Effects of excipients in nasal powder formulations for systemic drug delivery

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Meiner Familie gewidmet.

Ein Gelehrter in seinem Laboratorium ist nicht nur ein Techniker; er steht auch vor den Naturgesetzen wie ein Kind vor der Märchenwelt.

Marie Curie

The absence of a special marking or a corresponding reference to a trademark, utility model or patent protection does not allow the conclusion to be drawn that the items specified in this work can be freely disposed of.

Parts of this thesis have already been published as follows:

Journal articles

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1 Introduction

Nasal drug delivery is mainly associated with the use of liquid sprays for the treatment of rhinitis or allergy symptoms. Around 90 million packages of topical rhinologics were sold in German pharmacies in 2019, which corresponds to a share of almost 6% of total unit sales [1]. However, the nasal anatomy and physiology offers further options for drug delivery that have gained increasing attention in recent years. With the Covid-19 pandemic, nasal vaccination in particular has gained attention as a way to target immunocompetent cells in the nose. Given that the nasal mucosa is easy to reach, well supplied with blood and relatively permeable, the nose also offers an alternative for the administration of systemically acting drugs [2].

A major advantage of systemic drug delivery via the nose is a rapid onset of action, bypassing the gastrointestinal tract and first-pass metabolism. For some small drug molecules, similar plasma curves have been achieved after nasal administration as after intravenous administration with plasma peaks after about 5-30 min [3]. Therefore, nasal drug administration is particularly suitable for the treatment of pain and emergency situations. In emergency situations, nasal administration provides a non-invasive treatment of patients who are unable to swallow. Several nasal products in this therapeutic area have been approved by the Food and Drug Administration (FDA) and the European Medicines Agency (EMA) in recent years e.g., fentanyl nasal spray (Lazanda® in 2011 (FDA); Instanyl® in 2009 (EMA)), naloxon nasal spray (Narcan® in 2015 (FDA); Nyxoid® in 2017(EMA)), sumatriptan nasal powder (Onzetra® Xsail® in 2016 (FDA)) or glucagon nasal powder (Baqsimi® in 2019 (FDA and EMA)).

Apart from the opportunities offered by the nose as site of drug delivery, its anatomy and physiology also provide specific challenges. Since one of the main functions of the nose is the filtration of inhaled air and the protection of the lower airways from potentially noxious substances, deposited particles are removed from the nose by the mucociliary clearance mechanism with a half-life of clearance of 15-20 min [4]. Absorption of a drug introduced into the nose thus competes with the mucociliary clearance. The nose also functions as a sensory organ and is innervated by the olfactory and the trigeminal nerve [5]. Thus, the nasal application of drugs can constitute a stimulus that causes unpleasant odour perceptions or local irritation phenomena. If unpleasant sensory experiences are pronounced, this can severely limit the patient's acceptance.

To overcome challenges and optimise nasal drug delivery, different formulation strategies are conceivable. Several studies have shown a potential of nasal powders to increase the bioavailability of drugs compared to liquids [6]. Moreover, powder formulations allow the application of higher drug doses and show improved chemical and microbial stability. The

targeted use of excipients, such as mucoadhesives, permeation enhancers and fillers in powder formulations can facilitate further enhancement of systemic absorption of drugs and tailor the effect achieved. For an effective selection of excipients, a differentiated knowledge of the different effects induced by excipients is key. As the vast majority of nasal products are currently still liquid formulations, a gap in knowledge exists concerning these effects in nasal powders.

2 Objectives

The formulation of nasal products as powders and the targeted use of excipients potentially offers solutions to certain challenges of nasal drug delivery. Currently, however, nasal powders are still the absolute minority on the market. Consequently, a gap in knowledge exists regarding the effects of powder formulations in the nose. This work aims towards narrowing this gap by investigating the effect of excipients in nasal powder formulations with suitable in vitro characterisation methods for this purpose.

One of the most striking challenges of nasal drug delivery is the short residence time of the drug in the nose. The use of excipients is an applicable strategy to optimise the outcome of nasal drug delivery in this respect. This thesis therefore aims to answer the question of which excipient properties extend the nasal residence time, when applied as powders. Thereby, one focus is also on the use and assessment of methods that are suitable as screening tools in product development and enable robust differentiation of the effects of different excipients.

The sensation that the use of a nasal spray or powder triggers in the nose is a factor that is often underestimated in product development, but is of enormous importance in practical use. The assessment of sensory stimuli, such as pain, burning or itching often first occurs in clinical trials. This thesis aims to assess sensory effects caused by excipient powders with an in vitro method and to answer the question of which excipient properties trigger such effects. The toxicity of excipients for nasal cells will be additionally investigated.

Which excipient properties actually show advantages in a nasal powder formulation strongly depends on the drug to be formulated. The solubility and permeability of the drug are of great importance in this regard. In the second part of this thesis, I therefore prepared model formulations from the previously characterised excipients and active ingredients with different permeability properties with the aim to assess the effects and suitability of the excipients in the formulations. The influence of the formulations on the rheological properties of the nasal fluid, on the dissolution behaviour of the drugs in the nasal fluid, and on the permeability through the nasal mucosa are characterised separately in order to be able to distinguish the different effects on the excipients on these processes.

A differentiated knowledge of the effects is key for a targeted selection of excipients according to the requirements of the drug. Conclusively, the second part of this work shall result in a decision aid for the selection of excipients for nasal powder formulations based on the drugs characteristics.

3 Theoretical background

3.1 Anatomy and physiology of the nose

The nose consists of an external visible part and an internal part, which together form a cavity of about 5 cm in height and 10 cm in length. The nasal cavity is divided into two halves by the nasal septum. Both cavities cover a total volume of approximately 15 mL and a total surface area of 150 cm² [7]. The nasal cavity opens through the nostrils to the face and extends internally to the nasopharynx, where both halves of the nose join. The cavity can be divided into different areas, namely the nasal vestibule, the respiratory region and the olfactory region [2]. The nasal vestibule forms the anterior part of the nose and enters the inner part of the nose at the nasal valve [7]. The respiratory region forms the largest part of the inner nose and is divided into the lower, middle and upper turbinates, which originate from the nasal lateral wall and increase the surface area of the respiratory region to 130 cm² [2]. The olfactory region is located in the upper part of the nasal cavity below the cribriform plate of the ethmoid bone, which separates the nasal cavity from the cranial cavity. The olfactory receptor cells located there penetrate the cribriform plate and thus represent the only direct external contact of the central nervous system [8]. Additionally to olfactory innervation, the anterior and posterior parts of the nose are innervated by branches of the trigeminal nerve [5]. Figure 3-1 displays a schematic illustration of the nasal cavity in lateral view.

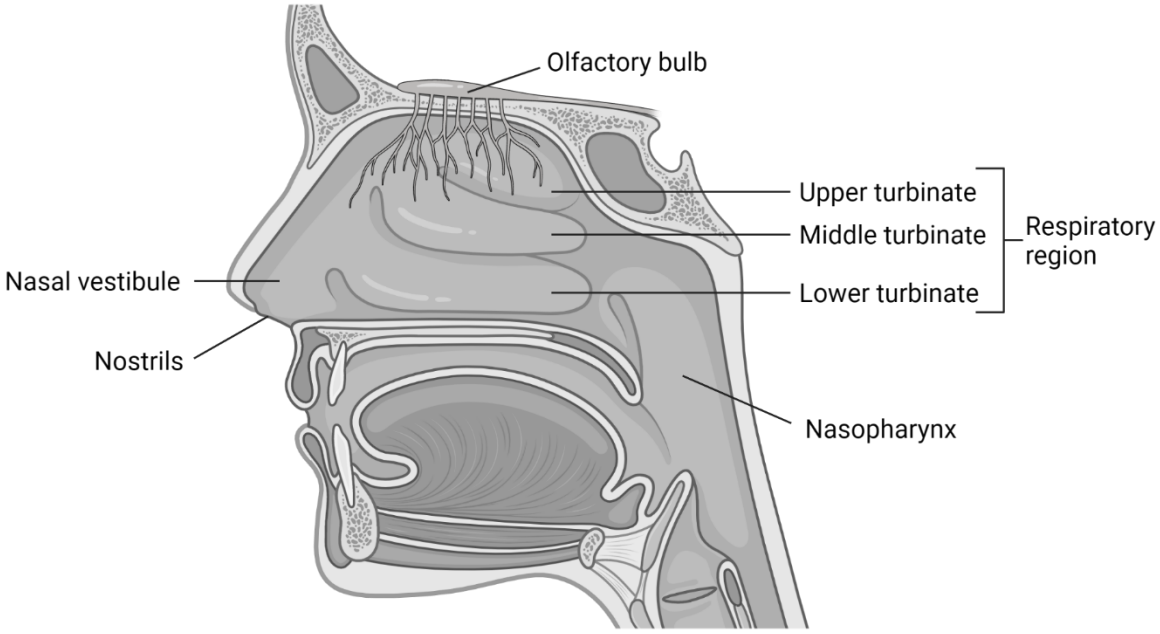

Figure 3-1: Anatomy of the upper respiratory tract in lateral view. Created with BioRender.com.

The nasal cavity is covered with different types of epithelia. The nasal vestibule is lined with squamous epithelium, which is dorsally turning into pseudostratified columnar epithelium.

The respiratory mucosa, which lines most of the nasal cavity, consists of basal cells, ciliated and non-ciliated columnar cells and goblet cells, attached to a basement membrane under which the highly vascularised lamina propria is localised (Figure 3-2). Goblet cells and submucosal glands produce secretions, which result in a 10-15 µm thick mucus layer, covering the epithelium [7,9]. The nasal mucus layer consists of a lower sol layer and an upper gel layer and is composed of 95% water, 2-3% mucins and proteins, 1% inorganic salts, 1% lipids and 0.02% DNA [10]. Secreted mucins are large glycoproteins with molecular masses of 10,000-40,000 kDa [11]. They consist of a polypeptide backbone, which comprises tandemly repeating regions with high amounts of the amino acids serin, proline and threonine [11]. These tandem repeat regions are highly glycosylated (40-80% mass fraction) with oligosaccharide side chains [11]. Mucin molecules form a three-dimensional network and are thus mainly responsible for the viscoelastic properties of the nasal mucus [10]. Due to sulphate and carboxyl groups in the terminal sugars of the oligosaccharide chains, mucins exhibit a negative overall charge at the physiological nasal pH, which is slightly acidic at 5.5-6.5 [9,11]. Due to these overall properties, the mucus layer forms an effective barrier for noxious substances.

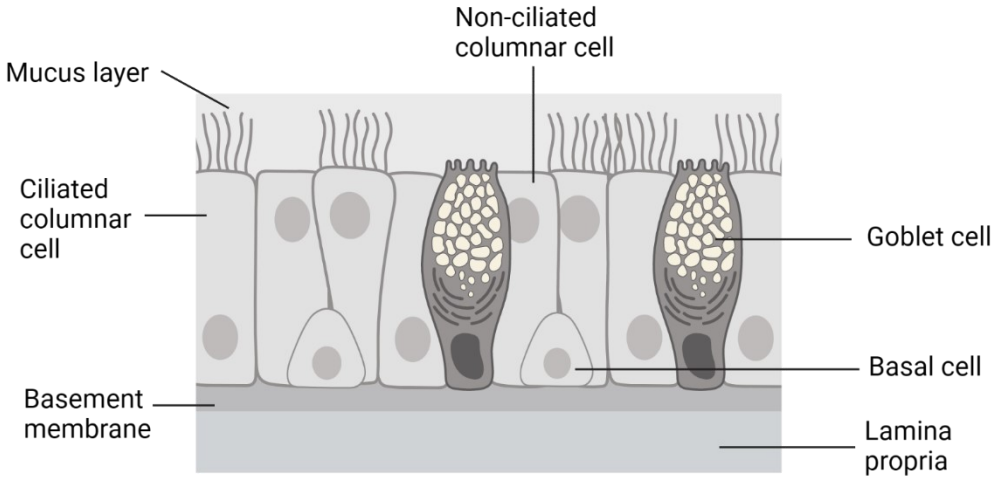

Figure 3-2: Schematic illustration of the respiratory mucosa. Created with BioRender.com.

The main functions of the nose include on the one hand olfaction and on the other hand filtering, warming and humidifying the inhaled air before it reaches the lungs [7]. The nasal anatomy, which passes the inhaled air through narrow pathways, facilitates the contact of air and mucosa and thus the exchange of heat and moisture. The heat exchange is additionally favoured by the high vascularisation of the submucosa [7]. Inhaled air at room temperature reaches a temperature of about 32 °C in the nasal cavity (middle turbinate) [12]. The narrow anatomy, which causes a turbulent air flow of the inspired air, and the lining mucus layer of the epithelium, which entraps impacting particles, provide an effective filter for particles larger than 10 µm [7]. Particles deposited on the mucus layer are removed from the nasal cavity due to the mucociliary clearance. In this process, the mucus layer is

transported towards the throat with an average velocity of 6 mm/min by coordinated ciliary movements, resulting in a replacement of the mucus layer every 15-20 min [7,8].

3.2 Nasal drug delivery

The nose provides an easily accessible mucosa that allows for non-invasive and easy-to-use drug delivery. While still mainly associated with local drug delivery, the nose also offers advantages for systemic drug delivery and targeted delivery to the central nervous system (CNS), as well as the possibility of mucosal vaccination.

3.2.1 Local delivery

The nasal administration of locally acting drugs is primarily used for the treatment of rhinitis and allergy symptoms. For this purpose, decongestant alpha-sympathomimetics, corticosteroids and antihistamines are applied. A major advantage of local therapy with antihistamines and corticosteroids is the effectiveness in low doses and the avoidance of systemic side effects [13].

3.2.2 Systemic delivery

The high vascularisation and good permeability of the nose in the area of the nasal turbinates leads to a high potential for rapid systemic drug absorption, bypassing the hepatic first-pass metabolism [3,14]. Nasal drug delivery is therefore a non-invasive and user-friendly alternative for the systemic administration of drugs that require a rapid onset of action, or are subject to gastrointestinal or hepatic degradation when administered orally. Areas of particular interest are therefore acute pain or emergency treatments, as well as the administration of substances with poor oral bioavailability like peptides. Table 3-1 summarises systemic acting drugs that are currently approved by the Food and Drug Administration (FDA) for nasal delivery.

Table 3-1: Currently FDA-approved systemic acting drugs for nasal delivery [15].

Drug	Indication	Class
Butorphanol	Pain management	Opioid
Cyanocobalamin	Vitamin B_{12} supplementation	Vitamin
Desmopressin	Diabetes insipidus	Peptide
Diazepam	Acute treatment of seizures	Benzodiazepine
Dihydroergotamine	Migraine	Alkaloid
Esketamine	Treatment-resistant depression	NMDA receptor antagonist
Glucagon	Severe hypoglycaemia	Peptide
Ketorolac	Pain management	Non-steroidal anti-inflammatory drug
Metoclopramide	Diabetic gastroparesis	D_2-receptor antagonist
Midazolam	Acute treatment of seizures	Benzodiazepine
Nafarelin	Central precocious puberty	Peptide
Naloxon	Opioid overdose	Opioid antagonist
Nicotine	Smoking cessation	Stimulant
Sumatriptan	Migraine	Triptane
Testosterone	Testosterone deficiency	Androgen
Zavegepant	Migraine	Calcitonin gene-related peptide receptor antagonist
Zolmitriptan	Migraine	Triptane

3.2.3 Vaccine delivery

Since the nose, as the natural entry portal for airborne pathogens, contains immunocompetent cells, it is suitable as an application site for vaccines. The nasal associated lymphoid tissue, which is located in the nasopharynx, can induce both a local and a systemic immune response [8]. Nasal vaccination is successfully used in influenza vaccines. FluMist®, a live attenuated influenza vaccine, was originally approved by the FDA in 2003 and Fluenz® by the EMA in 2011. During the Covid-19 pandemic, the nasal route of administration for vaccines has gained additional attention. Currently, 16 nasal Covid vaccines are investigated in clinical trials (April 2023) [16].

3.2.4 Targeting of the central nervous system

Nerve fibres of the olfactory nerve, which extend from the olfactory bulb in the brain to the olfactory region of the nasal cavity and penetrate the mucosa in that region, constitute the only direct connection of the brain to the outside [17]. The transport of drugs via this olfactory

pathway, as well as via the trigeminal nerve, which innervates the anterior and posterior nasal cavity, offers the possibility of a direct transfer of drugs into the central nervous system [17]. The direct nose-to-brain delivery of drugs holds huge potential for the treatment of CNS disorders as it bypasses the blood-brain barrier, which otherwise poses a major challenge to the effective delivery of drugs to the brain.

3.3 Influencing factors on systemic nasal drug delivery

The nose offers various opportunities for drug delivery, while at the same time posing specific challenges. These include the nasal mucociliary clearance, which limits the residence time of the drug in the nose, drug permeation through the mucosal membrane and the high sensitivity of the nasal mucosa [5,18]. In order to develop effective nasal drug products, these factors need to be taken into account.

3.3.1 Mucociliary clearance

The mucociliary clearance is an important cleaning and protective function of the nose. Inhaled particles are entrapped in the mucus layer covering the nasal epithelium. The mucus layer is transported towards the pharynx, where it is swallowed or expectorated, by coordinated movements of the underlying cilia [4]. Adequate ciliary function is therefore essential, as it reduces the risk of respiratory diseases. However, with regard to nasal drug delivery, nasal clearance also reduces the contact time of a formulation with the mucosa and thus the time for drug absorption or, in case of local drug delivery, for the drug effect. Under physiological conditions, the cilia beat at an average frequency of 10 Hz [10], thereby transporting the overlying mucus layer at an average velocity of 6 mm/min [7]. This transport results in a half-life of the nasal clearance of a simple liquid formulation of 15-20 min [4].

Changes in the ciliary beat frequency and in the viscoelasticity of nasal mucus can affect the mucociliary transport rate. The specific viscoelastic properties of nasal mucus enable an effective ciliary transport. The viscous properties enable the mucus layer to carry a load sufficiently, but an increase in viscosity can disrupt ciliary movement in the gel layer and decrease the beating frequency. Elasticity restores the mucus layer to its original shape after deformation by ciliary movement. A lack in elasticity hinders the transport of the mucus layer as continuous sheet, while an increase in elasticity inhibits the flow [10]. Elasticity is considered the most important for an efficient transport with an optimal range of the elastic modulus of 1-2 Pa [4,10]. Pathologic conditions, environmental factors and different substances can stimulate or impair the mucociliary clearance [4]. With regard to nasal drug delivery, a reduction in mucociliary clearance rate extends the time window for drug absorption. However, permanent impairment of the ciliary function is undesirable as it hinders the physiological function of the nose.

3.3.2 Drug permeation through the nasal mucosa

In order to induce systemic effects, a drug administered nasally must cross the nasal mucosa. In this process, the covering mucus layer as well as the epithelium constitute a barrier.

Airborne particles that deposit on the mucus layer can be entrapped by steric hindrance and chemical interactions [11]. The three-dimensional network of mucin molecules can immobilise particles with a diameter of 500 nm and larger. Smaller particles can additionally be filtered through interactions with the mucin molecules (e.g., electrostatic interactions of positively charged molecules with negative groups of mucin) [11,19].

After diffusion through the mucus layer, drug molecules need to permeate the epithelial membrane either transcellularly or paracellularly. Transcellular permeation can occur by passive diffusion or due to active processes. Paracellular transport occurs through the tight junctions, which interconnect the epithelial cells. The route by which the drug passes through the epithelium depends on its properties. Small lipophilic drugs (<1000 Da) show effective transport through passive diffusion, while the absorption sharply decreases with larger compounds over 1000 Da [13]. Small hydrophilic drugs can cross the epithelial membrane via the paracellular route. However, for larger compounds, the size of tight junctions of 3.9-8.4 Å prevents transport via this pathway [2]. While small lipophilic compounds, applied as nasal solutions, have shown a high bioavailability up to 100%, and pharmacokinetic profiles comparable to intravenous injection, the bioavailability of small hydrophilic compounds is considerably low in the order of 10% [3,20]. Since the transport of substances through the nasal mucosa competes with the nasal clearance, substances that show poor transport through the epithelial membrane are removed from the nasal cavity and are no longer available for nasal absorption.

A prerequisite for systemic absorption of drugs is the dissolution of the substance. Due to the narrow nasal geometry, the volume that can be applied from a liquid formulation is limited to 100-150 µL per nostril in order to prevent dripping [13]. If the drug is applied as a powder, it must dissolve in the nasal fluid volume. Drugs that exhibit low water solubility or require high doses may therefore pose a challenge for nasal administration.

3.3.3 Sensitivity of the nasal mucosa

The nose is innervated by the olfactory nerve, as well as by the ophthalmic branch (anterior part of the nose) and the maxillary branch (posterior part of the nose) of the trigeminal nerve. This innervation is responsible for olfactory sensations on the one hand and trigeminal sensations such as stinging, burning or pungent on the other [21]. These sensations can therefore be caused by the application of drug products to the nose.

The trigeminal innervation of the nose mediates sensations of touch, pressure, temperature and pain. Since pain receptors in the mucosa are not covered by squamous epithelium and are thus directly exposed to chemical stimuli, the mucosa appears to be particularly sensitive. High sensitivity is especially found in the anterior part of the nose [21]. During nasal drug administration, irritation can therefore be triggered by touch and pressure during application, as well as by chemical stimuli caused by the drug or excipients [5]. The occurrence of unpleasant sensations (irritative effects or unpleasant smell) can limit the patient's compliance, especially if repeated drug administration is required. If the nasal drug administration causes severe discomfort, even failure of the nasal product may result [22].

In addition to the sensations triggered directly in the nose, poor taste experiences that can occur if nasal formulations drip down the throat, can limit the acceptance of nasal products.

3.4 Formulation strategies for optimised nasal drug delivery

In order to optimise systemic drug application via the nose, different strategies are conceivable, which can involve the physicochemical properties of the drug, the formulation and the application technique. In the following, the application of nasal powders and the use of excipients will be discussed in more detail.

3.4.1 Nasal powder formulations

Nasal powders constitute a promising formulation strategy to optimise nasal systemic drug delivery. Compared to conventional liquid formulations, they offer improved stability and allow the administration of higher drug doses as no dispersion medium is required [6]. Several studies have also shown an improvement in the bioavailability of drugs when administered as powder formulations instead of liquid ones [23–26]. Tiozzo Fasiolo et al. comprehensively reviewed the improvements in absorption and bioavailability of various small drug molecules and macromolecules caused by the use of nasal powders [6]. A possible underlying mechanism for the improvement in bioavailability is a prolongation of the nasal residence time due to a higher resistance of the powders against the mucociliary clearance. Ishikawa et al. found a prolonged nasal residence time of a powder formulation, which contained the insoluble excipient calcium carbonate compared to a liquid formulation and a powder formulation containing lactose as soluble excipient [23]. In addition to a prolonged nasal residence time of the formulation, the high drug concentration that occurs when the solid drug dissolves on the mucosa can cause an absorption-promoting effect [23]. Studies comparing a sumatriptan powder formulation for the treatment of migraine with a conventional liquid formulation showed a faster onset of action and higher maximum plasma concentrations with the nasal powder [26]. This change in pharmacokinetic profile was associated with a higher deposition in the well-vascularised upper posterior nasal region, which allows for rapid absorption of the drug. The deposition profile of a formulation

in the nasal cavity may not only affect drug absorption due to differences in the blood supply, but can also affect the residence time of the formulation due to differences in the nasal clearance rate from ciliated an non-ciliated nasal regions [27]. The deposition depends partly on the formulation properties and partly on the application device used. Different application aids for nasal powders are available nowadays [5]. Thereby, three main function principles are applied. In powder sprays, the preparation is expelled from the device by pressure. Nasal powder inhalers use the patient's inhalation flow to deliver the preparation into the nasal cavity. In the case of nasal insufflators, exhalation through a mouthpiece creates an air stream that carries the preparation into the nose [5].

Despite the aforementioned advantages, nasal powders constitute the absolute minority of drug products on the market. Several of them are medical devices designed to create a physical barrier against pollen and viruses [28,29]. This barrier is created by the swelling of gel-forming polymers such as HPMC on contact with the moist nasal mucosa. Some locally-acting drug products for the treatment of hay fever are commercially available in Japan (Teijin Rhinocort®, beclomethasone dipropionate, Teijin; Erizas®, dexamethasone cipecilate, Nippon Shinyaku). Only two nasal powders for systemic drug delivery are currently approved by the FDA (sumatriptan powder, Onzetra Xsail® approved in 2016 and glucagon powder formulation, Baqsimi®, approved in 2019) and only one by the EMA (Baqsimi®, 2019). A possible hurdle in establishing nasal powders may be a lack of patient acceptance. Studies have shown a certain increase in nasal irritation after the administration of powders compared to liquid nasal sprays [30,31]. Nasal tolerance is therefore an aspect that should be considered in the development of powder formulations.

3.4.2 Excipients

Poor transport of the drug through the nasal epithelium combined with the limited absorption time due to mucociliary clearance pose the main challenges in nasal drug delivery. The use of mucoadhesive excipients, which slow down the nasal clearance of the formulation, as well as permeation-enhancing excipients, are thus reasonable strategies to optimise nasal drug delivery.

3.4.2.1 Mucoadhesives

Mucoadhesive excipients are intended to retain a formulation in close contact with the nasal mucosa as site of drug absorption. Typically, mucoadhesive materials are hydrophilic polymers containing multiple hydrogen bonding groups [32]. Interactions with mucus result in the formation of an adhesive bond.

The process of mucoadhesion comprises two steps [32]. The initial contact stage establishes a close contact between the mucoadhesive polymer and the mucosa. In the subsequent consolidation stage, the adhesive bond is strengthened by various interactions

occurring between the polymer and the mucosa. Depending on the dosage form of the mucoadhesive (solid, partially hydrated, fully hydrated, liquid), different types of interactions are involved in these processes. In nasal drug delivery, the intimate contact is established due to impaction of the applied particles onto the mucus layer, where dry or partly hydrated particles are wetted [32]. Hydration of the polymer frees the molecules for mucus interactions [32,33]. The hydration of dry or partly hydrated particles is accompanied with the dehydration of the surrounding mucus layer and forces intermixing and consolidation of the adhesive bond [32,34]. Dehydration of the nasal mucus layer additionally alters its rheological properties (increase of elastic and viscous moduli) and thus can decrease the mucociliary transport rate [34,35]. In the case of mucoadhesives, which are already administered as liquids, wettability and spreadability are decisive factors for the formation of the adhesive bond [32,33]. After hydration, the polymer chains of the mucoadhesive interpenetrate the mucus layer, entangle with the mucin chains and bind due to van-der-Waals, hydrogen, hydrophobic and electrostatic forces [32,33].

Polymer-related factors that influence the strength of the mucoadhesive bond include the molecular mass, the cross-linking density and the type of functional groups [33]. As the presence of free polymer chains is required for interpenetration and entanglement and thus the strengthening of an adhesive bond, an optimal molecular weight of about 10^4 Da to $4x10^6$ Da was found [32]. Polymers with higher molecular weight may not show sufficient hydration to free the polymer chains. If the free chain length is reduced due to a high cross-linking density, the adhesiveness of the polymer will also be reduced [32,33]. The functional groups of the mucoadhesive polymer determine the type of bonds that occur with the mucin molecules. For polymers with ionisable groups, the ambient pH can affect the type of interaction. In the presence of cationic groups, electrostatic bonds with negative groups of the mucin molecules and the cell surface can occur [32].

The use of mucoadhesive polymers has the potential to reduce the mucociliary clearance rate and thus prolong the residence time of a formulation in the nose. However, the clearance rate will be recovered over time due to the mucus turnover, causing the formulation to be removed. Earlier adhesive failure may occur at the weakest part of the adhesive system. Pronounced hydration of the mucoadhesive polymer thereby weakens the bond over time [32,33].

3.4.2.2 Absorption enhancers

Absorption enhancers are intended to improve the transport of poorly permeable drugs across the nasal epithelium. Substances from different chemical classes (e.g., surfactants, bile salts, fatty acids, phospholipids, chelating agents, cyclodextrins, cationic polymers) exhibit permeation enhancing effects [6]. Main mechanisms involved are the transient

opening of tight junctions, which increases paracellular permeability, and the disruption of the cell membrane structure, which increases transcellular permeability [36]. Based on the alteration of cell membrane permeability as mode of action, many permeation enhancing substances show cytotoxic effects and the enhancement in bioavailability correlates with the membrane damage caused [36,37]. When selecting absorption enhancers, it is therefore of great importance to consider whether cytotoxic effects occur, and if so whether they are reversible and tolerable. Substances that act through the transient opening of tight junctions may provide a higher potential for safe permeation enhancement [36]. A tight junction modulator, which is well studied with regard to nasal drug delivery is the cationic biopolymer chitosan [37]. Chitosan has been shown to transiently open tight junctions by the translocation of tight junction proteins and to provide a mucoadhesive effect due to electrostatic interactions with negative groups of the mucosa [38]. A nasal morphine formulation containing chitosan was investigated in phase 3 clinical studies (Rylomine™, Javelin Pharmaceuticals) [38].

In addition to substances that directly increase the permeability of the drug through the epithelium, substances that enhance the solubility of the drug, enzyme inhibitors that prevent the enzymatic degradation of the drug in the nose and the use of particulate muco-penetrating systems that promote the drug transport through the mucus gel, can increase the absorption [6,39].

3.4.2.3 Fillers

Fillers are used in powder formulations of potent drugs to improve powder handling and enable accurate dosing. In nasal powder formulations, however, the properties of filler materials used can also influence the absorption of the drug [6].

The use of small, soluble molecules as fillers has shown to increase the mucosal fluid volume due to the induced osmotic effect. This effect can on the one hand accelerate the dissolution of poorly soluble drugs, on the other hand the increased nasal fluid volume can cause an increase in the nasal clearance rate and thus reduce the time for absorption [40]. In contrast to that, the use of insoluble fillers has the potential to extend the residence time of a formulation in the nose and thus improve the absorption of active ingredients exhibiting low permeability [24].

4 Materials

In this work, numerous materials have been used. This section describes the most relevant ones in more detail. Table 10-1 of the appendix provides a list of all materials used with the respective suppliers.

4.1 Excipients

4.1.1 Fillers

4.1.1.1 Mannitol

Mannitol is a naturally occurring sugar-alcohol (Figure 4-1). It appears as white or almost white crystalline powder, which is freely soluble in water and practically insoluble in ethanol 96%. The molecular weight of mannitol is 182.2 g/mol [41]. Mannitol can be extracted from plants, algae or fungi, but for commercial purposes it is produced by hydrogenation of fructose.

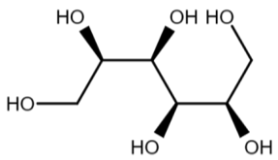

Figure 4-1: Chemical structure of mannitol.

Mannitol is used in pharmacy and medicine in a variety of applications. Therapeutically, mannitol is used primarily for its osmotic properties. Examples for the use of mannitol as active pharmaceutical ingredient (API) include its intravenous application in the treatment of intercranial hypertension [42] and its inhalative application in the treatment of cystic fibrosis [43]. Mannitol is widely used as inactive ingredient in pharmaceutical dosage forms. A common application is as an excipient in oral dosage forms, e.g., as filler in the production of tablets or capsules. However, mannitol is also listed by the FDA (inactive ingredient database) as an approved excipient in a nasal dosage form (nasal spray) with a maximum unit dose of 41.5 mg [44].

Two different mannitol qualities were used in this work. Pearlitol 160 C (Roquette, Lestrem, France) was the mainly used quality and will be referred to as mannitol hereafter. It appears as non-hygroscopic, crystalline powder, with an average mean particle diameter of 160 µm and a specific surface area of 0.25 m²/g [45]. The second used quality was Pearlitol 100 SD (Roquette, Lestrem, France), which is a spray dried mannitol quality, appearing as crystalline powder, with an average mean particle diameter of 100 µm and a specific surface area of 1.05 m²/g [46]. Pearlitol 100 SD will be referred to as spray dried mannitol in the following. Mannitol was characterised and used as water soluble filler for nasal powders in this work.

4.1.1.2 Lactose

Lactose is a disaccharide consisting of the monosaccharides glucose and galactose. In the European Pharmacopoeia (Ph. Eur.), lactose is monographed as the anhydrous form with its two anomers α- and β-lactose and as α-lactose monohydrate (Figure 4-2). It is described as a white to almost white crystalline powder, which is freely soluble in water and practically insoluble in ethanol 96%. The molecular weight of water-free lactose is 342.3 g/mol [41]. Lactose is obtained from milk and whey by crystallisation from a supersaturated solution. Crystallisation of lactose at temperatures below 93.5 °C results in α-lactose monohydrate crystals. B-lactose anhydride crystallises from supersaturated lactose solutions at temperatures above 93.5 °C. Additionally to the crystalline forms, lactose can be present as an amorphous material, containing the α- and β-form in the same ratio as the initial solution [47].

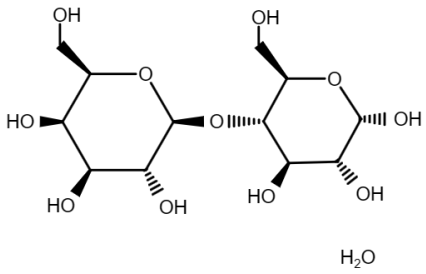

Figure 4-2: Chemical structure of α-lactose monohydrate.

Lactose is a commonly used excipient in the production of solid oral dosage forms e.g., as filler in tabletting. For respiratory delivery, α-lactose monohydrate is long established in powders for inhalation with a good safety profile [48]. However, with respect to nasal administration, it is not yet listed in the FDA inactive ingredient database, which provides information on excipients in FDA-approved drugs [44].

Two different lactose qualities were used in this work. Inhalac 230 (Meggle, Wasserburg am Inn, Germany) was the mainly used quality and will be referred to as lactose in the following. Inhalac 230 is a sieved quality of crystalline α-lactose monohydrate, with an average mean particle diameter of 97 µm and a specific surface area of 0.16 m²/g [49]. The second quality used in this work is FlowLac 100 (Meggle, Wasserburg am Inn, Germany). FlowLac 100 is spray dried from a suspension of milled α-lactose monohydrate crystals in dissolved lactose. Spray drying leads to the formation of spherical agglomerates of α-lactose monohydrate crystals and amorphous lactose. The average mean particle diameter of FlowLac 100 is 126 µm [50]. It will be referred to as spray dried lactose hereafter. Lactose was characterised as soluble filler for nasal powder formulations in this work.

4.1.1.3 Microcrystalline cellulose

Microcrystalline cellulose (MCC) is a purified, partially depolymerised cellulose (Figure 4-3). It is produced by treating α-cellulose, obtained as pulp from plant fibres, with mineral acid. This leads to a reduction in the degree of polymerisation to below 350 and to an increase in crystallinity [51]. MCC appears as white to almost white powder, which is practically insoluble in water [41].

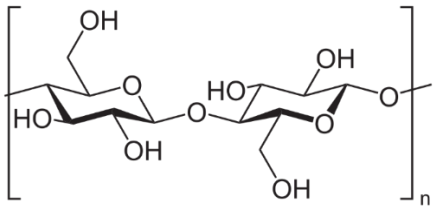

Figure 4-3: Chemical structure of cellulose.

MCC often serves as excipient in the production of solid oral dosage forms. It deforms plastically during compression and is considered one of the preferred direct compression binders [51]. Pure MCC is not yet used in nasal drug products, but it is listed as colloidal MCC (with sodium carboxymethyl cellulose) in the FDA inactive ingredient database for nasal sprays [44].

In this work, MCC (Vivapur 102, JRS Pharma, Rosenberg, Germany) was characterised as insoluble filler for nasal powder formulations. Vivapur 102 is a medium size standard MCC grade with an average mean particle diameter of 130 µm [52].

4.1.1.4 Colloidal microcrystalline cellulose

Colloidal microcrystalline cellulose is a co-processed synergistic composite of microcrystalline cellulose and 5-22% sodium carboxymethyl cellulose (CMC), which appears as white to almost white powder [41]. Within this synergism, CMC prevents re-aggregation of microcrystalline cellulose and leads to an easy dispersibility in water. After dispersion in water, colloidal MCC forms a white, opaque thixotropic gel [53].

In the pharmaceutical industry, colloidal MCC serves as stabilising agent for suspensions, reconstitutable powders, creams, lotions and sprays. It is listed in the FDA inactive ingredient database for nasal spray products [44].

The suitability of colloidal MCC (Vivapur MCG 811 P, JRS Pharma, Rosenberg, Germany) as filler for nasal powder formulations with additional mucoadhesive properties was assessed in this work. Vivapur MCG 811 P is a colloidal MCC grade with a CMC content of 11.3-18.8%, a viscosity of a 2.6% dispersion in distilled water of 2,400-5,600 mPas and an elasticity of a 3% dispersion in distilled water of 60 Pa [53].

4.1.2 Mucoadhesive polymers

4.1.2.1 Cellulose derivatives

Cellulose derivatives are derived from the chemical modification of cellulose. In this work, the derivatives hydroxypropyl methyl cellulose, hydroxypropyl cellulose, hydroxyethyl cellulose and sodium carboxymethyl cellulose (Figure 4-4) were used. They are characterised by the degree of polymerisation, the degree of substitution (average number of hydroxyl groups per anhydro glucose unit that are replaced by a substituent) and the molar substitution (average number of moles of substituents per mole of anhydro glucose). The degree of polymerisation directly affects the viscosity of a polymer solution. Cellulose derivatives are therefore often characterised by the viscosity (in mPas) of a 2 wt.% aqueous solution at 20 °C.

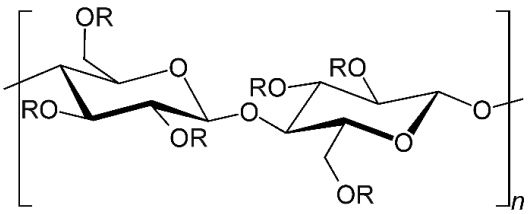

Figure 4-4: Chemical structure of cellulose derivatives. Hydroxypropyl methyl cellulose: R= —H, —CH_3, —$CH_2CH(OH)CH_3$; Hydroxypropyl cellulose: R= —H, —$(CH_2CH(CH_3)O)_xH$; Hydroxyethyl cellulose: —H, —$(CH_2CH_2O)_xH$, Sodium carboxymethyl cellulose: R= —H, —$CH_2COO^-Na^+$.

4.1.2.1.1 Hydroxypropyl methyl cellulose

Hydroxypropyl methyl cellulose (HPMC) is a non-ionic, partially O-methylated and O-(2-hydroxypropylated) cellulose derivative. It appears as white to yellow-white or grey-white powder that is colloidal soluble in cold water [41].

In the pharmaceutical industry, HPMC commonly serves as gelling and thickening agent, as matrix in extended release formulations, as film-forming substance, or as excipient in bioadhesive systems [54]. Due to its wide range of applications, it is used in many different dosage forms. The FDA lists HPMC as an approved inactive ingredient for nasal sprays and powders [44].

In this work, two different viscosity grades (400 mPas and 4000 mPas viscosity of a 2 wt.% aqueous solution) of Metolose 65 SH (Shin Etsu, Chiyoda, Japan) were characterised as mucoadhesive agents for nasal powder formulations. Metolose 65 SH is a HPMC grade with the substitution type 2906, which is defined by a methoxy content of 27.0-30.0% and a hydroxypropoxy content of 4.0-7.5% [55]. The two viscosity grades are referred to as HPMC 400 and HPMC 4000 hereafter.

4.1.2.1.2 Hydroxypropyl cellulose

Hydroxypropyl cellulose (HPC) is a non-ionic, partially O-(2-hydroxypropylated) cellulose derivative. The content of hydroxypropoxy groups in the dry mass is 53.4-80.5%. It appears as white to yellow-white powder that is colloidal soluble in cold water [41].

In the pharmaceutical industry, HPC is widely used in oral and topical formulations, e.g. as film former, binder, matrix in extended release formulations, thickener or gelling agent [56]. HPC does not occur in nasal drug products approved in Germany at present, but it is an ingredient of the medical device *"EMS Sinusitis Spray mit Eukalyptusöl"* (Emser, Bad Ems, Germany) [57].

In this work, two different viscosity grades of HPC were characterised as mucoadhesive agents for nasal powder formulations. The lower viscosity grade was Klucel GF Pharm (Ashland, Wilmington, Delaware, USA), with a typical viscosity of a 2 wt.% aqueous solution of 150-400 mPas and a typical molecular weight of 370,000 Da. This grade is referred to as HPC G hereafter. The higher viscosity grade was Klucel MF Pharm (Ashland, Wilmington, Delaware, USA), with a typical viscosity of a 2 wt.% aqueous solution of 4,000-6,500 mPas and a typical molecular weight of 850,000 Da. This grade is referred to as HPC M hereafter. Both qualities are characterised by a molar substitution of 2-4.1 [56].

4.1.2.1.3 Hydroxyethyl cellulose

Hydroxyethyl cellulose (HEC) is a non-ionic, partially O-(2-hydroxyethylated) cellulose derivative with a content of hydroxyethyl groups of 30-70%. It appears as white to yellow-white powder. In contrast to HPMC and HPC, HEC is colloidally soluble in hot and cold water [41].

In the pharmaceutical industry, HEC is most commonly used as viscosity modifier in liquid and semisolid drug products [58]. It is listed in the FDA inactive ingredient database for a nasal spray product [44].

In this work, two different viscosity grades of HEC were characterised as mucoadhesive agents for nasal powder formulations. The lower viscosity grade was Natrosol 250 G Pharm (Ashland, Wilmington, Delaware, USA), with a typical viscosity of a 2 wt.% aqueous solution of 250-400 mPas and a typical molecular weight of 300,000 Da. This grade is referred to as HEC G hereafter. The higher viscosity grade was Natrosol 250 M Pharm (Ashland, Wilmington, Delaware, USA), with a typical viscosity of a 2 wt.% aqueous solution of 4,500-6,500 mPas and a typical molecular weight of 720,000 Da. This grade is referred to as HEC M hereafter. Both qualities are characterised by a molar substitution of 2.5 [58].

4.1.2.1.4 Sodium carboxymethyl cellulose

Sodium carboxymethyl cellulose (CMC) is the sodium salt of anionic partially O-carboxymethylated cellulose. It appears as white powder, which is colloidally soluble in water [41].

In the pharmaceutical industry, CMC commonly serves as binder in the production of solid oral dosage forms, as matrix in extended-release formulations and as viscosity modifier. CMC is listed in the form of colloidal MCC (microcrystalline cellulose and CMC) in the FDA inactive ingredient database for nasal spray formulations [44].

In this work, a medium viscosity grade of CMC (Sigma-Aldrich, St. Louis, Missouri, USA) was assessed as mucoadhesive agent for nasal powder formulations. Characteristics of the used CMC grade are a viscosity of 400-800 mPas of a 2 wt.% aqueous solution and a degree of substitution of 0.65-0.90 [59].

4.1.2.2 Pectin

Pectin is a polysaccharide with varying molecular structure, consisting of a backbone of α-(1,4) linked galacturonic acid and additional neutral sugars like galactose, rhamnose or arabinose as part of the backbone or as sidechains. The carboxyl groups of the galacturonic acid are partly methylated (Figure 4-5). Pectins with a degree of esterification (DE) above 50% are defined as high methoxyl pectins and those with a DE below 50% as low methoxyl pectins. Pectin occurs in different plant materials, but for commercial purposes it is commonly extracted from apple pomace and citrus peel [60]. The dried material appears as beige powder that is colloidally soluble in water [61].

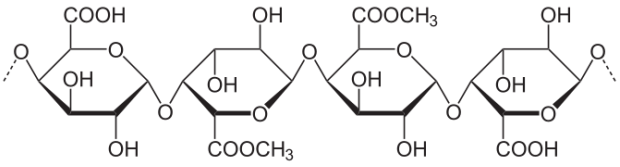

Figure 4-5: Partially esterified segment of the poly-α-(1→4)-galacturonic acid backbone of pectin.

The most common use of pectin is in the food industry as gelling or thickening agent. Archimedes Pharma introduced pectin to nasal drug delivery with the PecSys™ technology. This system bases on the gelling properties of low methoxyl pectin, which forms gels in the present of divalent cations, mainly calcium. Calcium ions bind to free carboxyl groups of the galacturonic acid and thus link different polymer chains to form a three-dimensional network. Since calcium ions are present in the nasal fluid, low methoxyl pectin gels in situ when getting in contact with the nasal mucosa [60]. PecSys™ is used in the fentanyl nasal spray product PecFent® (Kyowa Kirin GmbH, Düsseldorf, Germany) to optimise the

absorption profile of fentanyl. Pectin is listed in the FDA inactive ingredient database for nasal spray products with a maximum unit dose of 10 mg [44].

In this work, low methoxyl pectin was assessed as mucoadhesive agent for nasal powder formulations. The used pectin (Classic CU-L 045/18, Herbstreith & Fox, Neuenbürg, Germany) has a galacturonic acid content of 88% with a degree of esterification of 29% [61].

4.1.2.3 Chitosan derivatives

Chitosan is a polysaccharide consisting of randomly ordered β-(1,4) linked units of N-acetyl-D-glucosamine and D-glucosamine (Figure 4-6), which is derived from chitin from crustaceans and mushrooms via basic deacetylation [62]. Chitosan is available in different grades with different physicochemical properties that are derived from the degree of deacetylation (amount of free amino groups in the molecule), the molecular weight and possibly chemical modifications. Unmodified chitosan is soluble at acidic pH due to protonation of the amino groups but insoluble at neutral or basic pH.

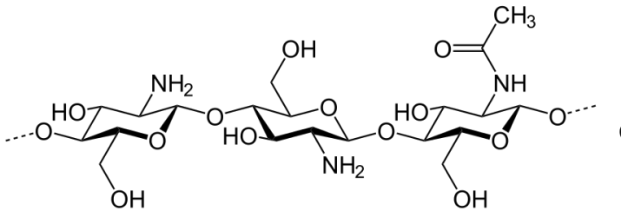

Figure 4-6: Chemical structure of chitosan with acetylated and deacetylated glucosamine units.

Its cationic character makes chitosan attractive for mucosal and transmucosal drug delivery [62–64]. The positively charged amino groups are able to interact with negatively charged groups of the mucosa and by this, retain the formulation at the mucosal surface. Additionally, chitosan has the ability to promote the permeation of drugs through the mucosa by opening tight junctions, which interconnect the epithelial cells. Chitosan is not yet listed in the FDA inactive ingredient database for drug products, but its biocompatibility and low toxicity have been demonstrated in literature [65].

The solubility of chitosan only at acidic pH limits its application in formulations and its effect in the pH conditions in the human body. To overcome this, chitosan derivatives with modified solubility are under investigation. In this work, the two chitosan derivatives N,O-carboxymethyl chitosan and chitosan glutamate were assessed as mucoadhesives and permeation enhancers for nasal powder formulations. Carboxymethyl (CM) chitosan is partially N,O-carboxymethylated chitosan with amphoteric properties, due to the presence of both, amino and carboxylic groups. The amphoteric character results in an optimised solubility of CM chitosan in basic conditions based on the deprotonation of the carboxylic

groups [62]. The CM chitosan used is characterised by a degree of deacetylation of 80-95% and a viscosity of 5-300 mPas according to the supplier (Heppe Medical Chitosan GmbH, Halle, Germany). Chitosan glutamate is a water-soluble salt of chitosan and glutamic acid. The used substance is characterised by a degree of deacetylation of 80-95% and a viscosity of 2-200 mPas according to the supplier (Heppe Medical Chitosan GmbH, Halle, Germany).

4.2 Model drugs

4.2.1 Metoprolol tartrate

Metoprolol is a small lipophilic drug in the class of cardioselective beta-blockers, which is commonly used in its tartrate or succinate form. In this work, the tartrate form of metoprolol was used (Figure 4-7). The molecular weight of metoprolol base is 267 g/mol (685 g/mol for metoprolol tartrate) and its logP of 1.6 [66] is associated with a high and transcellular permeation [14]. The Ph. Eur. 10.0 classifies the water-solubility of metoprolol tartrate as very soluble (>1000 mg/mL) [41]. Based on its permeation and dissolution behaviour, metoprolol is classified in class I of the biopharmaceutics classification system (BCS), which includes drugs with high solubility and high intestinal permeability. The FDA Guidance for Industry *M9 Biopharmaceutics Classification System-Based Biowaivers* suggests metoprolol as high permeability model drug for permeability assays [67]. This classification bases on data on the behaviour of metoprolol in oral dosage forms, but metoprolol is also considered as model drug in respiratory drug delivery. In their approach for an establishment of a pulmonary biopharmaceutical classification system, Eixarch et al. considered metoprolol as quality control marker [66] and Sibinovska et al. showed a high permeability of metoprolol in a nasal permeation model [68]. Therefore, metoprolol was used as high permeability model drug in this work.

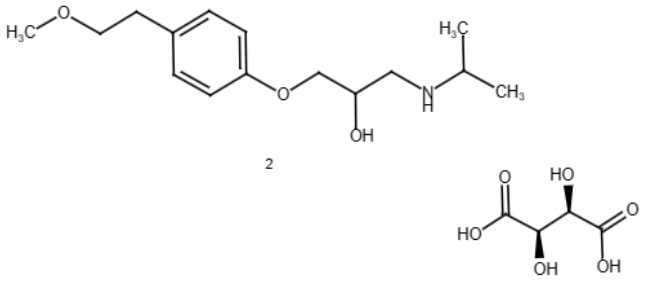

Figure 4-7: Chemical structure of metoprolol tartrate.

4.2.2 Atenolol

Atenolol is a small molecule with a molecular weight of 266 g/mol in the class of cardioselective beta-blockers (Figure 4-8). With a logP of 0.5, atenolol shows higher hydrophilicity than metoprolol [66]. LogP values below 1 are associated with decreased permeability through epithelial barriers [14]. The Ph. Eur. 10.0 classifies the water-solubility

of atenolol as sparingly soluble (10-33.3 mg/mL) [41]. Even though the solubility is considerably lower than that of metoprolol tartrate, it is still classified as high in the BCS related to oral use. Differences in the solubility, however, can have great impact in the small fluid volume in the nose and thus, this classification may not be transferrable to nasal administration. Based on its intestinal permeability atenolol is classified in BCS class III (high solubility, low permeability) and the FDA suggests atenolol as moderate permeability model drug for permeability assays [67]. In the study of Sibinovska et al. a lower permeability of atenolol compared to metoprolol was also confirmed in a nasal permeation model [68]. Atenolol was therefore used as low permeability model drug is this study, for which a paracellular transport can be assumed.

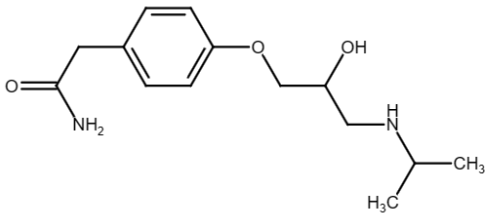

Figure 4-8: Chemical structure of atenolol.

4.3 Cell line

4.3.1 RPMI 2650

RPMI 2650 cells are nasal septum squamous cell carcinoma cells obtained from the pleural effusion of a 52-year-old man in 1962. They appear as adherent, epithelioid, small cells [69]. Different from human nasal mucosa, RPMI 2650 cells show multi-layered cell growth and do not exhibit cilia activity [70,71]. However, different studies revealed, that RPMI 2650 cells, grown at an air-liquid interface, show permeation barrier properties comparable to human nasal mucosa [68,71–73]. RPMI 2650 cell models proved to show a transepithelial electric resistance comparable to human nasal mucosa [70], presence of four tight junction proteins (ZO-1, occluding, claudin-1 and E-cadherin) [72] and mucus production [73]. Based on this, the RPMI 2650 cell line was considered as appropriate cellular model for the nasal epithelium. Cells were purchased from the German Collection of Microorganisms and Cell Cultures GmbH (ACC 287, Braunschweig, Germany).

5 Methods

This section describes the methods used in this work. Table 10-2 of the appendix provides a list of the used equipment with the respective manufacturers.

5.1 Preparation methods

5.1.1 Preparation of sieve fractions from the excipient raw materials

This work investigates the effect of selected excipients on nasal drug delivery from powder formulations. Since the particle sizes of the raw materials of the excipients differed considerably, the excipient powders were fractioned on a laboratory sieve shaker in order to reduce the influence of particle size on the investigated processes. Sieve fractions of 32-90 µm, 90-150 µm and 32-150 µm were prepared.

5.1.2 Preparation of model formulations

In order to investigate the effect of excipients in nasal powder formulations, different powder blends containing model APIs and selected excipients were prepared using the Turbula blender (Willy A. Bachofen, Muttenz, Switzerland). Excipients (sieve fraction 32-150 µm) and APIs were weighed into the mixing vessel using a double-sandwich-method. The blending process consisted of three blending steps of 5 min each at 42 rpm with sieving steps (355 µm mesh size) in between, which were intended to destroy formed agglomerates. In order to assess blend homogeneity, the drug content of six randomly picked samples of 10 mg each was quantified according to section 5.2.3. Powder blends with a relative standard deviation (RSD) of drug content below 5% and a mean drug recovery of 90-110% were considered as homogeneous.

5.2 Characterisation methods

5.2.1 Particle size distribution

The determination of particle size distributions of powder samples was conducted using laser diffraction (Helium Laser Optical System, HELOS, Sympatec GmbH, Clausthal-Zellerfeld, Germany). The powders were dispersed in air and injected into a laser light beam using the RODOS dispersing system with a dispersion pressure of 3 bar. The laser light beam, which is diffracted by the particles at different angles, is recorded by a detector. The evaluation was conducted according to the Fraunhofer theory and results in a volume-based particle size distribution. For non-spherical particle shapes, a size distribution equivalent to spherical particles is obtained. The measurements were conducted in triplicate. The characteristic particle sizes x_{10}, x_{50} and x_{90}, which express the particle diameters, where 10%, 50% and 90% of the particles are smaller, and the span value (equation 5-1), which indicates the width of the particle size distribution, were used for the comparison of different samples.

Equation 5-1: Calculation of the span value. With x_{10}, x_{50}, and x_{90} as particle diameters, where 10%, 50% and 90% of the particles are smaller.

$$\text{span} = \frac{x_{90} - x_{10}}{x_{50}}$$

5.2.2 Particle imaging

Particle morphologies and powder compositions were visualised using scanning electron microscopy (Phenom World XL, Thermo Fisher Scientific Inc., Waltham, USA). For this purpose, the powders were attached to an aluminium stub with double-sided carbon tape and coated with a thin gold layer using a sputter coater (BAL-Tec SCP 050, Leica Microsystems, Wetzlar, Germany). The samples were visualised with the scanning electron microscope using an acceleration voltage of 10 kV and a vacuum of 10 Pa.

5.2.3 Drug quantification

The drug content was quantified using high performance liquid chromatography (HPLC, Agilent 1100 Series LC, Agilent Technologies, Santa Clara, USA). Table 5-1 summarises the analytical parameters of the respective methods. External calibration curves (R^2>0.999) of the drugs were analysed for drug quantification. The limit of detection and limit of quantification were calculated according to ICH guideline CMP/ ICH/381/95 based on the standard deviation of response and the slope.

Table 5-1: Parameters of quantification methods (HPLC).

Parameter	Metoprolol tartrate	Atenolol
Column	LiChrospher® 100 RP-18-5	LiChrospher® 100 RP-18-5
Mobile phase	75% potassium dihydrogen phosphate buffer (0.067 M) with 0.2% triethylamine adjusted to pH 3 25% acetonitrile	90% potassium dihydrogen phosphate buffer (0.067 M) with 0.2% triethylamine adjusted to pH 3 10% acetonitrile
Dissolution medium	Mobile phase	Mobile phase
Flow rate	0.8 ml/min	0.8 ml/min
Injection	40 µL Double injection of each sample	20 µL Double injection of each sample
Wavelength	224 nm	224 nm
Retention time	3.3 min	3.2 min
Limit of detection	0.03 µg/mL	0.06 µg/mL
Limit of quantification	0.08 µg/mL	0.18 µg/mL

5.2.4 Rheological testing

Rheological studies were used in order to evaluate the potential of powder samples to cause changes in the rheological properties of the nasal fluid, which should lead to a resistance against the mucociliary clearance. A rotational viscosimeter (CVO 120 HRF, Bohlin Instruments GmbH, Pfortzheim, Germany) equipped with parallel plate setup (plate diameter 40 mm) was used in this work for viscosity and oscillatory measurements. Simulated nasal fluid (SNF [74], table 5-2) served as dispersion medium for the powder samples. The measurements were conducted at the nasal temperature of 32 °C [12].

Table 5-2: Composition of simulated nasal fluid.

Ingredient	Concentration	pH
NaCl	7.45 g/L	6.4
KCl	1.29 g/L	
$CaCl_2$ x 2 H_2O	0.32 g/L	
Double-distilled water	q.s.	

5.2.4.1 Viscosity measurements

For measuring the shear viscosity of pure excipients in SNF, 30 mg of the powder sample was added to 1.5 mL of SNF and vortexed for 20 s. The formed dispersions were allowed to rest for 1 min or 15 min before the measurement, in order to mimic the viscosity directly after contact with the nasal fluid, and at the end of the physiological nasal residence time of a formulation in the nose.

In order to compare the viscosity of the dispersions, a controlled shear rate test was conducted using a shear rate of 1 s^{-1}. This shear rate was selected in order to display the effective shear rate applied to the mucus layer by ciliary beating in the nose [10]. Prior to data acquisition, the sample was pre-sheared for 30 s at a shear rate of 1 s^{-1} in order to distribute the sample evenly in the measuring gap. The gap size was set between 150 µm and 500 µm, depending on the sample measured (i.e., size of powder particles, sample viscosity). The experiment was conducted in triplicate.

5.2.4.2 Oscillation measurements

Oscillatory tests were used to investigate the viscoelastic behaviour of the samples. Thereby, the sample is subjected to a small, oscillating deformation, which causes a periodic shear stress. The phase shift of the sinusoidal curves of the deformation and the resulting shear stress defines the viscoelasticity of the sample. The complex shear modulus (Equation 5-2) represents the relation of shear stress and deformation and is composed of the elastic and viscous parts of the sample. The storage modulus (G') represents the elastic

behaviour of the sample. It is a measure of the stored deformation energy that is fully available after relief and is the driving force for the elastic recovery. The loss modulus (G") represents the viscous behaviour of the sample. It is a measure of the deformation energy that is consumed by frictional processes during shearing.

Equation 5-2: Complex shear modulus (G^*) with the values of the sinusoidal functions of shear stress (τ) and deformation (γ).

$$G^* = \frac{\tau(t)}{\gamma(t)}$$

The frequency-dependent storage and loss moduli were recorded at a constant deformation within the linear viscoelastic region of the sample, which was determined with an amplitude sweep prior to the experiment.

Sample preparation for pure excipient samples was conducted according to section 5.2.4.1. The gap size of the parallel plate setup was set between 150 µm and 500 µm, depending on the sample measured. For the characterisation of the model formulations, the nasal fluid volume, in which one powder dose is distributed was estimated to be 200 µL, based on the total surface area of the nose of 150 cm² and the thickness of the covering mucus layer of 10-15 µm [75,76]. One powder dose was set to 50 mg (corresponds to 20 mg API) for the formulations and 20 mg for pure API controls. In order to obtain enough sample material for the measurement, powder mass and fluid volume were scaled up. 300 mg of the model formulations or 120 mg of the pure API were added to 1.2 mL of SNF and vortexed for 20 s. The dispersions were allowed to rest for 15 min before the measurement. The gap size of the parallel plate setup was set to 750 µm. The experiment was conducted in triplicate.

5.2.5 Displacement on agar-mucin gels

The mucoadhesiveness of excipients and the potential to interact with mucin was assessed by measuring the displacement of powder samples on pure agar gels and on agar-mucin gels on an inclined plane. The method was adapted from Nakamura et al. [77] and Bertram and Bodmeier [78]. For gel preparation, 45 g of a hot agar solution (1.5% w/w) with or without porcine mucin type II (2% w/w) in phosphate buffer pH 6.4 (Ph. Eur. 10.0, table 5-3) was casted on petri dishes (diameter 14 cm) and left for gelation in a refrigerator. Prior to the experiment, the gels were equilibrated for 1 h to the test temperature of 32 °C, which was selected to simulate the nasal temperature [12]. To start the experiment, 25 mg of the excipient powder (sieve fraction 32-150 µm) was placed on one side of the gel in a spot with a diameter of approximately 10 mm and the petri dish was turned up to an angle of 45°. The displacement of the powder spots was measured as a function of time up to a distance of 10 cm. Figure 5-1 displays the experimental setup.

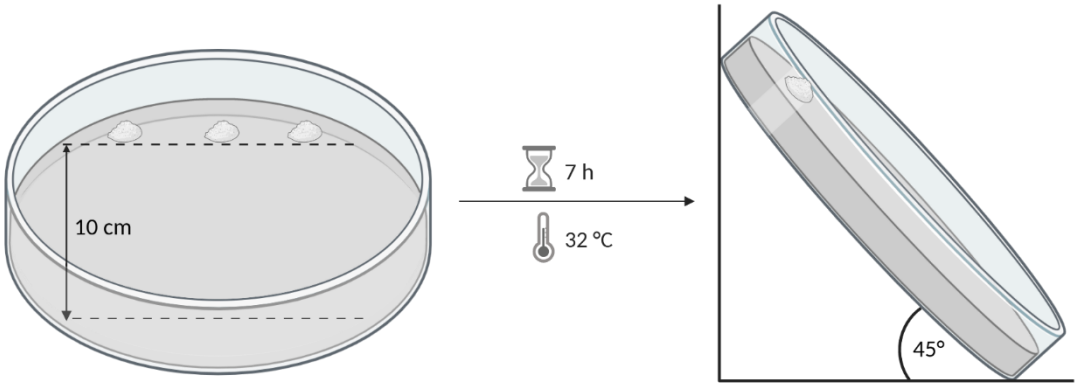

Figure 5-1: Schematic illustration of the experimental setup for assessing the displacement of powder samples on agar and agar-mucin gels [79]. Created with BioRender.com.

The measurements were conducted in triplicate and results are displayed as maximal displacement out of the three measurements.

In order to investigate the influence of calcium ions on the adhesiveness of the pectin powder, pectin was additionally tested on gels prepared in SNF (5.2.4, table 5-2) instead of phosphate buffer.

Table 5-3: Composition of phosphate buffered saline pH 6.4 (Ph. Eur. 10.0).

Ingredient	Concentration	pH
NaCl	8.2 g/L	6.4
Na_2HPO_4 x 12 H_2O	2.5 g/L	
NaH_2PO_4 x 2 H_2O	2.5 g/L	
Double-distilled water	q.s.	

5.2.5.1 Calcium quantification from porcine mucin type II

In order to evaluate the influence of calcium ions on the adhesiveness of pectin on mucin containing agar gels, atomic absorption spectroscopy (AAS) was used for the detection and quantification of calcium in porcine mucin type II. For the analysis, the mucin powder was dispersed in ultrapure water and shaken on a laboratory shaker for 24 h. The samples were centrifuged (Centrifuge 5430 R, 7197 rcf at 5 °C, multiple steps) and measured using a flame atomisation (air-acetylene flame) AAS system (AAS 3030). The analysis was conducted in triplicate.

5.2.6 Dynamic vapour sorption

The hygroscopicity of samples was assessed by measuring the dynamic vapour sorption (DVS, DVS Resolution, Surface Measurement Systems Ltd., Wembley, UK). The measurement bases on the recording of mass changes at varying humidities. The humidity was altered in two measuring cycles from 0% to 90% to 0% in 10% steps under isothermal conditions (25 °C). The samples were initially equilibrated for 180 min at 0% humidity. Each of the following steps was held until mass equilibrium (dm/dt<0.005 %/min) was reached. The hygroscopicity of the samples was classified according to Ph. Eur. 10.0., which rates the gain in mass at 80% humidity. A mass gain of 0.2-2% is classified as slightly hygroscopic, a mass gain of 2-15% is classified as hygroscopic and a mass gain of 15% and higher is classified as very hygroscopic.

5.2.7 Sensory effects in the nose

5.2.7.1 Slug mucosal irritation assay

The slug mucosal irritation assay (SMIA) bases on a correlation between the amount of mucus produced by slugs upon contact with a sample, and irritative effects caused by the sample on a human mucosa. Lenoir et al. provided a one-day protocol of the assay that correlates mucus production of slugs of the species Arion lusitanicus with stinging, itching and burning as short term sensations, occurring with nasal drug delivery in humans [80,81]. Based on this assay protocol, the potential of powder samples to cause nasal discomfort was assessed.

Slugs were obtained by wild harvesting and were kept under laboratory conditions. Two days before the experiment, slugs with a body weight between 3 g and 6 g were isolated on paper towels soaked with phosphate buffered saline (PBS, table 5-4). The body wall of the slugs was moistened daily with 1 mL PBS and checked for any damages of the mucosa. For the experiment, the samples to be tested were placed in petri dishes. For the assessment of powders, a sample amount of 50 mg was used in order to display a proper dose for nasal administration. As described by Lenoir et al., a 1% (w/v) benzalkonium chloride solution (100 µL) served as marker for severe irritation. In order to provide a particulate marker for no irritation pure sea sand (sieve fraction 32-150 µm, 50 mg) was used. Prior to the experiment, the initial body weight of slugs and the weight of the petri dishes containing the samples were determined. The slugs were placed on the samples for three contact periods of 15 min each (fresh sample for each contact period). Between the contact periods, the slugs rested and hydrated for 60 min in petri dishes containing 1.5 mL of PBS. The petri dishes containing the samples and the produced mucus were re-weighed in order to quantify the amount of mucus produced by the slugs. The total mucus production out of the three contact periods was calculated and expressed as percent of the initial body

weight of the slugs according to equation 5-3. Different from the protocol of Lenoir et al., the initial body weight of slugs before the first contact period was used as reference for the calculation. The classification of nasal discomfort proposed in [81] was therefore not applied in this work. Each experiment was conducted with three slugs that were not used in any experiment before.

Equation 5-3: Calculation of the total mucus production (TM) out of three contact periods (CP) expressed as percent of the initial body weight (BW) of slugs.

$$\mathrm{TM, \%} = \sum_{i=1}^{3} (\mathrm{Mucus\ per\ CP, g})_i \,/\, \mathrm{BW, g} \times 100\%$$

Table 5-4: Composition of phosphate buffered saline pH 7.4.

Ingredient	Concentration	pH	Osmolality
NaCl	8.0 g/L	7.4	290 mosmol/kg
KCl	0.2 g/L		
Na_2HPO_4	1.42 g/L		
KH_2PO_4	0.27 g/L		
Double-distilled water	q.s.		

5.2.7.1.1 Preparation of powder blends

In order to assess the effect of irritating substances in powder blends, blends of chitosan glutamate (5% and 20% (w/w)), which has shown to increase the mucus production of slugs, and MCC were prepared in a Turbula blender. The excipients were used as sieve fraction 32-150 µm and were weighed into the mixing vessel using a sandwich-method. The blending process consisted out of three blending steps of 5 min each at 42 rpm with sieving steps (355 µm mesh size) in between.

5.2.7.1.2 Assessment of pH and osmolality changes

The pH (Seven Compact pH meter, Mettler Toledo GmbH, Columbus, USA) and osmolality (Osmomat 030, Gonotec GmbH, Berlin, Germany) of dispersions of 10 mg of the excipients in 1 mL of PBS (table 5-4) were measured, in order to assess the effect of changes on the mucus production of slugs in the SMIA.

5.2.8 Drug dissolution and release

5.2.8.1 24 h solubility

In order to assess the saturation solubility of drugs, a sufficient amount of the powder to ensure an undissolved residuum was suspended in 2 mL of SNF (5.2.4, table 5-2) and stirred for 24 h. The drug content in the supernatant was quantified in duplicate according to 5.2.3.

5.2.8.2 Dissolution and release from model formulations

In order to assess the dissolution and release of the drug from model formulations, Franz diffusion cells (Permegear, Hellertown, USA) were used. Franz cells provide a donor and an acceptor compartment, which can be separated by a membrane. The setup therefore allows the assessment of drug dissolution on a wetted membrane in the donor compartment, and thus mimicking the air-liquid-interface in the nose, while the higher volume of the acceptor compartment maintains sink conditions. Franz cells with an acceptor volume of 8 mL and a diffusion area of 1 cm² were used in this work. SNF (5.2.4, table 5-2) served as acceptor medium and was thermostated to 32 °C to mimic the nasal temperature [12]. The acceptor compartment was separated from the donor compartment with a cellulose acetate membrane (pore size 0.45 µm, wetted in SNF). To avoid fluid being pushed up from the acceptor compartment into the donor compartment due to the hydrostatic pressure, the filling volume in the acceptor compartment was slightly adjusted to fit the height of the membrane. For the dissolution studies, 50 mg (corresponds to 20 mg API content) of the formulations or 20 mg of pure drug control were applied to the membrane. To apply the powders reproducibly to the total diffusion area of the membrane, the UDS powder device was used. In order to record dissolution and release curves of the drugs, 100 µL acceptor medium was sampled at defined time points and the drug content was quantified according to 5.2.3. The removed volume was replaced with fresh SNF. The experiment was conducted in triplicate.

5.2.9 Cell culture

RPMI 2650 cells were used for cell culture experiments in this study. Under standard cultivation conditions, the cells were grown in 75 cm² cell culture flasks at 37 °C and 5% CO_2. Supplemented Dulbecco's Modified Eagle's Medium (DMEM) or supplemented Minimum Essential Medium (MEM, both Sigma-Aldrich, St. Louis, USA) were used as standard cultivation media (Table 5-5). The medium was changed every two to three days. Cells that reached about 80% confluence were rinsed with PBS, detached by trypsin/EDTA treatment and seeded into new cell culture flasks. Cell viability was assessed by trypan blue staining and the cultures were routinely tested for absence of mycoplasma infection.

Table 5-5: Composition of cell culture media and buffer.

Abbreviation	Base Medium	Supplements
DMEM	Dulbecco's Modified Eagle's Medium, with 4500 mg/L glucose, L-glutamine, sodium pyruvate, and sodium bicarbonate	10% Fetal bovine serum (FBS) 1% Non-essential amino acids 1% Penicillin/streptomycin
MEM	Minimum Essential Medium Eagle, with Earle's salts, L-glutamine and sodium bicarbonate	10% FBS 1% Sodium pyruvate 1% Non-essential amino acids 1% Penicillin/streptomycin
PBS	Dulbecco's Phosphate Buffered Saline, modified, without calcium chloride and magnesium chloride	

5.2.9.1 Cytotoxicity

The toxicity of excipients was assessed using the MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazoliumbromide) assay. The assay bases on the reduction of yellow MTT to a water-insoluble purple formazan, which is catalysed by mitochondrial dehydrogenases of viable, metabolically active cells (Figure 5-2) [82]. For the assay, cells are seeded to 96-well-plates at a cell number of 4 x 10^4 cells per well (100 µL cell suspension with 4 x 10^5 cells/mL). After a growth time of 24 h the medium was removed and replaced with serum-reduced medium (2% FBS). 100 µL of the excipient solutions were added to the cells (triple determination per plate). The sample solutions were diluted from stock solutions, which were prepared by dissolving the excipient powder in a certain volume of PBS. 100 µL of pure PBS and 50 µL of Triton-X (1% in PBS) were used as negative and positive control, respectively. The cells were incubated with the excipient solutions for 24 h. After the incubation time, the samples were removed and replaced with 100 µL serum reduced medium and 25 µL MTT solution (5 mg/mL in PBS). After 4 h of incubation the solution was gently removed and replaced with 100 µL lysis solution (5% SDS in dimethylformamide + water 1+1, pH 4.7). After the dissolution of the formed crystals, the formazan was quantified using a plate reader (Tecan Spark, Tecan Group Ltd., Männedorf, Switzerland) at 570 nm (absorbance) and 650 nm (background). The viability of cells in contact with the samples is calculated by relating the measured absorption to the positive (0% viability) and negative (100% viability) control.

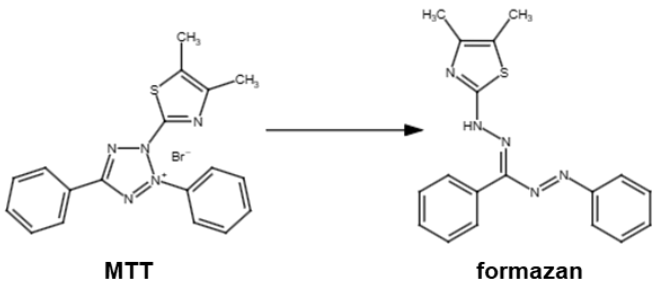

Figure 5-2: Metabolisation of MTT (yellow) to a violet formazan salt by viable cells.

5.2.9.2 Permeation

In order to assess the influence of excipients on the permeation of model drugs through RMPI 2650 cell layers, cells cultured in MEM were seeded on permeable filter inserts (PET, 1.13 cm³, pore size 3 µm) at a cell number of 4 x 10^5 cells per insert (500 µL cell suspension with 8 x 10^5 cells/mL). The cells were cultivated under submerged conditions for seven days with medium changes every two to three days. On day seven, the medium from the apical side was removed and the medium at the basolateral side was reduced from 1 mL to 0.5 mL to create an air-liquid interface (ALI). The cells were cultured at ALI for 14 further days to allow cell differentiation. The integrity of the formed cell layer was assessed by measuring the transepithelial electric resistance (TEER) with an Evom voltohmmeter (World Precision Instruments, Sarasota, USA). For TEER measurements, ALI cultures were overlayed with medium, which was removed again after the measurement. Cell layers with a net TEER (TEER of empty insert subtracted) above 75 Ω*cm² were used in the permeation studies. For the assessment of drug permeation, drug solutions with a concentration of 400 µg/mL were prepared in assay buffer (Hanks's balanced salt solution with 10 mM HEPES) and different concentrations of excipients were added to obtain the sample solutions. Prior to the experiment, the cells were rinsed with pre-warmed assay buffer and pre-incubated for 1 h with 0.5 mL assay buffer at the basolateral side. To start the experiment, the inserts were placed in 1.5 mL of fresh pre-warmed assay buffer and 0.5 mL of the sample solution was added to the apical side. At defined time points up to 3 h, 0.5 mL samples were withdrawn from the basolateral side and replaced with assay buffer. The drug content was quantified according to 5.2.3. At the last sample point, 100 µL were additionally sampled from the apical side for calculation of mass balance according to equation 5-4 and the TEER was measured to assess changes in cell layer integrity.

Equation 5-4: Calculation of mass balance with c_d and c_r as final concentrations in the donor and receptor compartment, respectively, V_d and V_r as corresponding volumes, M_s as drug amount, which was withdrawn at the sample points and c_0 and V_{0d} as initial donor concentration and volume.

$$\text{Mass balance} = \frac{c_d \times V_d + c_r \times V_r + M_s}{c_0 \times V_{d0}}$$

The permeation of the drugs was assessed at least in triplicate and is displayed as permeation coefficient, calculated according to equation 5-5.

Equation 5-5: Calculation of the permeation coefficient (P_{app}) with dQ/dt as flux of the API across the cell barrier, c_0 as initial donor concentration and A as area of the cell layer.

$$P_{app} = \frac{dQ}{dt \times c_0 \times A}$$

5.3 Statistical methods

Unless otherwise stated, the results of multiple determinations in this work were given as mean value and standard deviation. In graphs, the standard deviation is shown as error bars. Mean value and standard deviation were calculated according to equation 5-6 and equation 5-7, respectively.

Equation 5-6: Calculation of mean value.

$$\bar{x} = \frac{1}{n}\sum_{i=1}^{n} x_i$$

Equation 5-7: Calculation of standard deviation.

$$s = \sqrt{\frac{\sum_{i=1}^{n}(x_i - \bar{x})^2}{n-1}}$$

Statistical significance was tested using a two-tailed Student's t-test. The variances of the samples were tested for significant differences in advance using an F-test. If the variances were not significantly different, a homoscedastic t-test was used, and if the variances were significantly different, a heteroscedastic t-test was used. The significance value p was classified according to table 5-6.

The calculations and statistical tests in this work were conducted with Excel 2016 (Microsoft Corporation, Redmond WA, USA).

Table 5-6: Classification of significance value p.

Significance value p	Classification	Symbol
>0.05	Not significant	no
0.01<p<0.05	Significant	*
0.001<p<0.01	Very significant	**
≤0.001	Highly significant	***

6 Results and discussion

While the nose offers great potential as a site for drug delivery, it also poses specific challenges, such as the short residence time of the applied particles, which can cause nasal products to fail. The formulation of nasal powders with functional excipients may offer solutions to some of the challenges of nasal drug delivery. However, differentiated knowledge of the effects of excipients in powder formulations is essential, in order to develop successful products. To generate this knowledge, suitable screening methods must be used to characterise potential excipient and formulation candidates. The results of such investigations on pure excipients and model formulations are presented in this chapter.

6.1 Characterisation of excipients

This section characterises properties of different excipient powders that impact on nasal drug delivery. Thereby, one focus is on the characterisation of excipient properties that can prolong the nasal residence time of a formulation. The second focus is on the characterisation of potentially occurring irritative and toxic effects resulting from the use of the excipient powders in the nose.

6.1.1 Selection of excipients

Inactive ingredients can serve different purposes in nasal powder formulations. In order to depict different effects of excipients in the nose, substances with different functions and physicochemical properties were selected. Thereby, the focus was set on mucoadhesive substances and fillers.

Mucoadhesive polymers provide the opportunity to prolong the nasal residence time of a formulation. Different polymeric factors like the molecular weight and the presence of ionic groups can influence the mucoadhesive strength of a substance [32,83]. Therefore, polymers with differences regarding these factors were chosen to be investigated in this work. HPMC, HPC and HEC (two molecular weights, each) were assessed as neutral polymers, CMC and pectin were assessed as anionic polymers and the chitosan derivatives CM chitosan and chitosan glutamate were assessed as amphoteric and positively charged polymers, respectively. The chitosan derivatives were furthermore selected due to the reported absorption enhancing properties of chitosan, which will be investigated in the second part of this work [38].

Fillers are mainly used to facilitate powder handling by increasing the powder volume. However, depending on their physicochemical properties, fillers can also influence the outcome of nasal drug delivery. Tanaka et al. found a dissolution accelerating effect of water-soluble fillers, which was attributed to an increase in osmotic pressure, which withdraws water from underneath tissues [40]. Water-insoluble fillers on the other hand may

provide a prolonged residence time of a formulation in the nose, due to a higher resistance of the formulation against mucociliary clearance [23]. The influence of mannitol and lactose as water-soluble fillers and MCC and colloidal MCC as water-insoluble fillers was investigated in this work.

A selection criterion for all substances was non-toxicity. Therefore, mainly established excipients were selected that are already contained in products for nasal application and are listed in the inactive ingredient database of the Food and Drug Administration, are approved by the European Medicines Agency, or are available on the German market and can therefore be considered as safe when used in similar products. This selection of established substances is also intended to provide a basis for the classification of new excipients.

6.1.2 Particle size and morphology

In nasal formulations, particle size influences different steps between the application of the formulation and the final outcome. The nose effectively filters particles with an aerodynamic diameter above 10 µm, while smaller particles may bypass the nose and reach the lower airways [7]. Hence, nasal formulations require particle sizes above 10 µm to ensure the desired effect and avoid side effects due to lung deposition. Further, the particle size distribution of a powder formulation affects the dissolution of contained excipients and active ingredients and by that influences their function. While the maximal droplet size in liquid formulations is limited due to the occurrence of dripping [84], no defined limits exist for powder products. Assessments of the commercial nasal powder product Teijin Rhinocort® revealed a bimodal particle size distribution with peaks at 9.9 µm and 98 µm, which are assigned to the micronised drug and HPC as excipient, respectively [85].

The raw materials of the used excipients exhibit differences in the particle size distributions (Figure 6-1 A and figure 6-3 A), which may influence the processes assessed in this work. Three sieve fractions (32-150 µm, 32-90 µm and 90-150 µm) were prepared in order to investigate the influence of particle sizes on selected process, but also to minimise the effect of particle sizes among compared samples, thereby allowing correlation of the observed effects with other physicochemical properties of the excipients. Figure 6-1 B-D and figure 6-3 B-D show the distribution density of the particle size distributions of the sieve fractions. Table 6-1 and table 6-2 show characteristic values of the particle size distributions. The results show that the sieving steps have equalised the particle size distributions of the different excipients. However, the sieve fractions still show slight differences in the mean particle size and in the width of the particle size distribution. Scanning electron microscope images (Figure 6-2 and figure 6-4) show irregular particle morphologies for most of the

assessed substances. Due to the irregular particle shapes, oversized particles were found in the sieve fractions in different extents.

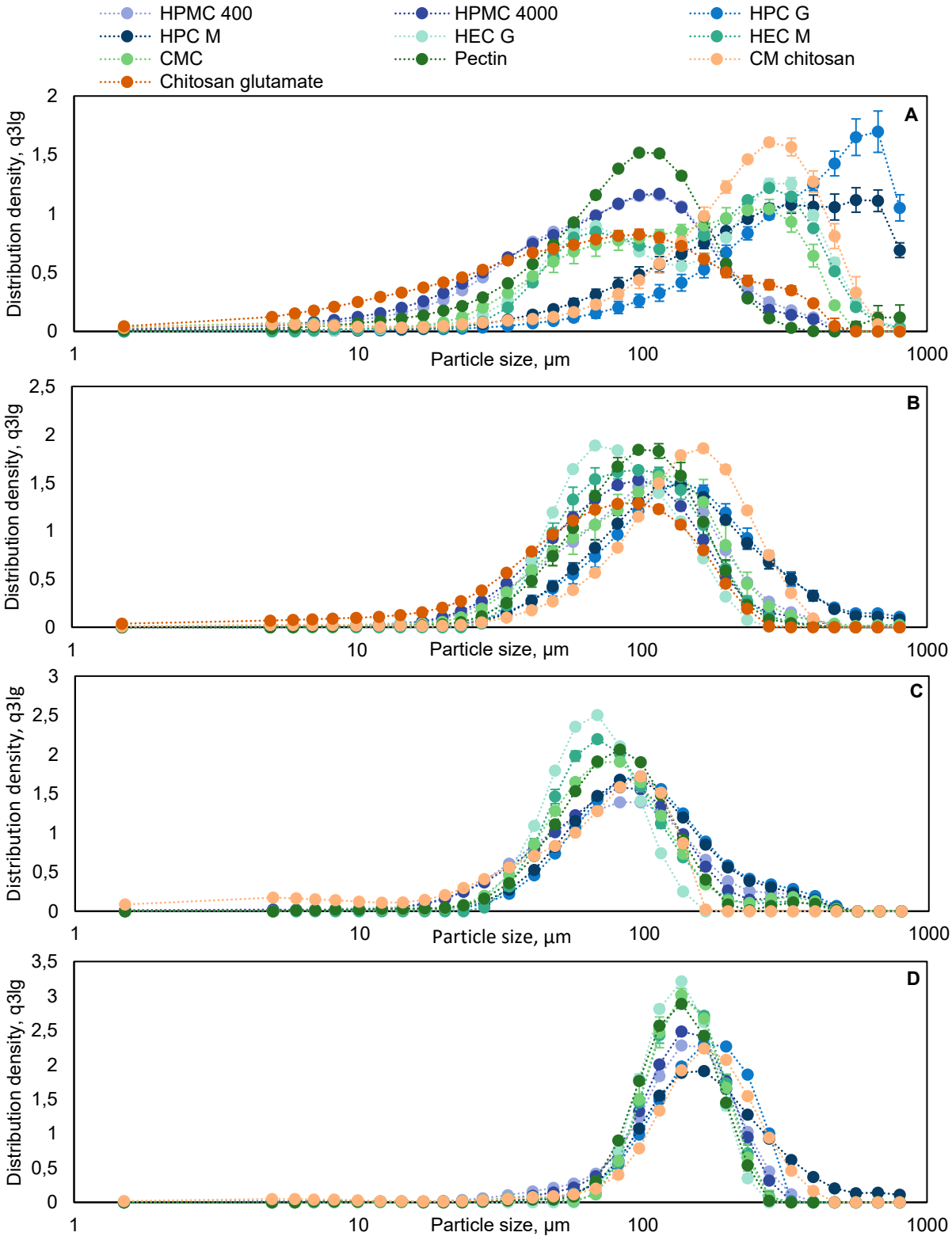

Figure 6-1: Particle size distributions of mucoadhesive excipients. A: raw material; B: sieve fraction 32-150 µm; C: sieve fraction 32-90 µm; D: sieve fraction 90-150 µm. n=3; error bars show standard deviation.

Table 6-1: Characteristic values of particle size distributions of the sieve fractions of mucoadhesive excipients. n=3; mean ± standard deviation.

Sample		x_{10}, µm	x_{50}, µm	x_{90}, µm	Span
HPMC 400	32-150 µm	39.4 ± 1.2	99.7 ± 1.6	200.7 ± 1.8	1.6 ± 0.0
	32-90 µm	31.6 ± 0.6	78.7 ± 1.1	178.3 ± 2.7	1.9 ± 0.0
	90-150 µm	72.4 ± 1.4	140.7 ± 0.9	225.8 ± 4.1	1.1 ± 0.0
HPMC 4000	32-150 µm	37.3 ± 0.1	86.5 ± 0.7	174.7 ± 0.7	1.6 ± 0.0
	32-90 µm	32.3 ± 0.4	78.1 ± 1.0	161.5 ± 7.3	1.7 ± 0.1
	90-150 µm	79.8 ± 0.7	140.2 ± 1.0	217.6 ± 5.2	1.0 ± 0.0
HPC G	32-150 µm	58.0 ± 4.4	136.8 ± 9.2	320.0 ± 23.2	1.9 ± 0.0
	32-90 µm	48.3 ± 0.4	96.0 ± 0.5	214.1 ± 1.6	1.7 ± 0.0
	90-150 µm	91.9 ± 0.0	162.1 ± 0.6	248.9 ± 0.7	1.0 ± 0.0
HPC M	32-150 µm	57.7 ± 1.6	131.4 ± 6.9	309.3 ± 21.3	1.9 ± 0.1
	32-90 µm	45.9 ± 0.2	92.1 ± 0.4	202.1 ± 3.0	1.7 ± 0.0
	90-150 µm	90.8 ± 0.5	163.1 ± 0.5	330.7 ± 7.7	1.5 ± 0.1
HEC G	32-150 µm	43.9 ± 0.6	78.9 ± 0.6	147.2 ± 0.9	1.3 ± 0.0
	32-90 µm	39.2 ± 0.2	64.7 ± 0.1	101.1 ± 0.1	1.0 ± 0.0
	90-150 µm	93.7 ± 0.8	134.5 ± 1.4	191.1 ± 2.6	0.7 ± 0.0
HEC M	32-150 µm	47.5 ± 0.4	88.7 ± 0.5	171.0 ± 0.5	1.4 ± 0.0
	32-90 µm	44.8 ± 1.0	73.4 ± 1.6	136.8 ± 3.9	1.3 ± 0.0
	90-150 µm	94.2 ± 1.5	140.3 ± 2.8	204.0 ± 4.8	0.8 ± 0.0
CMC	32-150 µm	41.6 ± 0.9	95.2 ± 1.9	188.5 ± 4.9	1.5 ± 0.0
	32-90 µm	40.1 ± 0.1	74.6 ± 0.5	142.9 ± 2.2	1.4 ± 0.0
	90-150 µm	92.9 ± 1.5	138.9 ± 4.4	201.8 ± 7.4	0.8 ± 0.0
Pectin	32-150 µm	47.6 ± 1.9	95.8 ± 5.4	170.5 ± 6.7	1.3 ± 0.0
	32-90 µm	42.1 ± 0.6	78.4 ± 0.6	136.7 ± 0.3	1.2 ± 0.0
	90-150 µm	87.0 ± 0.6	133.0 ± 2.1	196.6 ± 2.1	0.8 ± 0.0
CM chitosan	32-150 µm	62.1 ± 1.9	142.4 ± 2.4	251.9 ± 3.4	1.3 ± 0.0
	32-90 µm	5.9 ± 0.3	65.8 ± 1.9	119.8 ± 1.4	1.7 ± 0.0
	90-150 µm	80.2 ± 0.6	162.4 ± 1.1	267.0 ± 2.8	1.2 ± 0.0
Chitosan glutamate	32-150 µm	19.2 ± 1.0	72.3 ± 1.7	156.4 ± 2.8	1.9 ± 0.0

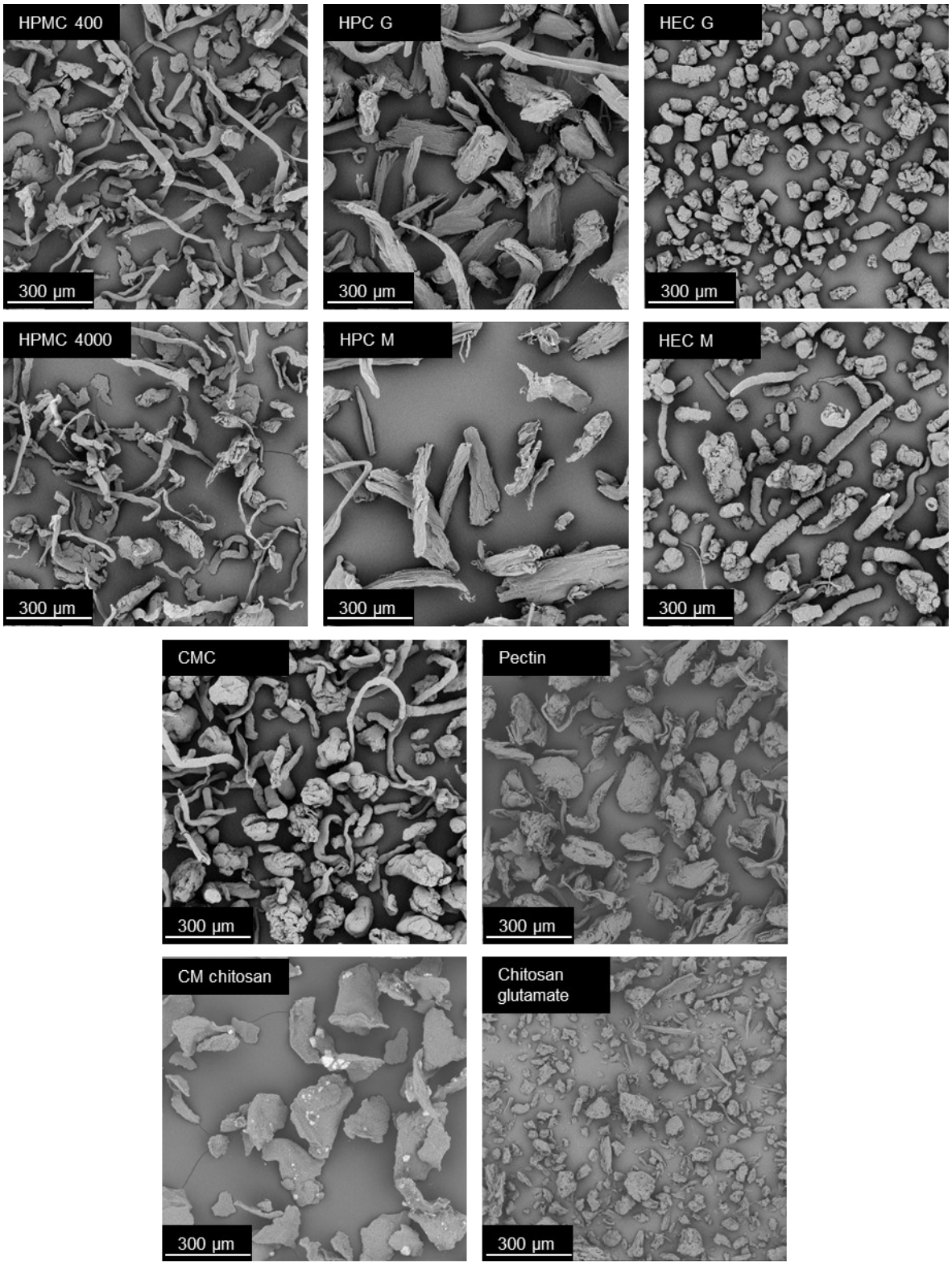

Figure 6-2: Scanning electron microscope images (250x magnification) of mucoadhesive excipients (sieve fraction 32-150 µm).

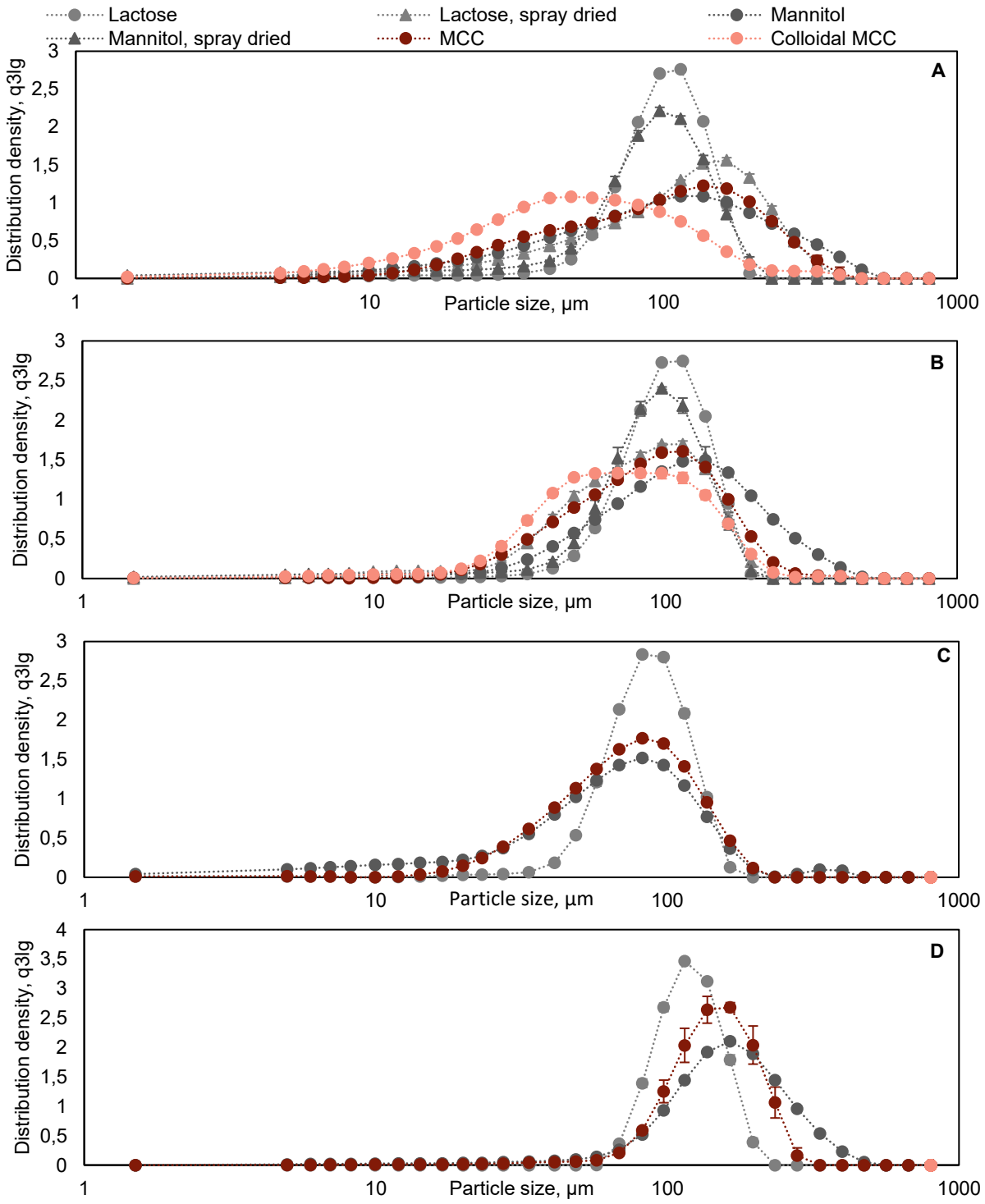

Figure 6-3: Particle size distributions of fillers. A: raw material; B: sieve fraction 32-150 µm; C: sieve fraction 32-90 µm; D: sieve fraction 90-150 µm. n=3; error bars show standard deviation.

Table 6-2: Characteristic values of particle size distributions of the sieve fractions of fillers. n=3; mean ± standard deviation.

Sample		x_{10}, µm	x_{50}, µm	x_{90}, µm	Span
Lactose	32-150 µm	62.6 ± 0.9	100.8 ± 0.5	146.8 ± 0.3	0.8 ± 0.0
	32-90 µm	55.3 ± 0.6	85.9 ± 0.7	124.3 ± 0.6	0.8 ± 0.0
	90-150 µm	84.7 ± 1.0	118.8 ± 1.1	165.1 ± 1.9	0.7 ± 0.0
Lactose, spray dried	32-150 µm	35.2 ± 0.8	81.4 ± 1.7	145.0 ± 2.0	1.4 ± 0.0
Mannitol	32-150 µm	44.8 ± 0.8	113.5 ±2.0	236.8 ± 3.1	1.7 ± 0.0
	32-90 µm	13.4 ± 0.3	66.0 ± 1.1	131.0 ± 2.0	1.8 ± 0.0
	90-150 µm	83.1 ± 0.3	160.3 ± 0.4	280.3 ± 2.0	1.2 ± 0.0
Mannitol, spray dried	32-150 µm	44.0 ± 2.4	91.2 ± 2.9	142.6 ± 3.4	1.1 ± 0.0
MCC	32-150 µm	35.7 ± 2.0	88.0 ± 0.5	167.1 ± 0.2	1.5 ± 0.0
	32-90 µm	32.9 ± 0.6	73.3 ± 0.6	132.1 ± 0.4	1.4 ± 0.0
	90-150 µm	91.2 ± 2.5	144.4 ± 2.9	211.6 ± 1.7	0.8 ± 0.0
Colloidal MCC	32-150 µm	31.3 ± 1.6	70.7 ± 2.6	146.9 ± 1.1	1.6 ± 0.1

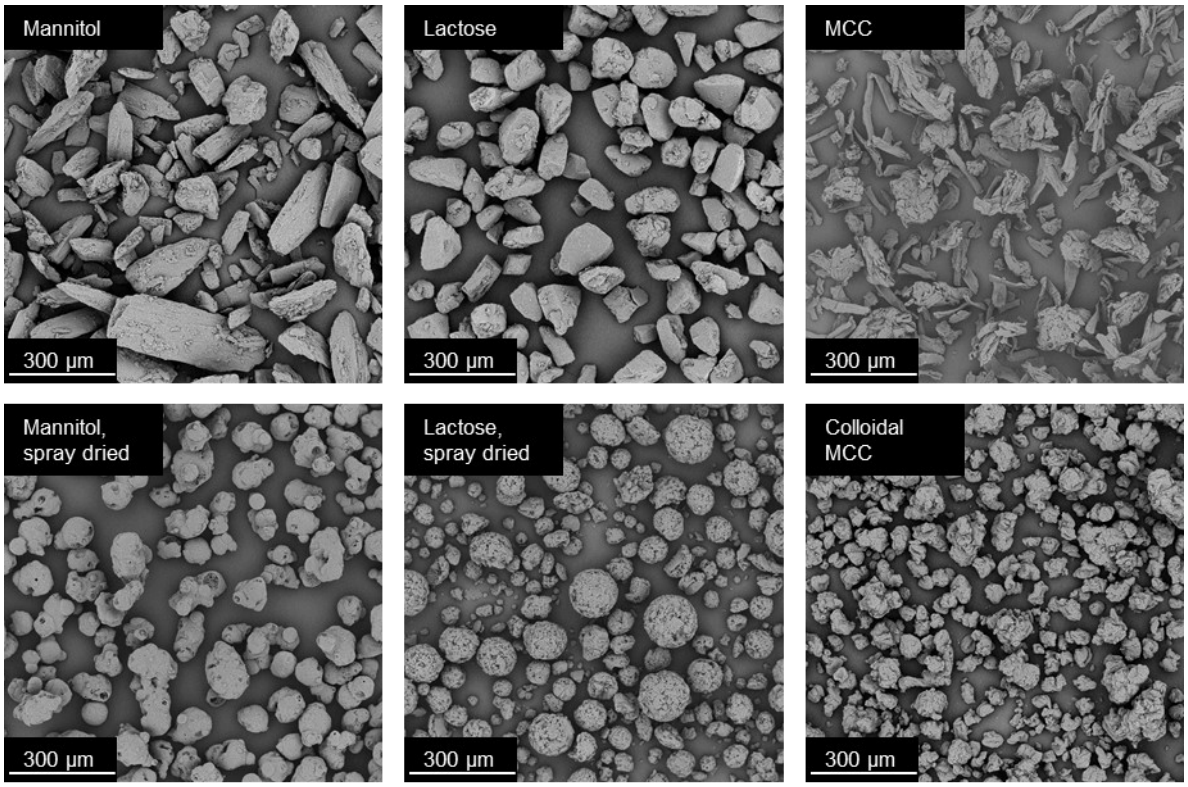

Figure 6-4: Scanning electron microscope images (250x magnification) of fillers (sieve fraction 32-150 µm).

6.1.3 Influence of excipients on the nasal residence time

One of the most striking challenges in nasal drug delivery is the short residence time of drugs in the nasal cavity of 15-20 min due to the mucociliary clearance [4]. The specific use of excipients is a strategy to enable successful drug delivery by prolonging the nasal residence time of a formulation. However, as nasal powders are still the minority on the market, there is a lack of data characterising this effect for excipient powders. This chapter therefore characterises selected excipient powders and evaluates, which properties lead to a high potential to extend the nasal residence time. One focus is on the use and discussion of methods, suitable for powders that are tailored for the comparison of different substances in early product development.

6.1.3.1 Viscoelastic properties

A formulation that is applied into the nose will deposit on the mucus layer, which covers the nasal epithelium. The interplay of viscous and elastic behaviour of the nasal mucus influences the rate at which the mucociliary cleaning mechanism subsequently removes the particles from the nasal cavity and thus the residence time of an applied drug. The viscosity enables the mucus layer to carry a load sufficiently, but an increase in viscosity can disrupt ciliary movement in the gel layer. Elasticity restores the mucus layer to its original shape after deformation by ciliary movement. A lack of elasticity hinders the transport of the mucus layer as continuous sheet, while an increase in elasticity inhibits the flow [10]. Elasticity is considered the most important aspect for an efficient transport. In ex vivo models on frog palates, an optimal range for the elastic modulus was estimated to 1-2 Pa [4,10,86]. The use of excipients that change the viscoelasticity of nasal secretions is therefore an approach to extend the nasal residence time by reducing the efficiency of the mucociliary transport.

The mucoadhesive excipients used in this work are expected to form viscoelastic gels upon fluid contact and thus, to alter the viscoelastic properties of nasal mucus. The addition of the selected fillers, with the exception of colloidal MCC, to nasal mucus is not expected to affect the viscoelastic properties of a fluid. In vivo, however, the soluble fillers mannitol and lactose may decrease the mucus viscosity due to the influx of fluid into the nasal cavity because of their osmotic activity [40]. Colloidal MCC forms colloidal gels in liquids when sufficiently dispersed by shearing [87]. A change in the viscoelasticity of the nasal mucus would therefore be conceivable with its use.

The aim of this section is to assess the gel-forming properties of the excipients in the nasal fluid using a reproducible screening method. This should enable a pre-selection of substances with suitable properties to extend the nasal residence time.

6.1.3.1.1 Selection of the dispersion medium

In order to assess the viscoelastic behaviour of the excipients in the nose in an in vitro setup, a dispersion medium is needed that mimics the properties of natural nasal secretions. Factors such as temperature, pH, presence of ions and ionic strength influence the gel forming behaviour of polymers. A salt solution (simulated nasal fluid, SNF) that corresponds to the nasal fluid in composition and concentration of ions, as well as in the pH was therefore selected. In nasal mucus, mucins are mainly responsible for the gel properties. To model the viscoelastic behaviour in vitro, 2% (w/w) commercially available mucin from pig stomach was added to the simulated nasal fluid to obtain simulated nasal mucus (SNM). Figure 6-5 displays the rheological properties of SNF and SNM.

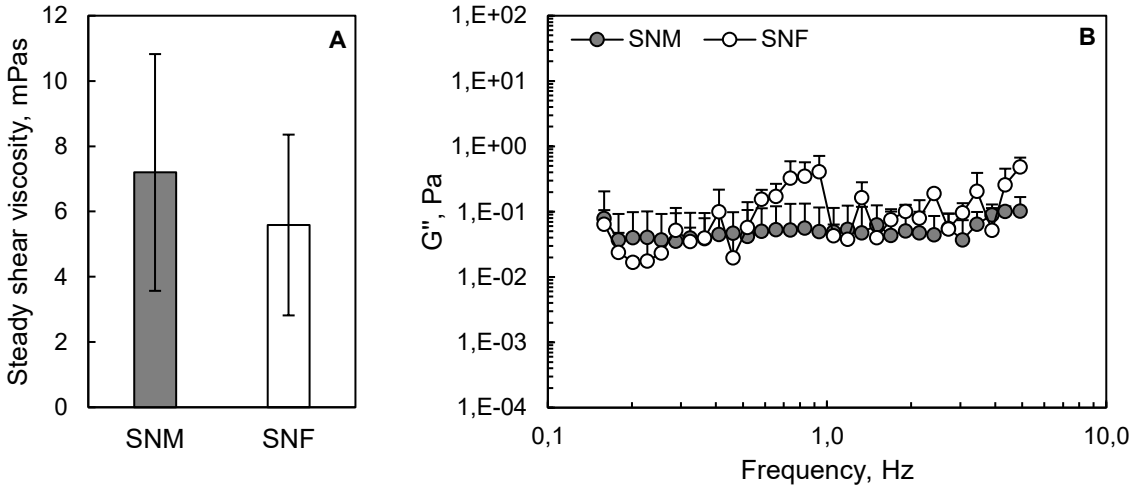

Figure 6-5: Rheological properties of simulated nasal mucus (SNM) and simulated nasal fluid (SNF). Figure A shows the steady shear viscosity at a shear rate of 1 s^{-1}. Figure B shows the frequency dependent viscous moduli (G''). n=3; error bars show standard deviation.

The data reveal that the addition of porcine gastric mucin to SNF did not significantly increase the measured steady shear viscosity at 1 s^{-1} (7.2 ± 3.6 mPas for SNM and 5.6 ± 2.8 mPas for SNF; p=0.57) or the frequency dependent viscous moduli. The elastic moduli are not displayed because of the occurrence of overlaying effects of instrument inertia. A studie on the nasal secretions on healthy subjects revealed substantially higher values [88]. Rubin et al. found a viscous modulus of nasal mucus of 46 ± 60 Pa and an elastic modulus of 140 ± 176 Pa at an oscillatory frequency of 0.16 Hz in ten healthy individuals [88]. Hence, the addition of commercially available mucin is not suitable to mimic the viscoelasticity of nasal mucus. One reason for the lack of gel formation is the source of the mucin used. Mucins that are commercially available in larger quantities are obtained from pig stomachs. Mucins from the stomach show a pH-dependent gel-forming behaviour [89]. The sol-gel transition occurs around pH 4. Hence, at the nasal pH of 5.5-6.5, a viscoelastic liquid is present instead of a gel. In addition, the purification process of commercial mucins leads to extensive loss of the natural gel-forming properties [11,90]. The addition of further gelling

polymers would therefore be necessary to adjust the rheological properties of simulated mucus [91]. However, these polymers may interact with the substances to be tested and thus alter the rheological properties. Due to these limitations of mucus surrogates, SNF was selected as dispersion medium in this study. In contrast to nasal mucus, SNF does not show viscoelastic behaviour but is a low viscous Newtonian fluid. Hence, the obtained data cannot reflect the in vivo rheological properties. However, its clearly defined composition enables a reproducible and comparative screening of substances in product development. The use of native mucus samples may be considered for additional characterisation in later stages of product development, but limited access to mucus samples and probably occurring batch variations make them unsuitable for early screening tests.

6.1.3.1.2 Influence of excipients on the steady shear viscosity of simulated nasal fluid

The steady shear viscosity is a commonly stated quality attribute for hydrogel-forming polymers. The Ph. Eur. requires its declaration for hydrogel-forming cellulose derivatives and corresponding methods are given in the monographs of the respective substances as purity tests. In this part of the work, these commonly used setups were adapted to nasal conditions in order to obtain a first indication of the rheological behaviour of the selected excipients in the nose. Since the viscosity of polymer dispersions depends on the applied shear rate, the effective shear rate in the nasal mucus layer influences the viscosity of a formulation in the nose. Ciliary movement with a beating frequency of about 10 Hz causes an effective shear rate of 1-3 s^{-1} in the mucus layer [10]. A shear rate of 1 s^{-1} was therefore selected for the measurements. Powder formulations that are applied to the nose need to hydrate in the nasal fluid. Hence, the viscosity of the surrounding nasal fluid will change over time and with progressing hydration of the particles. To assess the rheological behaviour of the excipients after the first contact with the nasal fluid and at the end of the residence time in the nose at a physiological clearance rate, measurements were conducted 1 min and 15 min after dispersion of the samples in SNF, respectively, at a temperature of 32 °C, which mimics the nasal temperature [12].

Figure 6-6 displays the steady shear viscosity of 2% dispersions of the mucoadhesive excipients in SNF after 1 min and 15 min.

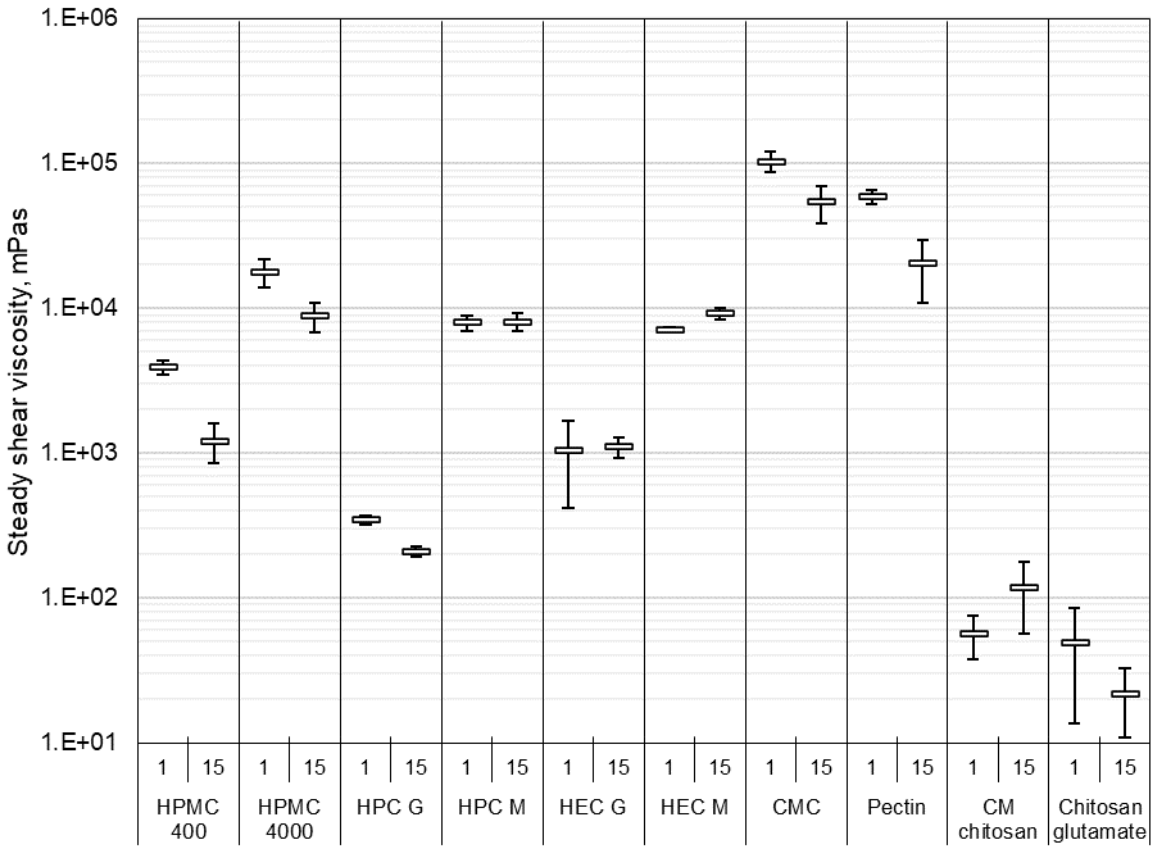

Figure 6-6: Steady shear viscosity of 2% dispersions of mucoadhesive excipients in SNF after 1 min (1) and 15 min (15) resting time at a shear rate of 1 s^{-1}. n=3; error bars show standard deviation.

All mucoadhesive excipients showed an increased steady shear viscosity compared to SNF (5.6 ± 2.8 mPas) after 1 min resting time as well as after 15 min resting time. The change in viscosity over the physiological half-life of clearance was not uniform among the samples, but dependent on the substance tested. While the viscosity significantly decreased in dispersions of HPMC (p=0.001 and p=0.02 for HPMC 400 and 4000, respectively), HPC G (p=0.002), CMC (p=0.02) and pectin (p=0.005), the dispersion of HEC M (p=0.02) showed a significant increase in viscosity over time. No significant changes (p>0.05) occurred for dispersions of HEC G, HPC M and the chitosan derivatives. No change or an increase in viscosity may be associated with samples that are easily dispersible in SNF and hence form homogenous gels immediately after fluid contact. In contrast, samples that are difficult to disperse in SNF may form high viscous areas around the particles that influence the measurement after 1 min and slowly decrease in viscosity as hydration progresses and the gels become more homogeneous. Especially with the anionic polymers CMC and pectin, this behaviour was visually observable. With regard to a prolongation of the nasal residence time of a formulation, a fast onset of gelation is advantageous in order to decrease the mucociliary transport rate and to fix the formulation to the deposition site. However, adequate hydration of the polymers is also required in order to make polymer chains

available for interactions with mucin. Considering these aspects, polymers that show good dispersibility and small changes in viscosity over time can be considered advantageous.

A direct comparison of the viscosities of the different mucoadhesive excipients reveals the lowest viscosity in SNF for the chitosan derivatives (56 ± 19 mPas to 117 ± 61 mPas for CM chitosan and 49 ± 36 mPas to 22 ± 11 mPas for chitosan glutamate after 1 min and 15 min, respectively). The highest viscosities in SNF were measured for the anionic polymers CMC (103,678 ± 16,314 mPas to 54,147 ± 15,627 Pas) and pectin (59,080 ± 6,992 mPas to 20,476 ± 9,487 mPas). However, these samples were not homogeneously gelled, but did form highly viscous areas around the particles, which led to higher measured values. For pectin, gel formation is dependent on the presence of divalent cations, especially calcium. Hence, dispersions of pectin in water are of low viscosity, while highly viscous gels can form in the calcium-containing nasal fluid. This specific gelling behaviour can be considered as beneficial regarding handling and storage of the powder product. Among the shorter-chain neutral cellulose derivatives (HPMC 400, HPC G, HEC G), HPMC 400 (3,914 ± 424 mPas to 1,220 ± 373 mPas) showed a significantly higher viscosity in SNF than HEC G (1,047 ± 629 mPas to 1,106 ± 185 mPas) after 1 min ($p=0.003$), which equalised after 15 min ($p>0.05$). Dispersions of HPC G (347 ± 25 mPas to 210 ± 18 mPas) showed the lowest viscosity among these samples. Within the longer-chain neutral cellulose derivatives (HPMC 4000, HPC M, HEC M), dispersions of HPMC (17,879 ± 3,905 mPas to 8,917 ± 2,086 mPas) in SNF again showed a significantly higher viscosity after 1 min ($p=0.01$ and $p=0.04$ compared with HPC M and HEC M, respectively) converging to a similar viscosity ($p>0.05$) to that of dispersions of HPC M (7,990 ± 985 mPas to 8,126 ± 1,210 mPas) and HEC M (7,181 ± 286 mPas to 9,192 ± 882 mPas) after 15 min.

While the rheological behaviour of mucoadhesive excipients is part of their functionality, the main function of fillers is to facilitate the handling of the powder product. Fillers are therefore not expected to gel upon contact with moisture or fluids, as this could complicate the handling and storage of the product. Among the fillers selected in this work, colloidal MCC is an exception in this respect, as it can form colloidal gels after sufficient dispersion by shearing. Figure 6-7 displays the steady shear viscosity of 2% dispersions of the fillers in SNF after 1 min and 15 min.

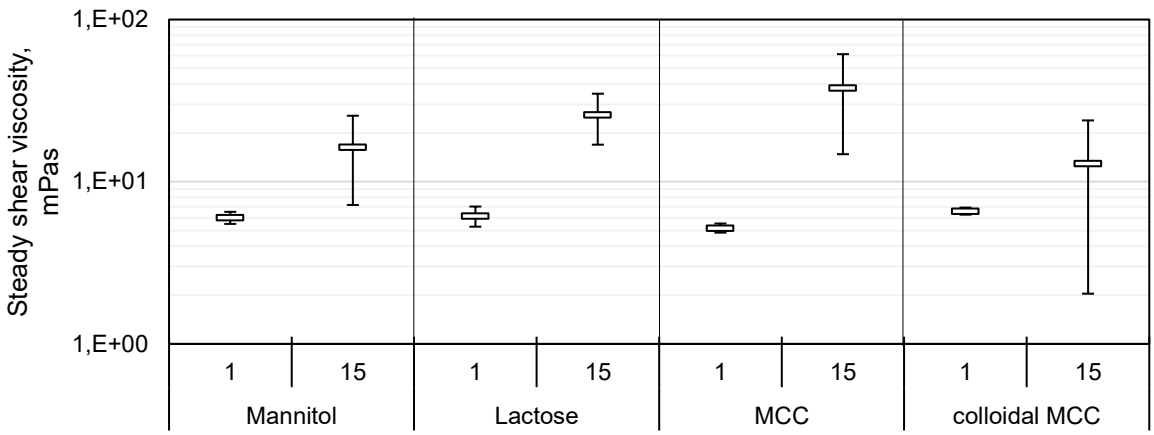

Figure 6-7: Steady shear viscosity of 2% dispersions of fillers in SNF after 1 min (1) and 15 min (15) resting time at a shear rate of 1 s^{-1}. n=3; error bars show standard deviation.

No relevant increase in viscosity was observed in dispersions of mannitol, lactose, MCC and, however, also colloidal MCC. After dispersion of colloidal MCC in fluids, the formation of a three-dimensional network of insoluble fibres of MCC and stabilising CMC requires sufficient shear forces. Dissolved salts, like present in the nasal fluid hamper the dispersion and therefore prevent gelling [53,87]. Since an adequate dispersion is not feasible after the application of the powder formulation to the nose, colloidal MCC shows no benefits as excipient in nasal powders. The other fillers tested can be considered as suitable for the facilitation of powder handling with regard to rheological properties.

Measuring the steady shear viscosity is a suitable method for gaining first impressions of the rheological behaviour of excipients in the nasal fluid. However, the elastic behaviour has a relevant influence on the residence time in the nose, which this method cannot assess. Oscillatory rheological measurements, instead, enable the characterisation of the elastic and viscous fraction of viscoelastic materials. The following section therefore discusses the additional information that these measurements can provide.

6.1.3.1.3 Viscoelastic behaviour of excipients in simulated nasal fluid

As described in section 6.1.3.1, the interaction of elasticity and viscosity of the nasal fluid is essential for mucociliary transport, with the elasticity being considered the most important factor with an optimal range estimated to 1-2 Pa [4]. Therefore, when assessing the influence of excipients on the rheological behaviour of the nasal fluid, it is necessary to consider not only the viscous but also the elastic properties. One approach to characterise the viscoelastic behaviour of samples is to subject the sample to a small, oscillating deformation that induces a periodic shear stress. The phase shift of the sinusoids of the deformation and the resulting shear stress defines the viscoelasticity of the sample. The storage modulus (G') represents the elastic behaviour of the sample. It is a measure of the stored deformation energy that is fully available after relief. The loss modulus (G'')

represents the viscous behaviour of the sample. It is a measure of the deformation energy that is consumed by friction processes during shearing and is no longer available. The dissipation factor (tan δ=G''/G') indicates the ratio of viscous to elastic behaviour. A dissipation factor of one corresponds to the intersection of storage and loss modulus. Samples with dominating G'' behave as viscoelastic liquids, while samples with dominating G' behave as viscoelastic gels. As an increased storage modulus of the nasal fluid (>2 Pa) may correspond to a decrease in mucociliary clearance rate, samples that show pronounced elastic behaviour in SNF (tan δ<1) can be considered advantageous. In oscillatory frequency sweeps, as conducted in this work, the frequency of the periodic deformation varies. With frequency being the reciprocal of time, this allows the assessment of time-dependent deformation behaviour. Thereby, high frequencies simulate short-term behaviour with fast motions, while low frequencies simulate long-term behaviour with slow motions. For an extension of nasal residence time due to changes in the viscoelasticity of the nasal fluid, high loss and storage moduli also at lower frequencies can therefore be considered advantageous.

Figure 6-8 and figure 6-9 display the frequency dependent loss and storage moduli of the neutral cellulose derivatives after 1 min and 15 min resting time after dispersion in SNF. Figure 6-10 displays the corresponding dissipation factors.

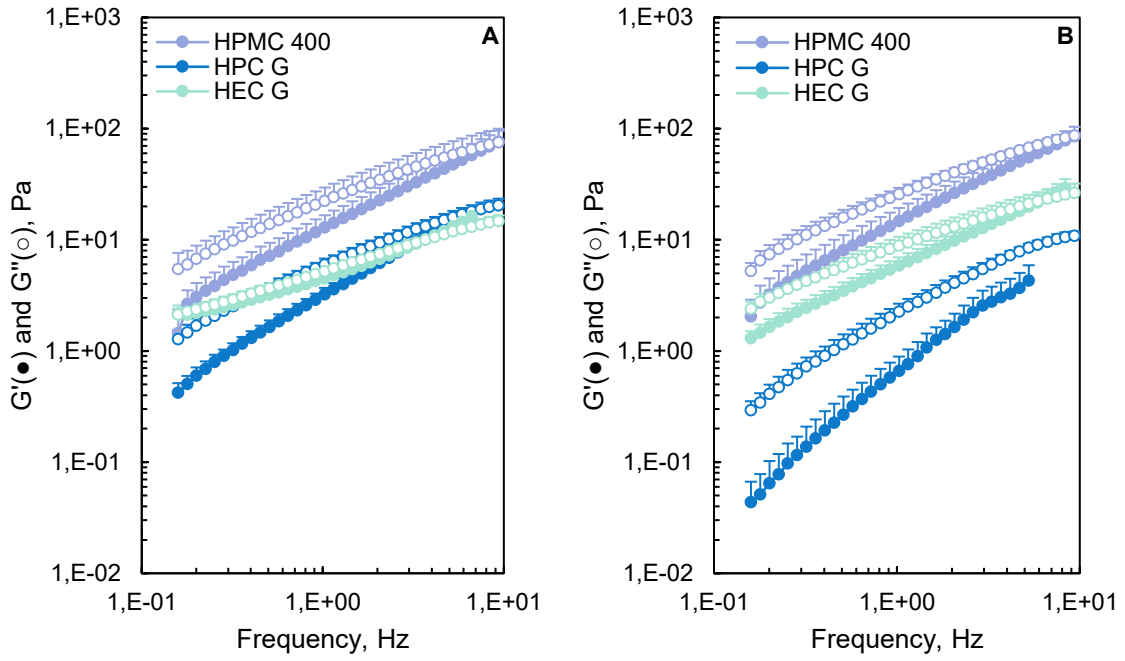

Figure 6-8: Frequency dependent storage (G', filled dots) and loss (G'', unfilled dots) moduli of 2% dispersions of the neutral cellulose derivatives HPMC 400, HPC G and HEC G in SNF after 1 min (A) and after 15 min (B) resting time. n=3; error bars show standard deviation; for the clarity of the graphs error bars are only displayed in positive direction. Shortened graphs are displayed for samples in which overlaying effects of instrument inertia occurred at higher frequencies.

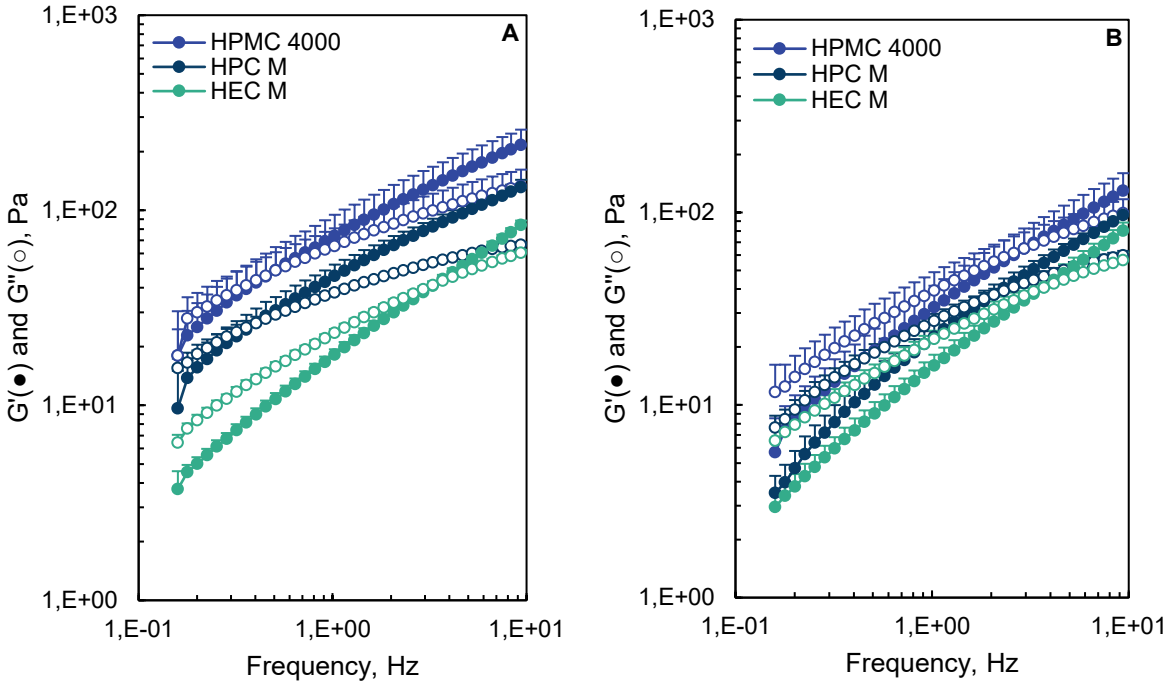

Figure 6-9: Frequency dependent storage (G', filled dots) and loss (G'', unfilled dots) moduli of 2% dispersions of the neutral cellulose derivatives HPMC 4000, HPC M and HEC M in SNF after 1 min (A) and after 15 min (B) resting time. n=3; error bars show standard deviation; for the clarity of the graphs error bars are only displayed in positive direction.

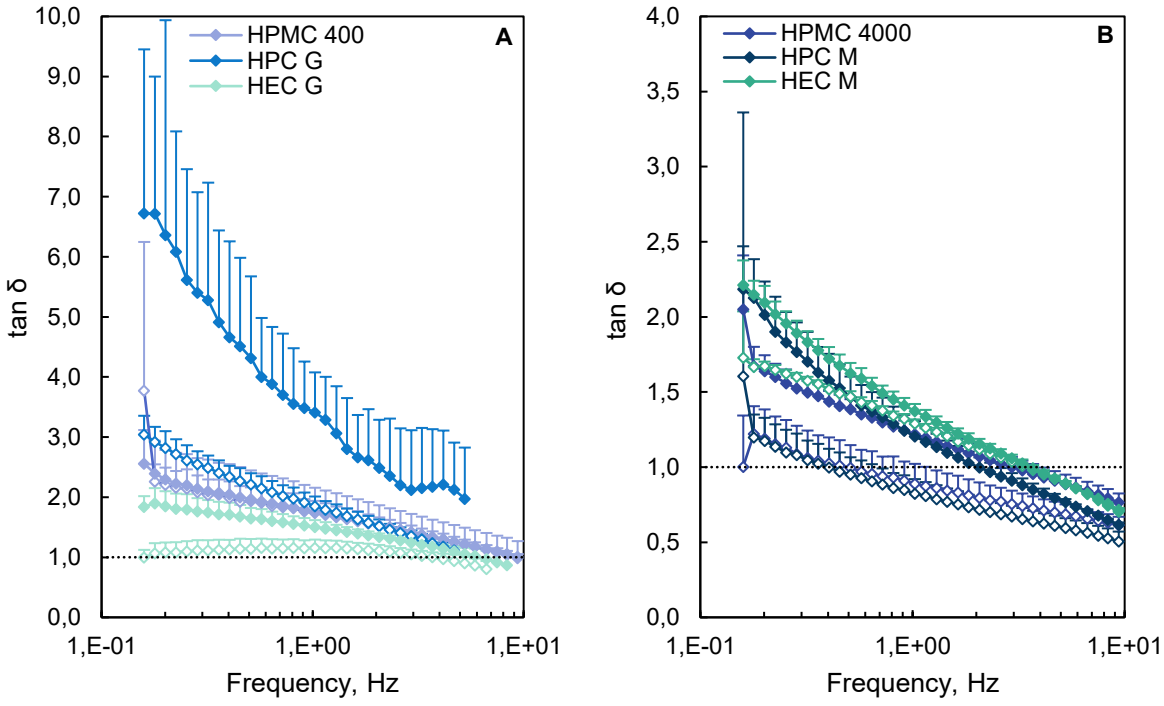

Figure 6-10: Frequency dependent dissipation factors (tan δ) of 2% dispersions of the neutral cellulose derivatives (A) HPMC 400, HPC G, HEC G and (B) HPMC 4000, HPC M, HEC M after 1 min (unfilled rhombuses) and after 15 min (filled rhombuses) resting time. A dissipation factor of 1 marks the point of intersection (G'=G''). n=3; error bars show standard deviation; for the clarity of the graph error bars are only displayed in positive direction.

Dispersions of the shorter-chain polymers (HPMC 400, HPC G, HEC G) showed dominant viscous behaviour over most of the mapped frequency range and hence behaved as viscoelastic fluid (G''>G'). Behaviour as viscoelastic gel (G'>G'') only occurred at high frequencies, as this is where the temporary entanglement network of the polymers becomes inflexible [92]. HEC G showed the earliest point of intersection (G'=G'') at a frequency of 4 Hz. As described above, mucoadhesives that increase the viscoelastic parameters of the nasal fluid with pronounced elastic behaviour may have a high potential to extend the nasal residence time of a formulation. Among the shorter-chain neutral cellulose derivatives, dispersions of HPMC 400 exhibited the highest net values for storage and loss moduli, while dispersions of HEC G provided the highest elastic fraction, displayed in the lowest dissipation factors. The lowest storage moduli were obtained with dispersions of HPC G, which also showed a further decrease in storage and loss moduli after 15 min. The decrease was relatively higher for the storage moduli than for the loss moduli, which corresponds to higher dissipation factors. A slight shift to a more pronounced viscous behaviour (higher dissipation factors) was also observed for HEC G after 15 min. A decrease of loss and storage moduli over time may occur with ongoing hydration of the polymers. Besides changing the viscoelasticity of the nasal fluid, a major mechanism of mucoadhesive substances is to provide an intimate contact of the formulation with the absorption site due to an adhesive attachment to the mucosa. For that, hydration of the polymers is essential in order to make the polymer chains available for interpenetration and interaction with mucin [4]. However, fast progressing hydration in an unrestricted fluid volume may lead to a decrease in viscoelasticity and decreased interaction of the polymer chains and thus to a decreased adhesive function [32]. Polymers that show sufficient initial hydration that is limited or slow as it progresses would be advantageous in this regard. Based on the oscillation rheological data, an advantage can be assumed for HPMC 400 and HEC G compared to HPC G in that respect. However, in this setup, the fluid volume was limited, while progressing hydration of the polymers may occur in the nasal cavity when water is withdrawn from underlying tissues.

Dispersions of the longer-chain polymers (HPMC 4000, HPC M, HEC M) exhibited higher values for storage and loss moduli compared to the shorter-chain polymers due to their higher molecular weight. For HPMC 4000 and HPC M, the elastic fraction increased relatively more, which is reflected in lower dissipation factors. Like the shorter-chain polymers, the longer-chain polymers behaved as viscoelastic liquids at low frequencies and as viscoelastic gels at higher frequencies. The intersection of loss and storage modulus already occurred at lower frequencies, especially in terms of HPMC 4000 and HPC M after 1 min resting time (point of intersection: 0.4-0.5 Hz). This suggests that the entanglement network of the longer chains behaves more rigid during movement. Interpenetration and

entanglement of the polymer chains of excipients and the mucin chains in the mucus layer are crucial steps in the consolidation state of mucoadhesion [32]. A higher tendency of the polymer chains to entangle, may therefore be a beneficial excipient characteristic. The storage and loss moduli of HPMC 4000 and HPC M dispersions decreased after 15 minutes, with a relatively stronger decrease in storage moduli. Thereby, the point of intersection shifted to higher frequencies in these samples. A progressive hydration of the polymers over time can therefore be assumed. This effect was less pronounced for HEC M.

Unlike the neutral polymers, the anionic polymers CMC and pectin showed dominant elastic behaviour over the whole frequency range and thus a behaviour as viscoelastic gels (Figure 6-11). Both formed high viscous areas around the particles immediately after contact with SNF, which still existed after 15 min resting time. However, the storage and loss moduli decreased and the loss factors increased over time (Figure 6-12), indicating progressive hydration. While the pronounced elastic behaviour and the high values for storage and loss modulus suggest a significant reduction in mucociliary clearance after application of CMC and pectin in the nose, the inhomogeneous gelation indicates limited hydration of the polymers, which could hinder interpenetration and thus adhesive bonding, as described above.

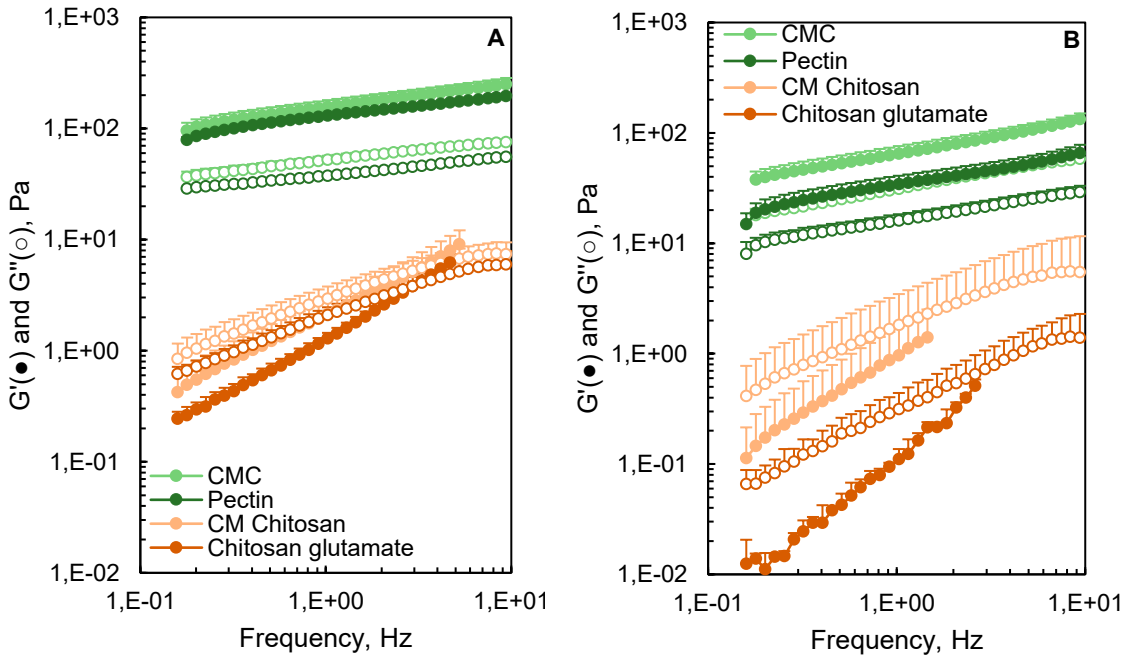

Figure 6-11: Frequency dependent storage (G', filled dots) and loss (G'', unfilled dots) moduli of 2% dispersions of CMC, pectin and the chitosan derivatives CM chitosan and chitosan glutamate in SNF after 1 min (A) and after 15 min (B) resting time. n=3; error bars show standard deviation; for the clarity of the graphs error bars are only displayed in positive direction. Shortened graphs are displayed for samples in which overlaying effects of instrument inertia occurred at higher frequencies.

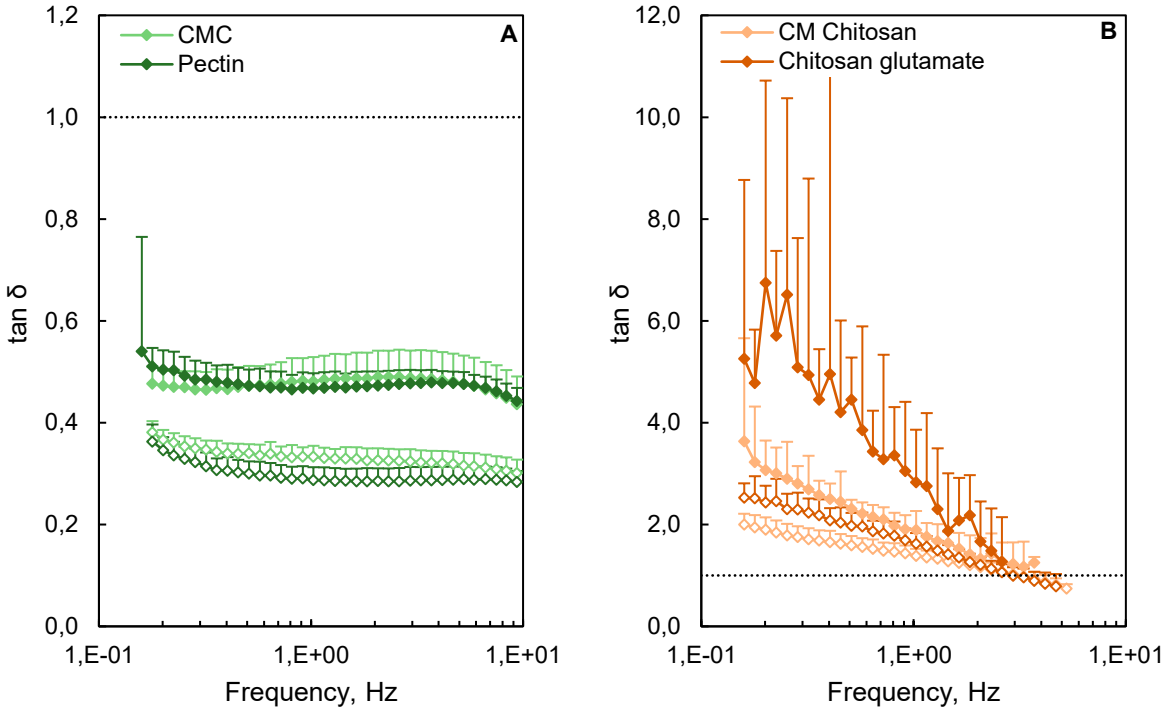

Figure 6-12: Frequency dependent dissipation factors (tan δ) of 2% dispersions of (A) CMC and pectin and (B) CM chitosan and chitosan glutamate after 1 min (unfilled rhombuses) and after 15 min (filled rhombuses) resting time. A dissipation factor of 1 marks the point of intersection (G'=G''). n=3; error bars show standard deviation; for the clarity of the graph error bars are only displayed in positive direction.

The assessment of the chitosan derivatives (Figure 6-11, CM chitosan, chitosan glutamate) revealed a similar frequency dependent change in viscoelastic behaviour as observed for the shorter-chain cellulose derivatives with sol character of the dispersions at low frequencies and gel character at higher frequencies. The intersection already occurred at lower frequencies (3 Hz) than observed with the shorter-chain cellulose derivatives, indicating less flexibility of the entanglement network. However, the dispersions of the chitosan derivatives provided relatively low storage and loss moduli, which may lead to a low resistance to mucociliary clearance.

The rheological characterisation of the fillers using the oscillation setup did not provide additional information compared to the measurements of steady shear viscosity. Overlaying effects of instrument inertia prevented the measurement of storage moduli and thus the assessment of viscoelastic behaviour. While ideal viscous flow behaviour (G'→0) can be assumed for solutions of lactose and mannitol in SNF, viscoelastic behaviour is expected for suspensions of the insoluble fillers MCC and colloidal MCC. However, the measurements did not allow the verification of these expectations. The viscous moduli of 2% dispersions of all fillers in SNF (Figure 6-13) were in the same range as for pure SNF.

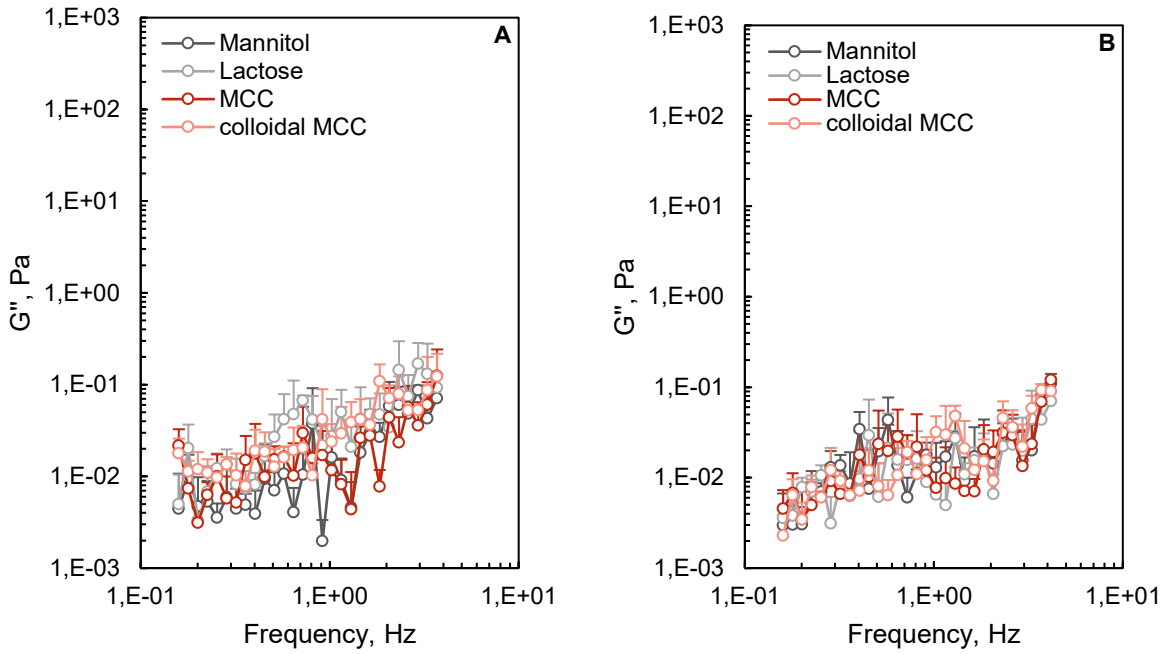

Figure 6-13: Frequency dependent loss moduli of 2% dispersions of the fillers mannitol, lactose, MCC and colloidal MCC in SNF after 1 min (A) and after 15 min (B) resting time. n=3; error bars show standard deviation; for the clarity of the graph error bars are only displayed in positive direction.

6.1.3.1.4 Influence of particle size

The rheological assessments revealed an influence of dispersibility and hydration of the excipient powders in SNF on the viscoelastic properties of the resulting dispersions. One factor that may influence these processes is the particle size. This section therefore assesses the effect of particle size on the viscoelastic behaviour of the excipients in SNF within the physiological clearance time. The assessment of the steady shear viscosity (Figure 6-14) of two sieve fractions (32-90 µm and 90-150 µm), did not allow a general statement. While HPMC, HEC and CM chitosan formed higher viscous dispersions with smaller particles, dispersions of HPC, CMC and pectin showed the reverse behaviour, i.e., larger particles resulted in higher steady shear viscosities. Two potential influences of the particle size, which can have opposite effects on the viscosity of the dispersion, are a possible explanation for this inconsistent behaviour. On the one hand, small particles that show good dispersibility in the fluid are expected to hydrate faster than larger particles. On the other hand, the larger surface area of smaller particles may lead to the immediate formation of a gel layer after contact with liquid, which makes subsequent dispersion of the particles more difficult. This could lead to the formation of particle aggregates, which in turn hydrate slower. Depending on the dispersibility and solubility of the powders, the behaviour may thus vary.

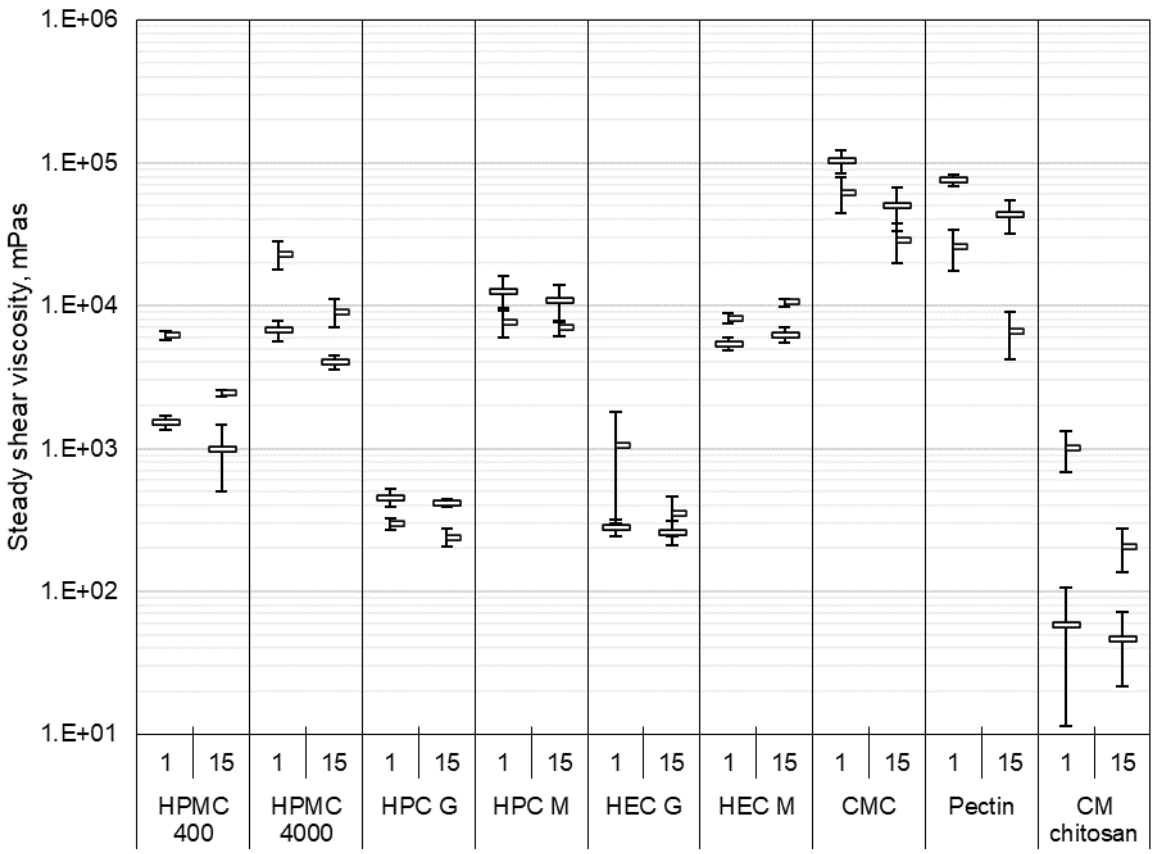

Figure 6-14: Steady shear viscosity of 2% dispersions of mucoadhesive excipients in SNF after 1 min (1) and 15 min (15) resting time at a shear rate of 1 s^{-1}. The viscosity of two sieve fractions is displayed: short rectangles display smaller particles (sieve fraction 32-90 µm), long rectangles display bigger particles (sieve fraction 90-150 µm). n=3; error bars show standard deviation.

The measurement of storage and loss moduli of the dispersions led to similar results as described for the steady shear viscosity. Figure 6-15 compares the storage and loss moduli of 2% dispersions of the mucoadhesive excipients at an oscillatory frequency of 1 Hz. Dispersions of HPMC, HEC and CM chitosan exhibited higher storage and loss moduli when the smaller particles where used. In contrast to that, dispersions of HPC initially showed higher storage and loss moduli with larger particles. This trend, however, reversed after 15 min. Dispersions of pectin exhibited higher loss and storage moduli with larger particles after 15 min resting time. No differences were observed with dispersions of CMC at either time points. Overall, the measurements did not allow a general prediction of the particle size dependence of the viscoelastic behaviour. In order to enable better comparability in further experiments, the previously characterised wider sieve fraction (32-150 µm) was selected.

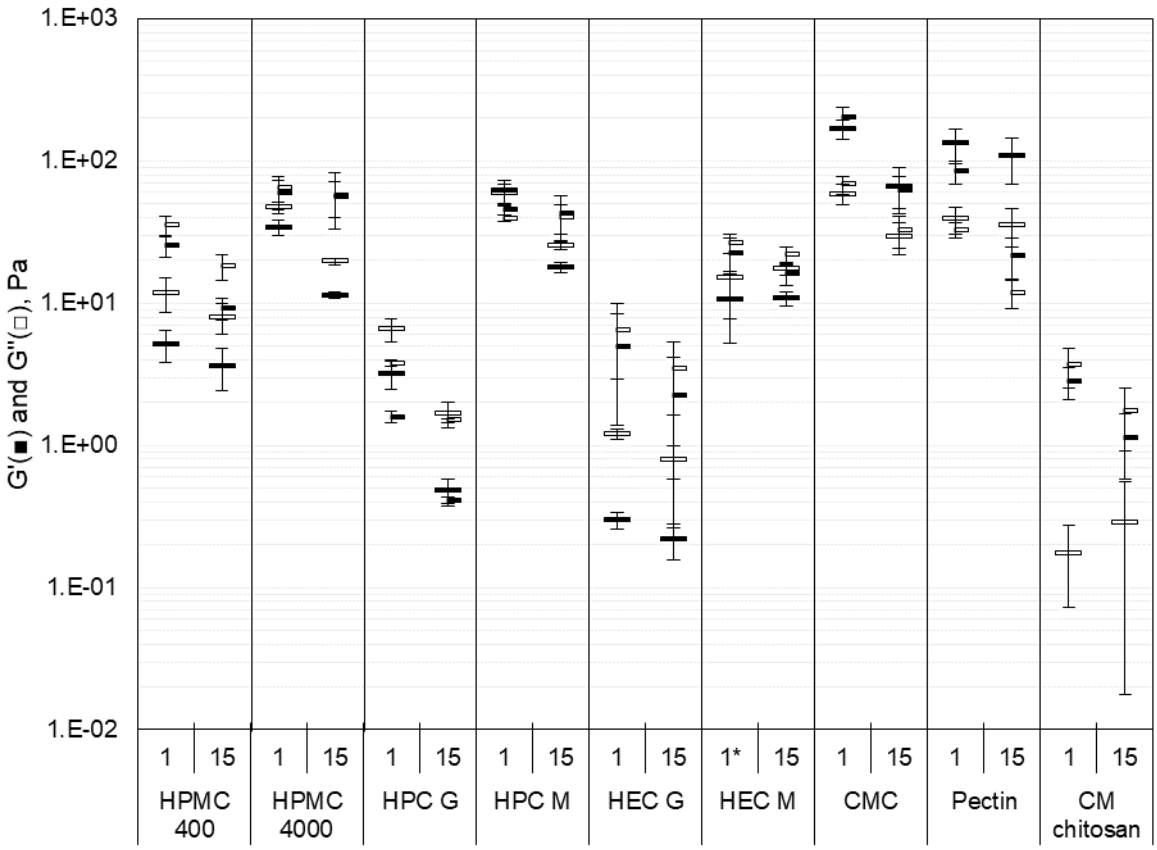

Figure 6-15: Storage (G', filled rectangles) and loss (G'', unfilled rectangles) moduli of 2% dispersions of mucoadhesive excipients in SNF after 1 min (1) and after 15 min (15) resting time at an oscillatory frequency of 1 Hz. The viscoelastic parameters of two sieve fractions are displayed: short rectangles display smaller particles (sieve fraction 32-90 µm), long rectangles display bigger particles (sieve fraction 90-150 µm). n=3, *n=2 (32-90 µm); error bars show standard deviation. Elastic modulus is not displayed when overlaying effects of instrument inertia occurred.

6.1.3.1.5 Conclusion on the rheological studies

The performed rheological tests allowed a comparative assessment of the potential of mucoadhesive excipients to reduce mucociliary clearance by influencing the viscoelasticity of the nasal fluid. Further, they provided information on polymer-related factors that may influence mucoadhesion. Measuring the steady shear viscosity is a convenient method to initially assess the gel formation behaviour of polymers under conditions that correspond to the nasal fluid in terms of temperature, pH and ionic composition. Oscillatory rheological measurements additionally allow to differentiate between the elastic and viscous fraction of viscoelastic samples. The interplay of elastic and viscous behaviour of the nasal mucus is important for an effective mucociliary transport, with the elasticity being considered the most important part [4]. Excipients, which exhibit high elastic moduli in the nasal fluid may therefore be advantageous in order to reduce the mucociliary clearance and prolong the residence time of a formulation in the nose. Among the mucoadhesive polymers tested, dispersions of the anionic polymers CMC and pectin showed the highest loss and storage moduli. Slightly lower but still high values were obtained for the longer-chain neutral

cellulose derivatives HPMC 4000, HPC M and HEC M. Frequency-dependent oscillation tests can additionally provide information about the time-dependent behaviour of the sample and the flexibility of the temporary entanglement network of the polymers. Interpenetration and entanglement of the polymer chains of the excipient and the mucin chains are steps during the consolidation stage of mucoadhesion [32]. While sufficient flexibility of the polymer chains is required for interpenetration, subsequent entanglement of the chains may enhance adhesive binding. Polymers, that undergo sufficient initial hydration to make the polymer chains available for interpenetration and show a rigid entanglement network during faster movements, may therefore be advantageous. CMC and pectin, which were promising due to their high measured storage modulus, showed inhomogeneous gel formation and a decrease in storage and loss moduli over time, which suggests an initially limited hydration of the polymers. As fewer polymer chains are available for interactions with mucin, this behaviour may be associated with weaker adhesive binding. Although the rheological assessments allow some assumptions about polymer-related factors of mucoadhesion, they do not reflect the actual process. The setup, in which polymers are actively dispersed in a larger volume of SNF is rather artificial and does not display the actual conditions in the nose, where dry powder particles deposit on the moist mucosa. For a comprehensive assessment of mucoadhesive excipients, complementary methods that characterise the wetting of the excipients on a moist surface and interactions with mucin are therefore needed. Section 6.1.3.2 of this thesis describes and discusses the results of relevant complementary methods.

6.1.3.2 Wetting and mucoadhesion

Mucoadhesive excipients are intended to form an adhesive bond upon contact with a mucosa. The mucoadhesive process involves two main steps that affect the resulting adhesive strength. The first step, the contact stage, establishes an initial contact between the mucoadhesive and the covering mucus layer of the mucosa. In the case of nasal powder formulations, dry particles are administered into the nasal cavity, deposit on a 10-15 µm thick mucus layer due to impaction and are “activated” by the presence of moisture [32,75]. In the subsequent consolidation step, the created bond is strengthened. The polymer chains hydrate under dehydration of the surrounding mucus layer. Interpenetration of the mucoadhesives and mucin glycoproteins and resulting physicochemical interactions further strengthen the adhesive bond [32]. The occurring interactions thereby depend on the mucoadhesive material used. The aim of this section is to evaluate the wetting and the mucoadhesive potential of the selected excipients.

6.1.3.2.1 Adhesion on agar and agar-mucin gels

In order to compare the processes of wetting and mucoadhesion of the different excipients, their adhesion behaviour on pure agar gels, as well as on agar-mucin gels was investigated. When dry polymer particles deposit on the nasal mucosa, the polymer particles hydrate, while the surrounding mucus gel dehydrates. This process on the one hand changes the rheological properties of the nasal fluid and thus the mucociliary clearance rate, and on the other hand frees the polymer chains of the mucoadhesive excipients and forces interactions between the excipient molecules and mucin glycoproteins. To characterise the extent and velocity of the hydration process, the adhesion of the excipients to pure agar gels was investigated. Stronger adhesion to agar-mucin gels than to pure agar gels indicates an enhancement of mucoadhesive binding due to interactions between the excipient molecules and mucin. Figure 6-16 depicts the displacement of the mucoadhesive powders on an inclined plane of agar and agar-mucin gels. Minor displacement of the powders indicates strong adhesion to the gels, while rapid displacement indicates low adhesion.

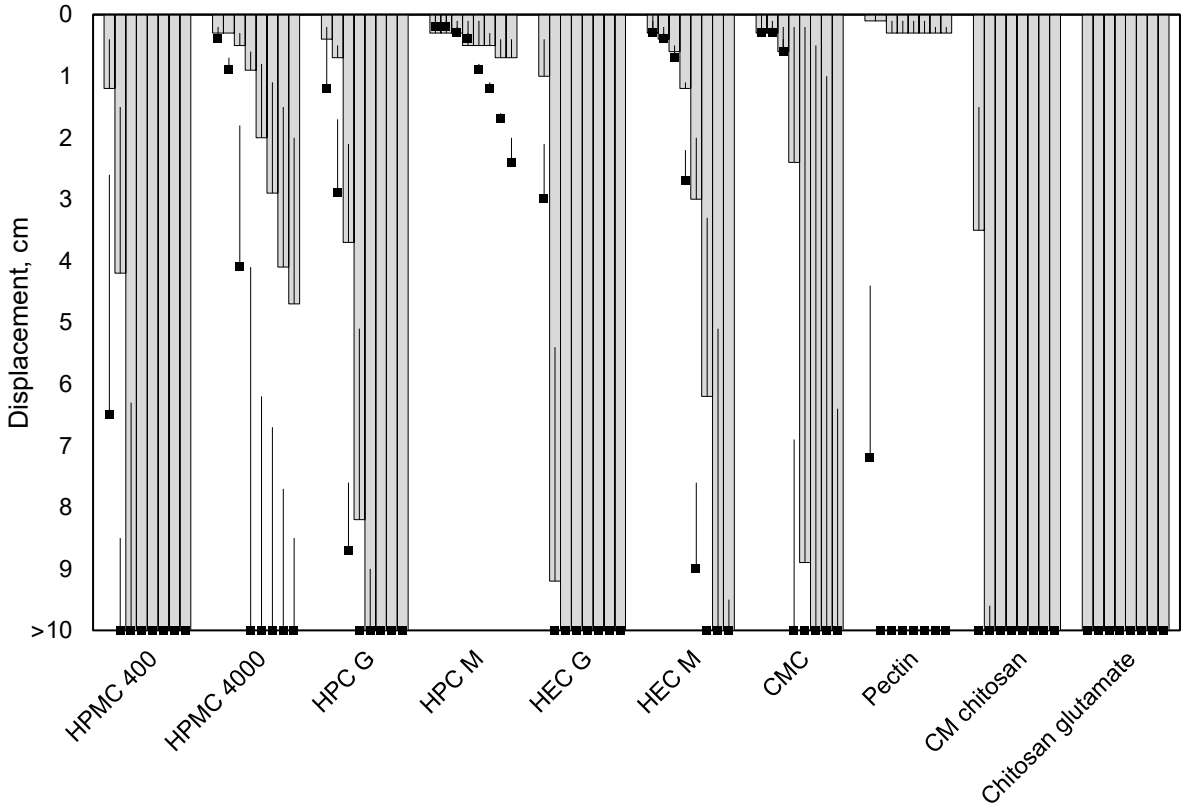

Figure 6-16: Displacement of mucoadhesive powders (sieve fraction 32-150 µm) on an inclined plane of agar-mucin gels (bars) and pure agar gels (squares) after 0.5 h, 1 h, 2 h, 3 h, 4 h, 5 h, 6 h and 7 h (from left to right). The maximal displacement out of n=3 is shown; error bars show range.

The assessment of the neutral cellulose derivatives (HPMC, HPC, HEC) showed a faster displacement of the shorter-chain polymers, compared to the longer-chain polymers. This difference in adhesive duration is in accordance with the expectations, since the degree of

polymerisation of the polymers is proportional to the viscosity of their solutions. Hence, shorter-chain polymers form weaker gels upon hydration that are more prone for adhesive failure. Within the shorter-chain polymers, HPC G powders dislocated the slowest on agar gels and reached the maximal displacement (> 10 cm) within 2-3 h. HPMC 400 and HEC G powders reached the maximal displacement already within 0.5-2 h and 0.5-1 h, respectively. A possible reason for the earlier adhesive failure of HPMC and HEC gels would be a higher rate or extent of hydration and thus earlier reached overhydration. The displacement of all samples was slower on agar-mucin gels compared to pure agar gels. Possibly, the higher total concentration of macromolecules in the mucin-containing gels reduced the hydration of the polymeric excipients, resulting in a slower displacement. However, as the reduction in displacement varied among the substances tested, it is likely that additional interactions of the excipients with mucin contribute to the effect. The time at which maximal displacement was reached shifted to 3-5 h for HPC G, to 1-3 h for HPMC 400 and to 1-2 h for HEC G. Hence, the shift was slightly greater for HPC G and HPMC 400 indicating stronger interactions with mucin for these polymers. The assessment of the longer-chain polymers revealed similar results. HPC M dislocated the least, with a maximal displacement on pure agar gels of 2.4 cm after 7 h. Within the HPMC 4000 samples, one gel reached the maximal displacement of above 10 cm on pure agar gels already within 2-3 h, while the other two samples dislocated slower and reached the maximal displacement between 5 h and more than 7 h. The formed HEC M gels reached the maximal displacement within 4-5 h. The rank order of adhesive failure again suggests higher extend of hydration for HPMC and HEC compared to HPC. The movement on agar-mucin gels was expectedly slower than on pure agar gels. The time at which maximal displacement was reached shifted to 5->7 h for HEC M. For HPMC 4000 samples, the maximal reached distance after 7 h was 4.7 cm. Almost no movement was observed with HPC M. The maximal reached distance there was 0.7 cm. As with the shorter-chain polymers, the results suggest stronger interactions for HPMC and HPC with mucin than for HEC.

The movement of the anionic cellulose derivative CMC on pure agar gels supports the hypothesis of overhydration being a cause of adhesive failure. While there was little movement of the samples within the first two hours, a rapid displacement was seen after 2-3 h. After this time, the increasing hydration of the polymer may have exceeded a threshold, which then led to loss of adhesion. The adhesive failure occurred later at mucin-containing gels. Only one sample reached the maximal displacement after 4-5 h. Two other samples showed less movement and reached a maximal displacement of 6.7 cm after 7 h. This suggests that interactions of CMC with mucin increased the strength of the adhesive bond.

The second anionic polymer, pectin, exhibited a clear difference in the strength of adhesion on pure agar gels and agar-mucin gels. On pure agar gels, maximum displacement was already reached after 1 h, while almost no displacement occurred on agar-mucin gels. After 7 h, the maximal displacement there was 0.3 cm. The strong adhesion to the mucin-containing gels indicates strong interactions of pectin with the mucin used. This finding is supported by the study of Hagesaether et al. [93]. Their investigation on the mucoadhesive properties of different types of pectin revealed pronounced specific interactions of pectins with a degree of methoxylation of ≈ 35% with mucin from porcine stomach type II. These interactions were attributed to a high ability of these types of pectin for hydrogen bonding, since the adhesive potential decreased, when hydrogen bonding was disrupted. Another factor that may influence the adhesiveness of pectin is the presence of calcium ions. Calcium ions induce the gelation of low methoxylated pectin and thus, an increase in adhesive strength can be assumed. Since the mucin used for gel preparation originates from porcine stomachs, the presence of residual calcium in the mucin powder is conceivable. Atomic absorption spectroscopy was therefore used to detect calcium in the commercial mucin powder. A calcium content of 0.356 ± 0.002 mg/g mucin was measured, which corresponds to a calcium concentration of 7.1 mg/L in the solution used for gel preparation. In contrast to that, no calcium ions are present in the pure agar gel. The gels used in this experiment were prepared in phosphate buffer pH 6.4 in order to keep the pH constant during gel preparation. Unlike the nasal fluid, this buffer, however, did not contain calcium salts. A higher viscosity of the hydrated sample on the mucin-containing gels due to the presence of calcium ions may therefore contribute to the stronger adhesion on these gels. To investigate the interaction with mucin, the experiment was repeated with gels prepared in simulated nasal fluid. On these gels, pectin showed only minor displacement, which was slightly smaller on the mucin-containing gels than on the pure agar gels after 7 h (Table 6-3). Interactions with mucin may thus increase the adhesive strength of pectin, which, however is already high in the presence of calcium ions.

Table 6-3: Displacement of pectin (sieve fraction 32-150 µm) on an inclined plane of agar-mucin gels and pure agar gels prepared with simulated nasal fluid after 0.5-7 h; n=3.

		0.5 h	1 h	2 h	3 h	4 h	5 h	6 h	7 h
Agar gels	**Maximal displacement, cm**	0	0	0	0	0	0	0.2	0.4
	Range, cm	0	0	0	0	0	0	0.2	0.3
Agar-mucin gels	**Maximal displacement, cm**	0	0	0	0	0	0	0.2	0.2
	Range, cm	0	0	0	0	0	0	0.2	0

The chitosan derivatives showed a rapid displacement on agar and agar-mucin gels. The early adhesive failure may be attributed to overhydration and a resulting low viscosity of the samples, which is consistent with the rheological characterisation. Only CM chitosan on

agar-mucin gels did not reach the maximal displacement already within 30 min, which indicates interactions with mucin that strengthened the adhesive joint. At a pH close to neutral, the carboxylic groups of CM chitosan are expected to be deprotonated [94], thus the interactions are most likely based on hydrogen bonding. The displacement of the chitosan glutamate samples on the inclined gels did not indicate interactions with mucin. However, different in vivo studies have shown mucoadhesive effects for drug delivery systems based on chitosan glutamate, which most likely base on ionic interactions of negatively charged sialic acid groups of mucin and positively charged amino groups of chitosan [35,95]. Rapid movement of samples that show low viscosity upon hydration could mask interactions with mucin in the inclined plate setup.

6.1.3.2.2 Assessment of polymer hydration by dynamic vapour sorption

Dynamic vapour sorption (DVS) was measured to evaluate the hydration behaviour of mucoadhesive polymers and to support the hypothesis that overhydration is a cause of early adhesive failure. Baumgartner et al. investigated the swelling behaviour of cellulose ethers using DVS and dynamic equilibrium swelling studies [96]. Both methods ranked the investigated polymers in the same order. When screening mucoadhesive polymers, the measurement of dynamic water vapour sorption can therefore provide a rapid and reproducible method for assessing the hydration and swelling behaviour.

Figure 6-17 displays the sorption isotherms of the mucoadhesive polymers, recorded in the first cycle of the DVS measurements.

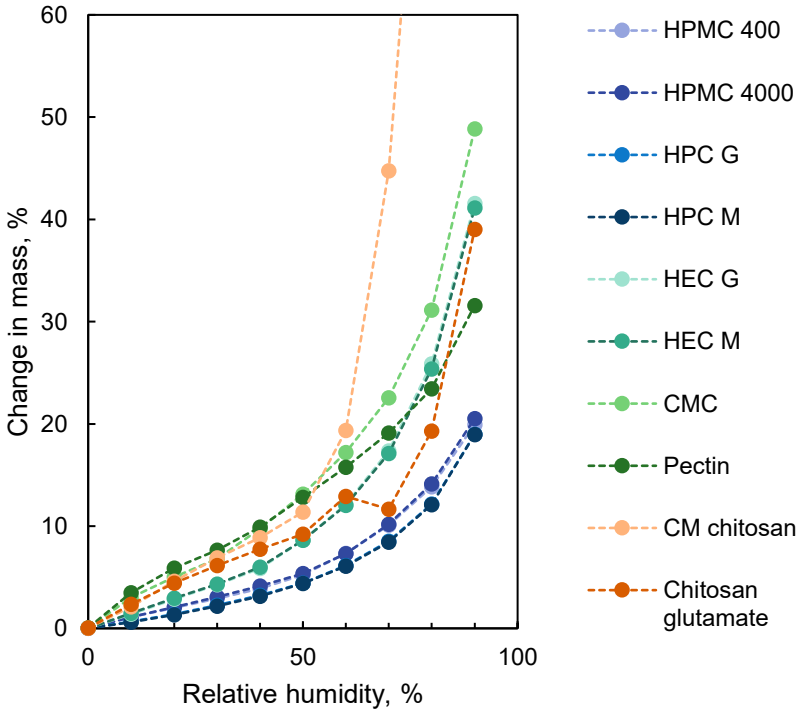

Figure 6-17: Sorption isotherms (first cycle) of the mucoadhesive polymers. n=1.

HPC (G and M) showed the smallest changes in mass and thus, the least water sorption among the assessed polymers. This finding is in accordance with the displacement of HPC samples on agar gels (Section 6.1.3.2.1) and the swelling studies of Baumgarner et al. [96]. Limited hydration and swelling of HPC may result in higher concentrated and thus more stable gels on the agar plates. Cohesive failure within the hydrated mucoadhesive or adhesive failure at the interface between the agar and HPC gel therefore occurs later. Within the neutral cellulose derivatives, water sorption increased in the order HPC, HPMC, HEC. This order was also found for the displacement of the polymers on agar gels, which supports the hypothesis, that overhydration induces adhesive failure. Compared to the neutral cellulose derivatives, the anionic polymers CMC and pectin showed a higher mass increase due to water vapour sorption already at low relative humidities, which increased comparatively more for CMC at higher relative humidities (>60%). Overhydration thus may limit the duration of adhesive binding especially for CMC. The chitosan derivatives showed similar curves with strong mass increase at high humidities. Overhydration is therefore a probable explanation for the early failure of the adhesive joint in these samples as well.

Since powders impinge on a moist surface in the nose and the ambient air in the nasal cavity is humidified to a humidity above 80% [97], water sorption at higher relative humidities should be considered to assess hydration and swelling of the polymers in the nose. The Ph. Eur. 10.0 classifies the hygroscopicity of powders according to their gain in mass at a relative humidity of 80%. This classification could be used to assess mucoadhesive polymers for their potential of adhesive failure due to overhydration. Table 6-4 displays the classification of the mucoadhesive polymers according to Ph. Eur. A mass gain of 2-15% is classified as hygroscopic and a mass gain of 15% and higher is classified as very hygroscopic. Polymers that are classified as very hygroscopic showed a higher risk for overhydration as polymers that are classified as hygroscopic.

Table 6-4: Gain in mass of the mucoadhesive polymers at 80% relative humidity (first cycle) and hygroscopicity according to Ph. Eur. 10.0. n=1.

Mucoadhesive polymer	Gain in mass at 80% relative humidity, %	Hygroscopicity
HPMC 400	13.84	hygroscopic
HPMC 4000	14.14	hygroscopic
HPC G	12.17	hygroscopic
HPC M	12.09	hygroscopic
HEC G	25.87	very hygroscopic
HEC M	25.36	very hygroscopic
CMC	31.13	very hygroscopic
Pectin	23.42	very hygroscopic
CM chitosan	97.7	deliquescent
Chitosan glutamate	19.29	very hygroscopic

6.1.3.3 Conclusion on the assessment of excipient properties that extend the nasal residence time

The rational selection of excipients requires reproducible methods to evaluate and compare excipient properties that result in a prolonged residence time in the nose. In this chapter, changes in the viscoelasticity of the nasal fluid, interactions with mucin and hydration of the powder particles were discussed as factors that are crucial for the mucoadhesive potential of powder formulations. Rheological tests, determination of adhesion to agar and agar-mucin gels and evaluation of dynamic water vapour sorption were used as methods to characterise the mucoadhesive potential of the excipients. The combination of these methods provided a suitable setup to screen and compare excipients for their potential to prolong the residence time of a powder formulation in the nose.

Oscillatory rheological tests allowed a comparative evaluation of the ability of mucoadhesive excipients to reduce the mucociliary clearance by influencing the viscoelasticity of the nasal fluid. The elasticity of nasal mucus is considered particularly important for effective mucociliary transport. Excipients that increase elasticity therefore have a high potential to reduce mucociliary clearance and thus increase the residence time of a formulation in the nose. Among the mucoadhesive polymers tested, CMC and pectin showed the most pronounced elastic behaviour in SNF with the highest elastic moduli. However, the gels formed were inhomogeneous, so that incomplete hydration of the polymer particles can be assumed. Homogeneous gels with slightly lower elastic moduli were obtained with the

neutral cellulose derivatives HPMC 4000, HPC M and HEC M. Adequate initial hydration of the polymer particles is a prerequisite for interactions of the polymers with mucin.

Applying the excipient powders to agar and agar-mucin gels and evaluating the adhesion to these gels in an inclined plane depicted the hydration of the powders on a wet surface and the occurring interactions with mucin. The limited hydration, which occurred in the rheological setup for CMC and pectin, did not prevent interactions with mucin on agar-mucin gels. Hydration of these polymers on a wet surface therefore seems adequate to free the polymer chains for interactions. Pectin actually showed the lowest displacement among the tested polymers on mucin and calcium-containing gels, indicating a strong mucoadhesive effect in the nose. Besides pectin, HPC M showed little movement on both agar and agar-mucin gels. The rheological properties of the hydrated polymers, as well as interaction possibilities with mucin due to chain entanglements and intermolecular forces influence the adhesive strength. Overhydration of the polymers leads to weaker gel structures and reduction of interactions and can therefore be the cause of adhesive failure. To confirm this hypothesis, dynamic water vapour sorption of the polymers was measured. Among the neutral cellulose derivatives with similar rheological and structural properties, HEC showed the highest water uptake and the strongest displacement in the agar plate model, which supports the theory of overhydration. The assessment of dynamic water vapour sorption in excipient screenings may therefore indicate the probability of adhesive failure due to overhydration.

The application of these methods enables a reproducible and comparative screening of different excipients, especially in early product development. Other methods described in literature for this purpose are based on the adhesion to nasal tissue, such as sheep or rabbit mucosa. The assessment of mucoadhesiveness in these methods relies on the washability of the formulation from the nasal mucosa [98], or on the detachment force required to separate the formulation from the mucosa after a defined contact time [99]. However, the stickiness of a formulation on nasal tissue does not necessarily correspond to its effect on ciliary movements, and therefore cannot be correlated with the effect on clearance in vivo. Hence, these methods may also be considered mainly as a screening tool and allow the comparison of different formulations. The use of tissues has the disadvantage that these are subject to inter-individual differences, which affect the reproducibility of the methods. The methods described in this work allow a higher degree of standardisation and may therefore be advantageous for screening purposes. Methods that directly address the mucociliary clearance use ciliated cells and reconstituted airway epithelia to determine the ciliary beat frequency [100,101], or airway tissue models like animal trachea [102] to determine the mucociliary transport rate. The need for specialised imaging techniques to record the cilia beat frequency and the need of animal or human material limit the suitability

of these methods for broad-scale screening. A comparative characterisation of excipients using such methods and the screening methods used in this work, however, can provide additional information that improves the interpretation of obtained data and would therefore be an aspect for future research. In the development of nasal products, broad-scale excipient screening can form a basis that can be complemented by the inclusion of more specific methods in the course of further product development.

6.1.4 Sensory effects caused by excipients

The development of sophisticated nasal formulations usually aims for overcoming the physiological challenges of nasal drug administration. One aspect that is often underestimated is the high sensitivity of the nasal mucosa, due to trigeminal innervation. Since nociceptors in the nose are not covered by squamous epithelium, stimuli have almost direct access to the free nerve endings [5,21]. Nasal drug delivery requires the deposition of the formulation in the nasal cavity and a close contact with the mucosa. These processes can constitute mechanical, physical and chemical stimuli, which cause unpleasant sensations and thus reduce the patient compliance. Limited acceptance of patients may cause the failure of a nasal product, especially if a regular use is intended. Sensory effects, however, are not detected in usual in vitro or animal studies and are most often only considered in clinical studies. The aim of this chapter is to assess irritative effects caused by excipients in nasal powder formulations and to identify influencing powder characteristics. The slug mucosal irritation assay (SMIA) served as predictive tool. The assay bases on a correlation between the amount of mucus produced by slugs upon contact with the substance, and stinging, itching and burning sensation upon contact with human mucus membranes [81].

Figure 6-18 displays the total mucus production of slugs in the SMIA, upon contact with the fillers and mucoadhesives characterised in this work. The assessment of the fillers revealed a significantly increased mucus production of slugs after contact with mannitol (4.70 ± 0.63%; p=0.011) and colloidal MCC (4.77 ± 1.66%; p=0.049) compared to pure sea sand, which was used as marker substance for no irritation, while the increase in mucus production was not significant with lactose (2.85 ± 0.54%; p=0.141) and MCC (3.52 ± 0.93%; p=0.077). Compared to benzalkonium chloride (BAC 1% w/v) as marker for severe irritation, however, the contact with all tested fillers resulted in clearly lower mucus production, which suggests only a mild irritation potential of these substances. Within the investigated mucoadhesives, there was no significantly increased mucus production in slugs after contact with HPMC 400, HPC and HEC M, while contact with the other investigated substances resulted in significantly higher mucus productions compared to sea sand. The increase of mucus production compared to sea sand tended to be smaller after contact with powders of the neutral polymers HPMC (2.61 ± 0.87%; p=0.272 and

3.77 ± 0.77%; p=0.044 for HPMC 400 and 4000, respectively), HPC (2.21 ± 0.33%; p=0.401 and 2.74 ± 0.62%; p=0.181 for HPC G and M, respectively) or HEC (4.03 ± 0.58%; p=0.023 and 6.11 ± 3.05%; p=0.07) than when in contact with the negatively charged polymers CMC (5.56 ± 0.76%; p=0.006) and pectin (5.21 ± 1.05%; p=0.002) and the chitosan derivatives (17.52 ± 0.63%; p<0.001 and 9.90 ± 0.87%; p<0.001 for CM chitosan and chitosan glutamate, respectively). An outstanding high mucus production in the range of BAC was observed with CM chitosan, which suggests a severe irritation potential in the nose.

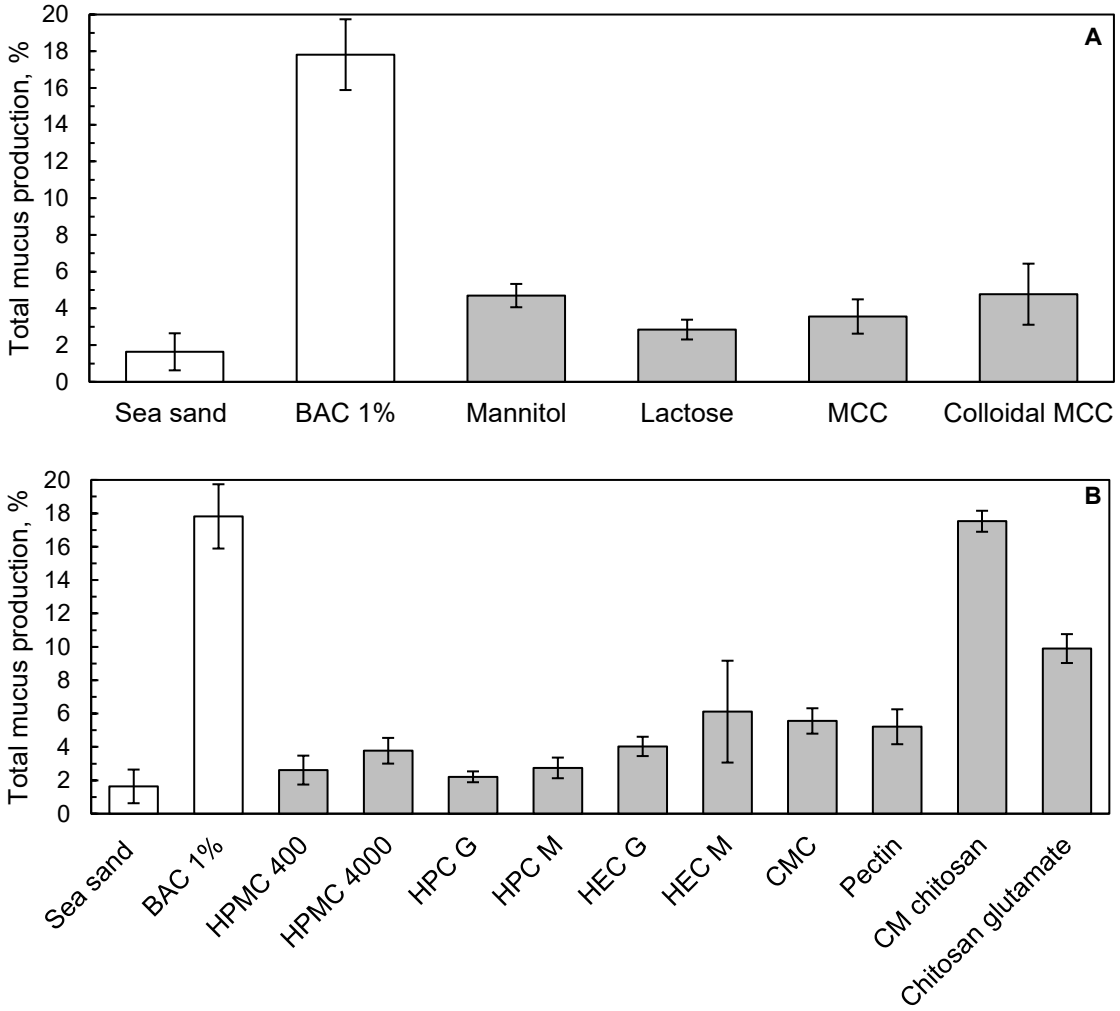

Figure 6-18: Sensory effects of fillers (A) and mucoadhesives (B) (tested sieve fraction: 32-150 µm) displayed as total mucus production of slugs in the slug mucosal irritation assay. Pure sea sand and benzalkonium chloride (BAC 1% w/v) served as markers for no and severe irritation, respectively. n≥3; error bars show standard deviation.

Irritations that are detected with the slug mucosal irritation assay are assumed to be caused by chemical stimuli, which will therefore be investigated in the next sections. Factors that may cause trigeminal mediated sensations at the nasal epithelium will also include exposure to touch, temperature and pressure [5,21], which can be caused by the impaction of the formulation in the nose and the mechanism of the device used. These effects are not

covered by the SMIA, but should be considered in subsequent product development and in the selection of the delivery device.

6.1.4.1 Influence of pH and osmolality

Changes in the physiological pH and tonicity are considered as stimuli, which can cause nasal irritation. The influence of the different fillers and mucoadhesives on the pH and osmolality of PBS is therefore compared in figure 6-19.

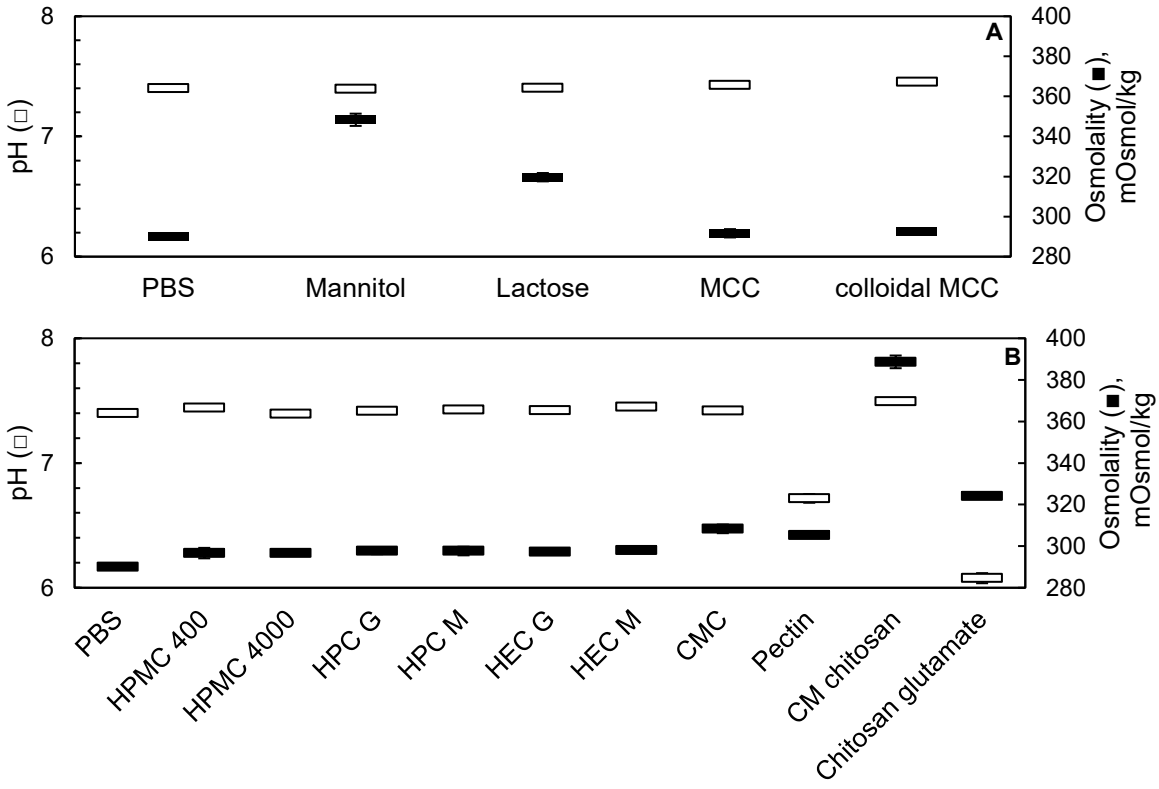

Figure 6-19: pH (unfilled rectangles) and osmolality (filled rectangles) of 1% solutions of fillers (A) and mucoadhesives (B) in phosphate buffered saline (PBS, 12 mM total phosphate). n=3; error bars show standard deviation.

The assessment of the fillers revealed no pH changes. An increase in osmolality was observed with the soluble fillers mannitol and lactose, but was more pronounced in mannitol solutions. While lactose did not cause an increased mucus production in the slug mucosal irritation assay, a significantly increased mucus production was observed with mannitol when compared to the negative control or lactose. The higher osmotic pressure, which is generated by dissolving mannitol particles may be a possible reason for this difference. An increased tendency of hyperosmolar saline solutions to cause nasal irritation, when compared to isotonic saline solutions was already shown in different studies, which supports this hypothesis [103,104]. The effect of osmotic active substances may however be even stronger in nasal powder formulations than in the assessed liquids. Powder particles that get in contact with a moist surface, like the nasal mucosa or the wet body wall of the slugs

in the SMIA, dissolve according to their water solubility. Depending on the sample amount, solubility, and dissolution velocity, highly concentrated solutions are thus formed, which then cause a correspondingly high osmotic pressure. In contrast to that, the osmolality of liquid formulations is predetermined in the formulation. However, the mucus production of slugs upon contact with mannitol was not significantly higher than with the insoluble fillers MCC and colloidal MCC. Hence further factors need to affect the mucus production of slugs. A possible influencing factor for the insoluble fillers may be moisture sorption due to hygroscopicity. The gain in mass at 80% relative humidity (DVS measurement) was 9.5% and 12.3% for MCC and colloidal MCC, respectively, which is classified as hygroscopic according to the Ph. Eur. Resulting dehydration of the body wall of slugs may have caused a slight increase in mucus production.

The assessment of the mucoadhesives showed no relevant changes in pH and osmolality in solutions of the neutral cellulose derivatives HPMC, HPC and HEC, which is consistent with the low mucus production in the SMIA. The total mucus production of slugs upon contact with HEC M was slightly higher than with the other neutral cellulose derivatives, but the standard deviation was high for this sample, so there was no statistically significant difference when compared to HPMC 4000 and HPC M. The anionic mucoadhesives CMC and pectin caused a slightly higher mucus production compared to HPMC and HPC. The assessment of osmolality showed an increase compared to PBS; however, the osmolality was still below the osmolality of the lactose solution. Since the contact with lactose did not cause an increase in mucus production in the SMIA, further factors need to contribute to the effect of CMC and pectin. In terms of pectin, this may be the slightly acidic behaviour, which may cause increased irritation especially when higher concentrated solutions are formed during dissolution of the powder. A more pronounced acidic behaviour was found for chitosan glutamate, which also showed a significantly increased mucus production in the SMIA compared to the cellulose derivatives and pectin. CM chitosan, which caused an outstanding high mucus production in the range of benzalkonium chloride as irritating marker in the SMIA, was found to show the highest osmolality of the tested substances in solution. The influence of hyperosmolar saline solutions on the mucus production in the SMIA was studied by Lenoir et al. [80]. 10% NaCl solutions (2912 mOsmol/kg) resulted in a mucus production that was classified as severe, and thus in the same category as BAC 1%. For nasal powders, dissolution of the particles can result in concentrated and thus highly hyperosmolar solutions that cause severe irritation. In case of the tested excipients in this work, however, substance specific toxicity may also contribute to the irritation potential. Further investigations on this are therefore conducted in section 6.1.5.

The results suggest that changes in osmolality and pH of the nasal fluid caused by powder formulations can cause irritation. In contrast to liquid formulations, these parameters are

difficult to adjust for powder formulations, as they depend on the solubility and dissolution rate of the powder ingredients and the fluid volume in the nose. Therefore, to avoid irritation, substances that are strongly osmotically active, acidic or basic should be used with caution. In addition to the stimuli examined in this section, however, substance-specific toxicity (pH independent) can also trigger irritation symptoms.

6.1.4.2 Influence of particle size and morphology

Changes in the particle size or morphology (e.g., spray dried powders) can affect the irritation potential of powder formulations. For example, changes can be triggered by differences in the particle surface. An increase in the particle surface area results in an increased contact area between powder and mucosa and can accelerate the dissolution of the particles, which in turn can lead to the formation of higher concentrated solutions at the contact site. Figure 6-20 displays the influence of particle size and morphology on sensory effects caused by selected excipients.

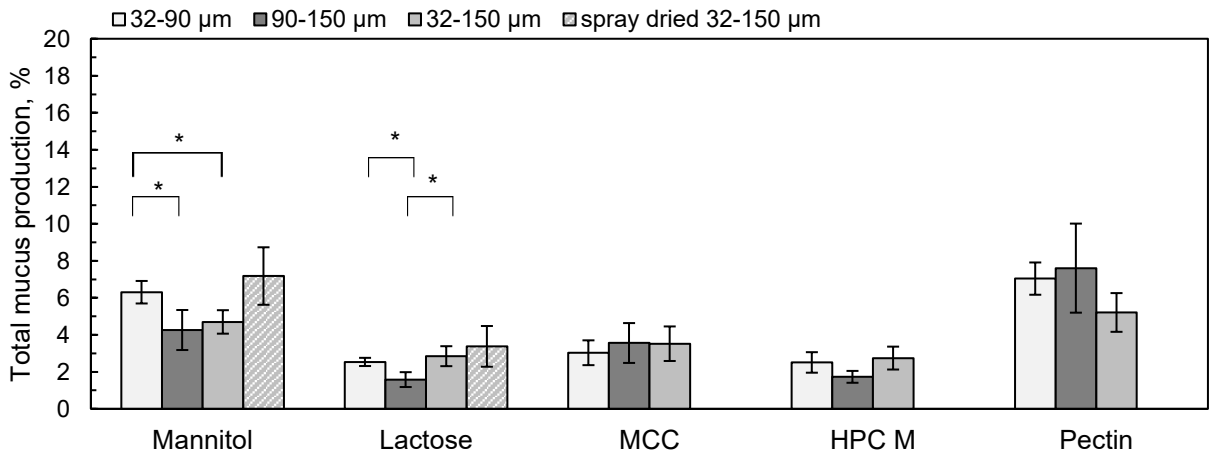

Figure 6-20: Influence of particle size and quality on sensory effects. Displayed as total mucus production of slugs in the slug mucosal irritation assay. n≥3; error bars show standard deviation; *=p<0.05.

The assessment showed different results for the soluble and insoluble fillers. While the total mucus production of slugs, and thus the irritating potential was not significantly affected for the insoluble filler MCC, the mucus production upon contact with the soluble fillers was particle size dependent. Contact with smaller particles (sieve fraction 32-90 µm) of mannitol and lactose caused a significantly higher total mucus production than contact with larger particles (sieve fraction 90-150 µm; p=0.046 and p=0.023 for mannitol and lactose, respectively). This increased irritation potential may be due to faster dissolution of the smaller particles upon contact with moisture. The higher concentrated solutions that are thus formed cause a higher osmotic pressure. Spray dried qualities of mannitol and lactose, which show a larger surface area compared to sieved qualities, and thus faster dissolution, also caused higher mucus production, but the difference was not statistically significant in

this case. However, it can be assumed that the manufacturing process of powders can affect the irritation potential of the formulation in the nose.

The assessment of the gelling mucoadhesive polymers HPC and pectin showed no particle size dependence of the irritating potential. In case of gelling substances, the gel barrier, which is formed after contact with moisture may decelerate further dissolution of the powder and thus decrease the effect of smaller particles.

6.1.4.3 Influence of irritating substances in powder blends

Since functional excipients, such as absorption enhancers, may be needed only in small quantities in powder formulations, the concentration dependence of sensory effects is of great practical interest. Figure 6-21 therefore displays the total mucus production of slugs caused by chitosan glutamate, as irritating substance in powder blends with MCC, as non-irritating substance.

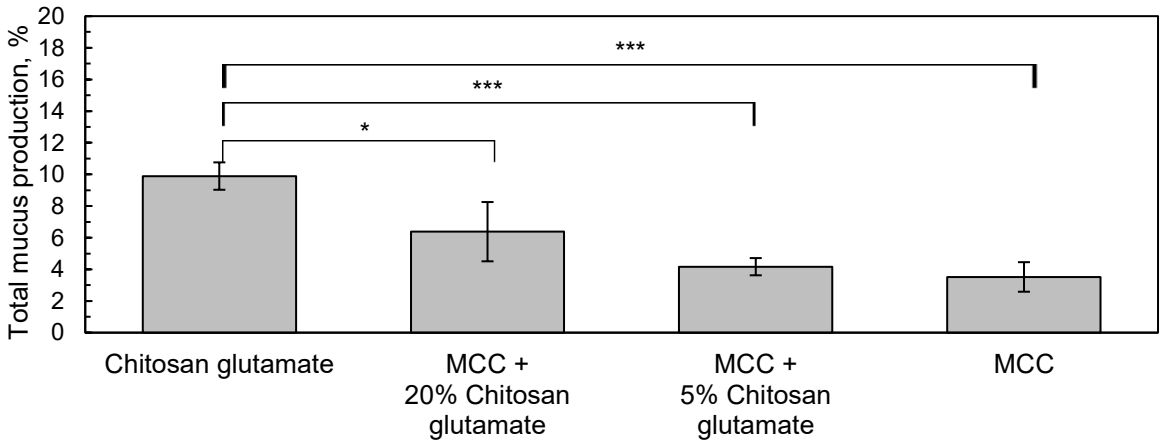

Figure 6-21: Sensory effects caused by irritating substances in powder bends. Displayed as total mucus production of slugs in the slug mucosal irritation assay. n=3; error bars show standard deviation; *=p<0.05, ***=p<0.001.

The assessment showed that the irritation potential of the blend decreased with decreasing content of chitosan glutamate. At a chitosan glutamate content of 5%, which is possibly sufficient for an permeation enhancing effect [105], the total mucus production of slugs (4.17 ± 0.54%) was in the same range as with pure MCC (3.52 ± 0.93%). Hence, the expected concentration of a substance in the formulation should be regarded when evaluating the irritating potential and the intended formulation should be tested. The formulation of irritating substances together with inert substances in powder formulations can be considered.

6.1.4.4 Conclusion on the assessment of sensory effects

It was possible to distinguish the irritation potential of different excipient powders and powder blends by using the slug mucosal irritation assay. The SMIA is therefore a useful tool for the characterisation of powder formulations, which allows an early assessment of sensory effects. Data on that is otherwise only obtained in clinical studies that have high regulatory requirements, are cost-intensive and are therefore only conducted at a later stage of product development. Lack of patient acceptance due to sensory effects, however, could cause failure of nasal products, especially if a regular use of the product is required. An early assessment of sensory effects is therefore advantageous. This study shows that osmotic activity and pH changes as well as solubility and dissolution accelerating factors like particle size and morphology are influencing factors on sensory effects. Since the adjustment of tonicity and pH is difficult in powder formulations because the obtained values depend on the dissolution in the nasal fluid volume, this should be considered already during the selection of excipients. In addition to the factors mentioned, specific substance toxicity can also cause nasal irritation. However, irritancy and toxicity of a formulation are not necessarily concomitant [20]. Cytotoxicity of the excipients is therefore investigated in the next section.

6.1.5 Cytotoxicity of excipients

Toxic effects can limit the use of excipients in nasal formulations. Especially in powders, where the effective substance concentration in the nose is not precisely known, but depends amongst others on the dissolution in the nasal fluid, knowledge on the concentration dependent toxicity of used substances is essential. In this section, the toxicity of the excipients for nasal epithelial cells was therefore assessed using the RPMI 2650 cell line. Figure 6-22 and figure 6-23 display the concentration dependent toxicity of the excipients after a contact time of 24 h.

The evaluation of the soluble fillers revealed a higher toxicity (assessed as LC_{50}) of mannitol compared to lactose. The calculated LC_{50} of mannitol was 38.7 ± 4.0 mg/mL and thus about half the LC_{50} of lactose (74.2 ± 11.1 mg/mL; $p<0.001$). Considering cell viability as a function of concentration shows similar slopes for both substances, and therefore a comparable concentration dependency of toxicity. Since the maximum concentration reached in the nose is not precisely known when using nasal powders, a large concentration interval between non-toxicity and total cell death is advantageous and offers a higher degree of safety in use. The investigation of the toxicity of the substances in the in vitro cell experiment led to the same rating of the soluble fillers as the assessment of sensory effects with the slug mucosal irritation assay (Section 6.1.4). The higher osmotic activity of mannitol was discussed there as a possible cause for the higher irritation potential. As cell viability also

depends on the conditions of the surrounding medium, such as osmolality, this may also contribute to the higher cell toxicity found [82]. However, a substance specific toxicity cannot be ruled out. The assessment of further sugars and sugar alcohols may provide additional information in this regard.

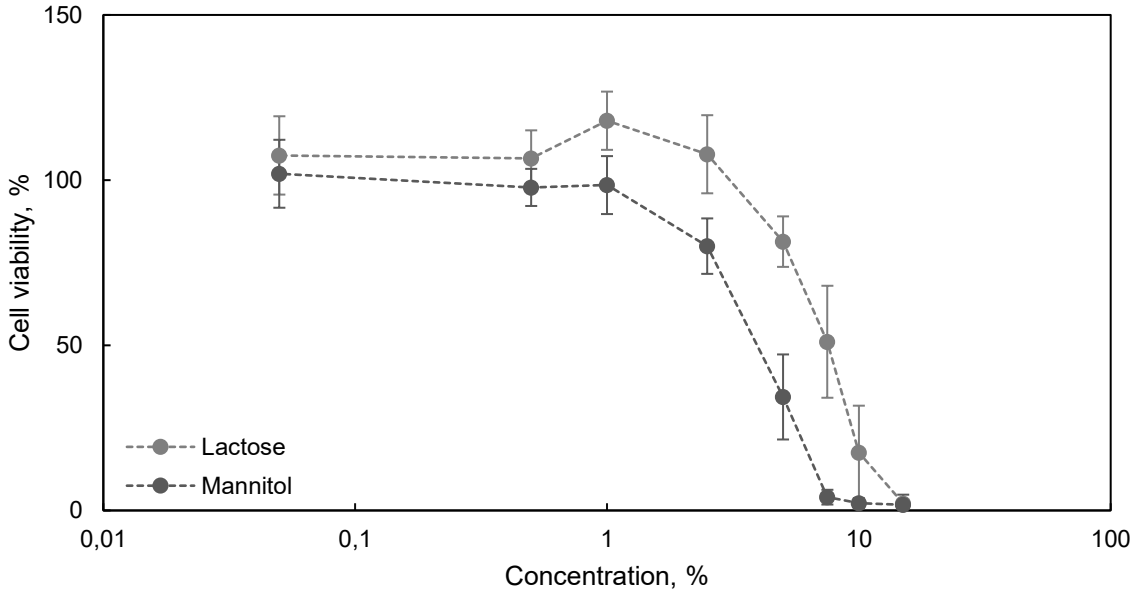

Figure 6-22: Concentration dependent toxicity of the soluble fillers lactose and mannitol on RPMI 2650 cells after a 24 h contact period. n=9; error bars show standard deviation.

The investigation of the insoluble fillers was not possible in the cell culture setup, because the undissolved particles could not be completely removed from the cells and would thus impair the measurements.

The assessment of the mucoadhesives revealed no toxic effects (viability above 80%) for the tested neutral cellulose derivatives (HPMC 400, HPC G, HEC G), anionic polymers (CMC, pectin) and CM chitosan in the assessed concentration range. It was not possible to test higher concentrations of these excipients due to an increase of viscosity. The resulting gels could not be pipetted. Chitosan glutamate showed toxic effects to the nasal epithelial cells in the tested concentration range with an LC_{50} of 3.8 ± 0.1 mg/mL. It thus exhibited the highest toxicity among the substances tested. Considering cell viability as function of concentration also revealed a smaller interval from non-toxicity to cellular death compared to lactose and mannitol. However, due to the different functions of chitosan glutamate and the fillers in nasal formulations, smaller amounts of chitosan glutamate would be used.

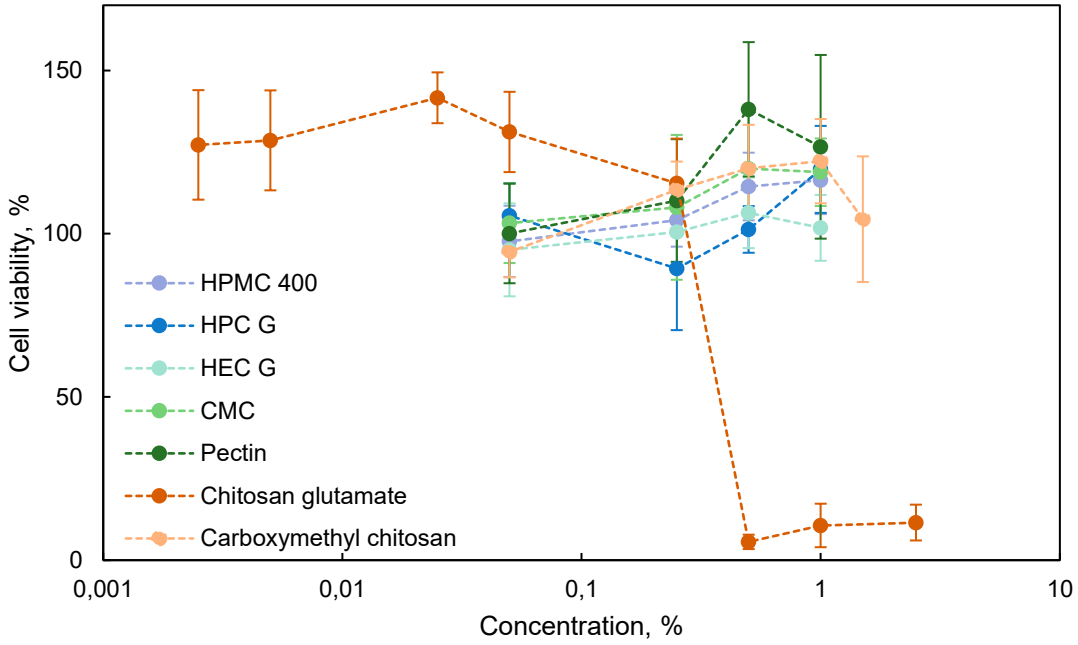

Figure 6-23: Concentration dependent toxicity of mucoadhesives on RPMI 2650 cells after a 24 h contact period. n=9; error bars show standard deviation.

Comparing cell toxicity of the substances with the assessment of sensory effects using the slug mucosal irritation assay (Section 6.1.4), reveals differences in the rating order. While CM chitosan showed the highest irritation potential in the SMIA, no toxic effects could be seen in the cell studies. However, as the substance was used as powder in the SMIA and dissolved only in the moist of the body wall of the slugs, higher concentrated solutions may have formed, while the concentration in the cell toxicity study was limited due to the viscosity of the solution. It is therefore possible that toxic effects on nasal epithelial cells occur at higher concentrations. Chitosan glutamate, which showed the highest toxicity to RPMI 2650 cells also showed an increased irritation potential in the SMIA and the influence of pH changes was discussed in section 6.1.4.1. However, the as well slightly acidic reacting pectin did not show cell toxic effects. Figure 6-24 compares the pH of the test solutions of pectin and chitosan glutamate that were applied to the cells in the toxicity studies.

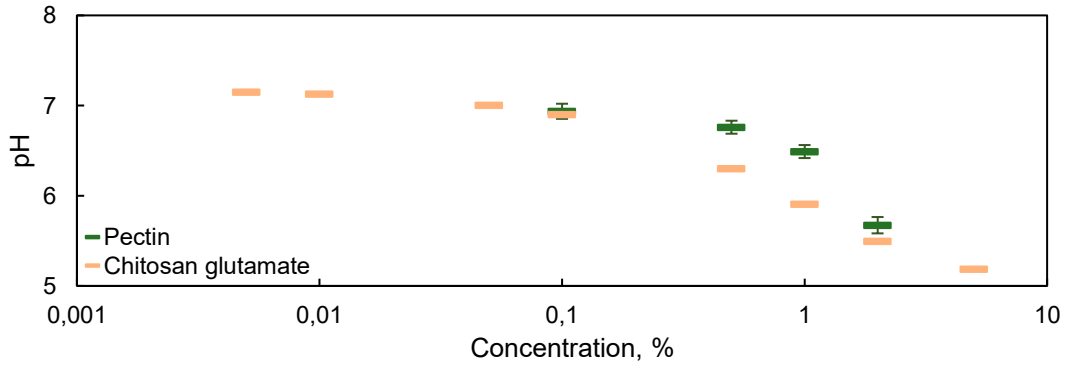

Figure 6-24: pH of test solutions of pectin and chitosan glutamate applied to RPMI 2650 cells in the MTT assay. n=3; error bars show standard deviation.

The comparison shows that slightly acidic pectin solutions are non-toxic, while comparably or less acidic chitosan glutamate solutions caused cell death. It can therefore be assumed that substance-specific factors contribute to the toxicity of chitosan glutamate.

6.1.6 Conclusion on the characterisation of excipients

Powder formulations have the potential to serve unmet needs in nasal drug delivery. However, they are currently the absolute minority on the market, which is accompanied by a knowledge gap regarding their characterisation and performance. The aim of this chapter was therefore to identify and address existing hurdles. The potential of selected mucoadhesive excipients (HPMC 400 and 4000, HPC G und M, HEC G und M, CMC, low methoxylated pectin, carboxymethyl chitosan and chitosan glutamate) to enhance the nasal residence time of a formulation was assessed. The assessment of rheological properties in simulated nasal fluid, adhesiveness on agar-mucin gels and water vapour sorption facilitated the characterisation of processes, affecting mucoadhesion in powders, including wetting and hydration after contact with a moist surface. The combination of these methods provides an easy-to-use in vitro setup for the comparison of excipients. However, for the selection of beneficial excipients for drug powder formulations, the properties of the API to be formulated need to be taken into account. Tanaka et al. showed an enhanced absorption of sumatriptan, as model drug with high solubility and low permeability, in powder formulations with different types of HPC, but not of warfarin as model drug with already high permeability [106]. In order to investigate the influence of excipients in formulations, model formulations with active ingredients with defined properties are prepared and characterised in the next part of this work.

This chapter additionally addressed sensory effects of powders in the nose as factor that can cause the failure of nasal drug products. It was possible to distinguish the irritation potential of the mucoadhesive excipients and selected fillers (lactose, mannitol, MCC, colloidal MCC) by using the slug mucosal irritation assay. The irritation potential of excipients should be regarded when selecting substances in formulation development.

The characterisation of the selected excipients with the described methods provides a basis for the classification of new substances.

6.2 Influence of excipients in model formulations

6.2.1 Preparation of model formulations

The effect of excipients on the outcome of nasal drug administration will depend on the properties of the drug substance. Drugs with different physicochemical properties require different formulation strategies in order to optimise nasal drug delivery and hence, benefit from different types of excipients. This part of the work therefore investigates the effect of excipients in powder blends on the resistance of the formulation against the mucociliary clearance and the dissolution as well as release of the drug. Furthermore, the effect of excipients on drug permeation is investigated.

6.2.1.1 Selection of excipients

Excipients with different properties and promising results in chapter 6.1 were selected for the preparation of model formulations.

For the examination of the benefit of fillers in nasal formulations, the effects of water-soluble and water-insoluble substances was compared. MCC was selected as water-insoluble compound. Since colloidal MCC did not show adequate dispersion in simulated nasal fluid and thus no gelation, it is not considered as beneficial for nasal powder formulations, and will thus not be assessed further in the model formulations. For further investigations of the influence of water-soluble fillers, mannitol was selected. Indeed, mannitol has shown a higher irritation potential in the slug mucosal irritation assay and higher cell toxicity than lactose, however, these effects were mainly attributed to the higher osmotic activity of mannitol. Since a probable function of soluble fillers in nasal powders is the facilitation of drug dissolution due to the generation of an osmotic pressure that withdraws water from underneath tissues [40], mannitol was selected, as a greater effect of osmotically induced processes is expected.

For the examination of the influences of mucoadhesive excipients in powder formulations, charged and uncharged polymers were selected. HPC M was selected as neutral polymer, as it exhibited high storage and loss moduli in simulated nasal fluid, and the strongest adhesion to agar (-mucin) gels among the tested neutral polymers. Even stronger adhesion to mucin gels was found for pectin, which was therefore selected as anionic polymer. Its specific gelation in presence of calcium ions is also attractive for nasal delivery, as it results in mucoadhesive properties in the calcium-containing nasal fluid (commercially used in the PecSys™ technology [60]). Additionally, handling and storage of powder products may be facilitated compared to other gelling polymers, due to the specific gelation behaviour. According to the screening results, the tested chitosan derivatives showed the least potential to prolong the nasal residence time. However, they possess a special status among the mucoadhesive polymers tested, as chitosan is also regarded as a permeation

enhancer due to the opening of tight junctions. Since the cationic charge of chitosan is assumed to contribute to its permeation enhancing effect [107], chitosan glutamate was selected for further investigations instead of CM chitosan, which would exhibit a negative net charge at nasal pH.

6.2.1.2 Properties of model drugs

In order to take the requirements of drugs with different physicochemical properties into account, model drugs with different permeability properties through epithelial barriers were selected for the preparation of model formulations. The permeability of different drugs through the nasal epithelium is well described in literature [13]. Similar to other absorption sites, small lipophilic molecules show a high, transcellular permeability in the nose, while more hydrophilic molecules show a lower permeability and tend to pass the cell barrier paracellularly. Since the focus of this work is on characterising the effects of excipients in the nose and not on developing a specific formulation, the model drugs were not selected for their suitability for nasal application, but as commonly used quality control markers in permeability studies [108]. Metoprolol tartrate was selected as high and transcellular permeability model drug (logP: 1.6 [66]) and atenolol (logP: 0.5 [66]) was selected as moderate and paracellular permeability model drug.

Since the drug substances need to dissolve for permeation through the epithelium, the solubility of APIs is another factor affecting nasal drug delivery. While in liquid formulations the required solubility is determined by the volume of one spray puff, in powder formulations the drug must dissolve in the nasal fluid volume. The 24 h-solubility of atenolol in simulated nasal fluid was determined to be 16 mg/mL, which is classified as sparingly soluble in the Ph. Eur. 10.0. The solubility of metoprolol tartrate was above 1000 mg per mL SNF, which is classified as very soluble. A higher amount of metoprolol will thus dissolve in the nasal fluid, which may enhance the absorption of the drug. However, since the residence time of the drug in the nasal cavity is limited due to the mucociliary clearance, the systemic absorption is not only affected by the saturation solubility of the drug, but also by the dissolution velocity of the powder. In this regard, the particle size distribution of the drug powders should be taken into account, as the larger particle surface of small particles accelerates the dissolution rate. Figure 6-25 shows the distribution density and table 6-5 characteristic values of the particle size distribution of the APIs. The comparison of the two drug powders shows a larger mean particle size for atenolol, suggesting slower dissolution. While for atenolol (x_{10}=18 µm) a mainly nasal deposition of the drug powder would be expected, the metoprolol powder contains a considerable proportion of particles below 10 µm, which could probably lead to post-nasal deposition. However, as the nasal deposition profile was not the focus of this work, the drug powder was used without separating the fine fraction. The different particle sizes of the two drug powders potentially

allow the investigation of different influences of excipients on fast- and slow-dissolving drugs.

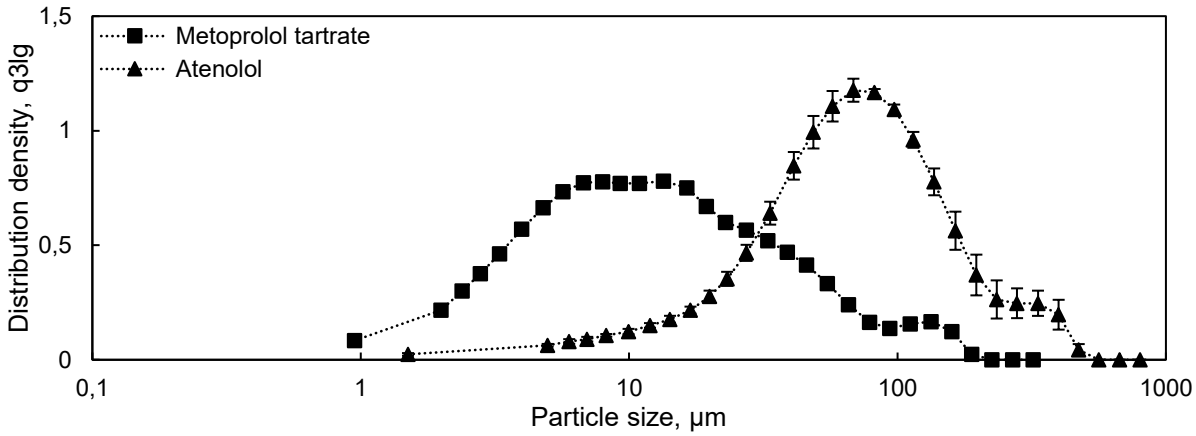

Figure 6-25: Particle size distribution of APIs. n=3; error bars show standard deviation.

Table 6-5: Characteristic values of the particle size distribution of APIs. n=3; ± standard deviation.

Sample	x_{10}, µm	x_{50}, µm	x_{90}, µm	Span
Metoprolol tartrate	2.8 ± 0.1	11.4 ± 0.4	51.6 ± 1.5	4.3 ± 0.0
Atenolol	18.4 ± 1.6	68.3 ± 4.8	186.1 ± 24.1	2.5 ± 0.2

Scanning electron microscope images (Figure 6-26) of the drug powders confirm the smaller particle sizes of metoprolol and show a needle-shaped particle form, while a round, platelet-shaped particle form is observed for atenolol.

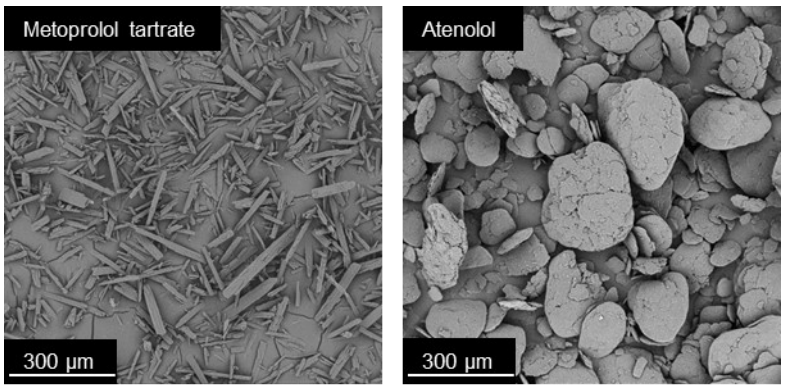

Figure 6-26: Scanning electron microscope images (250x magnification) of APIs.

6.2.1.3 Model formulations

For the preparation of model formulations, the model drugs were blended with the sieve fraction 32-150 µm of the selected excipients. Powder blends of the drug and soluble or insoluble fillers with or without mucoadhesive polymers were prepared. The pure drugs served as control in the following experiments. Table 6-6 summarises the composition of the assessed samples and the selected characteristics of the components.

Table 6-6: Composition of samples for the assessment of the influence of excipients in powder blends.

Sample	Composition	Substance	Selected characteristic
Control	100% API	Metoprolol tartrate (MET)	High permeability, very soluble
		Atenolol (ATN)	Moderate permeability, sparingly soluble
Blends without mucoadhesive	40% API	Metoprolol tartrate	
		Atenolol	
	60% filler	Mannitol	Water-soluble
		MCC	Water-insoluble
Blends with mucoadhesive	40% API	Metoprolol tartrate	
		Atenolol	
	50% filler	Mannitol	
		MCC	
	10% mucoadhesive	Pectin (PEC)	Anionic
		Chitosan glutamate (CHIT)	Salt of positively charged chitosan
		HPC M	Neutral

All powder blends met the defined specifications for drug content (90-110%) and homogeneity (RSD < 5%). Scanning electron microscope images of the blends (Figure 6-27 metoprolol tartrate as model drug and figure 6-28 atenolol as model drug) confirm the uniform distribution of drug and excipients and do not show agglomerates of pure API or pure excipients.

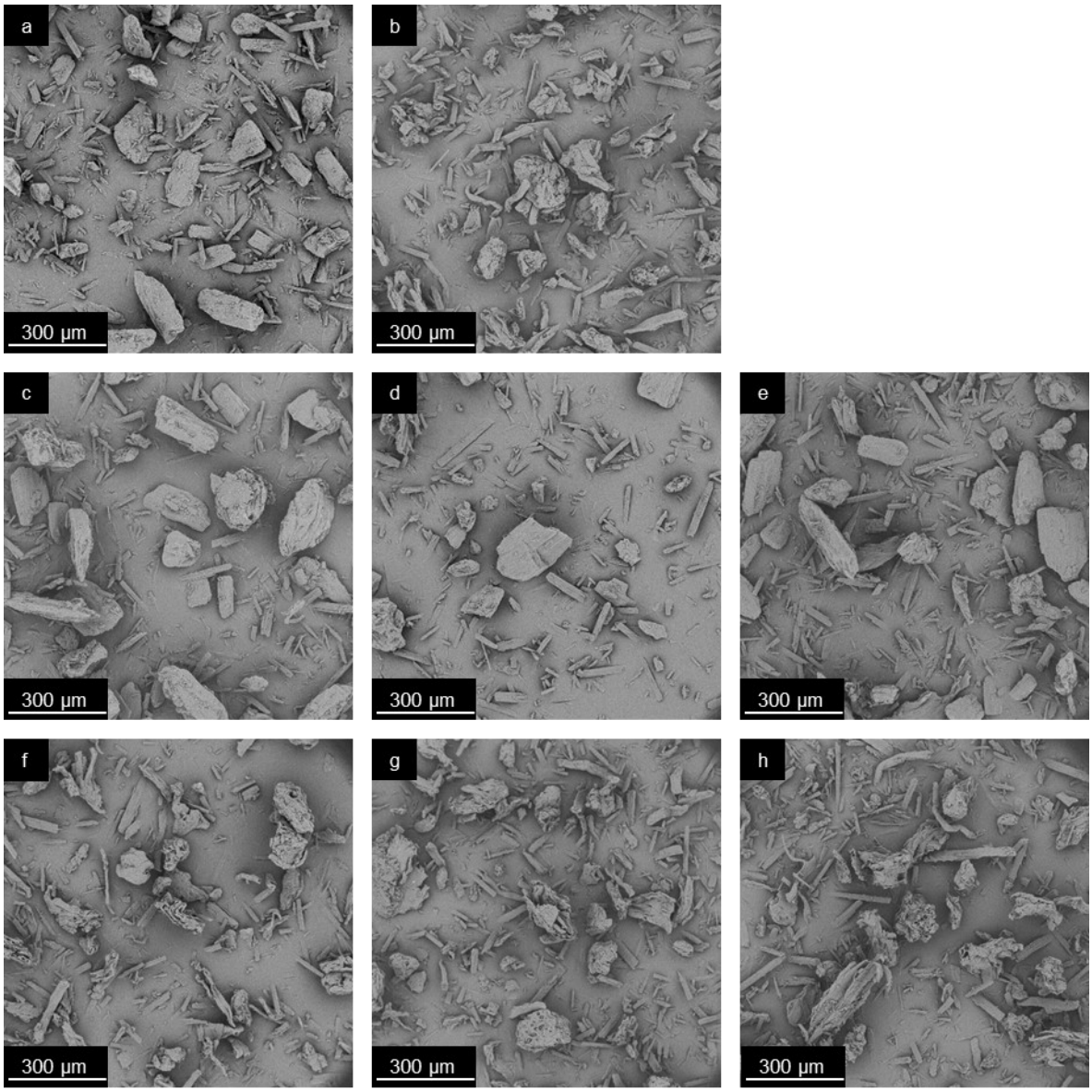

Figure 6-27: Scanning electron microscope images (250x magnification) of powder blends with metoprolol tartrate as model drug. a-b: blends without mucoadhesives, a: mannitol as filler, b: MCC as filler. c-e: blends with mannitol as filler with mucoadhesive, c: pectin, d: chitosan glutamate, e: HPC. f-h: blends with MCC as filler with mucoadhesive, f: pectin, g: chitosan glutamate, h: HPC.

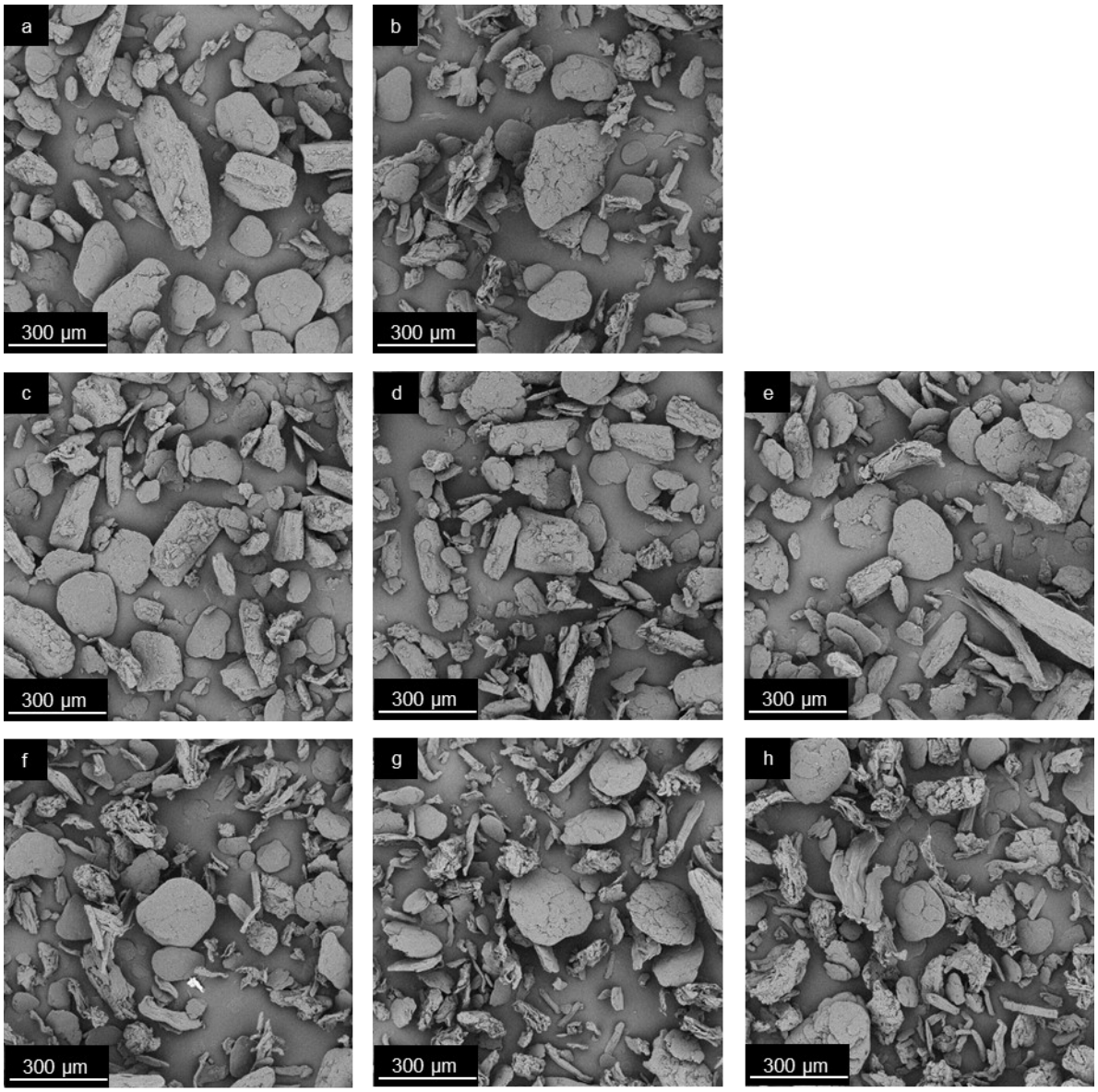

Figure 6-28: Scanning electron microscope images (250x magnification) of powder blends with atenolol as model drug. a-b: blends without mucoadhesives, a: mannitol as filler, b: MCC as filler. c-e: blends with mannitol as filler with mucoadhesive, c: pectin, d: chitosan glutamate, e: HPC. f-h: blends with MCC as filler with mucoadhesive, f: pectin, g: chitosan glutamate, h: HPC.

6.2.2 Characterisation of model formulations

For an effective selection of excipients in the development of nasal powder formulations, a comprehensive knowledge of their influences on drug absorption is essential. It must be noted that formulation strategies, such as the use of excipients with the aim of prolonging the nasal residence time, do not solely affect the desired process, but can also influence other factors, such as the dissolution of the powder and the diffusion of dissolved drug molecules. Major processes that influence the total outcome of systemic nasal drug delivery from powder formulations include the nasal clearance and thus the residence time of the formulation on the nasal mucosa, the dissolution and release of the drug from the formulation and the permeation of dissolved drug molecules through the nasal epithelium. Thereby, excipients can provide benefits to one or more processes, but they can also influence processes in such a way that the effect on drug absorption is counteracting. This chapter therefore characterises the influence of excipients in model formulations on the viscoelasticity of the nasal fluid, the dissolution and release of the drug and the permeation of the dissolved drug separately, in order to gain a comprehensive knowledge of the effects of the selected excipients and to detect additive and counteracting effects regarding drug absorption in the nasal cavity.

6.2.2.1 Resistance of powder formulations against the nasal clearance

This section investigates the ability of the prepared model formulations to prolong the nasal residence time of the drugs. Changes in the rheological behaviour of simulated nasal fluid served as surrogate for the provided resistance against the mucociliary clearance. As described in section 6.1.3, the viscoelastic properties of the nasal mucus can strongly influence the effectiveness of mucociliary transport. Thereby, elasticity is described to be most important for a sufficient mucus transport, with an optimal storage modulus of 1-2 Pa [4,10,86]. To model the influence of one powder dose in the nose, dispersions in SNF were prepared using an estimated nasal fluid volume of 200 µL. This estimation bases on the total surface area of the nose of 150 cm² and the thickness of the covering mucus layer of 10-15 µm [75,76]. One powder dose was set to 50 mg (20 mg drug content) for the formulations and 20 mg for pure API controls. In order to obtain enough sample material for the measurement, powder mass and fluid volume were scaled up. Figure 6-29 shows the frequency dependent storage and loss moduli of metoprolol tartrate-containing samples.

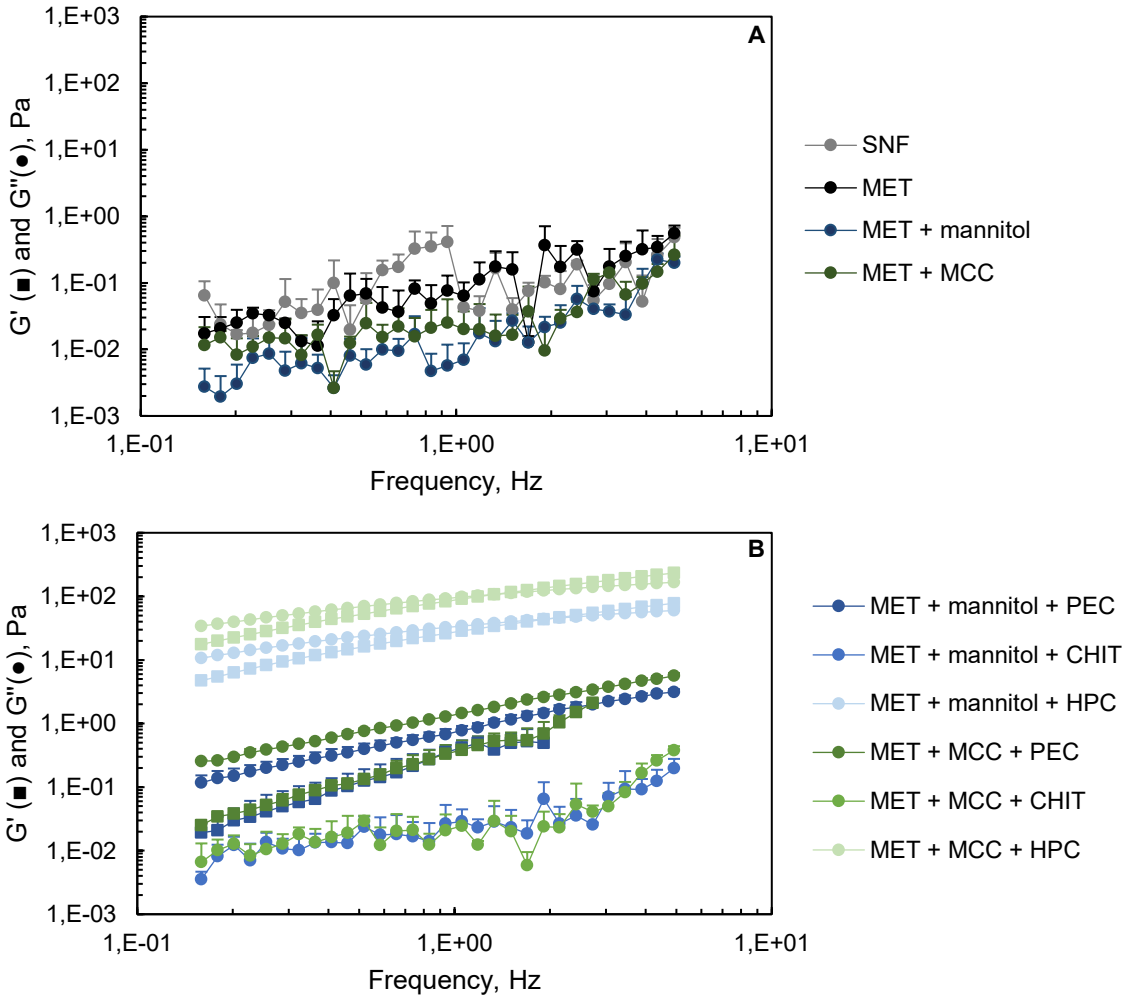

Figure 6-29: Frequency dependent storage (G', squares) and loss (G'', dots) moduli of metoprolol tartrate (MET)-containing model formulations in SNF. A: model formulations without mucoadhesive polymers and pure API and SNF as control (only G'' displayed). B: model formulations with mucoadhesive polymers. n=3; error bars show standard deviation; for the clarity of the graphs error bars are only displayed in positive direction. Storage moduli are not displayed when overlaying effects of instrument inertia occurred.

The pure API control showed no differences in the frequency dependent loss moduli compared to SNF. Since one dose of metoprolol tartrate dissolves completely in the used fluid volume, this finding is within the expectations. Powder blends without mucoadhesives did not cause changes in the loss moduli of SNF as well. While the mannitol-containing sample was completely dissolved, solid particles were present in the MCC-containing sample. However, the suspended MCC particles did not cause observable changes in the viscoelastic behaviour. Powder blends with mucoadhesive excipients showed differences in the viscoelastic behaviour depending on the mucoadhesive polymer used. Blends with chitosan glutamate did not cause observable changes compared to SNF, irrespective of the filler used. The addition of pectin or HPC-containing powder blends to SNF resulted in an increase of loss and storage moduli, which was more pronounced with HPC. Dispersions of the pectin-containing powder blends in SNF behaved as viscoelastic liquids (G'<G'') in

the assessed frequency range. The use of MCC as filler instead of mannitol resulted in increased loss moduli and thus, a more pronounced viscous behaviour. Dispersions of the HPC-containing powder blends behaved as viscoelastic liquids at lower frequencies and as viscoelastic gels (G'>G'') at higher frequencies, which indicates a more rigid entanglement network of the polymer chains at higher frequencies. Blends with MCC as filler provided higher loss and storage moduli and a slightly earlier point of intersection (G'=G''), as blends with mannitol as filler. Compared to the results of the rheological tests of the pure mucoadhesive excipients (Section 6.1.3.1.3), a similar behaviour of HPC and chitosan glutamate is found in the formulations, whereas pectin shows clearly lower loss and storage moduli when the formulations were used than as individual substance. This difference is probably due to the more uniform dispersion of pectin particles in SNF when using the formulations. No highly viscous areas were apparent, but rather homogeneous looking gels.

Figure 6-30 displays the frequency dependent loss and storage moduli of atenolol-containing samples. Pure atenolol showed an increase in loss and storage moduli compared to SNF, which can be attributed to suspended, undissolved atenolol particles. The dispersion showed a dominating elastic behaviour over the assessed frequency range. Powder blends with mannitol or MCC but without mucoadhesive showed similar frequency dependent loss and storage moduli. Hence, undissolved MCC particles did not show an additional effect. As in the metoprolol-containing blends, the effect of mucoadhesive excipients depended on the used substance. While the presence of chitosan glutamate in the powder blends did not cause observable changes in the viscoelastic behaviour compared to the pure drug, changes were observable in samples with pectin and HPC. All pectin-containing blends showed dominating viscous behaviour in the assessed frequency range. When mannitol was used as filler, the storage moduli were decreased compared to the pure drug dispersion. Blends with MCC as filler showed higher loss and storage moduli, with storage moduli in the range of the pure drug sample. An increase of loss and storage moduli with MCC as filler was also observed with the dispersions of HPC-containing powder blends. Samples which contain mannitol as filler behaved as viscoelastic fluid in the assessed frequency range and exhibited storage moduli in the same range as the pure drug. MCC-containing samples behaved as viscoelastic fluid at low frequencies and as viscoelastic gel at higher frequencies, which indicates a less flexible entanglement network of the polymer chains when MCC was present in the samples.

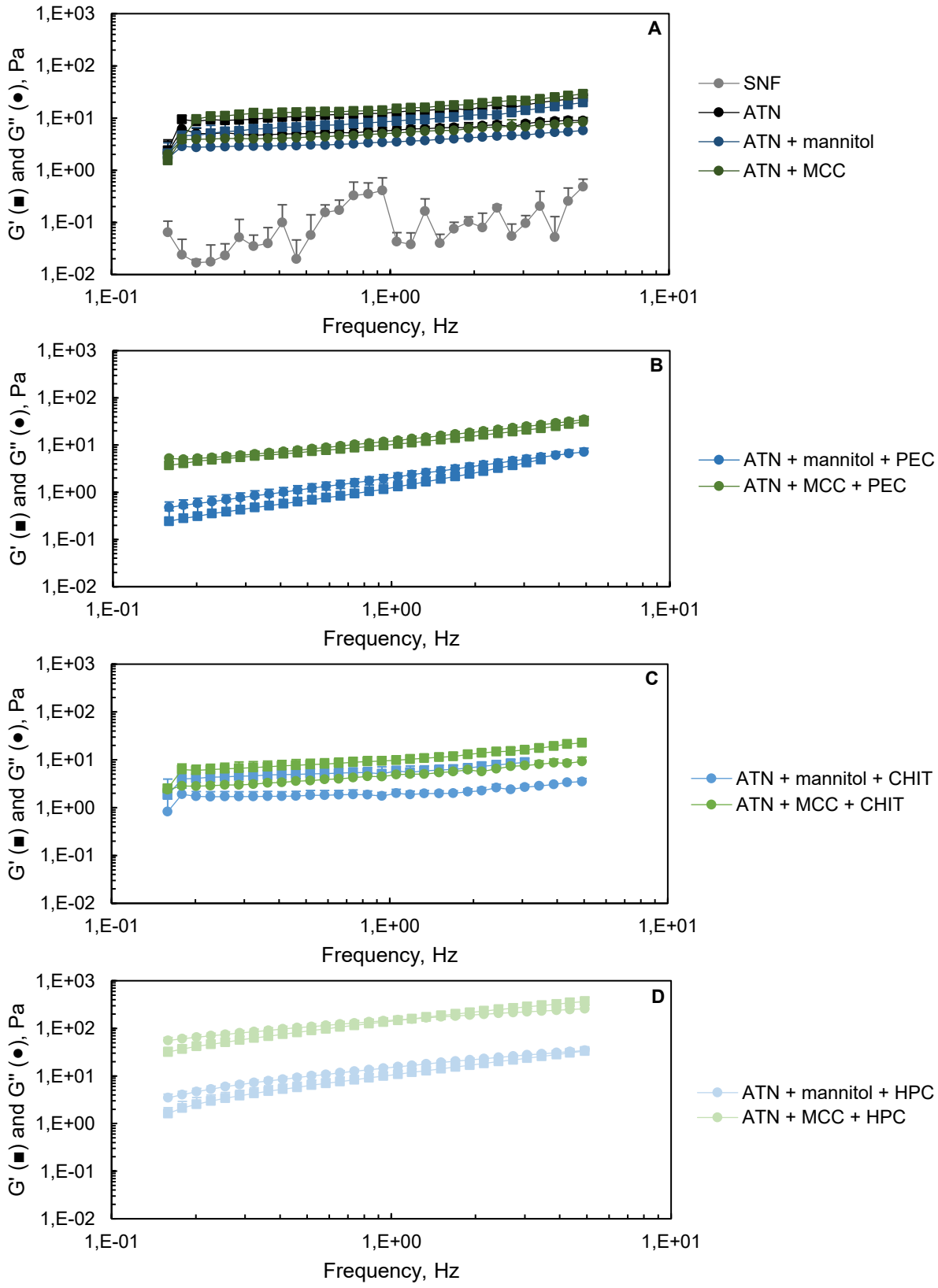

Figure 6-30: Frequency dependent storage (G', squares) and loss (G'', dots) moduli of atenolol (ATN)-containing model formulations in SNF. A: model formulations without mucoadhesive polymers and pure API and SNF as control. B-D: model formulations with mucoadhesive polymers. n=3; error bars show standard deviation; for the clarity of the graphs error bars are only displayed in positive direction. Storage moduli are not displayed when overlaying effects of instrument inertia occurred.

Figure 6-31 shows a comparison of storage and loss moduli of all samples at an oscillation frequency of 1 Hz in order to estimate a ranking of the formulations in terms of resistance to nasal clearance.

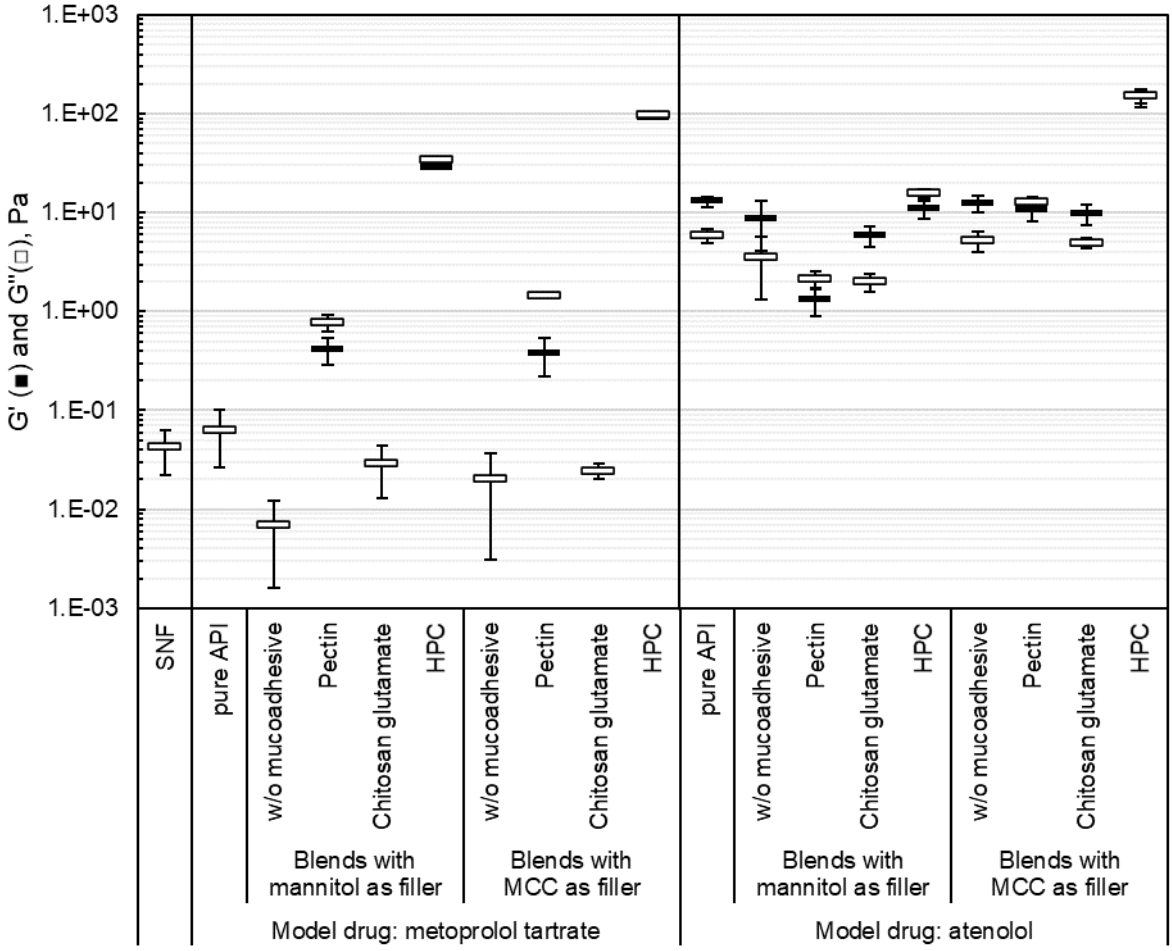

Figure 6-31: Comparison of the influence of different model formulations on the rheological properties of SNF. Storage (G', filled rectangles) and loss (G'', empty rectangles) moduli at an oscillation frequency of 1 Hz are displayed. G' is not displayed when overlaying effects of instrument inertia occurred. n=3; error bars show standard deviation [109].

The comparison shows that atenolol-containing samples exhibited generally higher storage and loss moduli than metoprolol-containing samples. The pure drug sample exhibited dominant elastic behaviour with a storage modulus of 13.0 ± 1.6 Pa and a loss modulus of 5.8 ± 0.9 Pa. With the exception of the blend containing mannitol as filler and pectin as mucoadhesive all blends that contained atenolol showed storage moduli of above 2 Pa, which suggests a decrease of the nasal clearance rate with these formulations [86]. Blends with mannitol as filler and pectin or chitosan glutamate as mucoadhesive exhibited significantly lower values of G' than the pure drug control (p=0.0003 and p=0.004 for pectin and chitosan glutamate, respectively). The only formulation that caused a significant increase in storage modulus compared to the pure drug was the formulation that contained MCC as insoluble filler and HPC as mucoadhesive (G'=146.9 ± 31.0 Pa; p=0.02). This

formulation is therefore assumed to have the greatest resistance to nasal clearance and thus the longest residence time in the nose. The measurement of storage modulus of the pure metoprolol tartrate sample was not possible due to overlaying effects of instrument inertia, which impaired the measurement. However, since the sample was a solution of small molecules in water, ideal viscous behaviour, and thus G'→0 Pa can be assumed. The recorded loss modulus of the sample was similar to pure SNF (0.06 Pa ± 0.04 Pa and 0.04 ± 0.02 Pa for metoprolol and SNF, respectively). Therefore, no changes in the nasal clearance due to the deposition of the metoprolol tartrate powder in the nose can be assumed. The addition of both fillers with or without chitosan glutamate as mucoadhesive did not cause measurable changes of the rheological properties. The measurement of storage moduli was possible for samples that contained pectin or HPC. Higher storage (28.6 ± 1.1 Pa and 90.6 ± 1.7 Pa with mannitol and MCC, respectively) and loss moduli of the HPC-containing blends indicate the strongest resistance of these formulations against the nasal clearance among the metoprolol-containing blends.

The comparison of the different model formulations shows that undissolved drug particles, as present in atenolol-containing formulations, may cause sufficient resistance to the mucociliary clearance to prolong the nasal residence time (G'>2 Pa), due to interactions of the particles as an elastic network. Prolonged nasal residence time of formulations containing insoluble components has also been shown by Ishikawa et al. in in vivo experiments in rats [23,24]. Powder formulations containing precipitated calcium carbonate as insoluble excipient significantly prolonged the residence time of elcatonin [24] and fluorescein isothiocyanate dextran [23] in the nasal cavity of rats compared to liquid formulations or formulations with lactose as soluble excipient. This suggests a higher resistance of the insoluble powder against the mucociliary clearance. In this work, however, an elastic behaviour of samples, and thus probably higher resistance against nasal clearance, was not observable when MCC was used as insoluble component. No measurable changes in the elasticity occurred with powder blends with metoprolol tartrate or atenolol and MCC compared to the pure drug. Whether particle interactions occur, which cause an elastic behaviour of the sample therefore not only depends on the presence of insoluble particles, but also on the further properties, like particle size and shape, occurring particle interactions and the concentration of particles in the suspension [110]. If a resistance against the nasal clearance is caused by undissolved drug particles, as found in this work, the effect decreases with progressing dissolution of the drug in the nasal fluid. Elastic behaviour due to insoluble excipients would cause a more constant effect and may therefore be considered beneficial. Screening of insoluble excipients with regard to their rheological behaviour may help identifying suitable excipients. While a longer nasal residence time may be achieved by using insoluble excipients, different studies have shown

opposite effects when soluble substances were used [23,27,40]. Tanaka et al. found an accelerated clearance of powders containing lactose or sodium chloride compared to pure drug powders [40]. This effect was attributed to an increase in fluid volume and thus a reduction in viscosity of the nasal mucus, due to the osmotic activity of the used substances. Djupesland and Skretting describe a decelerated clearance of a lactose powder compared to a liquid spray in the first minutes after deposition, which then accelerated to a faster overall clearance [27]. The authors attributed the initially slower clearance to the time required for the lactose powder to dissolve, while the overall faster clearance was assigned to a higher deposited amount in ciliated regions of the nose for the powder formulation in this study. The rheological assessments of powder formulations in this work observed no differences in the rheological behaviour of completely dissolved formulations (pure metoprolol tartrate and powder blend with mannitol) compared to pure SNF, indicating no increase in resistance against the nasal clearance with these formulations. However, reduced resistance, due to water influx from underneath tissues cannot be displayed in the used setup, but is conceivable in vivo. The rheological tests in this work have not only shown the influence of soluble and insoluble powder components, but also the influence of mucoadhesive polymers. Depending on the substance used, mucoadhesive polymers can lead to an increase in storage and loss modulus and thus increase the resistance of the formulation to nasal clearance. Among the excipients tested in this work, this effect was particularly strong for HPC. An increase in the nasal residence time of powder formulations when HPC was used as an excipient was also shown by Tanaka et al. in in vivo studies in rats [106]. The effect was more pronounced with HPC grades, showing higher viscosity.

As described in section 6.1.3.1.1, SNF was chosen as the dispersion medium for the rheological experiments in this work to assess the gel-forming properties of the excipients and formulations at a physiological pH and ionic composition. However, SNF does not reflect the viscoelastic properties of nasal mucus. Therefore, the data obtained cannot reflect the rheological properties in vivo, but is particularly suitable for the comparison of different formulations. In this context, the use of SNF offers a high degree of standardisation compared to mucus sources.

6.2.2.2 Influence of excipients on drug dissolution

Substances used in nasal formulations to prolong the residence time of the drug on the nasal mucosa, such as mucoadhesive and insoluble substances, will not solely affect this process, but will influence other processes in the course of drug absorption via the nose. Especially for formulations containing swelling polymers, a retardation of the dissolution and release of the drug in the nasal fluid can be expected. On the contrary, the use of soluble substances has the potential to increase the nasal fluid volume by inducing an osmotic effect, thus favouring the dissolution of the drug [40]. This section therefore assesses the

influences of the selected fillers and mucoadhesive polymers on the dissolution and release of the drugs in the prepared model formulations. Franz cells were used to model the dissolution of the drug on a wet surface. The setup allows the separation of a donor and an acceptor compartment with a membrane. In the conducted experiments, the dissolution of the powder occurred only on the wetted membrane, while the larger acceptor medium allowed the uptake of the already dissolved drug. In the experiments, the maximum final concentration of the drugs in the acceptor medium did not exceed one third of the saturation concentration (16 mg/mL for atenolol and >1000 mg per mL SNF for metoprolol tartrate), thus sink conditions can be assumed.

In order to characterise the dissolution processes, the obtained data was fitted to the Weibull model and linearised according to equation 6-1. The dissolution time t_d ($a=t_d^b$), which represents the time to dissolve and release 63.2% of the drug is used to compare the dissolution velocity of the formulations (Table 6-8).

Equation 6-1: Linearisation of the dissolution and release data according to the Weibull model. m=dissolved fraction of the drug at time t; a=time scale, b=shape parameter.

$$\log(-1\ln(1-\mathrm{m})) = \mathrm{b}\times\log(\mathrm{t}) - \log(\mathrm{a})$$

In order to compare the dissolution profiles of the formulations with the dissolution curve of the pure drug, the similarity factor f_2 was calculated according to equation 6-2. Table 6-7 displays the corresponding f_2-values. f_2-values of 50-100 indicate similarity of the dissolution profile.

Equation 6-2: Calculation of the similarity factor f_2. n=number of time points; R(t)=mean percent reference drug dissolved at time t; T(t)=mean percent test drug dissolved at time t.

$$\mathbf{f_2 = 50\times\log\left(\frac{100}{\sqrt{1+\frac{\sum_{t=1}^{t=n}(R(t)-T(t))^2}{n}}}\right)}$$

Figure 6-32 displays the dissolution profile of pure metoprolol tartrate and the dissolution and release from the model formulations. The dissolution curve of the pure drug control reached a plateau after 20 min, indicating the complete dissolution of the drug powder. Thus, the drug is completely dissolved within the physiological half-life of nasal clearance of 15-20 min [4]. This finding is in accordance with the small particle size of the powder and the very high solubility of metoprolol tartrate in SNF, which suggested a fast dissolution.

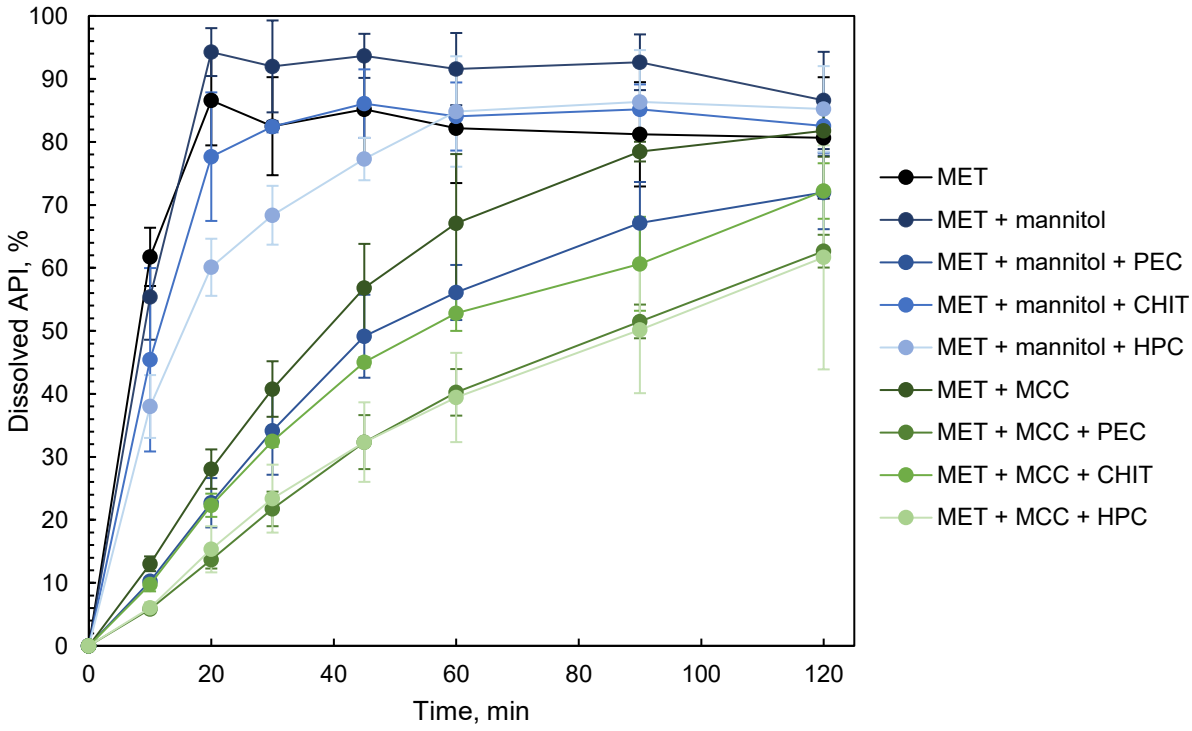

Figure 6-32: Dissolution and release of metoprolol tartrate from the model formulations in comparison to the pure API. n=3; error bars show standard deviation [109].

The powder blend containing mannitol as soluble filler exhibited a similar dissolution profile to the pure drug (f_2>50). A study by Tanaka et al. describes an accelerated dissolution of active ingredients in powder formulations with sodium chloride and lactose as soluble fillers, which is attributed to the osmotic pressure created by the dissolved molecules and thus an increase in the nasal fluid volume [40]. An accelerated dissolution of metoprolol tartrate in the presence of the soluble filler mannitol was not detected in this work. However, this may be attributed to the experimental setup. The donor and acceptor compartment of the Franz cells were separated by a cellulose acetate membrane with a pore diameter of 0.45 µm. The membrane was intended to physically separate the powder from the acceptor medium in this study, but not to provide a permeation barrier. Dissolved mannitol molecules therefore diffuse into the acceptor compartment in the same way as dissolved drug molecules. Hence, the osmotic pressure in the donor compartment will get reduced over time. In contrast to that, mannitol shows a low permeability through the nasal epithelium due to its hydrophilicity [72] and thus can cause an increase in fluid volume in vivo. Hence, the used setup may underestimate the effect of osmotically active substances. The influence of mucoadhesive excipients depended on the substance tested. The dissolution and release from chitosan glutamate-containing formulations was still similar to the pure drug (f_2>50, t_d=15.6 min), while the presence of pectin or HPC in the powder blends resulted in a stronger decrease of the dissolution rate of metoprolol tartrate. The swelling of the polymers upon contact with fluid causes a gel barrier from which the drug molecules in

solution have to be released. The decrease in dissolution rate was more pronounced with pectin than with HPC, which is shown by a mean dissolution time, derived from the Weibull function, of 25.2 min with HPC and of 78.7 min with pectin (f_2-factors of 19.78 and 41.09 with pectin and HPC, respectively). If diffusion of the dissolved drug molecules through the gel layer is assumed to be the mechanism causing the decrease, a stronger effect with HPC would have been expected based on the results of the rheological study (Section 6.2.2.1), which showed higher viscous moduli in formulations with HPC. Ionic interactions between anionic pectin and protonated metoprolol molecules may therefore be a possible mechanism that contributes to the reduced release from the pectin-containing formulation. Prolonged release from other drug-polyelectrolyte complexes has already been described in literature [111,112]. Powder blends containing MCC as insoluble filler with or without mucoadhesives showed differences in the dissolution curves (f_2<50) due to a decrease in dissolution rate compared to the pure drug. For the formulation without mucoadhesive (t_d=59.8 min), this effect can be attributed to the smaller amount of drug particles that are directly in contact with the wetted membrane due to the presence on insoluble excipient particles. It can be assumed that this effect is less pronounced in vivo, since the total area on which a powder dose is distributed is significantly larger than the diffusion area of the Franz cell of 1 cm². The presence of mucoadhesives in the MCC-containing formulations further decelerated the dissolution and release of the drug, with pectin or HPC-containing blends showing the slowest dissolution and release rates among all tested samples (t_d=113.7 and 117.0 min and f_2=14.81 and 14.97 with pectin and HPC, respectively).

Figure 6-33 displays the dissolution and release curves of pure atenolol and atenolol-containing formulations. Pure atenolol showed slower dissolution (t_d=99.3 min) compared to pure metoprolol tartrate, which is in accordance with the expectations due to the lower saturation solubility and the larger mean particle size of atenolol. Within the physiological half-life of clearance of 20 min, only 17.3 ± 2.4% was dissolved in the acceptor medium, whereas pure metoprolol tartrate was already completely dissolved. Formulations of atenolol and mannitol with or without chitosan glutamate resulted in similar dissolution profiles as the pure drug (f_2=62.50 and 72.48 with and without chitosan glutamate, respectively), but slightly increased values for the Weibull derived dissolution time (t_d=125.4 min and 112.4 min with and without chitosan glutamate, respectively). The dissolution curves from all other formulations differ (f_2<50) from the dissolution of the pure API. The differences are based on a reduced dissolution and release rate from these formulations, represented in increased t_d values (see table 6-8). The effects of the individual excipients used in these formulations are comparable to the results of the metoprolol-containing formulations. However, the dissolution curves are less distinguished. A comparison of the formulations revealed similarity in the release profiles (f_2>50).

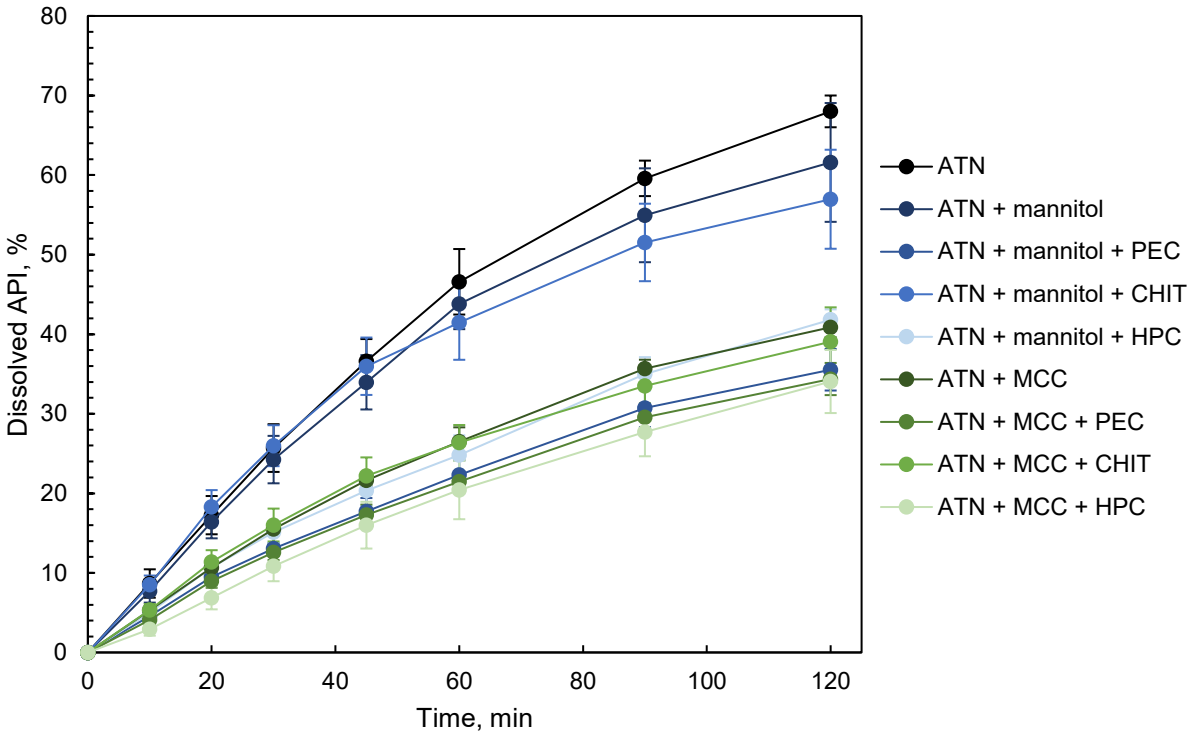

Figure 6-33: Dissolution and release of atenolol from the model formulations in comparison to the pure API. n=3; error bars show standard deviation [109].

Table 6-7: Similarity factors f_2 of the dissolution profiles. Model formulations are compared with the pure drugs as reference. F_2-factors of 50-100 indicate similarity of the dissolution curves. Mean values of three dissolution curves were used for calculation [109].

Formulation			Reference	
			Metoprolol	**Atenolol**
Blends without mucoadhesive	Mannitol as filler		53.16	72.48
	MCC as filler		22.78	38.07
Blends with mucoadhesive	Mannitol as filler	Pectin	19.78	33.92
		Chitosan glutamate	56.71	62.50
		HPC	41.09	37.54
	MCC as filler	Pectin	14.81	33.15
		Chitosan glutamate	18.87	37.18
		HPC	14.97	32.08

Table 6-8: Correlation coefficient of the linearised Weibull function and derived dissolution time t_d. Mean values of three dissolution curves were used for calculation.

Formulation			R^2		t_d, min	
			Metoprolol	Atenolol	Metoprolol	Atenolol
Pure drug			N/A	0.9980	N/A	99.3
Blends without mucoadhesive	Mannitol as filler		N/A	0.9934	N/A	112.4
	MCC as filler		0.9837	0.9954	59.8	221.9
Blends with mucoadhesive	Mannitol as filler	Pectin	0.9783	0.9960	78.7	281.6
		Chitosan glutamate	0.9421	0.9832	15.6	125.4
		HPC	0.9893	0.9958	25.2	223.7
	MCC as filler	Pectin	0.9934	0.9932	113.7	282.7
		Chitosan glutamate	0.9823	0.9879	85.8	241.6
		HPC	0.9872	0.9929	117.0	253.0

N/A: Data not available due to lack of datapoints before the plateau is reached.

A successful enhancement of drug absorption from powder formulations requires a balance of nasal residence time and sufficient dissolution and release of the drug. To compare the potential of the formulations to extend the nasal residence time against the impact on dissolution, figure 6-34 and figure 6-35 plot the storage and loss moduli of the formulations in SNF against the dissolved and released amount of drug after 20 min, which marks the end of the physiological half-life of nasal clearance.

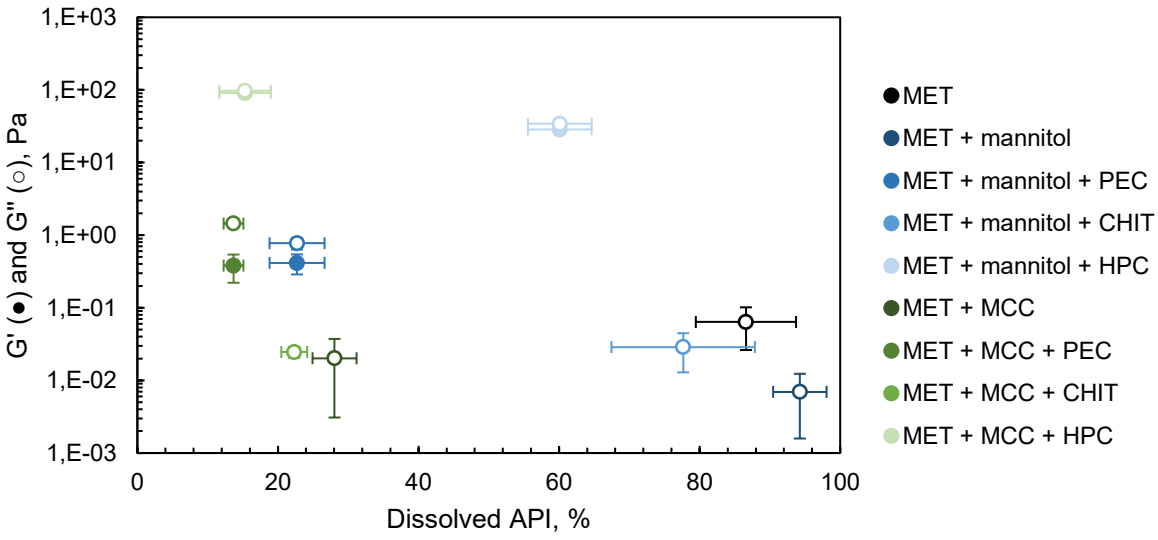

Figure 6-34: Storage (G', filled symbols) and loss (G'', empty symbols) moduli of metoprolol-containing formulations in SNF plotted against the dissolved and released amount of API after 20 min. n=3; error bars show standard deviation.

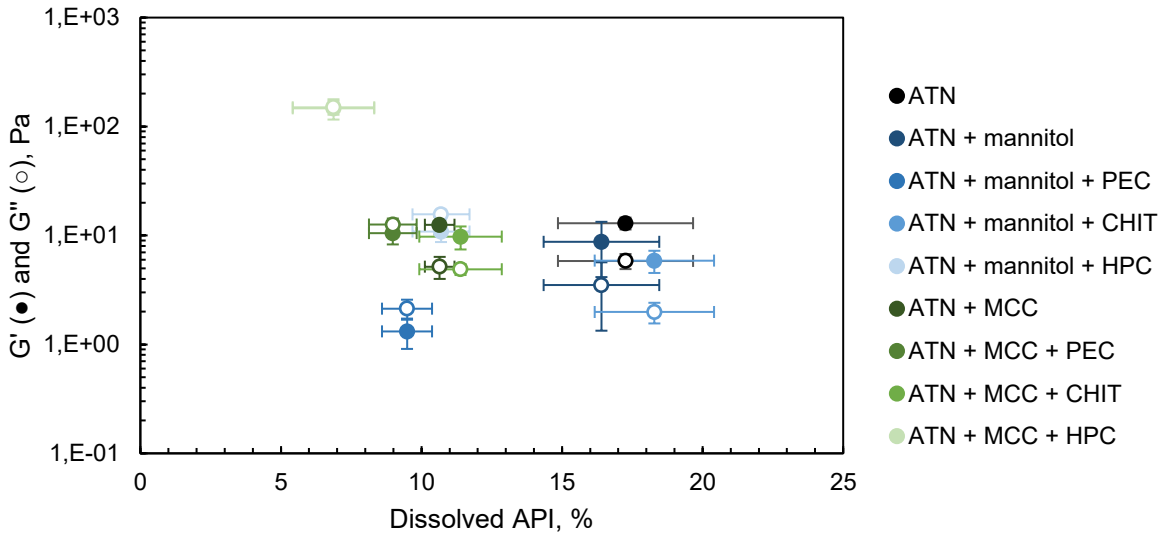

Figure 6-35: Storage (G', filled symbols) and loss (G'', empty symbols) moduli of atenolol-containing formulations in SNF plotted against the dissolved and released amount of API after 20 min. n=3; error bars show standard deviation.

With metoprolol tartrate as model drug, the comparison shows sufficient drug dissolution of above 75% with the pure drug, and with formulations that contain soluble excipients that did not increased the storage or loss modulus. The use of excipients, which increase the storage and loss modulus is thus not required and potentially disadvantageous if only drug dissolution is considered. For systemic nasal drug delivery, however, the dissolved drug molecules need to permeate through the nasal epithelium. In terms of metoprolol, which is considered as high permeability drug (BCS class I), the physiological nasal residence time may be sufficient for drug permeation as well. If the results are transferred to drugs, which show fast dissolution, but low permeability (BCS class III), however, the use of excipients can be required for sufficient absorption. Two strategies to improve the absorption are conceivable in this case. Since the dissolution of the drug is fast, an adequate absorption may be achieved, if the permeation through the epithelium is enhanced. Among the excipients used in this study, chitosan glutamate is reported to show permeation enhancing effects, while it only showed minor effects on drug dissolution in this study. A second option for an increased drug absorption is the prolongation of the nasal residence time and thus, the time for permeation. Figure 6-34 reveals beneficial properties for that purpose for the formulation containing mannitol as soluble filler and HPC as mucoadhesive, as it provided high storage and loss moduli while decreasing the dissolution and release of the drug to a smaller extent than other formulations with impact on the rheological properties. The considerations based on this study for the use of excipients with fast-dissolving drugs are supported by a study of Tanaka et al., which found an positive effect of HPC on the absorption of sumatriptan as drug with high solubility and low permeability, but not for warfarin as drug with high solubility and high permeability [106]. The use of excipients can also be considered for well absorbed drugs (fast dissolution and fast permeation) in order

to adjust the pharmacokinetic profile. Excipients, which extend the drug release and prolong the nasal residence time can be used in this regard to attenuate peak plasma concentrations and to enhance the duration of action. This strategy is for example used in nasal fentanyl formulations, containing pectin as dissolution and release modulator [60].

In contrast to metoprolol tartrate, the pure atenolol powder did not show sufficient dissolution within the physiological nasal residence time (17.3 ± 2.4% dissolved after 20 min, Figure 6-35). Hence, a prolongation of the nasal residence time is required in order to allow adequate absorption. Figure 6-35 reveals that the blend, which contains MCC as insoluble filler and HPC as mucoadhesive is the only formulation that increased storage and loss moduli compared to the pure drug. However, this formulation is also causing the strongest retardation of drug dissolution and release. The use of the soluble filler mannitol with HPC increased only the loss modulus but not the storage modulus compared with the pure drug, but the dissolution and release of the drug from this formulation after 20 min was significantly higher ($p=0.02$) than with MCC as filler. In vivo studies are needed to assess whether the potential of the formulations to increase the nasal residence time outweighs the reduction in drug dissolution and release and whether the respective formulation is therefore of overall benefit.

6.2.2.3 Influence of excipients on drug permeation

Systemic drug delivery via the nose requires permeation of the drug through the nasal epithelium. While small, lipophilic molecules with high permeability have been shown to have a high bioavailability even in simple liquid formulations, small polar molecules or biomacromolecules with low permeability tend to require more sophisticated formulations [3]. The permeability of the drug is therefore a crucial factor in determining the need for excipients in nasal formulations. For low-permeability drugs, the use of excipients may increase bioavailability, e.g., by increasing the nasal residence time and thus the time for permeation, or by directly improving the permeation of the drug through the epithelium. This section investigates the effect of the excipients selected for the model formulations on the permeation of the model drugs. In order to enable a differentiation of the effects of the excipients on the dissolution (see section 6.2.2.2) and on the permeation of the drug, the model formulations were not used as powders in this study, but drug solutions, containing single excipients were used.

6.2.2.3.1 RPMI 2650 cell line as permeation model

In order to assess drug permeation through the nasal mucosa in in vitro experiments, the use of primary and immortalised cells is conceivable. Primary cells from human donors provide high histological similarity to the nasal mucosa, however, showing higher barrier properties as excised tissue, reflected in a higher transepithelial electric resistance (TEER)

and lower permeability of hydrophilic compounds [70]. Primary cultures may also be subject to inter-individual differences, making the use of immortalised cell lines more standardisable [70]. The human nasal immortal cell line RPMI 2650 shows some histological differences compared to the nasal mucosa, however, several studies have found that RPMI 2650 cells, grown at an air-liquid interface, show permeation barrier properties comparable to human nasal mucosa in terms of TEER and the permeation of marker substances [70,71,73,113]. Furthermore, the presence of four tight junction proteins (ZO-1, occluding, claudin-1 and E-cadherin) [72,73] and mucus production [73] was proven in RPMI 2650 cell models. RPMI 2650 cells grown at an air-liquid interface were therefore selected for the permeability studies in this work. Cultivation conditions can strongly influence the outcome of cell experiments. A study of Reichl and Becker has shown an impact of growth medium and filter material, used as growth substrate, on the barrier expression of RPMI 2650 cells [70]. Cultivation with serum-containing Minimal Essential Medium on PET inserts resulted in the highest TEER and permeation properties similar to nasal mucosa in [70] and was therefore used for the permeation studies in this work.

Figure 6-36 displays the development of TEER of RPMI 2650 cells seeded on PET inserts. At the end of the growth period in liquid covered culture on day seven, a mean TEER of 37 ± 11 Ω*cm² was reached. After the cells were lifted to an air liquid interface (ALI), the TEER further increased and reached a stable plateau at day 18 (11 days of ALI) with a mean TEER of 84 ± 2 Ω*cm², which is in the range of human nasal mucosa of 75-180 Ω*cm² [70] and in accordance with TEER values reported from other studies [70,71,113]. Visual inspection of the cells grown in the ALI under the light microscope (Figure 6-37) showed a evenly distributed, homogeneous cell layer with similar morphology as shown in [72].

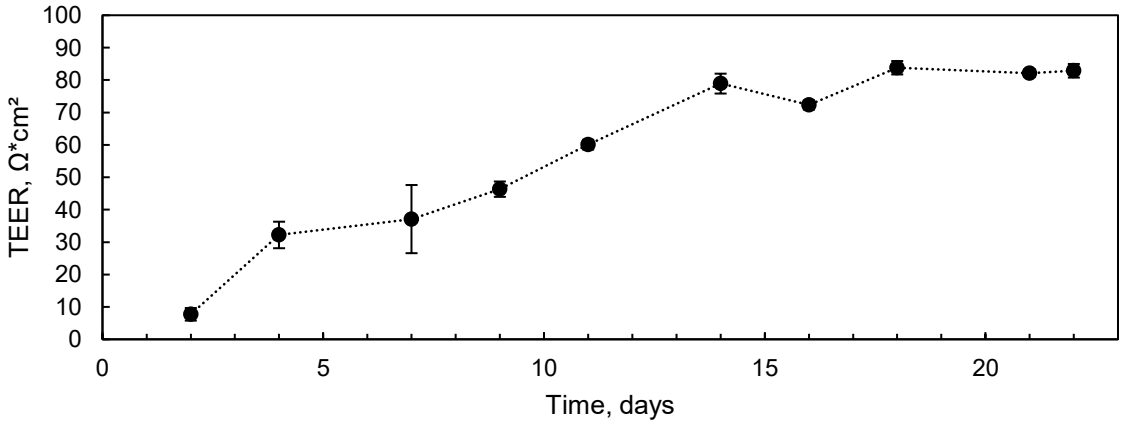

Figure 6-36: TEER values of RPMI 2650 cells over time. Cells were lifted from liquid covered culture to air liquid interface on day 7. n=6; error bars show standard deviation.

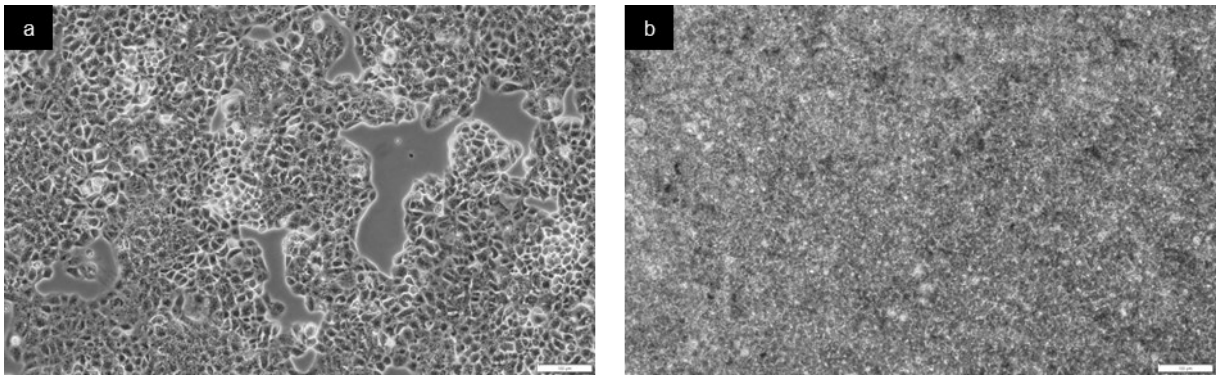

Figure 6-37: Microscopic image (10x magnification) of RPMI 2650 cells grown in a 75 cm² cell culture flask (a) and on PET inserts at an air liquid interface (14 days ALI culture) (b).

Figure 6-38 compares the permeation of the selected model drugs metoprolol and atenolol through the RPMI 2650 cell layer and through the blank filter inserts without cells.

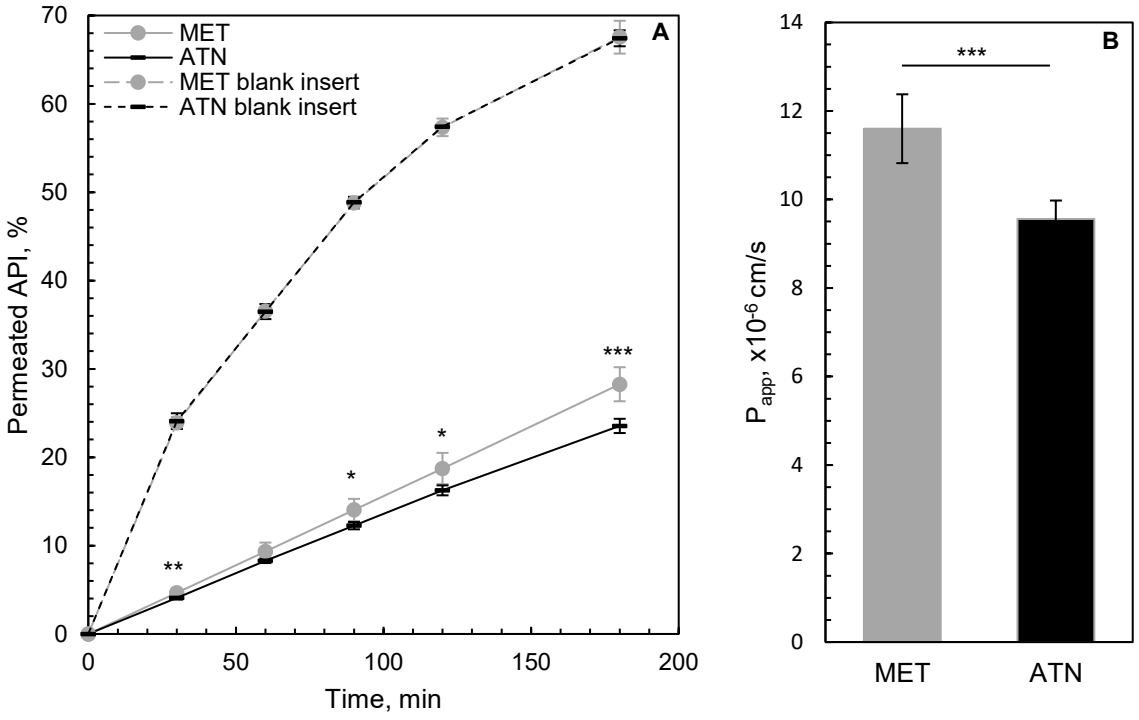

Figure 6-38: A: Permeation of metoprolol (MET) and atenolol (ATN) through RPMI 2650 cell layer or blank filter inserts without cells. B: Permeability coefficients (P_{app}) calculated for the permeation of drugs through the cell layer. n=6; n=3 for blank inserts; error bars show standard deviation; *=p<0.05; **=p<0.01; ***=p<0.001.

Both drugs showed no difference in permeation through the blank filter insert, which proves, that the filter did not retain one of the drugs specifically. The permeation of the drugs through the cell layer was clearly decreased, with metoprolol showing a faster permeation than atenolol. The permeability coefficient (P_{app}) of metoprolol was 11.6 ± 0.8 x10^{-6} cm/s and thus significantly (p=0.0002) higher than the permeability coefficient of atenolol 9.6 ± 0.4 x10^{-6} cm/s. This permeation behaviour is within the expectations due to the higher lipophilicity of metoprolol. Based on its intestinal permeability metoprolol is classified as high

permeability drug by the FDA, while atenolol is classified as moderate permeability drug [108]. A study of Sibinovska et al. obtained a similar permeability coefficient for metoprolol through a RPMI 2650 cell model (12.8 ± 0.9 x10^{-6} cm/s) and a slightly lower permeability coefficient for atenolol (6.6 ± 0.4 x10^{-6} cm/s).

6.2.2.3.2 Permeation studies

Figure 6-39 shows the influence of the selected excipients in solution or dispersion on the permeability of metoprolol and atenolol.

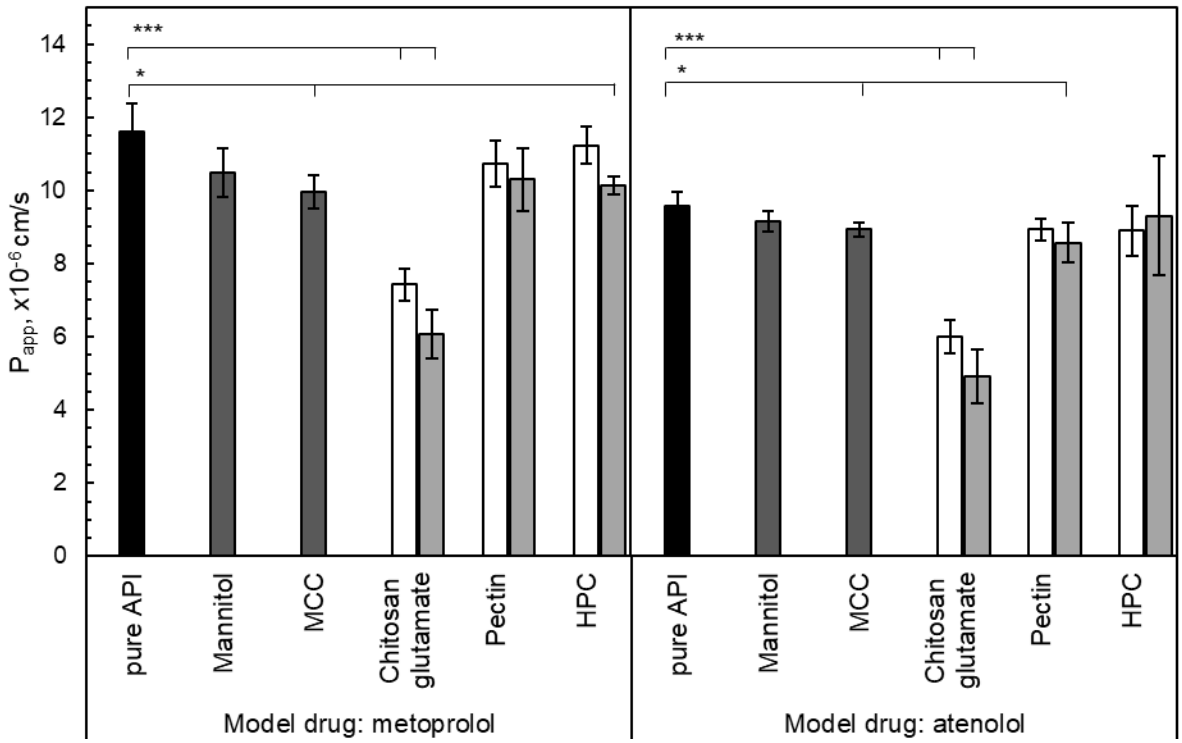

Figure 6-39: Permeability coefficients of metoprolol and atenolol in presence of different excipients. Colouring of the bars shows the excipient concentration: black – no excipient, dark grey – 1%, light grey – 0.5%, white – 0.25%. n=6 for pure APIs and n=3 for samples with excipients; error bars show standard deviation; *=p<0.05; ***=p<0.001 [109].

The assessment of the selected fillers mannitol and MCC showed no influence on the permeability of both model drugs with the soluble filler mannitol, while MCC caused a significant reduction of the permeability coefficients. In contrast to the other tested excipients, MCC stayed undissolved in the drug solution due to its insolubility in water. Therefore, the undissolved MCC particles deposited on the cell layer and caused an additional barrier through which drug molecules had to diffuse before getting in contact with the cell surface. Hence, this diffusion barrier is assumed to have caused the reduction in permeation rate. In samples with atenolol and MCC, a significant reduction of TEER was observed after the experiment. Since no increase in permeation rate occurred, this change, however, may be due to remaining MCC particles on the cell layer, which may have

impaired the measurement. The mean TEER after the experiment was still above 75 Ω*cm² (Table 6-9).

The investigation of the influence of the mucoadhesive excipients on drug permeability showed a small influence of pectin and HPC in the tested concentrations. A significant decrease in the permeability coefficient was found in samples containing metoprolol and HPC (0.5%; p=0.018) and atenolol and pectin (0.5%; p=0.018). In the case of HPC (0.5%), this reduction in permeability may be due to an entrapment of the drug molecules in a gel layer on the cell surface, which acted as a diffusion barrier, as the calculated mass balance yielded a recovery of only 82 ± 3% with metoprolol as model drug and 88 ± 6% with atenolol as model drug. For all other samples, the mass balance showed a recovery of 90-110%. Decelerated diffusion due to an increase in viscosity of the drug solution would also be conceivable as mechanism with pectin-containing samples. However, an entrapment of drug molecules, resulting in a reduced mass recovery, was not observed. Another process that influences the permeability of the model drugs in the presence of pectin is its slightly acidic reaction in aqueous solutions. Table 6-10 summarises the pH of drug solutions in the assay buffer with the different excipients used. Since metoprolol and atenolol are weak bases (pk_a=9.67 [114]), at lower pH a greater proportion is in the ionised form, which contributes less to permeation. In a study of Jacobsen et al., the permeability coefficient of metoprolol through an artificial lipid barrier was reduced from 12.4 x 10^{-6} cm/s at pH 7.4 to 5.98 x 10^{-6} cm/s at pH 6.5 [115]. However, a study of Hagesaether, which investigates permeation modulation effects of natural polymers on human colon adenocarcinoma cells (HT29-MTX) describes a permeation decreasing effect of low methoxylated pectin also in solutions with an adjusted pH of 7.4. This influence of pectin is attributed to a cell membrane protective effect by the author [116]. The presence of chitosan glutamate significantly (p<0.001) decreased the permeability coefficients of metoprolol and atenolol through the RPMI 2650 cell layer. This finding was against the expectations, as, different from the other mucoadhesives tested, chitosan glutamate is reported to show permeation enhancing effects due to the opening of tight junctions [64]. TEER measurements after the permeation studies revealed a significant decrease of TEER after contact of the cell layer with 0.25% (p=0.009 and p<0.001 with metoprolol and atenolol, respectively) or 0.5% chitosan glutamate (p=0.002 and p=0.013 with metoprolol and atenolol, respectively). This reduction of the cellular barrier possibly indicates tight junction opening, however, without resulting in an increase in transport rate of the model drugs. Toxic effects of the excipients can also lead to a reduced integrity of the cell layer and thus to a reduction of the TEER. Therefore, concentrations of excipients that did not show any toxic effects after an incubation time of 24 h in the MTT assay were generally used for the permeability studies. In a study by Illum et al. optimal permeation-increasing effects were obtained in in vivo studies with chitosan

glutamate at concentrations of 0.2% and 0.5% in rats and sheep [117]. For chitosan glutamate, a concentration of 0.5% was therefore additionally chosen, that was toxic to cells at a contact time of 24 h, in order to see possible differences between the lower and higher concentration. Since the contact time in the permeation studies was considerably shorter (3 h), it can be assumed that the substances have less influence on cell viability. The results of the TEER measurements showed no difference between the lower concentration, which showed no cell toxic effects in the MTT assay, and the higher concentration. The mean reduction in TEER was 23.0 ± 6.8 Ω*cm² and 25.2 ± 5.3 Ω*cm² with 0.25% and 0.5% chitosan glutamate, respectively, in metoprolol-containing samples and 23.4 ± 3.3 Ω*cm² and 22.2 ± 9.6 Ω*cm² with 0.25% and 0.5% chitosan glutamate, respectively, in atenolol-containing samples. However, in order to better assess the influence of toxic effects, toxicity studies need to be conducted that represent the setup of the permeability studies more closely. As described for pectin, the reduction of the permeation coefficients in the presence of chitosan glutamate can be explained by an acidic reaction of the excipient, which increases the proportion of the model drugs in ionised form (see table 6-10). Compared to pectin, chitosan glutamate lowers the pH to a greater extent, resulting in a stronger deceleration of permeation. For better comparability of the direct effect of the substances on the permeation of the model drugs studies with adjusted pH of the drug solution would be required. However, the acidity of chitosan glutamate may limit its use as permeation enhancer in nasal powder formulation with drugs that are weak bases, since the adjustment of pH is difficult in powders, as the pH depends on the dissolution volume and velocity. A further process that possibly influences the permeability of drugs in presence of chitosan glutamate is the potential of positively charged chitosan to interact with negatively charged mucin. Based on this interaction, it can be assumed, that chitosan tightly sticks to the mucus layer, which is produced by RPMI 2650 cells [73,113]. An accumulation of positive charge due to the chitosan molecules on the cell surface may provide an additional diffusion barrier for charged drug molecules.

In general, the permeation studies have shown that the physicochemical properties of excipients have the potential to strongly influence permeation of drug molecules through epithelial barriers. It is therefore of great relevance to consider these effects when selecting excipients in formulation development.

Table 6-9: TEER of cell layer before (start) and after (end) the permeation studies. n=6 for pure APIs and n=3 for samples with excipients; mean ± standard deviation; *significant reduction in TEER [109].

Sample	Model drug: metoprolol		Model drug: atenolol	
	TEER start, Ω*cm²	TEER end, Ω*cm²	TEER start, Ω*cm²	TEER end, Ω*cm²
Pure API	85.2 ± 7.8	85.6 ± 9.5	87.6 ± 4.2	89.1 ± 8.2
Mannitol (1%)	81.6 ± 1.3	82.0 ± 2.4	83.3 ± 0.0	84.8 ± 1.3
MCC (1%)	80.5 ± 1.3	77.1 ± 4.7	83.7 ± 0.7	76.6 ± 1.1*
Chitosan glutamate (0.25%)	93.3 ± 3.3	70.3 ± 7.8*	91.8 ± 1.1	68.5 ± 2.4*
Chitosan glutamate (0.5%)	90.3 ± 5.8	65.1 ± 0.7*	88.4 ± 7.8	66.2 ± 4.6*
Pectin (0.25%)	89.9 ± 4.7	86.9 ± 1.7	91.4 ± 7.5	92.6 ± 4.7
Pectin (0.5%)	85.0 ± 4.1	83.5 ± 4.7	92.9 ± 3.0	92.6 ± 5.6
HPC (0.25%)	85.4 ± 4.6	87.3 ± 4.5	92.2 ± 1.3	89.6 ± 3.0
HPC (0.5%)	85.4 ± 2.6	86.9 ± 3.3	82.8 ± 4.5	79.8 ± 8.3

Table 6-10: pH of drug solutions in Hanks' balanced salt solution + 0.01 M HEPES. n=1.

Sample	pH	
	Model drug: metoprolol	Model drug: atenolol
Pure API	7.03	7.22
Mannitol (1%)	7.04	7.22
MCC (1%)	7.04	7.22
Chitosan glutamate (0.25%)	6.46	6.66
Chitosan glutamate (0.5%)	6.09	6.31
Pectin (0.25%)	6.86	7.09
Pectin (0.5%)	6.72	6.98
HPC (0.25%)	7.07	7.26
HPC (0.5%)	7.07	7.27

6.2.3 Conclusion on the influence of excipients in model formulations

The benefit of different excipients in nasal powder formulations for systemic drug delivery strongly depends on the properties of the drug to be formulated. Therefore, this chapter investigated influences of selected excipients in model formulations with metoprolol tartrate and atenolol as model drugs with different permeability and dissolution properties. For that purpose, powder blends containing the API and a soluble (mannitol) or insoluble (MCC) filler with or without a mucoadhesive excipient (pectin, chitosan glutamate or HPC) were characterised regarding their influence on the viscoelasticity of the nasal fluid and thus the nasal residence time and the dissolution and release of the drug. The influence of the excipients on the permeation behaviour of the dissolved drug molecules was additionally investigated from drug solutions. The used methods enable the distinction of different processes that strongly influence drug absorption in the nasal cavity and therefore allow the detection of additive or counteracting excipient effects on drug absorption.

Rheological studies revealed a potential of undissolved particles to cause sufficient resistance to the mucociliary clearance to prolong the nasal residence time, due to interactions of the particles as an elastic network. This effect, however, was only observed with undissolved atenolol particles, but not with undissolved MCC particles and thus does not only depend on the presence of undissolved particles, but also on particle properties. Among the model formulations that contained mucoadhesive excipients, blends with chitosan glutamate did not cause measurable changes in the rheological properties of SNF compared to the pure drug control, while blends with HPC provided the highest increase in storage and loss moduli. Thereby, higher values were obtained when MCC was used as insoluble filler instead of mannitol as soluble filler.

The assessment of drug dissolution showed a decreased dissolution and release rate of the drugs in presence of insoluble or swelling excipients, which provided a matrix through which dissolved drug molecules need to diffuse. The decrease in dissolution rate did not show a direct correlation with the increase in storage or loss moduli. Therefore, it is essential to assess both processes separately. Plotting of the rheological characteristics against the dissolved drug amount can help to identify promising formulation candidates for increased drug absorption, which show a high potential to prolong the nasal residence time due to an increase in storage and loss moduli, but minimal decrease in the dissolution and release rate of the drug.

The assessment of drug permeation in presence of the excipients revealed a permeation decreasing effect of MCC, pectin, HPC and chitosan glutamate, which was strongest for chitosan glutamate. The strong decrease of the drug permeability in presence of chitosan glutamate was attributed to a reduction of the pH of the drug solution and the characteristic

of the model drugs as weak bases, which show a higher ionised fraction at lower pH. Permeation increasing effects of chitosan, as described in literature, could thus not be shown in this study. The physicochemical properties of drug and excipients must therefore be considered when selecting suitable permeation enhancing excipients for nasal powder formulations.

7 Conclusion and outlook

The formulation of nasal products as powders and the targeted use of excipients potentially offers solutions to certain challenges of nasal drug delivery. This work therefore investigated different influences that excipients can have in nasal powders, aiming for a better understanding of how to successfully use excipients in formulation development and providing suitable characterisation methods.

The short residence time of the drug in the nose is one of the most striking challenges of nasal drug delivery. The first part of this work therefore characterised excipient properties that can influence the residence time of the powders. Rheological testing, investigation of the adhesiveness of the powders to agar (mucin) gels and measurement of dynamic water vapour sorption were selected as methods to represent changes in the viscoelasticity of the nasal fluid, hydration of the excipients and interactions with mucin as core processes affecting the residence time of the powders in the nose. Excipients, which increase the storage and loss moduli of simulated nasal fluid in the rheological tests have a high potential to decrease the efficacy of the mucociliary clearance, while interactions with mucin can improve the contact of the drug to the mucosal surface. Excipient powders that show extensive hydration, are, however, more prone for adhesive failure. Dynamic vapour sorption measurements have been found as good surrogate to predict the probability of the tested excipients to show early adhesive failure.

The use of excipients and powder formulations can not only improve the performance of nasal drug delivery, but can also cause sensory or toxic effects in the nose. The slug mucosal irritation assay served as predictive tool for irritative effects caused by excipient powders in this work. The assessment showed that osmotic activity and pH changes caused by the excipients as well as solubility and dissolution accelerating factors like particle size and morphology are influencing factors on sensory effects. Additionally, substance specific toxicity can cause nasal irritation. However, toxicity and sensory effects are not necessarily concomitant. The evaluation of cell toxicity and sensory effects did not lead to the same ranking of the excipients in all cases.

Which excipient properties actually show advantages in a nasal powder formulation depends strongly on the drug to be formulated. The second part of this work therefore investigated the effect of selected excipients in model formulations with metoprolol tartrate and atenolol as model drugs with different dissolution and permeation behaviour. The nasal residence time of a formulation, the dissolution and release of the drug and the permeation of dissolved drug molecules were identified and assessed as influencing factors for nasal drug delivery from powder formulations. Based on the results of these assessments, the following formulation considerations for nasal powders can be derived (Figure 7-1).

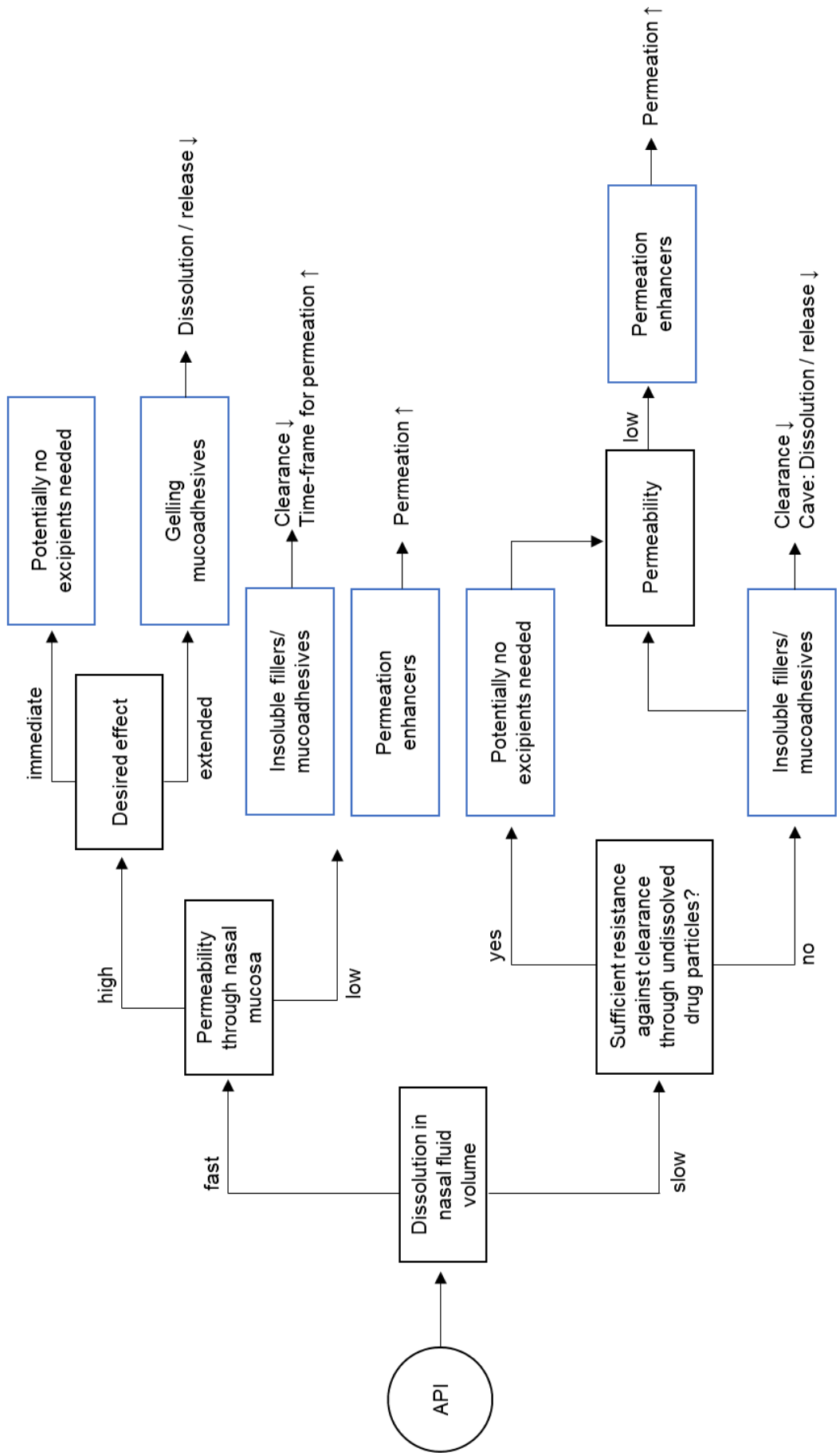

Figure 7-1: Formulation considerations for nasal powder formulations proceeding from the API to be formulated [109].

For drugs applied to the nasal cavity in powder form, drug dissolution is a prerequisite for drug absorption and thus important to consider in formulation development. The dissolution behaviour with regard to nasal drug delivery can be described as fast or slow, depending on whether the drug dissolves completely on a moist surface within the physiological nasal residence time of 15-20 min. API properties that influence this process include particle size and morphology, and saturation solubility. While the particle size and morphology can potentially be adjusted through the selection and control of the production process, the use of solubility-modifying excipients is conceivable to influence the saturation solubility. If the dissolution of the drug powder is judged as fast, further formulation considerations depend on the permeability of the dissolved drug molecules thought the nasal mucosa and the desired effect. If the drug shows high permeability in comparison with model compounds, such as metoprolol and an immediate effect of the drug is required (emergency indications), the use of excipients may not be required in order to achieve an adequate effect. For drugs with different indications, the adaption of the pharmacokinetic profile can be beneficial. The use of gelling mucoadhesives can extend the duration of action and reduce plasma concentration peaks by decreasing the dissolution and release of the drug and prolonging the nasal residence time of the formulation. If the drug shows low permeability on the other hand, the fast-dissolving drug can be cleared from the nasal cavity before the permeation is completed, which reduces its bioavailability. Two formulation options based on the use of excipients are conceivable in this case in order to enhance drug permeation. The more direct approach in this respect is to use excipients that directly increase permeability, e.g., by opening tight junctions. For drugs that require a rapid onset of action, this approach is preferable. Another option is to increase the time for permeation of the drug through the mucosa, which can be achieved by using mucoadhesive or insoluble excipients that reduce nasal clearance.

Drugs that show a slow dissolution require slightly different considerations during product development. If the drug particles do not dissolve completely within the physiological residence time, either formulation strategies that accelerate dissolution are required, or the nasal residence time needs to be prolonged by providing a resistance against the nasal clearance at best with good diffusion properties of the formulation through the mucus layer. Rheological studies can be used to assess, whether the undissolved drug particles themselves do already exhibit sufficient resistance against the nasal clearance to prolong the residence time. However, if the resistance of a formulation against the nasal clearance is due to undissolved drug particles, the effect will decrease with progressing dissolution. A longer lasting effect can be achieved by using insoluble excipients or mucoadhesives, which, however, can further decrease the dissolution velocity of the drug. If the permeability of the drug is low, permeation enhancing excipients can increase the absorption and should

be preferred over mucoadhesives for that purpose, as a further reduction in dissolution rate is disadvantageous. A new class of excipients that may unite dissolution enhancement and mucoadhesion is the 3rd generation of thiolated cyclodextrins [118]. Thiolation causes the molecules to bind covalently to mucin molecules via disulphide bonds, while the low reactive S-protection of the 3rd generation leads to good mucopenetration properties. The investigation of such thiolated excipients in nasal powder formulations was not covered in this work but is an interesting subject for future research.

Conclusively, the assessments in the second part of this work revealed that excipients do not affect drug absorption one-dimensional, but can exhibit counteracting effects. Effective selection of excipients in formulation development therefore requires a distinguished knowledge about the different influences that excipients may have, which need to be evaluated based on the properties of the drug to be formulated. Among the mucoadhesive excipients characterised in this work, HPC M exhibited the overall most promising properties for the formulation of drugs, which require an extended nasal residence time. The additional use of the insoluble filler MCC increased the effect, however, compared to mannitol as soluble filler, it caused a reduction in the dissolution velocity of the drugs. The use of pectin as mucoadhesive requires a more detailed consideration, as it caused greater retardation of the dissolution and release of the model drugs. This behaviour may be attributed to ionic interactions between drug and excipient. Therefore, the formulation of pectin with cationic drugs should be evaluated with particular care. A close examination of the interaction of drug and excipient is also required when using chitosan glutamate as absorption enhancer in powder formulations. In this work, chitosan glutamate caused a strong reduction of the permeability of the model drugs. This behaviour was attributed to the slightly acidic reaction of chitosan glutamate and the property of the drugs as weak bases, which were thus increasingly present in their protonated form.

The in vitro methods used in this work aimed to provide a high degree of standardisation and to be suitable for the testing of high numbers of samples in early formulation development. They are therefore subject to simplifications. Table 7-1 displays the significance and limitations of the methods used. Interactions of excipients and formulations with the nasal mucus gel and the actual ciliary function influence the nasal residence time in vivo, but are not directly addressed in the in vitro studies. Mucus properties do also influence the absorption of drug molecules in vivo, as the drug needs to penetrate and diffuse through the mucus layer to reach the epithelium. In order to better interpret the results obtained with the used in vitro methods with regard to the in vivo significance, a comparison with ex vivo and in vivo experiments is therefore necessary and further optimisation of the in vitro models based on these results can be an interesting subject for future research.

Table 7-1: Significance and limitations of used in vitro methods.

Characterisation method		Significance	Limitations
Nasal residence time of the formulation	**Rheological testing**	- Examination of viscoelasticity of excipients at nasal conditions in terms of temperature, ionic strength and pH as surrogate for the provided resistance against the mucociliary clearance - Assessment of polymer-related factors that may affect mucoadhesion (e.g., rigidity of the chain entanglement network) - Simulated nasal fluid as dispersion medium provides high degree of standardisation for comparative studies	- In vivo rheological properties are not reflected (only comparative screening) - No assessment of interactions with mucin, which can affect viscoelasticity in vivo - Influence on ciliary beat is not directly assessed
	Displacement on agar-mucin gels	- Examination of wetting and hydration of powders on a wet gel surface - Assessment of interactions with mucin	- Only applicable for excipients/ formulations that move on an inclined plane due to the formation of a slippery layer (no active transport mechanism) - No assessment of mucus turnover
	Dynamic vapour sorption	- Surrogate for the hydration behaviour of mucoadhesive polymers	- In vivo situation of hydration and swelling on a wet surface is not displayed
Drug dissolution	**Franz cells**	- Assessment of drug dissolution on a wet surface or in small volumes, while remaining sink conditions	- Small diffusion area can increase retardation effects - Dissolution accelerating effects of osmotic active substances are not displayed, as dissolved molecules diffuse into acceptor compartment - Diffusion of dissolved drug through the mucus layer is not displayed
Drug permeation	**RPMI 2650 cell line**	- Standardisable permeation model with barrier properties similar to existing nasal mucosa	- Histological differences compared to nasal mucosa (e.g., multilayered cell grow, absence of cilia)
Sensory effects	**Slug mucosal irritation assay**	- Assessment of stinging, itching and burning sensations	- Unpleasant smell or taste of the formulation is not assessed

The characterisation of excipients and formulations with meaningful in vitro methods that map the different processes involved in nasal drug delivery can help to better exploit the potential of the nose as drug delivery site and prevent the failure of nasal products in the future. In addition, the number of in vivo studies needed can be reduced. The optimisation of nasal drug delivery through the targeted use of excipients is not only an important strategy for the application of systemically acting drugs, but should also be considered with regard to further drug delivery strategies like mucosal vaccination via the nose or nose-to-brain delivery, which are gaining increasing attention.

8 Abstract

The nose as a site of drug delivery offers therapeutic opportunities for a variety of indications, due to the presence of immunocompetent cells, direct contact with the central nervous system and easy access to a permeable and highly vascularised mucosa. However, nasal drug delivery is primarily associated with locally acting drugs, being applied as simple liquid sprays or drops. A new generation of nasal products that takes advantage of the opportunities offered, however, will likely require more sophisticated formulations, as it will need to address specific challenges of the nose, such as the short residence time of inhaled particles. The formulation of nasal powders and the targeted use of excipients are conceivable strategies in this regard. The aim of this work is the characterisation of influences of excipients in powder formulations that affect systemic absorption of nasally administered drugs, in order to enable effective formulation development of nasal powder products.

The first part of the work characterises selected mucoadhesive excipients (hydroxypropyl methyl cellulose, hydroxypropyl cellulose, hydroxyethyl cellulose, carboxymethyl cellulose, low methoxyl pectin, carboxymethyl chitosan, chitosan glutamate) and fillers (mannitol, lactose, microcrystalline cellulose, colloidal microcrystalline cellulose) regarding their potential to extend the nasal residence time of a formulation and regarding the occurrence of sensory and toxic effects on the nasal mucosa. A potential of the excipients to increase the elasticity of the nasal fluid and to interact with mucin glycoproteins was regarded as beneficial excipient property to extend the nasal residence time, while sufficient initial, but later limited hydration of polymeric excipients was found to prolong the duration of the effect. Excipients that cause changes in pH or osmolality of the nasal fluid upon dissolution were found to provide a higher potential for causing sensory effects in the nose. Dissolution accelerating factors, like a small particles size, can further enhance this effect. The occurrence of sensory effects was not necessarily concomitant with cell toxic effects, hence both factors need to be assessed in product development.

Since the benefit of excipient properties depends on the drug to be formulated, in the second part of the work the effect of selected mucoadhesives (hydroxypropyl cellulose, pectin, chitosan glutamate) and fillers (mannitol, microcrystalline cellulose) was investigated in model formulations. The influences of the formulations on the rheological properties of the nasal fluid, and thus the nasal residence time, on the dissolution and release of the drug, as well as on the permeation of the drug through the epithelium were assessed separately, in order to detect additive and counteracting effects on drug absorption. A differentiated knowledge about the different influencing effects is essential to optimise drug absorption.

The consideration of the characterised processes in product development can avoid failures and enable the development of successful nasal products.

9 Zusammenfassung

Die Nase bietet als Ort der Arzneimittelgabe durch das Vorhandensein von immunkompetenten Zellen, dem direkten Kontakt zum zentralen Nervensystem und die leichte Erreichbarkeit einer gut durchbluteten, permeablen Schleimhaut Therapiemöglichkeiten für eine Vielzahl von Indikationen. Trotzdem wird die nasale Arzneimittelgabe aktuell in erster Linie mit der Verabreichung lokal wirksamer Arzneistoffe als Nasensprays oder -tropfen in Verbindung gebracht. Da eine neue Generation nasaler Arzneimittel, die sich die gebotenen Möglichkeiten zunutze macht, spezifische Hürden, wie zum Beispiel die kurze Verweildauer nasal verabreichter Partikel in der Nasenhöhle, überwinden muss, werden in Zukunft komplexere Formulierungen erforderlich sein. Denkbare Strategien in diesem Zusammenhang sind die Formulierung nasaler Pulver und der gezielte Einsatz von Hilfsstoffen. Ziel dieser Arbeit ist es, Einflüsse von Hilfsstoffen in Pulverformulierungen, die die systemische Absorption nasal verabreichter Arzneistoffe beeinflussen, zu charakterisieren und damit eine effektive Formulierungsentwicklung zu ermöglichen.

Im erste Teil der Arbeit werden ausgewählte mukoadhäsive Hilfsstoffe (Hydroxypropylmethylcellulose, Hydroxypropylcellulose, Hydroxyethylcellulose, Carboxymethylcellulose, Pektin, Carboxymethylchitosan, Chitosanglutamat) und Füllstoffe (Mannitol, Laktose, mikrokristalline Cellulose, kolloidale mikrokristalline Cellulose) hinsichtlich ihres Potenzials, die nasale Verweildauer einer Formulierung zu verlängern, und hinsichtlich des Auftretens von sensorischen und toxischen Effekten auf der Nasenschleimhaut charakterisiert. Als vorteilhafte Hilfsstoffeigenschaften zur Verlängerung der nasalen Verweildauer wurden die Erhöhung der Elastizität der Nasenflüssigkeit, sowie die Fähigkeit zur Interaktion mit Mucin angesehen, während eine anfänglich ausreichende, aber im Verlauf limitierte Hydratation polymerer Hilfsstoffe die Dauer der Wirkung verlängerte. Für Hilfsstoffe, die bei der Auflösung Veränderungen im pH-Wert oder in der Osmolalität des Nasensekrets bewirken können, wurde ein höheres Potenzial für das Auftreten sensorischer Effekte in der Nase festgestellt. Faktoren, wie eine geringe Partikelgröße, die die Auflösung beschleunigen, können diesen Effekt verstärken. Da das Auftreten sensorischer Effekte nicht immer mit einer zelltoxischen Wirkung einherging, müssen beide Faktoren in der Produktentwicklung getrennt bewertet werden.

Ob bestimmte Hilfsstoffeigenschaften von Vorteil sind, hängt von dem zu formulierenden Arzneistoff ab. Deshalb wurde im zweiten Teil der Arbeit die Wirkung ausgewählter mukoadhäsiver Hilfsstoffe (Hydroxypropylcellulose, Pektin, Chitosanglutamat) und Füllstoffe (Mannitol, mikrokristalline Cellulose) in Modellformulierungen mit Arzneistoffen untersucht. Einflüsse auf die rheologischen Eigenschaften der Nasenflüssigkeit und damit

auf die nasale Verweildauer, auf die Auflösung und Freisetzung des Wirkstoffs, sowie auf die Permeation durch das Epithel wurden separat untersucht, um additive sowie gegenläufige Effekte auf die Wirkstoffaufnahme zu identifizieren. Ein differenziertes Wissen über die unterschiedlichen Einflussfaktoren ist unerlässlich, um die Arzneistoffaufnahme gezielt zu optimieren. Die Berücksichtigung der in dieser Arbeit charakterisierten Prozesse in der Produktentwicklung kann Misserfolge vermeiden und zur Entwicklung erfolgreicher nasaler Produkte beitragen.

10 Appendix

10.1 Abbreviations

AAS	Atomic absorption spectroscopy
ALI	Air liquid interface
API	Active pharmaceutical ingredient
ATN	Atenolol
BAC	Benzalkonium chloride
BCS	Biopharmaceutics classification system
CHIT	Chitosan glutamate
CM chitosan	Carboxymethyl chitosan
CMC	Carboxymethyl cellulose sodium salt
CNS	Central nervous system
DVS	Dynamic vapour sorption
EMA	European Medicines Agency
F_2	Similarity factor
FDA	Food and Drug Administration
G’	Storage/elastic modulus
G’’	Loss/viscous modulus
HBSS	Hanks’ balanced salt solution
HEC	Hydroxyethyl cellulose
HELOS	Helium Laser Optical System
HEPES	4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid
HPC	Hydroxypropyl cellulose
HPLC	High performance liquid chromatography
HPMC	Hydroxypropyl methyl cellulose
LC	Lethal concentration
MCC	Microcrystalline cellulose
MET	Metoprolol tartrate
MTT	3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazoliumbromide
P_{app}	Permeability coefficient
PBS	Phosphate buffered saline
PEC	Pectin
PET	Polyethylene terephthalate
Ph. Eur.	European Pharmacopoeia
RSD	Relative standard deviation
SMIA	Slug mucosal irritation assay
Tan δ	Dissipation factor

t_d	Dissolution time, derived from Weibull function
TEER	Transepithelial electric resistance
UDS powder	Unidose powder nasal spray system
$x_{10/50/90}$	Particle diameter, where 10/50/90% of particles are smaller

10.2 Substances

Table 10-1: Substances used with the respective suppliers.

Substance	Supplier
Acetonitrile	Honeywell International Inc., Charlotte, USA
Aqua bidest.	In-house production, FinnAqua 75-E-4, San Asalo Sohlberg Corp., Helsinki, Finnland
Atenolol	Lot: 19J08-F03-367167, Fagron, Thias, France
Benzalkonium chloride	Caelo, Hilden, Germany
Calcium chloride dihydrate	Merck KgaA, Darmstadt, Germany
Carboxymethyl cellulose sodium salt	Lot: SLCB7664, Sigma-Aldrich, St. Louis, USA
Carboxymethyl chitosan	Lot: 312-120213-01, Heppe Medical Chitosan GmbH, Halle (Saale), Germany)
Chitosan glutamate	Lot: 312-050320-01, Heppe Medical Chitosan GmbH, Halle (Saale), Germany)
Dimethylformamide	Merck KgaA, Darmstadt, Germany
Dulbecco's Modified Eagle's Medium, with 4500 mg/L glucose, L-glutamine, sodium pyruvate, and sodium bicarbonate	Sigma-Aldrich, St. Louis, USA
Dulbecco's Phosphate Buffered Saline, Modified, without calcium chloride and magnesium chloride	Sigma-Aldrich, St. Louis, USA
Fetal bovine serum	Sigma-Aldrich, St. Louis, USA
Hanks' Balanced Salt Solution, Modified, with sodium bicarbonate, without phenol red	Sigma-Aldrich, St. Louis, USA
HEPES (4-(2-Hydroxyethyl)piperazine-1-ethanesulfonic acid)	Biochrom, Berlin, Germany
Hydroxyethyl cellulose (Natrosol™ 250 G Pharm)	Lot: S0297, Ashland Inc. Wilmington, USA
Hydroxyethyl cellulose (Natrosol™ 250 M Pharm)	Lot: S0292, Ashland Inc. Wilmington, USA

Substance	Supplier
Hydroxypropyl cellulose (Klucel™ GF Pharm)	Lot: 177246, Ashland Inc. Wilmington, USA
Hydroxypropyl cellulose (Klucel™ MF Pharm)	Lot: 188495, Ashland Inc. Wilmington, USA
Hydroxypropyl methyl cellulose (Metolose® 65 SH 400)	Lot: 7036200, Shin-Etsu Chemicals, Chiyoda, Japan
Hydroxypropyl methyl cellulose (Metolose® 65- SH 4000)	Lot: 6106586, Shin-Etsu Chemicals, Chiyoda, Japan
Lactose (FlowLac® 100)	Lot: L101502019A537, Meggle GmbH & Co. KG, Wasserburg am Inn, Germany
Lactose (InhaLac® 230)	Lot: L1327A9859, Meggle GmbH & Co. KG, Wasserburg am Inn, Germany
Mannitol (Pearlitol® 100 SD)	Lot: E253D, Roquette, Lestrem, France
Mannitol (Pearlitol® 160 C)	Lot: E131X, Roquette, Lestrem, France
MEM Non-essential Amino Acid Solution (100x)	Sigma-Aldrich, St. Louis, USA
Metoprolol tartrate	Lot: 275411501, Chemos GmbH & Co. KG, Altdorf, Germany
Microcrystalline cellulose (Vivapur® 102)	Lot: 56102195325, JRS Pharma GmbH& Co. KG, Rosenberg, Germany
Microcrystalline cellulose, colloidal (Vivapur® MCG 811 P)	Lot: 33811190129, JRS Pharma GmbH& Co. KG, Rosenberg, Germany
Minimum Essential Medium Eagle, with Earle's salts, L-glutamine and sodium bicarbonate	Sigma-Aldrich, St. Louis, USA
Mucin from porcine stomach type II	Sigma-Aldrich, St. Louis, USA
Orthophosphoric acid 85%	Merck KGaA, Darmstadt, Germany
Pectin (Classic CU-L 045/18)	Lot: 01806579, Herbstreith & Fox KG, Neuenbürg, Germany
Penicillin-streptomycin (100x)	Invitrogen, Thermo Fisher Scientific Inc., Waltham, USA
Potassium chloride	Roth, Karlsruhe, Germany
Potassium dihydrogen phosphate	Merck KGaA, Darmstadt, Germany
RPMI 2650 cells, ACC 287	German Collection of Microorganisms and Cell Cultures GmbH, Braunschweig, Germany
Sea sand	Walter GmbH & Co. KG, Kiel, Germany
Sodium chloride	Roth, Karlsruhe, Germany
Sodium dihydrogen phosphate dihydrate	AppliChem, Darmstadt, Germany
Sodium dodecyl sulphate	Roth, Karlsruhe, Germany
Sodium hydrogen phosphate	Merck KgaA, Darmstadt, Germany

Substance	Supplier
Sodium hydrogen phosphate dodecahydrate	Merck KgaA, Darmstadt, Germany
Sodium pyruvate solution 100 mM	Sigma-Aldrich, St. Louis, USA
Thiazolyl Blue Tetrazolium Bromide (MTT)	Sigma-Aldrich, St. Louis, USA
Triethylamine	Merck KGaA, Darmstadt, Germany
Triton® X-100	Merck KgaA, Darmstadt, Germany
Trypan Blue solution 0.4%	Sigma-Aldrich, St. Louis, USA
Trypsin 0.25% / 1mM EDTA-Na in HBSS, w/o Ca, Mg, w: Phenol red	Pan Biotech, Aidenbach, Germany

10.3 Equipment

Table 10-2: Equipment used with the respective manufacturers.

Equipment		Manufacturer
Analytical sieves and sieve shaker	N/A	Retsch GmbH & Co. KG, Haan, Germany
Atomic absorption spectroscopy	AAS 3030	Perkin Elmer Inc., Waltham, USA
Cell culture plates	TC-plate 96 well, Standard, F	Sarstedt AG & Co. KG, Nümbrecht, Germany
	Cellstar 12-well	Greiner Bio-One, Frickenhausen, Germany
Centrifuge	Centrifuge 5430 R	Eppendorf SE, Hamburg, Germany
	Biofuge 28 RS	Heraeaus, Hanau, Germany
Drying oven	Heraeus	Thermo Fisher Scientific Inc., Waltham, USA
Dynamic vapour sorption	DVS Resolution	Surface Measurement Systems Ltd., Wembley, UK
Franz cells	8 ml volume; 1 cm² area	Permegear, Hellertown, USA
High permformance liquid cromatography	Agilent 1100 Series LC	Agilent Technologies, Santa Clara, USA
Incubator	HERAcell 150	Thermo Fisher Scientific Inc., Waltham, USA
Laser diffractometer	HELOS in combination with RODOS module	Sympatec GmbH, Clausthal-Zellerfeld, Germany
Microscope	Olympus CKX 53	Olympus, Shinjuku, Japan
Nasal powder applicator	Unidose Powder Nasal Spray System	Aptar, Louceciennes, France
Osmometer	Osmomat 030	Gonotec GmbH, Berlin Germany

Equipment		**Manufacturer**
Permeable cell culture inserts	TC-PTP 1.13 cm³, pore size 3 µm	Greiner Bio-One, Frickenhausen, Germany
pH meter	WTW pH 540 GLP	Xylem Analytics Germany Sales GmbH & Co. KG, Weilheim in Oberbayern, Germany
	Seven Compact	Mettler Toledo GmbH, Columbus, USA
Plate reader	Tecan Spark	Tecan Group Ltd., Männedorf, Switzerland
Rheometer	CVO 120 HRNF, parallel plate setup, diameter 40 mm	Bohlin Instruments GmbH, Pfortzheim, Germany
Scanning electron microscope	Phenom World XL	Thermo Fisher Scientific Inc., Waltham, USA
Sputter coater	BAL-Tec SCP 050 Sputter Coater	Leica Microsystems, Wetzlar, Germany
Transepithelial electric resistance	Evom voltohmeter with chopstick electrode	World Precision Instruments, Sarasota, USA
Turbula blender	N/A	Willy A. Bachofen, Muttenz, Switzerland
Vortex	IKA Vortex 4 basic	IKA-Werke GmbH & Co. KG, Staufen im Breisgau, Germany

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Erklärung nach § 9 der Promotionsordnung

Hiermit erkläre ich gemäß § 9 der Promotionsordnung der Mathematisch-Naturwissenschaftlichen Fakultät der Christian-Albrechts-Universität zu Kiel, dass ich die vorliegende Abhandlung, abgesehen von der Beratung durch meine Betreuerin, nach Inhalt und Form eigenständig und ohne fremde Hilfe verfasst habe. Weiterhin habe ich keine anderen als die angegebenen Quellen oder Hilfsmittel benutzt und die den benutzten Werken wörtlich oder inhaltlich entnommenen Stellen als solche kenntlich gemacht. Die vorliegende Arbeit ist unter Einhaltung der Regeln guter wissenschaftlicher Praxis der Deutschen Forschungsgemeinschaft entstanden. Sie wurde weder ganz noch in Teilen an einer anderen Stelle im Rahmen eines Prüfungsverfahrens vorgelegt, veröffentlicht oder zur Veröffentlichung eingereicht. Weiterhin wurde mir kein akademischer Grad entzogen, noch habe ich an dieser oder einer anderen Fakultät einen früheren Promotionsversuch unternommen.

Marie Trenkel

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