The concentration of calcium in the reference solutions was 6 and 10 mg/l. Here for sample preparation with approximately 9 mg/l nominal content, 10.0 ml of the infusion solution were diluted to 100 ml.
As ionisation interferences and physical interferences are described as common problems in the analysis of alkali (Na^+, K^+) and alkaline earth metals (Ca^{2+}) (Technical Documentation, Perkin Elmer) the matrix influence of the samples was analysed before actual sample measurements took place. Ionisation interferences can take place when thermal dissociation of a sample not only lead to atoms, but when these atoms are transferred into higher excited states or are ionized. Atoms with electrons in higher energy levels can not absorb at the same wavelength as atoms in ground state and are therefore not detectable. This is a common occurrence with alkali and alkaline earth metals. It diminishes the sensitivity and depends on the concentration. The addition of another element, which is easy to ionize, can correct this interference through the law of mass action as it represses the ionization of the actual element to detect. Physical interferences can be overcome by adding the same main components to all sample and reference solutions to keep the overall number of formed atoms alike in all solutions. To investigate the occurrence of these interferences in the infusion samples, solutions with the analysed substance and solutions where the other two components were added separately as well as together were analysed for each element, e.g. for calcium solutions with just calcium chloride, calcium chloride and potassium chloride, calcium chloride and sodium benzoate as well as a solution with calcium chloride, potassium chloride and sodium benzoate were measured and their absorption was compared. As can be seen from Figure 61, the adding of the single components led to a considerable increase

in absorption. The effect is stronger for sodium than for potassium as the actual fraction was kept alike to the initial formulation where sodium benzoate is the main component with 20 g/l whereas the other two components add up to less than 1g. This further explains why the matrix effect was least prominent in the detection of sodium, the main component, where the addition of small concentrations of either calcium or potassium did not influence the absorption strongly (see Figure 63). The matrix effect was less pronounced in potassium than in calcium, but again the stronger influence of sodium in comparison to calcium was clear to see, as displayed in Figure 62. As a result, all reference solutions were prepared with a set concentration of one component and the addition of the two other components in concentrations alike to the tested samples. Calcium, potassium and sodium concentrations were analysed for all vials with different stopper varieties separately over 24 months for the vials stored at room temperature and 12 months for the ones stored under accelerated conditions.

2.2.3 Osmometry

The osmolality of the infusion solution was measured with a semi-micro freezing point osmometer (Knauer, Berlin, Germany). Calibration was performed before every measuring cycle using a 0 mosmol/kg and a 400 mosmol/kg standard solution. About 150 µl of the solution was inserted into the measuring cap and super cooled by a Peltier device. Freezing was initiated by a stir wire. The results could be read off directly in mosmol/kg from the scale.

2.2.4 pH-Measurement

The pH-value of the infusion solutions was measured with pH-electrodes Calimatic® 766 (Knick, Berlin, Germany) and Knick Type 507 (Berlin, Germany).

2.3 Pellet production

2.3.1 Sieving

Powdered excipients were desagglomerated by sieving through a sieve with 1400 µm mesh size before further processing.

2.3.2 Blending

After weighing, the powdered materials were transferred into a laboratory scale blender (LM 40, Bohle, Enningerloh, Germany) and blended for 15 minutes at 25 U/min. Afterwards the mixture was transferred into the gravimetric powder feeder of the extruder (KT20, K-Tron, Soder, Lenzhard, Switzerland).

2.3.3 Extrusion

For wet and solid lipid extrusion, a Micro 27GL-28D extruder (Leistritz, Nuremberg, Germany) with co-rotating twin-screws was used. The extruder was equipped with an axial screen plate with 23 dies of 1 mm and 2.5 mm length for all experiments. The length of the screws was 790 mm and as a screw configuration, a set up of only conveying elements was chosen to allow easier adaptation of processes to other extruder types. As seen in Figure 64, where the applied screw configuration is shown schematically, the conveying elements were inserted in order of decreasing pitch size (from 40 to 20). Due to easier adaptation the temperature for five out of the seven cylinders of the extruder were set to 25 °C during the cold lipid extrusion. The cylinder next to the axial screen was cooled to 20 °C as no direct temperature control of the screen plate was possible and during process, a continuous heating up of the screen plate occurred due to the applied pressure and frictional heat. The cylinder where the powder was fed in was not equipped with a

temperature control. For wet extrusion, all six cylinders were cooled to 25 °C. The extrusion of lipid mixtures was performed at a constant screw speed of 50 rpm and a powder feed rate of 40 g/min. With MCC and κ-carrageenan the extrusion was performed at a constant screw speed of 100 rpm or 200 rpm (for one mixture containing benzoic acid and MCC), a powder feed rate of 33 g/min and a suitable liquid supply. The granulation liquid was purified water continuously pumped by a membrane pump (Cerex EP-31, Bran und Lübbe, Norderstedt, Germany) with a flow through metering device (Corimass MFC-081/K, Krohne, Duisburg, Germany). During process time the powder and liquid feed rate, screw speed, driving power, temperature and pressure at the screen plate was recorded. 300 g batches of extrudate were collected. Spheronisation was conducted immediately for wet extrudates and on the next day for lipid extrudates. During wet extrusion, three samples each of about 1-2 g were collected during the process for the determination of the loss on drying.

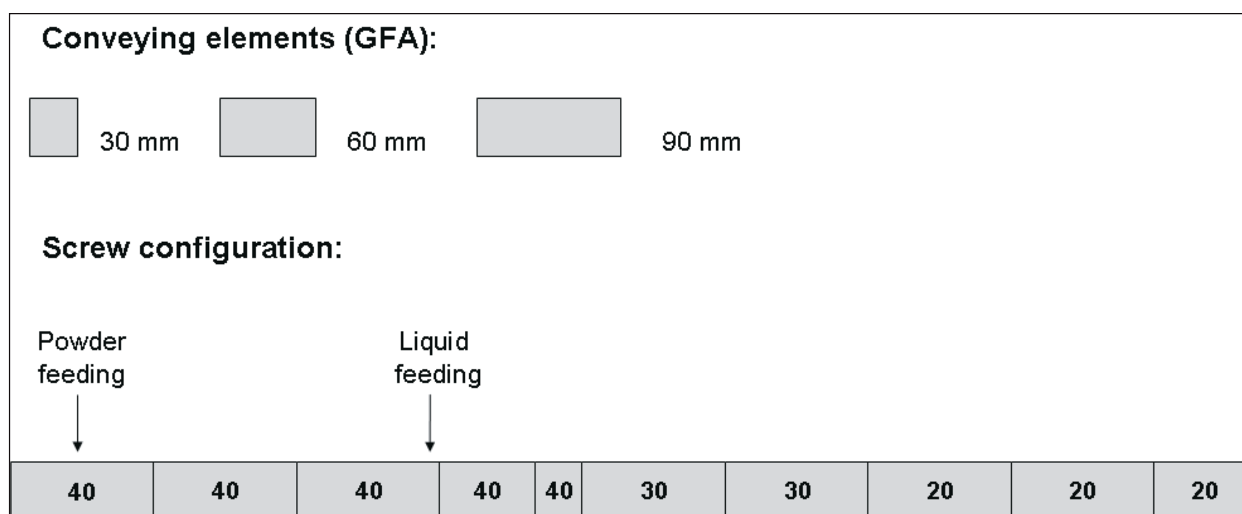

Figure 64: Extruder configuration (numbers indicating the pitch size of the respective element).

2.3.4 Spheronisation

Batches of 300 g extrudate were transferred into a Schlueter spheroniser RM 300 (Schlueter, Neustadt am Ruebenberge, Germany). The apparatus was equipped with a 300 mm diameter cross-hatched rotor plate (see Figure 65 for a detail drawing). Compressed air was used to keep the gap between rotor plate and spheroniser jacket open. The speed of the rotor plate, jacket temperature and spheronisation time were varied for the spheronisation of lipid formulations and are explained in detail in chapter C2.3.3. For wet extrusion, 300 g of wet extrudates were collected and then spheronised for 5 min at 1000 rpm at 25 °C.

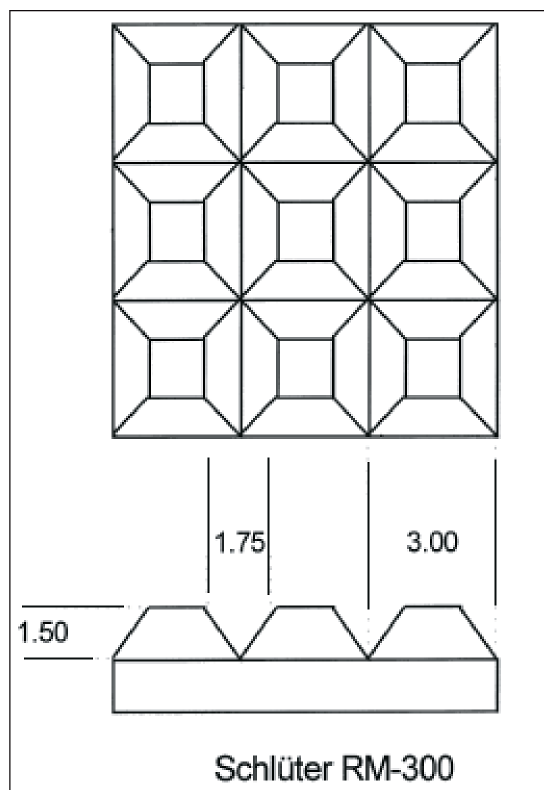

Figure 65: Detail drawing of the cross-hatched rotor plate (Schmidt and Kleinebudde 1998).

2.3.5 Pellet drying

The pellets produced by wet extrusion, with either MCC or κ-carrageenan as a binder, were dried in a fluid bed apparatus (GPCG 1.1, Glatt, Dresden, Germany) with an inlet air temperature of 60 °C and an air flow of 100 m^3/min for 10 minutes. For the determination of the loss on drying, the pellets were stored in a vacuum oven (Heraeus VT 6060 M, Kendo, Hanau, Germany) at 70 °C and barometric pressure of < 10 mbar for 24 h. The humidity (H) of the pellets were determined by the weight of the sample before (e_B) and after (e_A) drying.

$$H[\%] = \frac{e_B - e_A}{e_A} \times 100 \quad \textbf{(Eq. 3)}$$

Pellets made from lipids were not subjected to a drying process.

2.3.6 Pellet preparation for SEM

A sample of pellets was put in an evaporating dish and doused in liquid nitrogen. After the evaporation of the nitrogen, the frozen pellets were tapped on with a pestle. Suitable fragments of split pellets were attached onto the brass specimen holder for scanning electron microscopy.

2.3.7 Pellet milling

For further pellet characterisation through dissolution testing, one lipid pellet batch was ground using the Ultra Centrifugal Mill ZM 200 (Retsch, Haan, Germany). The size reduction of the pellets took place through impact and shearing effects between the 12 wedge-shaped rotor teeth and the fixed ring sieve with conidur holes. This ensured a fast processing of the samples without altering through the milling process. The size of the conidur holes was 1.5 mm and the speed was set to 6000 rpm.

2.4 Pellet Characterisation

2.4.1 Pellet shape, size and size distribution

Pellet yield was defined as the sieved fraction from 0.63 to 2.0 mm. Representative samples of suitable volume were obtained from the yield using a rotary sample divider (Retschmühle PT, Retsch, Haan, Germany). Image analysis was conducted using a system consisting of a stereomicroscope (Leica MZ 75, Cambridge, UK), a ring light with cold light source (Leica KL 1500, Cambridge, UK), a digital camera (Leica CS 300 F, Cambridge, UK) and a computer with data logging card and the software Image C (QWin, Leica, Cambridge, UK). Images of at least 500 pellets of each sample at a suitable magnification (1 pixel = 17.5 µm) were translated into binary images. Contacting pellets were separated by the software algorithm. If the automatic separation failed, pellets were deleted manually. For each pellet, 64 independent Feret diameters were determined. As a method to characterise the shape of the obtained granules the aspect ratio was used. The aspect ratio was calculated from following equation:

$$AR = \frac{d_{max}}{d_{90}} \qquad \textbf{(Eq. 4)}$$

with maximum Feret diameter (d_{max}) of each individual pellet and the Feret diameter perpendicular to it (d_{90}). The pellet size was characterised by the equivalent diameter (d_{eq}). It was calculated using the projected pellet surface (A) (Voigt 2000).

$$d_{eq} = \sqrt{\frac{4A}{\pi}} \qquad \textbf{(Eq. 5)}$$

The ratio of the mean Feret diameter (d_F) and the median of all mean Feret diameters (d_{F50}) was used to calculate the dimensionless particle size (d)(Kleinebudde 1997) to guarantee comparability between the particle size distributions of different pellet batches. The distribution of the particle size was characterised by the fraction of the particles in the interval $0.9 < d < 1.1$. The size distribution was characterised as “good” if the fraction of this 10 % interval exceeded 50 % (Thommes et al. 2006).

2.4.2 Pellet storage

To observe changes in release profiles through thermal treatment of lipid pellets, samples of lipid pellet batches were stored at 32 °C in a drying oven (ET 6130, Heraeus, Hanau, Germany) for four weeks. A temperature of 10 °C below the melting point of the lowest melting lipid component of the formulations was adjusted. Hence, in the case of Witocan 42/44, 32 °C was chosen.

2.4.3 Dissolution tests, UV/VIS-spectroscopy

The dissolution tests were performed using the Ph. Eur. basket apparatus method at 150 rpm (Sotax AT7, Sotax, Lörrach, Germany). The dissolution media were purified water, 0.1 N hydrochloric acid and phosphate puffer solution pH 6.0 R2 (Ph. Eur) continuously pumped (Sotax piston pump CY 7, Sotax, Lörrach, Germany) to a UV/VIS-Photometer (Lambda 2, Perkin-Elmer, Überlingen, Germany). The concentration of sodium benzoate in the dissolution fluid was determined by measuring the absorption at 273 nm. Preliminary investigations showed that the extrusion aids do not have an impact on the absorption at the wavelength 273 nm. All experiments were conducted with five replicates. Calibration of the UV/VIS- photometer was performed using 6 solutions (Absorption = 0.1 – 1.1; n = 3).

2.4.4 Drug release in food stuff

2.0 g of pellets were mixed into 100.0 g of different food stuff (milk, apple sauce, vanilla yoghurt and vanilla pudding) and samples of 1.0 g were taken after 5, 10 and 20 minutes. The samples were dissolved in about 50 ml of HPLC eluent (see 2.2.1) and poured through a paper filter into a 100 ml volummetric flask, which was then filled with eluent. Prior to injection into the HPLC, the samples were filtered through a 0.45 µm filter. The same HPLC assay as for the analysis of the infusion samples was used (2.2.1).

2.4.5 Taste panel test

Samples of 2.0 g of pellets were stirred in 100 ml of distilled water until batch dependent time points (for MCC and κ-carrageenan samples were taken after 1, 3, 5, 8 and 10 minutes, for uncoated lipid pellets sample points were 5, 8, 10, 15 and 30 minutes and for a saliva-resistant coated formulation the sample points were 5, 10, 30, 60 and 120 minutes) and then the pellets were sieved off. The solutions were tasted by adult volunteers and rated on a scale from 0 to 3.5 indicating no taste (0) to very bad taste (3.5). Each volunteer was given unlimited amount of time between testing the different samples and water to rinse the mouth before testing the next sample. The same samples were also analysed through UV-spectroscopy (Spekol 1200, Analytik Jena, Jena, Germany) to determine the sodium benzoate concentration of the solution.

2.4.6 Electronic tongues

Electronic tongues are analytical systems, based on the principal of potentiometry, for taste measurement. The two different systems available on the market have been used in this work.

Astree e-tongue

A αAstree liquid and taste analyzer (E-Tongue) connected with a LS16 autosampler unit and equipped with a data acquisition and analysis software package (Alpha-MOS, Toulouse, France) was used to conduct the studies (Figure 66).

Figure 66: α Astree Electronic Tongue (Alpha MOS).

As a sensor set, the sensor array #2 for pharmaceutical purposes with the sensors ZZ, AB, BA, BB, CA, DA and JE was selected. The sensors are silicon transistors with an organic coating, details on sensor material have not been disclosed by the manufacturer. The measurement principle is based on the potentiometric difference between each individually coated sensor and the Ag/AgCl reference electrode. Sensors show cross-selectivity i.e. each sensor is enabled to react to several different chemical substances, but with a different sensitivity. Sensors were stored in air and the reference electrode in a washing solution. Before the actual test series, several system checks were performed. Sensors were stored in water to allow conditioning, i.e. hydrating of the matrix. The reproducibility of sensor data was checked in a conditioning phase where three 0.01 mol/l hydrochloric acid test solutions were measured three times. This allowed checking the stability of sensor response, dispersion of each sensor response and maximum dispersion of the average between the three runs. If parameters failed to meet the set criteria for any of the parameters, the conditioning phase was repeated or the set criteria changed to a lower limit. Calibration was performed with one sample of 0.01 mol/l hydrochloric acid test solution, which was measured three times. Stability of sensor response, dispersion of sensor response and the difference between target and real values of the sensor response were tested. The diagnostic test with three standard solutions of 0.01 mol/L of hydrochloric acid, sodium chloride and sodium glutamate, which were analysed six times, was done to test the discrimination of the sensors. The results were displayed in a principal component analysis (PCA) with a minimal discrimination index of 94. In case of a lower discrimination index the test was repeated. The sample volume was 80 ml and sample preparation was done as described in chapter 2.4.5.

Figure 67: Sensor head with attached sensor array (Alpha-MOS).

For each tested pellet formulation, a corresponding placebo formulation (matrix without an active ingredient) was prepared. This was necessary, as the Astree e-tongue is only able to detect taste differences between formulations. The sensor head with the attached sensor array and a mechanical stirrer (see Figure 67) was dipped in a beaker containing the sample. Each sample was analysed for 180 s and the data acquisition time was 120 s. In between samples, the sensors were rinsed for 20 s in water. First all placebo formulations and then all active containing samples were analysed, the whole process was repeated 8 times. The measured output represents the aggregate potential difference for each sensor vs. the reference electrode. Data collected by the e-tongue were reviewed, and replicates (starting with the first out of 8) as well as sensors were taken out until the RSD for all samples and sensors reached a minimum. A multivariate analysis, i.e. PCA was used by the α-Astree software to reduce the dimensional spaces, without losing in-

formation. For each sample, a cluster could be obtained in a PCA map. The PCA was only evaluated if the discrimination index (DI) was > 80. The distance between a pair of data cluster (the placebo sample and the corresponding sample with the active ingredient) was determined and used to assess the taste intensity. The bigger the distance on a PCA map, the bigger the difference in taste between the samples.

Insent Taste Sensing System

The Insent Taste Sensing System SA402B (Intelligent Sensor Technology, Atsugi, Japan), as seen in Figure 68, equipped with four sensors (SB2CT0, SB2CA0, SB2C00, SB2AE1) and reference electrodes as well as analysing software was used to detect taste and taste differences in different formulations.

Figure 68: Insent Taste Sensing System SA402B (Intelligent Sensor Technology, Ltd.)

The sensors are comprised out of a sensor probe body and a terminal with an attached silver wire, which is plated with Ag/AgCl. The inner cavity, which is open to one side, is filled with 3.33 M potassium chloride (KCl) solution saturated with AgCl (inner solution) and closed by a lipid/polymer membrane. The reference electrode consists of the terminal part and a glass tube, which is also filled with inner solution, with a porous ceramic field at the tip (see Figure 69). Before the test series, the stored sensors were filled with inner solution and left to saturate for half a day. The sensors were then attached to the sensor heads (2) of the system according to their membrane charge (positive or negative). A standard solution of 30 mM KCl and 0.3 mM tartaric acid and different washing solutions for sensors with positive and negative charged membranes were prepared and beakers with them positioned on the sampling rack. A sensor check was performed before each test series. The sensors were dipped repeatedly in standard solution as a cleaning procedure (90 s dipping into the first beaker set, 120 s each into the 2nd and 3rd set) and then conditioned in the standard solution for 30 s. None of the sensors should exceed the stability of 0.5 mV difference from the set value otherwise the procedure is repeated. Furthermore, all sensors should be in their respective mV range or should be replaced.

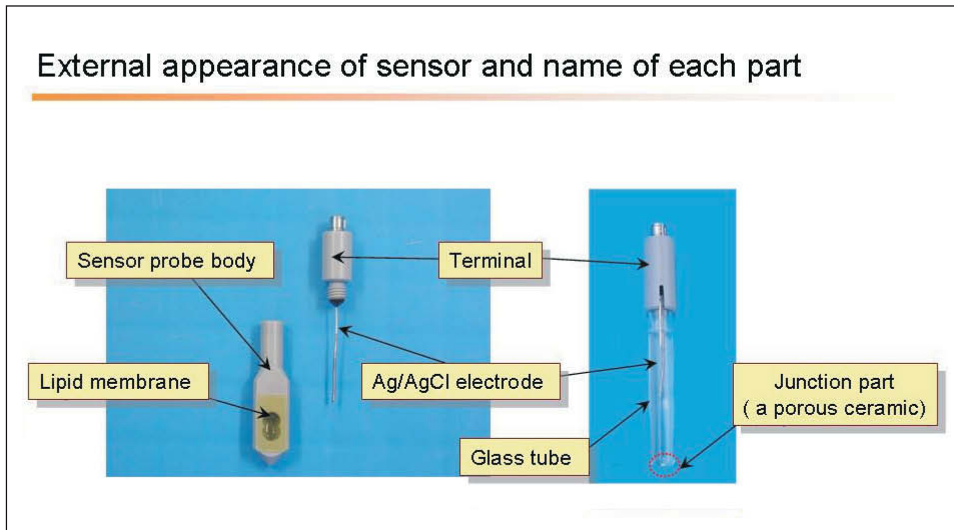

Figure 69: Insent Sensors; left: Taste Sensor right: Reference electrode (Intelligent Sensor Technology, Ltd.)

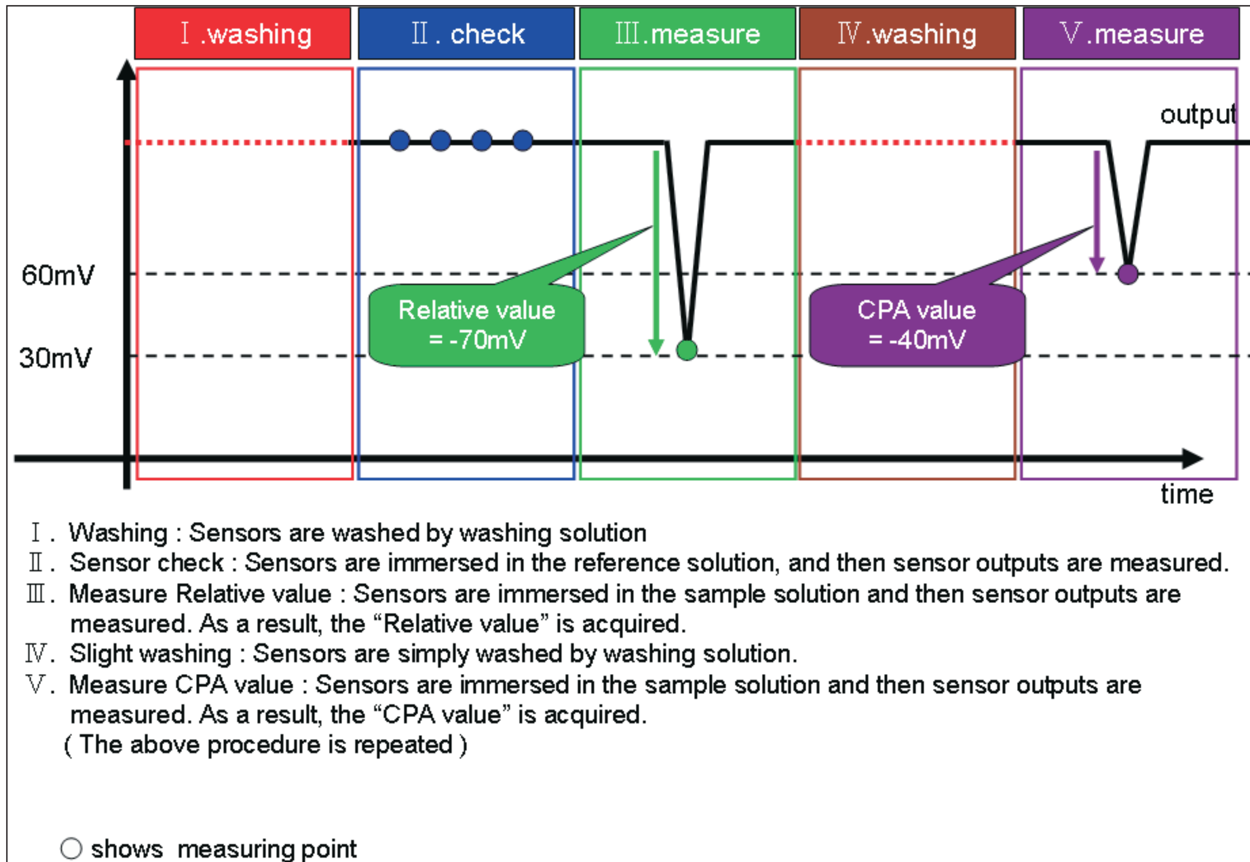

Figure 70: Measurement details (Intelligent Sensor Technology, Ltd.).

Sample measurement followed a standard procedure, also displayed in Figure 70. The first phase included the washing of the sensors for 330 s in three different beaker sets (the first with ethanolic washing solutions, the others with standard solution). In the second phase the sensor output was conditioned for 30 s in the next set of standard solution, then the actual sample was measured for 30 s and the sensor outputs after this time recorded, afterwards the sensors were dipped shortly (3 s) in two standard solution beaker sets for cleaning. The last step was again the measurement of a standard solution similar to phase two.
This procedure was repeated for every sample. Each sample was tested four times by rotation procedure (i.e., the first round of measurements of all samples was completed before the next round was started). The Insent sensors react, due to their specific lipid/polymer membrane, particularly to functional groups. In this study, four sensors detecting taste information for bitterness, saltiness, sourness and astringency were used (see Table 19 for the property of the different sensors).

Table 19: Property of sensors.

Sensor	Relative Value	CPA Value
SB2C00	Bitterness	Aftertaste from bitterness
SB2AE1	Astringency	Aftertaste from astringency
SB2CA0	Sourness	None
SB2CT0	Saltiness	None

The sample value (= relative value) from each sensor was calculated from the actual mV response of the sensor in the sample solution minus the value V_r, which is the actual mV response of the sensor in standard solution. For two sensors, a CPA value (aftertaste) was recorded from the mV response of the last measurement in standard solution minus V_r. For data analysis, mean relative values for each sample and sensor were calculated from the last three out of four runs. For direct correlation with other data, these mean values were used. Using the Insent Software further data analysis was possible. In a process called “interpolating addition process”, the values obtained from the standard solution were used to define the zero point and all data from the samples were displayed in relation to it. Sample data could be depicted in a radar plot or a PCA where the allocation of the components could be chosen from the sensors. Further data analysis was possible with “transformation to taste information” by substituting the relative values into taste specific equations, through this process the mV data was changed into dimensionless “taste values”.

Table 20: Equations for the transformation of raw data into taste information by the Insent Software (Intelligent Sensor Technology, Ltd.).

Taste information	Equation for taste information
Sourness	= 0.332 x sensor SB2CA0 + 12.0
Bitterness	= -0.140 x sensor SB2C00 + 0.084 x sensor SB2CT0
Adstringency	= -0.1575 x sensor SB2AE1 + 0.1575 x sensor CT0
Saltiness	= -0.1575 x sensor SB2AAE
Aftertaste from bitterness	= -0.210 x CPA (C00)
Aftertaste from adstringency	= -0.252 x CPA (AE1)

These values could again be displayed in either radar or PCA plots. If correlation with other data sets was required, a MRA data file was built through adding the additional data e.g. data from dissolution tests or human taste panel results. The multivariate data analysis was then displayed in a PLS.

2.4.7 Multivariate analysis of taste prediction models

For the correlation of taste data from both e-tongues with either the actual sodium benzoate concentration determined by UV-spectroscopy or the human taste impression obtained through human taste panel test, the multivariate data analysis tool "projection to latent structures by means of partial least squares"(PLS), as implemented in Simca-P version 10.0.4 (Umetrics AB, Umeå, Sweden) was used. All variables (e-tongue data) were mean centred and scaled to unit variance. The predictability of the models was judged by the R^2 and the RMSECV (root mean square error of cross validation), calculated according to following equation:

$$RMSECV = \sqrt{\frac{\sum (obs - pred)^2}{N}} \quad \textbf{(Eq. 6)}$$

where *obs* and *pred* were the observed and the predicted values for the observations (taste), respectively, and N the number of observations. All PLS models were validated using the leave-one-out cross validation (Q^2) test. Simple variable selection was applied to decrease the complexity of the models and ease interpretation. If the exclusion of the least important variable resulted in a model with a higher Q^2, then that descriptor was permanently left out of the model. This procedure was repeated until no further improvement was accomplished. The aim of the variable selection was to achieve a high predicitvity and removing information not directly related to the response variable thus increasing the robustness of the model.

2.4.8 Helium density

The gas pycnometric density (ρ_{He}) of dry pellets was determined by a helium pycnometer (AccuPyc, Micromeritics, Norcross, USA). The measuring temperature was 25 ± 0.1 °C, the filling and equilibration pressure 134 kPa and each sample was measured five times in a 10 cm^3 sample chamber. For each tested pellet batch, three samples were analysed.

2.4.9 Mercury density

The determination of the mercury density (ρ_M) was performed with a mercury porosimeter (Pascal 140, Thermo Finigan, Milan, Italy) on the same pellet samples that were used before for the helium density. All samples were evacuated for 20 minutes at 0.01 kPa to assure equal conditions for the addition of mercury. Volume determination was done automatically after evacuation and filling of the dilatometer.

2.4.10 Porosity

The porosity of the different pellet batches was calculated using the helium and mercury density according to following equation:

$$\varepsilon = \left(1 - \frac{\rho_{He}}{\rho_M}\right) \quad \textbf{(Eq. 7)}$$

2.4.11 Scanning electron microscopy (SEM)

Pellets were visualised by the scanning electron microscope Leo 1430 VP (Leo Electron Microscopy, Cambridge, UK). Working voltages between 15 and 20 kV were used. The samples were gold sputtered by the Agar Manual Sputter Coater B7340 (Agar Scientific, Stansted, UK) prior to electron microscopic investigations. The sputtering was done in 12 steps of 15 s with 30 s breaks in between to avoid partial melting of the samples.

2.4.12 X-ray diffraction

For each batch, pellets were compressed to receive the smooth surface required for X-ray diffraction measurements. The compacts made from lipid pellets and sodium benzoate powder, before and after storage at 32 °C for 4 weeks in an ET 6130 drying oven (Hanau, Germany) were used for qualitative x-ray diffraction measurements performed by a Rigaku Miniflex (Rigaku Denki Co. Ltd., Tokyo, Japan) powder diffractometer with Bragg-Brentano-geometry. The anode consisted of copper and the acceleration voltage was 30 kV at which a current flow of 10 mA emerged. The theta-angle bracket was 3 to 50°, the measuring range 1000 cps, the scan speed 2°/min and the distance between two data points 0.02°.

2.4.13 Raman spectroscopy

For these studies, a Raman spectrometer (Control Development Inc., South Bend, IN, USA) equipped with a thermoelectrically cooled CCD detector and a fiber optic probe (Raman Probe RPS785/12-5, InPhotonics, Norwood, MA, USA) was used. A 500 mW laser source (Starbright 785S, Torsana Laser Technologies, Skodsborg, Denmark) provided the light at 785 nm. The spectra were recorded between 200 and 2200 cm^{-1} with an integration time of 1 s and by averaging 30 scans per spectrum. The samples were gently compressed into the sample holder to obtain a smooth surface. The sample holder was put on a rotor (80 rpm) to prevent sub-sampling. Each experiment was conducted in triplicate to avoid artefacts.

2.4.14 Karl-Fischer titration

For the determination of the water content in extrudates prepared through wet extrusion a Karl-Fischer titrator type DL 18 (Mettler, Gießen, Germany) was used. The operating medium was Hydranal®-methanol dry (Lot. 5095A) and Hydranal®-formamide dry (Lot. 5081A) in equal shares at room temperature. The one-component-reagent was Hydranal®-composite 5 (Lot. 5081A) and as calibrating solution Hydranal-Water Standard 10.0 (Lot. 52980) was used. One gram of the calibrating solution contained 10.07 mg of water. Measurements were performed at room temperature (n = 3).

2.4.15 Differential scanning calorimetry

Thermal characteristics of lipids, lipid mixtures and lipid pellets were studied using a Mettler DSC 821e (Mettler Toledo, Giessen, Germany) in duplicate. DSC scans were recorded at a heating rate of 10 K/min. Samples with an initial weight of approximately 3 mg were heated from 20 to 120 °C in a sealed and pierced aluminium pan. Data analysis was performed with the $STAR^e$ program Version 6.01 (Mettler-Toledo, Giessen, Germany). An empty aluminium pan was used as reference.

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