3. Results and Discussion

3.1. Influence of the polymer type

The sponge-like structure of in situ gelling nasal inserts is an important parameter to ensure rapid hydration and gelation of the inserts at the nasal mucosa. Fast water uptake by capillary forces guarantees rapid gelation and thus reduces the foreign body sensation when compared to other solid dosage forms, such as tablets. Initial studies were therefore performed to identify polymers, which form sponges during freeze-drying. Further on, the properties of in situ gelling nasal inserts prepared from different polymers were investigated.

3.1.1. Sponge-structure formation

Different water-soluble polymers were analyzed as received and after freeze-drying from a 2% (w/w) aqueous solution. The appearance of the freeze-dried product was observed visually and the physical state of the polymer was characterized by X-ray diffraction (Figure 3. 1 and Table 3. 1). The polymers did not change their physical state after freeze-drying. PEG 4000 and Lutrol® F127 and F68, which remained crystalline after lyophilisation, did not form the desired sponge-structure, but formed an incoherent, dense powder (e.g. PEG 4000 in Figure 3. 1). Even higher solution concentrations (25% w/w) of the crystalline polymers did not form sponges after freeze-drying. Crystalline polymers were therefore not suitable as carrier materials for the nasal inserts.

All other polymers tested, which were amorphous after freeze-drying, formed the desired sponge-like structure. The inserts had a layered structure with varying degree of fineness (Figure 3. 1). Carrageenan, Na-alginate, and NaCMC inserts (2% w/w) were characterized by larger continuous polymeric layers with pronounced cavities in between, while HPMC E5, HPMC K15M, PVP 90 and chitosan inserts had a more network-like structure. Xanthan gum and Carbopol® inserts showed an intermediate appearance. Fine pores were not visible within the actual polymer layer (maximum magnification factor 5000; resolution of pores to approx. 100 nm) Higher magnifications were not possible due to melting of the polymers under the

electron beam. The polymer layer density increased with higher polymer content of the inserts as shown with carrageenan inserts (1, 2 and 3% w/w) (Figure 3. 1).

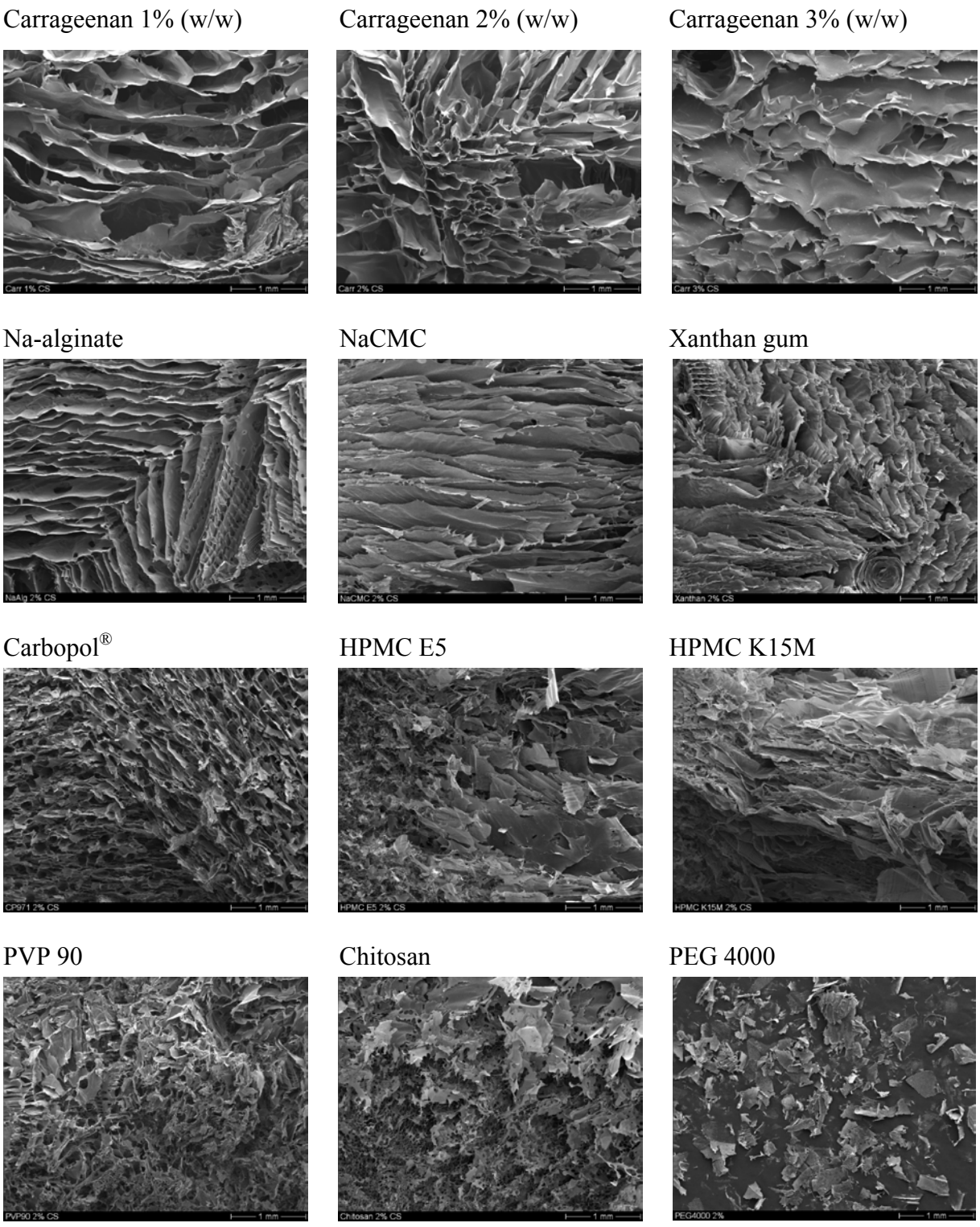


Figure 3. 1 Scanning electron microscopic pictures of insert cross sections (polymer 2% w/w if not otherwise stated, V = 1.5 ml).

Table 3. 1 Physical state (X-ray analysis) and appearance of freeze-dried polymers (freeze-dried from a 2% w/w polymer solution).

Polymer	Untreated polymer	Freeze-dried polymer	
	Physical state	Physical State	Appearance
Carrageenan	Amorphous	Amorphous	Sponge
Carbopol®	Amorphous	Amorphous	Sponge
Chitosan glutamate	Amorphous	Amorphous	Sponge
HPMC E5	Amorphous	Amorphous	Sponge
HPMC K15M	Amorphous	Amorphous	Sponge
Na-alginate	Amorphous	Amorphous	Sponge
NaCMC	Amorphous	Amorphous	Sponge
PVP 90	Amorphous	Amorphous	Sponge
Xanthan gum	Amorphous	Amorphous	Sponge
PEG 4000	Crystalline	Crystalline	Powder
Lutrol® F127	Crystalline	Crystalline	Powder
Lutrol® F68	Crystalline	Crystalline	Powder

3.1.2. Polymer solution rheology

Viscosity. In situ gelling nasal inserts are supposed to take up fluid from the mucosa and to form a gel. The viscosity of this gel, and thus, respectively, the viscosity of the polymer solution for insert preparation, are of utmost importance for the performance of inserts with respect to drug release, water uptake and corresponding polymer mass loss, as well as bioadhesion. The solutions of polymers (2% w/w) were therefore investigated concerning their rheological behavior (Table 3. 2). The viscosity of the polymers followed the order: HPMC E5 < PVP 90 < chitosan < Na-alginate < NaCMC < xanthan gum < carrageenan < Carbopol® < HPMC K15M. HPMC E5 and PVP 90 were Newtonian solutions while all other tested polymers exhibited pseudoplastic behavior (Casson characteristics). In addition, NaCMC, carrageenan, and Carbopol® gave thixotropic solutions.

Table 3. 2 Viscosity and rheological properties of polymer solutions (C60/1°, D = 30 s^{-1}, 22°C, n = 3).

Polymer	Concentration, % (w/w)	Viscosity, mPas	Characterization (R = goodness of fit)	Thixotropy
HPMC E5	2	4.1 ± 0.2	Newton (0.9981)	No
PVP 90	2	6.1 ± 0.5	Newton (0.9992)	No
Chitosan	2	182.9 ± 1.7	n.d.	n.d.
Na-alginate	2	345.2 ± 8.6	Casson (0.9999)	No
NaCMC	2	721.5 ± 16.0	Casson (0.9992)	Yes
Xanthan gum	2	835.5 ± 5.6	Casson (0.9999)	No
Carrageenan	2	1177.7 ± 20.9	Casson (0.9991)	Yes
Carbopol®	2	1359.2 ± 82.2	Casson (0.9999)	Yes
HPMC K15M	2	2992.3 ± 55.6	Casson (0.9973)	No

n.d. = not determined.

Viscoelasticity and spreading behavior. Viscoelasticity is the collective name for the behavior of especially semisolid materials, which results from the properties of viscosity and elasticity (Schramm, 1995). Viscoelasticity can be measured by applying a small periodic stress to the sample in a sinusoidal function and by registering the resulting time-dependent deformation of the sample. A phase separation between stress and deformation results, which is characterized by the phase angle δ. If δ = 0° there is no separation, meaning the sample is ideally elastic, i.e. solid. A phase angle of δ = 90° stands for an ideally viscous sample. Angles of 0° < δ < 90° mark viscoelastic behavior. Simultaneously to the phase angle, the magnitude of the complex modulus G*, representing the total resistance of the sample against periodic deformation, is recorded. G* comprises of the storage or elasticity modulus G', which stands for the solid component of the sample, and the loss or viscosity modulus G'', reflecting the viscous element of the sample (G* = G' + i G''; i…imaginary unit). The loss tangent tan δ is the ratio of G'' and G' (tan δ = G'' / G'). A loss tangent tan δ < 1 marks a sample with predominantly solid characteristics and tan > 1 a sample with predominantly viscous characteristics. In addition, the complex viscosity η* can be calculated

($\eta^* = G^* / i \cdot \omega$; ω…angular velocity). Its magnitude $|\eta^*|$ describes the total resistance of the sample against dynamic shearing.

The measurement of viscoelasticity may be a useful tool to predict the spreading behavior of gels and thus of rehydrated inserts. The spreading ability of hydrated inserts seemed to be an important parameter determining the area of contact between insert and mucosa. An increase in the contact area would lead to an enhanced drug release rate.

For this reason, the viscoelastic properties of polymer solutions (2% w/w) were investigated in order to correlate them with the spreading behavior (Table 3. 3). Neither for PVP 90 nor for HPMC E5 a viscoelastic region could be found for measurements. As expected from previous viscosity measurements, the magnitude of the complex viscosity followed the order Na-alginate < NaCMC < xanthan gum < carrageenan < Carbopol® < HPMC K15M. However, there was no clear relation to the measured loss tangent. The loss tangent decreased in the order Na-alginate > NaCMC > HPMC K15M > carrageenan > xanthan gum > Carbopol®. The latter two even exhibited more elastic than viscous properties. Thus, it may be expected that xanthan gum and Carbopol® have the lowest tendency to spread followed by carrageenan and HPMC K15M and finally NaCMC and Na-alginate.

Table 3. 3 Viscoelastic properties of polymer solutions (polymer 2% w/w, 22°C, n = 3).

Polymer	**Stress, Pa**	**Complex viscosity, mPas, at ω = 9.2 rad/s**	**tan δ (mean over ω-range)**
PVP 90	No linear viscoelastic region detectable		
HPMC E5	No linear viscoelastic region detectable		
Na-alginate	3	610 ± 33	3.5
NaCMC	3	1010 ± 14	2.5
Xanthan gum	4	2250 ± 78	0.6
Carrageenan	10	2820 ± 164	1.4
Carbopol®	4	6683 ± 267	0.3
HPMC K15M	10	12233 ± 544	1.5

Spreading studies with polymer solutions (2% w/w) were performed to establish a possible relation between the measured viscoelasticity and the spreading behavior. The ability of polymer solutions to spread on a hydrated surface (agar gel) was highest for Na-alginate followed by NaCMC, HPMC K15M, carrageenan, xanthan gum, and Carbopol®. It was attempted to correlate the diameter of spreading (spreading time 5 min) with several rheological parameters. No correlation was found with the solution viscosity (R^2 = 0.358), the complex solution viscosity (R^2 = 0.145), and the storage modulus (R^2 = 0.284). The best correlation was obtained with the loss tangent (R^2 = 0.953) (Figure 3. 2). It was therefore concluded that the loss tangent can be used as a measure of the spreading ability of solutions and gels, and thus also for hydrated nasal inserts.

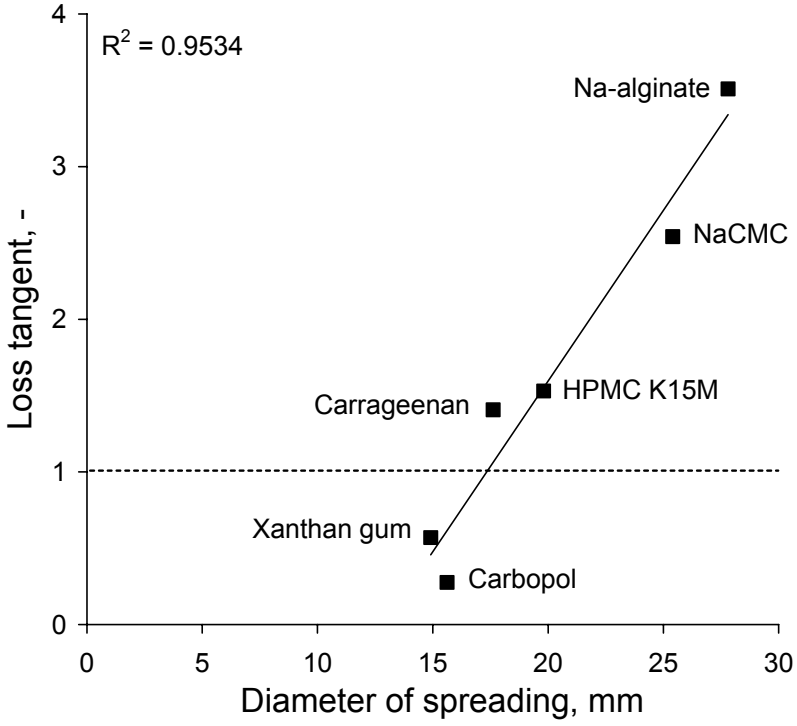


Figure 3. 2 Correlation between the spreading diameter (after 5 min) and the loss tangent of solutions (polymer 2% w/w).

Furthermore, the viscoelastic behavior of polymer solutions, whose polymer concentration corresponded to that of inserts after 8 h of water uptake studies (Table 3. 5), was investigated in order to estimate the spreading ability of nasal inserts. Similar loss tangents were determined for carrageenan, xanthan gum, and Carbopol® (Table 3. 4). The loss tangent of HPMC K15M was slightly increased but still predominantly elastic, while NaCMC showed more liquid-like behavior. From this data, similar spreading was concluded for carrageenan,

xanthan gum, and Carbopol®, slightly higher spreading for HPMC K15M, and significantly higher spreading for NaCMC.

The effect of the investigated spreading behavior of the polymer solutions on the drug release from in situ gelling nasal inserts will be discussed in section 3.1.3.

Table 3. 4 Viscoelastic properties of polymer solutions corresponding to inserts after 8 h of water uptake studies (polymer % w/w from Table 3. 5, 37°C, n = 3).

Polymer	**Concen-tration, %**	**Stress, Pa**	**Complex viscosity, Pas, at ω = 9.2 rad/s**	**tan δ (mean over ω-range)**
Carrageenan	6.0	150	103 ± 7	0.2
Carbopol®	12.0	30	83 ± 6	0.2
Xanthan gum	6.0	30	43 ± 1	0.2
HPMC K15M	14.0	100	2310 ± 262	0.4
NaCMC	4.2	30	8 ± 1	1.3

3.1.3. Effect of polymer type on nasal insert properties

Bioadhesion. Once administered into the nasal cavity, the inserts have to adhere to the nasal mucosa to take up water and to transform into a gel. The presence of water is a prerequisite for bioadhesion, which is a key factor for a successful prolonged nasal drug delivery. A new test to measure the bioadhesion potential of in situ gelling nasal inserts was developed (Werner and Bodmeier, 2002). The inserts were placed on a hydrated surface (agar / mucin) and the displacement on the vertical surface due to gravity was taken as an inverse measure of the bioadhesion potential.

Almost instantaneous displacement and therefore a low bioadhesion potential was obtained with PVP 90, Na-alginate, and HPMC E5 inserts due to their inability to interact with mucin either electrostatically or by entanglements because of their rather low molecular weight and therefore low solution viscosity (Figure 3. 3 and Table 3. 2). These inserts hydrated rapidly, dissolved, and flowed down the agar / mucin gel. A minimum molecular weight of 100 kDa is usually required for bioadhesion (Lee et al., 2000). Chitosan, a positively charged polymer of low viscosity, showed a relatively fast displacement. However, despite this displacement,

chitosan inserts formed a thin film on the agar / mucin gel due to its opposite charge to mucin and agar, which probably would also allow a prolonged contact with the mucosa. An intermediate displacement was measured for HPMC K15M, a neutral, viscous polymer. Although unable to interact electrostatically with mucin, it may do so by entanglement due to its high molecular weight. In addition, the solution viscosity of the HPMC K15M insert is high, thus inhibiting the flow of the hydrated insert. The differences between HPMC E5 and K15M inserts were attributed to the large differences in the molecular weight, water uptake behavior, and solution viscosity of the two HPMC grades. No or very little displacement was observed for carrageenan, xanthan gum, Carbopol®, and NaCMC inserts. All four polymers are negatively charged, have a relatively high solution viscosity (approx. 700 - 1400 mPas for 2% w/w polymer solutions) (Table 3. 2) and are known for good bioadhesion (Junginger, 1991b; Madsen et al., 1998; Nakamura et al., 1996). The positive bioadhesion performance of negatively charged polymers may be related to their good balance between available hydrogen bonding sites and an open expanded conformation (Madsen et al., 1998).

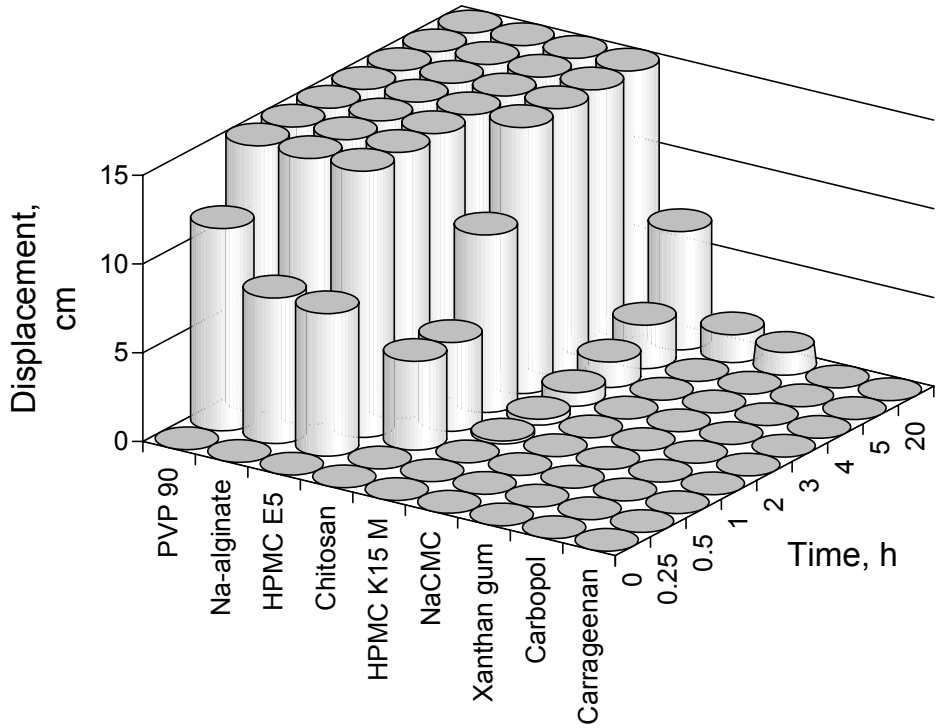


Figure 3. 3 Adhesion profiles of inserts prepared from different polymers (polymer 1% w/w, V = 0.1 ml, n = 3, CV = 13.6 ± 6.7%).

Water uptake and mass loss. As mentioned above, the uptake of water by in situ gelling inserts is a crucial step for the transformation into the gel and for adhesion to the mucosa. Dehydration of the mucosal tissue can widen tight junctions due to their connection to the cytoskeleton (Björk et al., 1995), but also trigger unwanted side effects. The ability of hydrogels to absorb water is due to the presence of hydrophilic groups such as -OH, -COOH, and -OSO_3H. The hydration of these functional groups results in water entry into the polymer network, which leads to expansion and consequently an ordering of the polymer chains. The swelling equilibrium (maximum water uptake) is reached when the osmotic forces of the functional groups are balanced by the restrictive forces of the higher ordering of the polymer chains (Peppas and Khare, 1993).

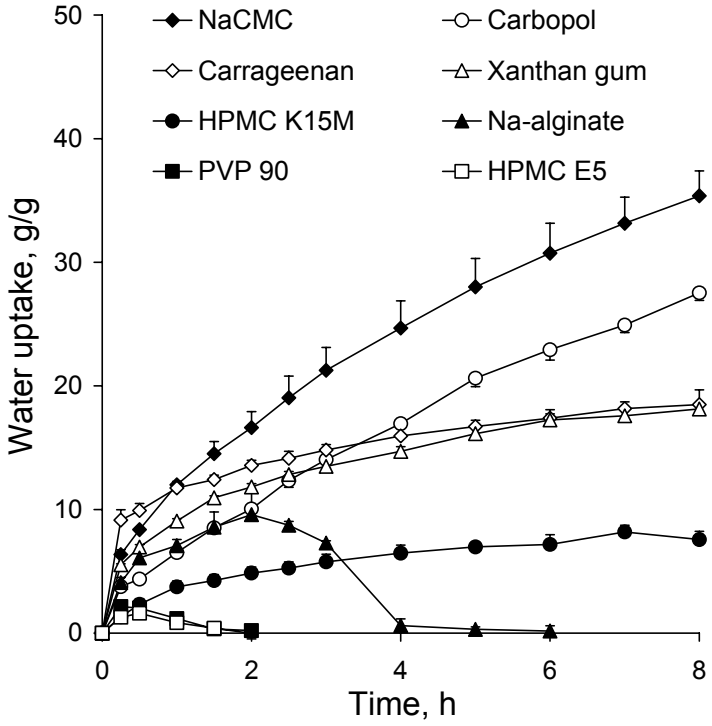


Figure 3. 4 Water uptake of inserts prepared from different polymers (polymer 2% w/w, V = 1.5 ml, n = 3).

The water uptake of inserts depended on the type of polymer used (Figure 3. 4). The balance of water uptake, polymer mass loss during the hydration, and the molecular weight of the polymer defined the viscosity of the resulting gel. Polymers with low solution viscosity dissolved during the study period with a complete disappearance after 2 h for PVP 90 and HPMC E5 inserts and 4 h for Na-alginate inserts. The dissolution of the inserts contributed to the poor bioadhesion potential. For all other inserts (HPMC K15M, xanthan gum, NaCMC,

Carbopol®, carrageenan), approximately 100% of the polymer could be recovered after 8 h of water uptake study (Table 3. 5), thus excluding mass loss over the study period. This indicated that the inserts gelled well without dissolution.

As expected, charged polymers led to a higher extent of water uptake compared to neutral polymers (Figure 3. 4). In contrast to most other polymers, carrageenan inserts showed an abrupt reduction in the water uptake rate after the first data point. The fast initial water uptake may be attributed to the high charge density of carrageenan and therefore high osmotic forces as well as rapid initial water imbibition due to capillary forces resulting from the highly porous structure of the inserts, while the slower second water uptake phase resulted from a restricted water influx, possibly due to delayed formation of a gel barrier, which reduced the diffusivity of water and thus its uptake rate.

Table 3. 5 Recovery and polymer content of nasal inserts after 8 h of water uptake studies (polymer 2% w/w, V = 1.5 ml, n = 3).

Polymer	**Recovery, %**	**Polymer content, %**
PVP 90	Dissolved within 2 hours	
HPMC E5	Dissolved within 2 hours	
Na-alginate	Dissolved within 4 hours	
Carrageenan	107 ± 4	6.0 ± 0.2
HPMC K15M	107 ± 4	13.7 ± 0.5
Xanthan gum	110 ± 8	5.9 ± 0.4
NaCMC	112 ± 3	4.2 ± 0.1
Carbopol®	113 ± 7	12.4 ± 0.1

Drug release. The water uptake of inserts partially correlated with the release of the model drug oxymetazoline HCl (solubility in water at 22°C: 150 mg/ml, pK_a 9.9) (Figure 3. 5). Inserts prepared from HPMC E5, PVP 90 and Na-alginate released the drug rapidly due to dissolution of the gel matrix. 100% release coincided with the time point for complete dissolution observed previously in the water uptake study. The other polymers, which did not dissolve during water uptake, exhibited extended release, which was slowest for carrageenan.

The small lag time (approx. 0.5 h) prior to the drug release was probably caused by the initiation of the hydration process.

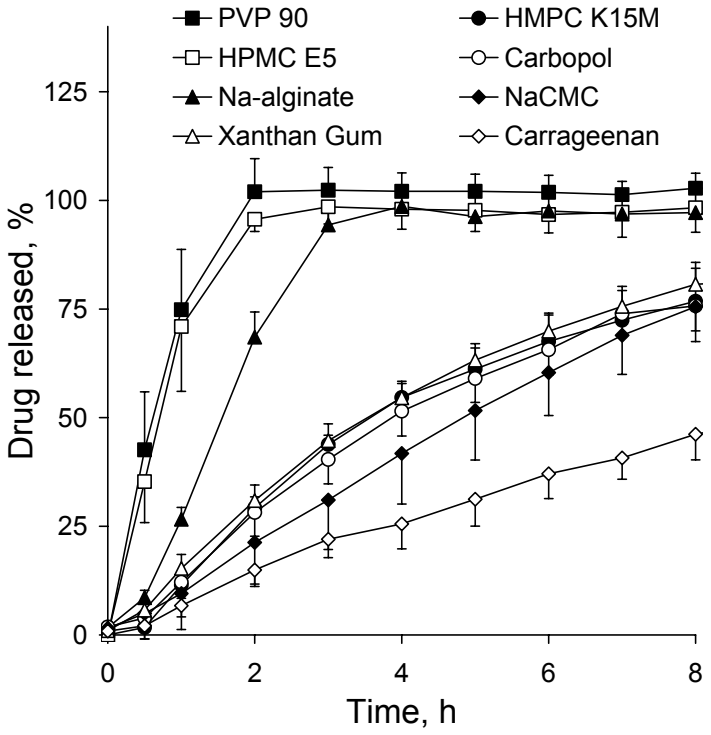


Figure 3. 5 Release profile of oxymetazoline HCl from inserts prepared from different polymers (polymer 2% w/w, drug 5% w/w based on polymer, V = 1.5 ml, n = 3).

Among the slow releasing polymers, carrageenan showed the lowest release rate with an apparent zero order kinetic over the study time. This may be attributed to salt formation between the negatively charged carrageenan and the positively charged drug. This drug-polymer interaction was already visually observed as precipitation in 2% (w/w) carrageenan solutions at oxymetazoline HCl concentrations of ≥ 19% (w/w based on polymer). The less soluble salt forms a drug depot keeping the concentration of free oxymetazoline in the gel constant with subsequent constant concentration gradient of the drug and constant release rate. The oxymetazoline-carrageenan salt, which was less soluble than the hydrochloride salt, was either formed due to the concentration increase during the freeze-drying or at the moment of rehydration of the insert.

Also HPMC K15M, Na-alginate, NaCMC, Carbopol®, and xanthan gum were investigated with respect to their ability to interact electrostatically with oxymetazoline HCl. The turbidity due to precipitation of 2% (w/w) polymer solutions with increasing drug concentrations

(% w/w based on polymer) was measured as the absorption at 500 nm (Figure 3. 6). The minimum drug concentration for precipitation (zero intercept for each polymer in Figure 3. 6) decreased with increasing charge density of the polymer (number of possible charges / molecular weight at pH 6) (Table 3. 6). Due to the high initial cloudiness of xanthan gum solutions, absorption at 500 nm exceeded the measurement range and precipitation was only observed visually in this case.

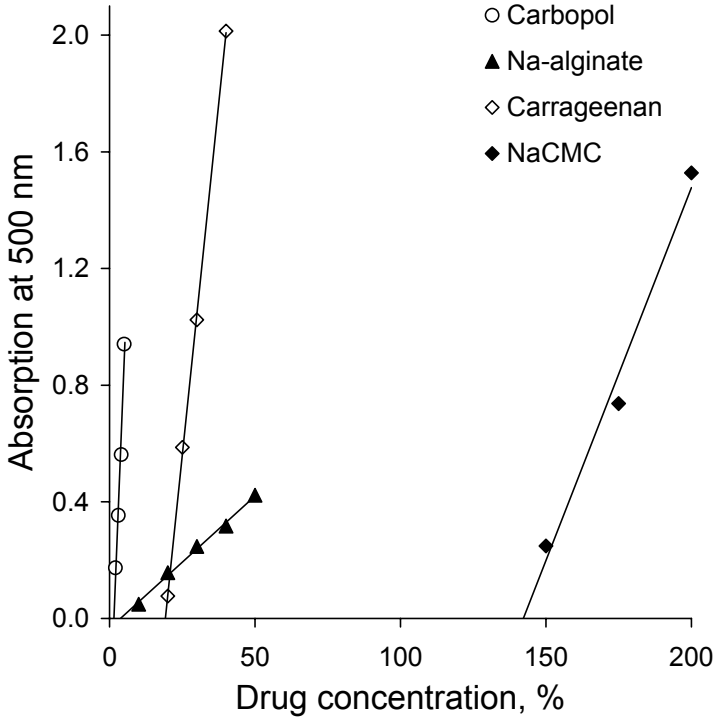


Figure 3. 6 Turbidity (absorption at 500 nm) of polymer solutions (polymer 2% w/w) loaded with oxymetazoline HCl (% w/w based on polymer) (Carbopol®: $y = 0.25\ x - 0.37$, $R^2 = 0.967$; Na-alginate: $y = 0.01\ x - 0.03$, $R^2 = 0.995$; Carrageenan: $y = 0.10\ x - 1.84$, $R^2 = 0.999$; NaCMC: $y = 0.03\ x - 3.64$, $R^2 = 0.982$).

If the drug-polymer-salt formation solely ruled the drug release, slowest release rates could have been expected with Carbopol® and Na-alginate inserts followed by carrageenan, NaCMC, xanthan gum and HPMC K15M. The observed faster drug release from NaCMC inserts compared to carrageenan may thus result from the lower charge density of the former. The apparent zero order release kinetic of NaCMC and carrageenan inserts over the study time (Figure 3. 5) may indicate the predominance of the drug-polymer salt in the release mechanism. However, in case of Na-alginate, the dissolution of the gel matrix (Figure 3. 4) had a stronger impact on the release mechanism. The high polymer viscosity (Table 3. 2)

combined with the low water uptake (Figure 3. 4) of HPMC K15M inserts may explain the relatively slow drug release from these inserts. No obvious explanation for the unexpectedly faster release of Carbopol® compared to carrageenan is available. Opposite to all other tested polymers, Carbopol® is a cross-linked polymer. Therefore, the its gel is not homogenous but has a "fuzzball" type structure of gel particles and interstitial water channels. This leads to a much lower microviscosity compared to the measured macroviscosity (Table 3. 2) which allows faster diffusion of the drug in the gel. Also it may be postulated that the drug does not come in contact with the carboxy-groups of the polymer, which are within a "fuzzball". In summary, this indicates that the release of oxymetazoline HCl from nasal inserts prepared from different polymers is a complex phenomenon composed of multiple single processes such as drug-polymer interactions, the viscosity of the hydrated inserts resulting from polymer molecular weight, water uptake and polymer mass loss during hydration, as well as possible spreading of the gel with subsequent increase of the release area.

Table 3. 6 Charge density of polymers and precipitation start point for polymer solutions (polymer 2% w/w) for oxymetazoline HCl.

Polymer	**Charge density, charges / Dalton**	**Precipitation starting point, % drug (w/w based on polymer)**
HPMC K15M	neutral	no precipitation
Xanthan gum	$2.3 \cdot 10^{-3}$	125-150%
NaCMC	$3.6 \cdot 10^{-3}$	143%
Carrageenan	$4.6 \cdot 10^{-3}$	19%
Na-alginate	$5.7 \cdot 10^{-3}$	4%
Carbopol®	$6.9 \cdot 10^{-3}$	1.5%

Another approach to explain the apparent zero order release from carrageenan inserts may be the limited contact area of the inserts with the dissolution medium, through which the release occurred. This was previously described for HPMC tablets, which were partially covered with an impermeable coating. This coating shifted the original release kinetics towards constant release (Colombo et al., 1990; Colombo et al., 1992). However, the authors attributed the constant release to the swelling restriction by the coating, while nasal inserts were free to

swell in every dimension and to spread on the sponge surface through which the release occurred.

Previously (see section 3.1.2), the viscoelastic properties of the investigated polymers were analyzed in order to quantify the increase in the drug release surface of nasal inserts and the subsequent enhanced drug release. It was attempted to correlate these viscoelastic properties, namely the loss tangent, with the drug release data. However, a correlation was neither found for the release of oxymetazoline HCl from inserts prepared of different polymers nor for the neutral drug species diprophyllin and APAP. This implies that the drug release process even in the absence of drug-polymer interactions is very complex and cannot be simplified to one major process, such as spreading.

Mechanical properties. The mechanical properties of inserts reflect the sensitivity of the inserts towards the handling by the patient. Strong, elastic inserts are desired to allow easy removal from blisters and application in the nose in order to avoid changes to the sponge-structure, which is a prerequisite for rapid water uptake, bioadhesion and drug release. Insert with a hardness of approximately ≥ 2 N are easily handled without damage.

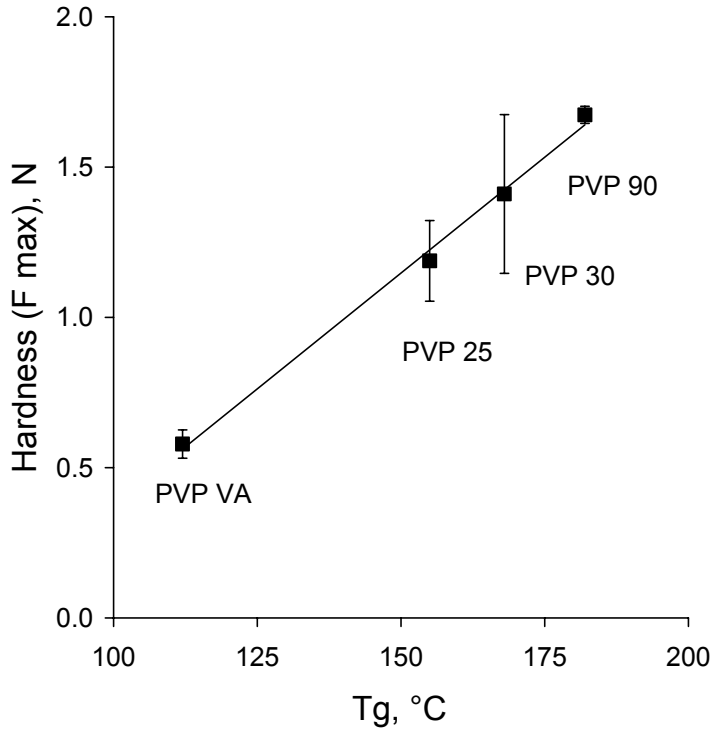


Figure 3. 7 Relationship between hardness and glass transition temperature T_g of inserts prepared from PVP grades of different molecular weight and PVP VA (polymer 2% w/w, linear correlation coefficient $R^2 = 0.996$, n = 6).

The hardness of the inserts was measured as the maximum force during compression with an Instron® force sensor. PVPs of different molecular weight (PVP 25: 28 - 30 kDa; PVP 30: 44 - 54 kDa; PVP 90: 1,000 - 1,500 kDa) and PVP VA (45 - 70 kDa) (Bühler et al., 1999) formed sponges by freeze-drying. The hardness of the PVP inserts increased with higher molecular weight, with PVP 25 and PVP VA resembling a spider web rather than a solid dosage form. The glass transition temperature T_g of the polymer (Bühler, 1999) correlated well with the hardness of the inserts (Figure 3. 7). A larger difference between the T_g and room temperature, at which the hardness test was performed, resulted in a decreased polymer chain flexibility and therefore a higher hardness. PVP had a higher T_g with increased molecular weight and, therefore, formed harder inserts with increasing molecular weight.

Table 3. 7 Mechanical properties of inserts prepared from different polymers and with varying polymer content (n = 6).

Polymer	**Concentration, % (w/w)**	**Hardness, N**	**Elasticity, %**
Carrageenan	1	1.4 ± 0.2	30.9 ± 2.4
Carrageenan	2	5.2 ± 0.8	35.2 ± 4.2
Carrageenan	3	16.7 ± 1.3	38.0 ± 2.2
NaCMC	2	4.8 ± 0.5	30.6 ± 4.7
Xanthan gum	2	3.0 ± 0.4	43.4 ± 8.7
Na-alginate	2	2.1 ± 0.2	39.7 ± 1.9
Carbopol®	2	1.9 ± 0.2	33.1 ± 5.7
PVP 90	2	1.7 ± 0.0	56.9 ± 2.4
HPMC K15M	2	1.4 ± 0.1	45.2 ± 2.6
HPMC E5	2	0.8 ± 0.0	53.5 ± 1.4

The type of polymer chosen for insert formation determined the mechanical properties of the inserts (Table 3. 7). The hardness of inserts prepared from a 2% w/w polymer solution ranged from 5.2 N for carrageenan inserts to 0.8 N for HPMC E5 inserts. Insert with a hardness of approximately ≥ 2 N were found to be easily handled without damage. The elasticity is a

measure of the ability of the inserts to recover their sponge-like structure after a compression, for example an accidental compression during the removal by the patient from blister packs. The sponge-like structure is an important feature of the inserts and a prerequisite for water uptake, bioadhesion and drug release. The elasticity of inserts varied with the polymer type (PVP 90 > HPMC E5 > HPMC K15M > xanthan gum > Na-alginate > carrageenan > Carbopol® > NaCMC). Inserts with a more pronounced network structure (PVP 90, HPMC E5, and HPMC K15M) appeared more elastic, while inserts with a larger, more layered structure (Carrageenan, Na-alginate, and NaCMC) had a lower elasticity (Figure 3. 1 and Table 3. 7). The network structure maintained its properties, while the more separate polymer layers were more prone to breakage upon an external force.

Summary. In situ gelling inserts can be prepared from various water-soluble polymers. The sponge-like structure of nasal inserts was formed by amorphous, but not by crystalline polymers during the freeze-drying process. The selected polymer dictated principal insert properties like water uptake behavior, bioadhesion potential, mechanical properties, and drug release profile. The bioadhesion potential was governed by the water uptake and dissolution of the polymer gel as well as by the ability of the polymer to interact with mucin / agar. Hydration of inserts made from low viscosity polymers resulted in polymer dissolution and consequent fast drug release (HPMC E5, Na-alginate, PVP 90). Water uptake and drug release of inserts from high viscosity polymers extended over more than 8 h (carrageenan, Carbopol®, NaCMC, xanthan gum, HPMC K15M). Taken together, the release of oxymetazoline HCl from nasal inserts prepared from different polymers was a complex phenomenon composed of multiple single processes such as drug-polymer interactions, water uptake and polymer mass loss during hydration, the viscosity of the hydrated inserts resulting additionally from the polymer molecular weight, and spreading of the gel with subsequent increase of the release area. The hardness of different dry PVP inserts was successfully correlated with the glass transition temperature of the polymers. The elasticity of nasal inserts depended on the structure of the inserts formed during freeze-drying. Among the polymeric carriers tested, carrageenan and xanthan gum had the most promising in vitro properties with respect to bioadhesion, water uptake and polymer dissolution, drug release profile, and mechanical properties of in situ gelling nasal inserts. Further studies concentrated on the manipulation of the drug release rate from inserts while maintaining the other insert properties, e.g. bioadhesion and mechanical properties, within acceptable ranges.

3.2. Drug release rate control

As previously shown, the drug release rate can be controlled by the polymer chosen for the formation of the in situ gelling nasal insert. Unfortunately, polymers which allow short and intermediate release rates, e.g. Na-alginate, lack reasonable bioadhesion or mechanical strength. Therefore, studies were directed towards the control of the drug release rate from in situ gelling nasal inserts while maintaining other insert properties within acceptable ranges (insert hardness > 2 N, elasticity > 30%, maximum bioadhesion). Several approaches were followed: (i) variation of the polymer content, (ii) addition of freely water-soluble additives, (iii) polymer molecular weight reduction, and (iv) use of polymer blends.

3.2.1. Polymer content

It can be expected that a higher polymer content in nasal inserts leads to a more viscous gel on the mucosa after hydration of the insert and thus to prolonged drug release.

Solution viscosity, viscoelasticity, and spreading behavior. As expected, the viscosity of carrageenan solutions increased with higher polymer concentration (Table 3. 8). The magnitude of the complex viscosity increased with carrageenan concentration, while the loss tangent decreased (Table 3. 9). Solutions with more the 3% (w/w) carrageenan had a loss tangent < 1 and thus behaved more like a solid than a liquid. It was not possible to drop accurate volumes of these carrageenan solutions (> 3% w/w) from a syringe to study the spreading behavior. However, the spreading diameters of the concentrations 1%, 2%, and 3% (w/w) could be well correlated with the loss tangent ($R^2 = 0.9988$).

Table 3. 8 Solution viscosity of carrageenan solutions with different polymer content (C60/1° (*C20/4°), D = 30 s^{-1}, 22°C, n = 3).

Polymer	Concentration,% (w/w)	Viscosity, mPas
Carrageenan	1	93.9 ± 1.0
Carrageenan	2	1177.7 ± 20.9
Carrageenan	3	8605.1 ± 703.0*

Table 3. 9 Viscoelastic properties of carrageenan solutions with different polymer content (37°C, n = 3).

Carrageenan concentration, %	**Stress, Pa**	**Complex viscosity, mPas, at ω = 9.2 rad/s**	**tan δ (mean over ω-range)**
1	5	218 ± 12	3.0
2	5	491 ± 93	2.6
3	5	2453 ± 90	2.0
4	50	18567 ± 2029	0.6
5	100	58200 ± 1296	0.3
6	150	103000 ± 6976	0.2

Water uptake. The uptake of water during the test period of 8 h was highest for inserts containing 1% (w/w) carrageenan followed by 2% (w/w) and 3% (w/w) (Figure 3. 8). None of the inserts seemed to have reached equilibrium swelling and no polymer dissolution was visually observed. The initial fast water uptake was reduced with higher polymer content probably due to the reduced porosity of the inserts (Figure 3. 1). Inserts with 1% (w/w) carrageenan showed a more gradual decrease of the water uptake rate than those of higher concentration. Due to their more porous structure and lower polymer content the water influx may be less effected by the postulated formation of a gel barrier (see section 3.1.3), which could restrict water influx.

Drug release. Viscosity and water uptake studies were mirrored in the release behavior of in situ gelling nasal inserts with increasing carrageenan content. The release of the model drug oxymetazoline HCl from nasal inserts increased with decreasing polymer content of the inserts (Figure 3. 9). This may be attributed to several factors: the decreased viscosity due to the lower polymer content, the accelerated and enhanced water uptake with subsequent lower viscosity of the rehydrated insert, and the enhanced spreading and thus higher surface area due to more liquid-like behavior of the hydrated inserts. In addition, the previously described electrostatic interactions between oxymetazoline HCl and carrageenan (see section 3.1.3) may lead to a reduced amount of free drug due to a shift of the dynamic equilibrium at increased polymer concentrations with constant drug loading, resulting in a more prolonged release.

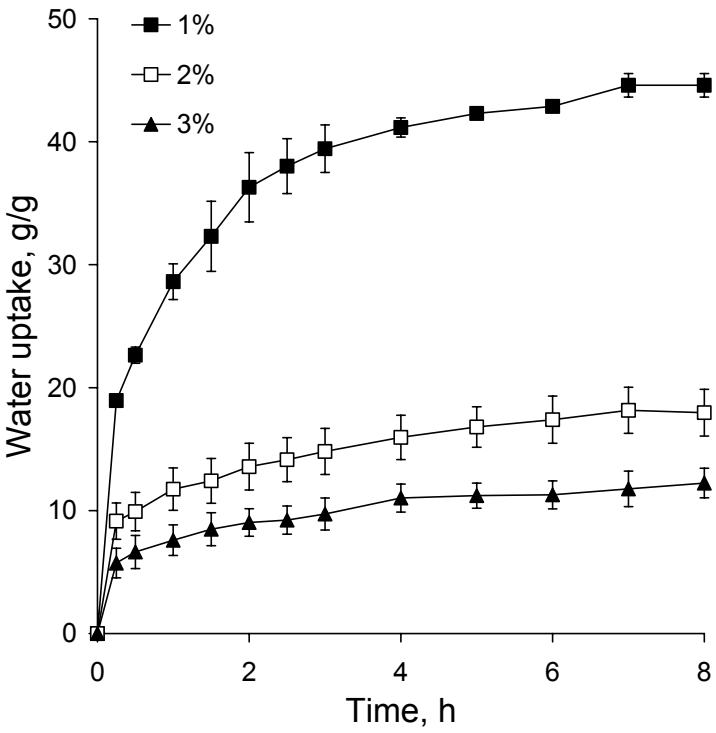


Figure 3. 8 Water uptake of carrageenan inserts of different polymer content (polymer % w/w, V = 1.5 ml, n = 3).

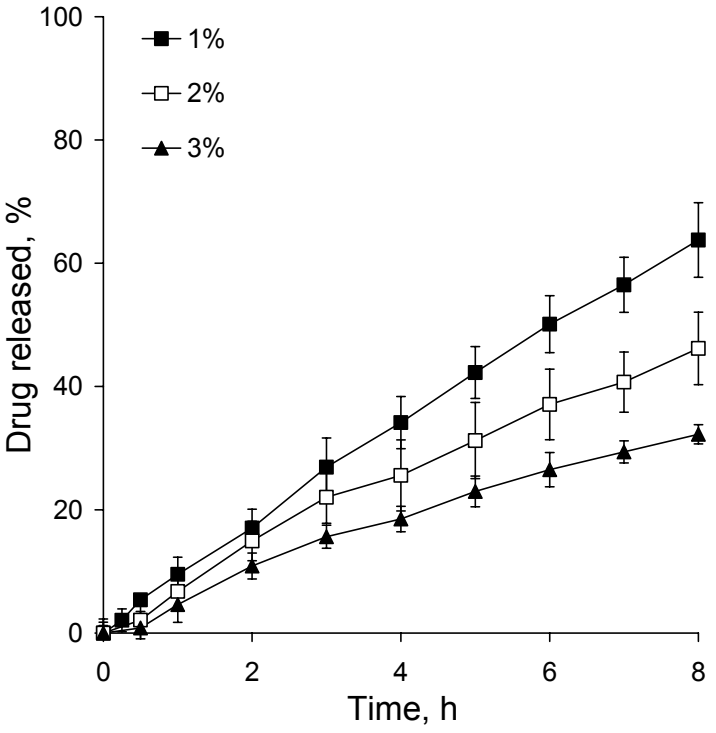


Figure 3. 9 Release profile of oxymetazoline HCl from carrageenan inserts with increasing polymer content (polymer % w/w, 1.5 mg drug / insert, V = 1.5 ml, n = 3).

Bioadhesion. The effect of the polymer content of inserts on the bioadhesion potential was investigated for HPMC K15M. This polymer had an intermediate bioadhesion potential so that slight changes in the polymer concentration would likely result in measurable variation in the displacement on the agar / mucin gel, opposite to carrageenan whose excellent bioadhesion potential was beyond the limits of the test. The displacement decreased with increasing polymer content (displacement after 4 h bioadhesion testing: 0.5% w/w HPMC K15M: 1.5 ± 0.1 cm, 1.0%: 1.4 ± 0.3 cm, 1.5%: 1.2 ± 0.0 cm, 2.0%: 0.9 ± 0.0 cm) likely due to a retarded hydration of the inserts at a higher polymer content and a higher gel viscosity. After 20 h bioadhesion testing the rank order was reversed (0.5% w/w HPMC K15M: 3.3 ± 0.2 cm, 1.0%: 6.7 ± 0.3 cm, 1.5%: 9.6 ± 0.1 cm, 2.0%: 11.8 ± 0.4 cm) because inserts of higher polymer content provided more polymer to go in solution and therefore formed quantitatively more polymer solution, which then resulted in higher displacements.

Mechanical properties. An increase in the carrageenan content of inserts resulted in harder inserts, and also in an increase in elasticity (Table 3. 7). The enhanced elasticity could be attributed to a denser sponge structure in the inserts at a higher polymer content (Figure 3. 1). The hardness of inserts, which was smaller than the desired 2 N at 2% polymer (w/w) (e.g. PVP 90 and HPMC), could therefore be increased by using more concentrated polymer solutions (> 2% w/w).

Summary. The drug release rate increased with decreasing polymer content of the nasal inserts as expected. Accelerating the drug release by reducing the polymer content was limited by the decreased mechanical stability of the inserts and possibly reduced bioadhesive properties. The upper limit in the solution concentration is determined by the processability of the polymer solutions, e.g. during polymer dissolution or the lyophilisation process, which primarily depends on the solution viscosity.

3.2.2. Freely water-soluble additives

Freely water-soluble additives were chosen to control the drug release rate from nasal inserts based on the rational that they may enhance the water uptake of inserts and thus lead to a less viscous gel. Sugar alcohols were studied as freely soluble additives because salts such as

those of Na^+ and Ca^{2+} are known to increase the swelling and the viscosity of carrageenan, which would be an unwanted effect (Akahane et al., 1982).

Viscosity. Addition of sorbitol up to 20% (w/w based on the polymer) to a 2% (w/w) carrageenan solution had no significant effect on the viscosity (see section 3.4 and Figure 3. 34A).

Water uptake. For the sake of comparison the water uptake of inserts with varying additive amounts was calculated as weight increase based on the initial dry polymer weight which was constant for one polymer level. Addition of 25% (w/w based on polymer) of either mannitol (solubility 18 g / 100 g water), xylitol (solubility 64 g / 100 g water), or sorbitol (solubility 83 g / 100 g water) did not change the water uptake and no difference was seen between them (data not shown). Even higher loadings (up to 100% w/w based on polymer) of sorbitol had no effect, independent of the carrageenan content of the inserts (Figure 3. 10A). Thus the above stated concept for using highly water-soluble additives to increase the water uptake of in situ gelling nasal inserts was not proven.

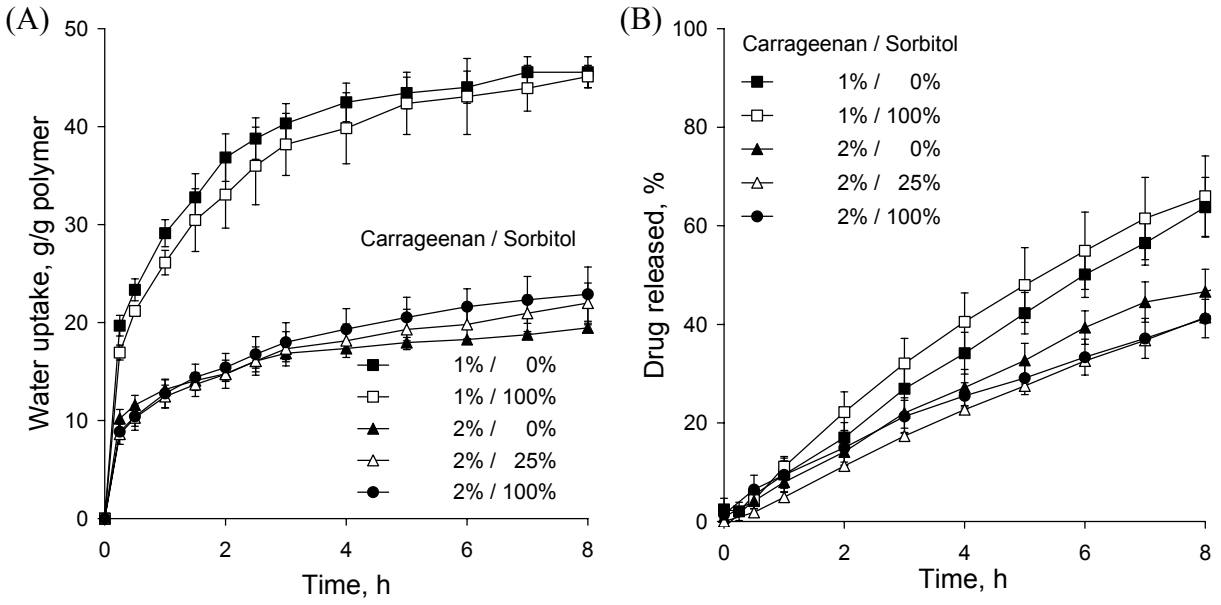


Figure 3. 10 (A) Water uptake of and (B) release profile of oxymetazoline HCl (5% w/w based on polymer) from carrageenan inserts with increasing amounts of sorbitol (polymer 1% or 2% w/w, sorbitol % w/w based on polymer, V = 1.5 ml, n = 3).

Drug release. The missing effect of freely soluble additives on the water uptake is also reflected in the release of the model drug oxymetazoline HCl from carrageenan inserts. The drug release rate was neither influenced by the sugar alcohol species (mannitol, sorbitol or xylitol; data not shown) nor by the concentration. Even a sorbitol content of 100% (w/w based on polymer) in inserts containing 1% (w/w) carrageenan did not effect the drug release rate (Figure 3. 10B).

Mechanical properties. Although the suitability of adding highly water-soluble additives to increase the drug release rate of inserts could not be shown, the effect of these additives on the mechanical properties on inserts was investigated. Different effects were found for mannitol and sorbitol (Table 3. 10). While mannitol addition did not influence the hardness of inserts, sorbitol led to a strong decrease of the insert hardness. The effects on the insert elasticity were less significant due to higher variability. However, again different trends were visible. Mannitol tended to decrease the elasticity while sorbitol made nasal inserts more elastic.

Table 3. 10 Mechanical properties of carrageenan inserts containing different amounts of either mannitol or sorbitol (polymer 1% w/w, additive % w/w based on polymer, n = 7).

Additive, %	**Mannitol**		**Sorbitol**	
	Hardness, N	**Elasticity, %**	**Hardness, N**	**Elasticity, %**
0	1.4 ± 0.2	30.9 ± 2.4	1.4 ± 0.2	30.9 ± 2.4
10	1.3 ± 0.2	29.5 ± 1.4	0.7 ± 0.2	27.2 ± 10.7
50	1.3 ± 0.1	27.2 ± 9.2	0.3 ± 0.1	36.4 ± 12.3
100	1.7 ± 0.3	15.3 ± 7.0	0.2 ± 0.1	51.0 ± 14.3

Moisture sorption studies were performed to understand this phenomenon. The moisture sorption was determined as weight increase of the insert over time normalized to the initial dry polymer weight of the inserts, which was kept constant for all inserts. Thus, it was shown that addition of sorbitol and mannitol increased the moisture sorption of nasal inserts with a much more pronounced effect for sorbitol (Figure 3. 11). Sorbitol behaved as a highly hygroscopic additive. The moisture attracted to the inserts acted as a plasticizer on the

polymer thus leading to a decrease of the glass transition temperature and consequently of the hardness as well as to an increase of the elasticity (see section 3.1.3; Schreder and Lee, 1996). Mannitol, which was less hygroscopic, had no significant effect on the insert hardness and reduced the elasticity because it is less elastic than the polymer.

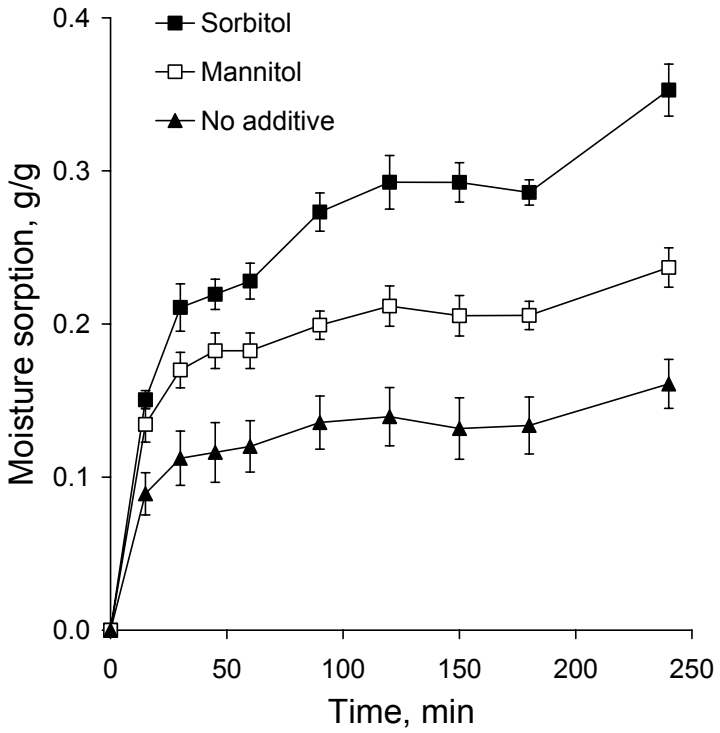


Figure 3. 11 Moisture sorption of carrageenan inserts containing 50% (w/w based on polymer) of either mannitol or sorbitol (polymer 1% w/w, V = 1.5 ml, n = 3).

Summary. Opposite to the assumption, the incorporation of freely water-soluble additives did not enhance the water uptake of in situ gelling nasal inserts. Also no effect on the drug release was observed. The additives influenced the inserts' mechanical properties depending on their hygroscopicity. This limited the amount of additives in the inserts. In conclusion, addition of freely water-soluble additives is not feasible to control the drug release rates of nasal inserts.

3.2.3. Polymer molecular weight

The rational to reduce the polymer molecular weight to control the drug release from in situ gelling nasal inserts lies in the direct connection between the molecular weight and the viscosity (Mark-Houwink equation). Thus, not only the drug diffusion through the hydrated gel matrix should be enhanced but also the water uptake may be increased. As the polymer is

also responsible for the bioadhesion potential and the mechanical stability of inserts, these properties may also be affected.

Solution viscosity. Carrageenan was used as carrier polymer to study the molecular weight dependence of various insert properties because it strongly retarded the drug release and had an excellent bioadhesion potential (see section 3.1.3). Carrageenan of varying molecular weight was not commercially available. Three methods, namely heat treatment, autoclaving, and sonication, were tested to reduce the molecular weight of carrageenan and therefore the solution viscosity. Simple heat treatment (30 min, 100°C) had no influence on the viscosity of a 2% (w/w) carrageenan solution, autoclaving (30 min, 121°C, 2 bar) resulted in a significant reduction of the viscosity, and sonication achieved the most drastic reduction. Sonication has been an effective method to degrade polysaccharides (Chen et al., 1997; Lii et al., 1999). The carrageenan solution viscosity decreased exponentially with ultrasonication time, as previously observed by Lii et al. (1999), because of the higher energy input into the system. A carrageenan solution viscosity range of approximately 50 - 300 mPas was covered (Table 3. 11). Sonication times longer than 10 min were not examined because longer times were less effective due to the reduction of the slope of the viscosity-sonication time-curve.

Table 3. 11 Comparison of different methods to reduce the viscosity of carrageenan solutions and the effect of sonication time (20 g solution) on the viscosity of carrageenan solutions (polymer 2% w/w, C20/4°, D = 10 s^{-1}, 37°C, n = 3).

Treatment	**Treatment time, min**	**Viscosity, mPas**
Untreated	0	298.1 ± 15.9
Heat: 100°C	30	315.1 ± 16.4
Autoclaving: 121°C, 2 bar	30	200.1 ± 9.3
Sonication	0.5	247.9 ± 11.3
	1	190.5 ± 13.4
	3	133.1 ± 10.4
	5	94.3 ± 10.4
	10	53.0 ± 9.3

The presence of the model drugs oxymetazoline HCl and diprophyllin had no effect on the viscosity reduction. The UV spectrum of both model drugs was not changed by 10 min of sonication. Smaller sample size during sonication led to stronger viscosity reductions of a 2% (w/w) carrageenan solution (20 g: 149 ± 12 mPas, 10 g: 65 ± 9 mPas, 5 g: 31 ± 9 mPas after 5 min sonication, D = 10 s^{-1}, 22°C) due to increased relative energy inputs. All further studies on the effect of carrageenan molecular weight were performed with sonicated carrageenan solutions.

Na-alginate was chosen as an alternative polymer to investigate the effect of the polymer molecular weight on the properties of in situ gelling nasal inserts. Three commercially available Na-alginate grades were investigated and characterized by their solution viscosity, which covered a large range from approximately 400 to 10,000 mPas (Table 3. 12).

Solutions of the sonication treated (0.5 - 10 min) carrageenan and Na-alginate of different molecular weight (HV, MV, and LV) were freeze-dried and in situ gelling nasal inserts were formed.

Table 3. 12 Molecular weight (supplier information) and solution viscosity (polymer 2% w/w, C20/4°, D = 5 s^{-1}, 22°C, n = 3) of different Na-alginate grades.

Na-alginate grade	Molecular weight	Viscosity, mPas
HV	high	10153 ± 518
MV	medium	1617 ± 49
LV	low	407 ± 27

Water uptake and mass loss. The water uptake profile of carrageenan inserts was biphasic with a fast initial phase due to rapid water uptake by capillary forces followed by a reduced water uptake rate, which was attributed to the formation a gel layer and thus a slower hydration (see section 3.1.3). Lyophilized nasal inserts prepared from sonicated carrageenan solutions took up more water with longer sonication times, which was attributed to the reduced viscosity of the hydrating inserts and therefore reduced diffusion barrier function of the gel matrix for the water influx (Figure 3. 12). However, the differences were not large and almost no effect was observed at sonication times longer than 3 min. The polymer mass of the hydrated inserts did not change over 8 h of hydration irrespective of the sonication time, indicating no mass loss due to dissolution of the polymer gel and subsequent diffusion

through the filter (polymer mass recovery for carrageenan inserts after 8 h of hydration: untreated - 107 ± 4 %, 1 min sonication - 110 ± 3 %, 3 min sonication - 107 ± 5 %, 5 min sonication - 109 ± 3 %, 10 min sonication - 106 ± 4 %). Thus the integrity of the gel matrix was maintained over the entire study period.

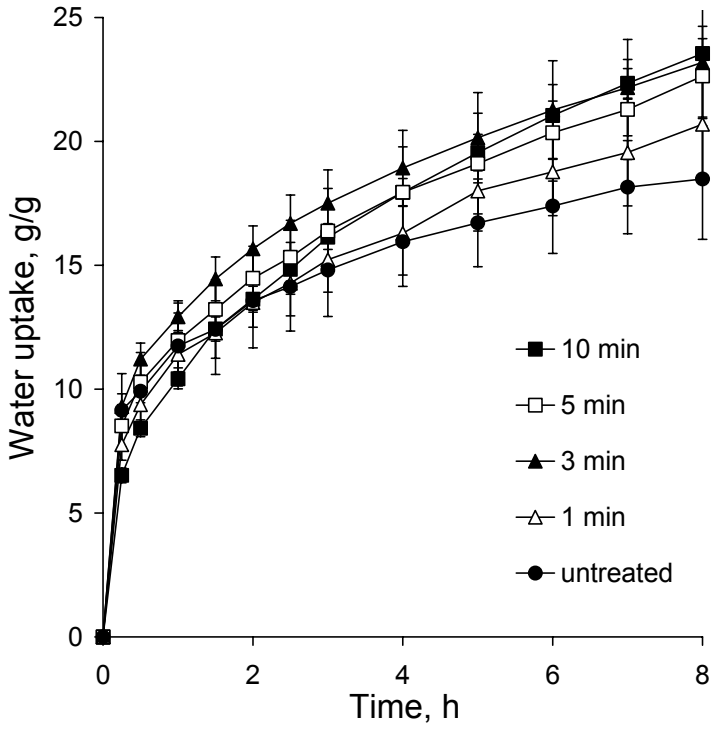


Figure 3. 12 Effect of sonication time on the water uptake of inserts prepared from sonicated carrageenan solutions (polymer 2% w/w, V = 1.5 ml, n = 3).

In contrast, the water uptake of Na-alginate inserts depended strongly on the polymer molecular weight. The extent increased with increasing polymer molecular weight (Figure 3. 13A). Longer polymer chains (higher molecular weight) have a higher probability for chain entanglements which results in a higher viscosity and enhanced integrity of the gel matrix so that more water can be bound without overhydration and subsequent mass loss due to polymer dissolution. Inserts prepared from the Na-alginate types LV and MV dissolved during the study period (Figure 3. 13B). No major differences between them were observed up to 2 h. At later time points, the mass loss of Na-alginate MV inserts was slower compared to LV. Na-alginate HV inserts showed no mass loss but the water uptake leveled off after an initial phase.

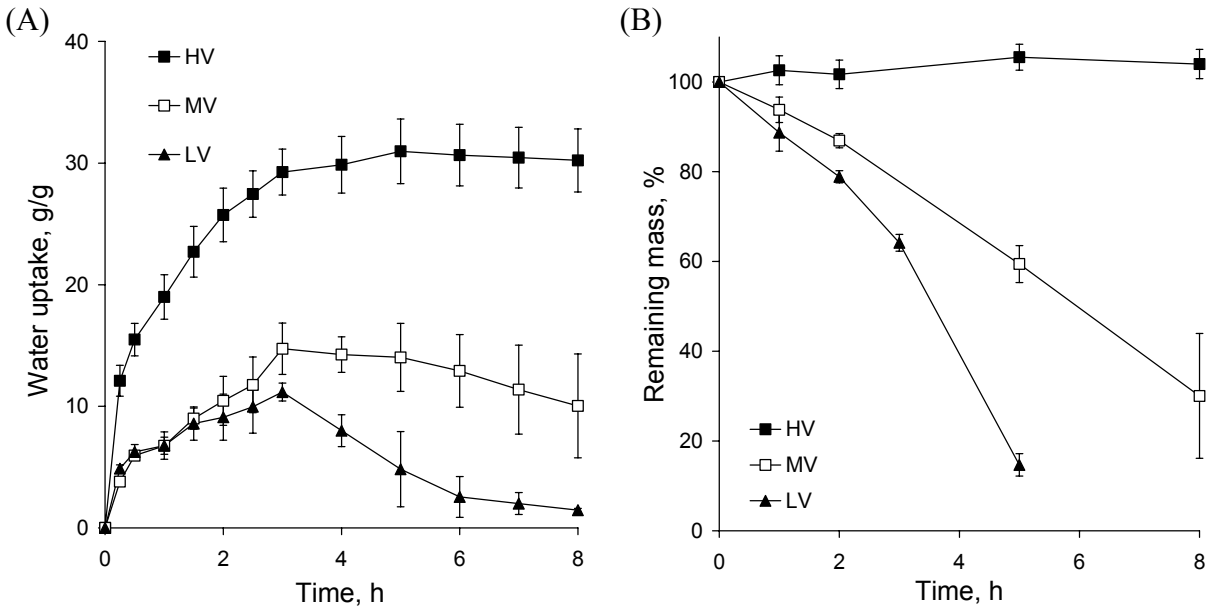


Figure 3. 13 Effect of Na-alginate molecular weight on (A) water uptake and (B) mass loss of inserts (polymer 2% w/w, V = 1.5 ml, n = 3).

The differences in water uptake and mass loss of carrageenan and Na-alginate inserts were likely to be related to the molecular weight range covered by the two polymer species. The carrageenan molecular weight was not sufficiently reduced to observe gel matrix disintegration / dissolution during the water uptake studies. It is important to note that the viscosity of the 2% (w/w) polymer solutions (Table 3. 11 and Table 3. 12) reflected only the behavior of the solutions under different treatments (sonication or molecular weight). The viscosity of the 2% (w/w) solutions was not identical to that of the rehydrated inserts. The dissolution of the rehydrated insert / gel matrix depended on the actual viscosity which was an interplay between the water uptake of the inserts and consequently the resulting polymer concentration in the gel, and the polymer molecular weight. From the water uptake and mass loss studies a polymer concentration after 8 h of hydration between 4.7 and 5.3% for sonication treated carrageenan inserts and 2.9 to 3.3% for Na-alginate HV and MV were calculated. Na-alginate LV inserts were completely dissolved after 8 h of hydration. The difference in the polymer concentration in the resulting gel and the corresponding viscosity, which was also related to the polymer molecular weight, were responsible for the observed gel disintegration / dissolution of the low molecular weight Na-alginate grades. Although sonication reduced the viscosity of carrageenan solutions, this did not sufficiently affect the extent of the water uptake. Thus, no overhydration with subsequent mass loss was observed opposite to the Na-alginate inserts.

Drug release. The drug release from nasal inserts is a complex phenomenon of water penetration, relaxation of the polymer chains, swelling and spreading of the insert, dissolution of the water-soluble polymer and drug, interactions of the drug and carrier, and drug diffusion through the rehydrated insert. The release of the model drugs oxymetazoline HCl (solubility in water at 22°C: 150 mg/ml, pK_a 9.9) and diprophyllin (solubility in water at 22°C: 330 mg/ml, neutral) from carrageenan and Na-alginate inserts of varying molecular weight was investigated. The model drugs were chosen because of their potential to interact electrostatically with the negatively charged polymers (oxymetazoline HCl) or the absence of these interactions (diprophyllin).

The oxymetazoline HCl release from inserts prepared from sonicated carrageenan solutions was linear and independent of the sonication time (Figure 3. 14A). This was in accordance with the water uptake and mass loss studies, which were also not affected by the sonication time (Figure 3. 12). The carrageenan gel matrix remained intact during the release study. Electrostatic interactions of the positively charged drug with the negatively charged polymers were previously investigated and also contributed to the release (see section 3.1.3). Diprophyllin was released faster than oxymetazoline HCl because of its higher water solubility and its inability to interact electrostatically with carrageenan. It was released slightly faster from inserts prepared from solutions with increasing sonication time and thus decreasing carrageenan molecular weight and solution viscosity (Figure 3. 14B). However, the effect of the sonication time was relatively small as it was expected from the very similar water uptake behavior of the inserts.

Similar to carrageenan, Na-alginate was also able to interact electrostatically with oxymetazoline HCl. Precipitates were formed at drug concentrations of 5% (w/w based on polymer) as determined by turbidity measurements at 500 nm of 2% (w/w) solutions of the Na-alginate grades with different drug loading. The differences between the different molecular weight grades were only minute. Thus, the drug release from Na-alginate inserts was mainly influenced by the dissolution behavior of the gel matrix. The release of oxymetazoline HCl from inserts made from the lower to medium molecular weight Na-alginate grades LV and MV was similar, rapid and complete within 3 - 4 h (Figure 3. 15A). This was related to the gel matrix disintegration observed during the water uptake and mass loss studies, which were similar for these two Na-alginate grades in the first few hours (Figure 3. 13A and B). The higher molecular weight Na-alginate HV inserts showed an extended release profile for oxymetazoline HCl as was expected from the water uptake studies, with an

apparently constant release rate over the time studied, similar to carrageenan (Figure 3. 14A and Figure 3. 15A). Na-alginate HV inserts did not show dissolution of the polymer matrix and the rehydrated inserts / gels contained a constant amount of polymer (plateau of water uptake) (Figure 3. 13A and B). The drug release was therefore only affected by the diffusion of the drug through the oppositely charged gel.

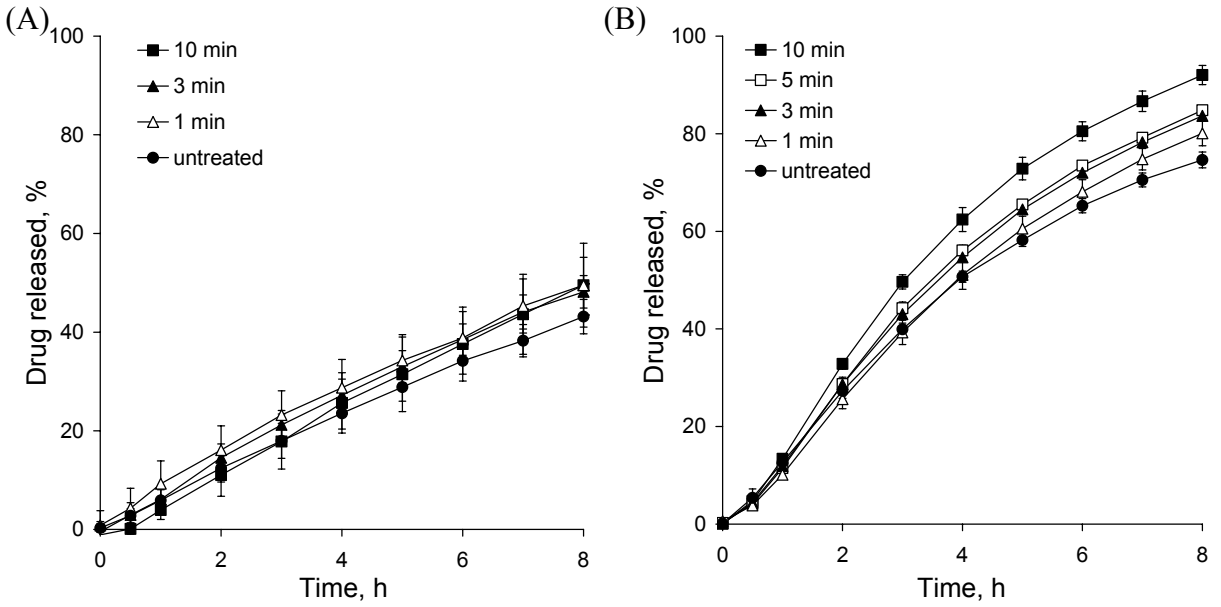


Figure 3. 14 Effect of sonication time on (A) oxymetazoline HCl and (B) diprophyllin release from inserts prepared from sonicated carrageenan solutions (polymer 2% w/w, drug 5% w/w based on polymer, V = 1.5 ml, n = 3).

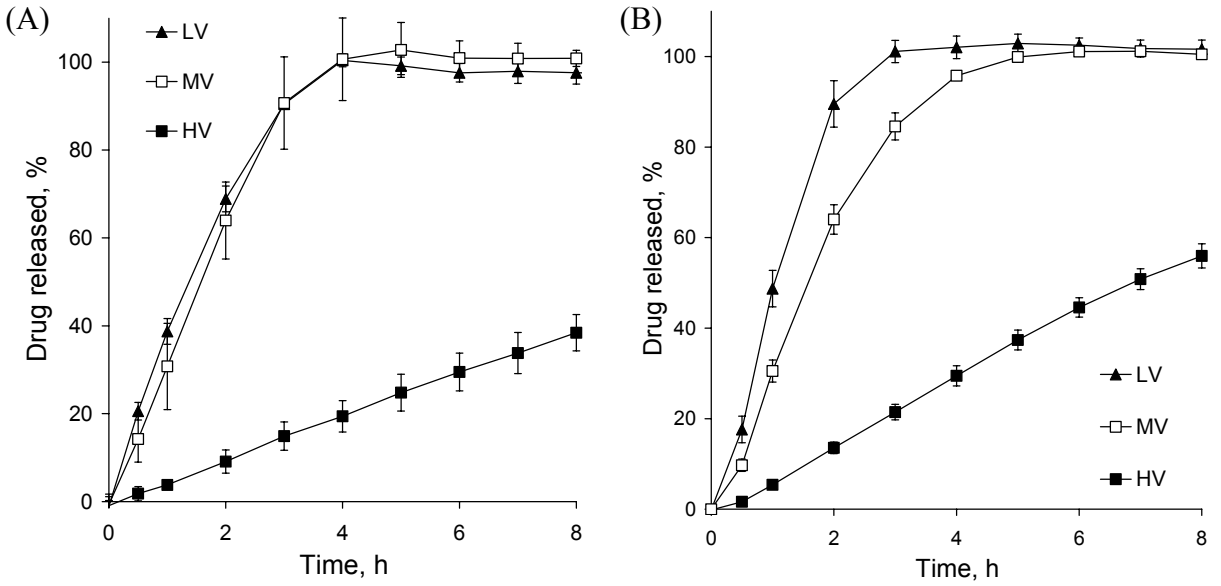


Figure 3. 15 Effect of Na-alginate molecular weight on (A) oxymetazoline HCl and (B) diprophyllin release from inserts (polymer 2% w/w, drug 5% w/w based on polymer, V = 1.5 ml, n = 3).

Diprophyllin was released faster from inserts prepared with decreasing Na-alginate solution viscosity (LV > MV > HV) (Figure 3. 15B). A large decrease in release rate was observed from the MV- to the HV-grade, which could be explained by the much lower solution viscosity and enhanced mass loss for the MV and LV-types but not the HV-type inserts (Table 3. 12 and Figure 3. 13B).

In summary, it could be shown for Na-alginate that the polymer molecular weight had a controlling influence on the drug release rate of both, charged and neutral drugs. Na-alginate inserts were therefore chosen to investigate the effect of the polymer molecular weight on the bioadhesion potential and mechanical properties.

Mechanical properties. As expected, the hardness of inserts decreased with decreasing solution viscosity (molecular weight) of Na-alginate (Table 3. 13). This agreed with previous studies, whereby the hardness of PVP inserts correlated with the glass transition temperature T_g of the polymer, which in fact increases with increasing polymer molecular weight (see section 3.1.3). The inserts' elasticity was only slightly affected by the viscosity grade of Na-alginate (Table 3. 13).

Table 3. 13 Mechanical properties of insert of different Na-alginate grades (polymer 2% w/w, n = 6).

Na-alginate grade	Hardness, N	Elasticity, %
HV	10.0 ± 2.4	47.8 ± 6.7
MV	3.9 ± 0.6	42.6 ± 4.1
LV	3.1 ± 0.3	41.4 ± 4.8

Bioadhesion potential. The bioadhesion potential of Na-alginate inserts, which is inversely related to the displacement, increased with increasing solution viscosity (molecular weight) (LV > MV > HV) (Figure 3. 16). It has been reported that a minimum polymer molecular weight of 100,000 is required for bioadhesion (Lee et al., 2000). Although exact molecular weight information for the Na-alginate grades investigated was not available, this study substantiated this hypothesis of a minimum molecular weight. The bioadhesion potential results were in good agreement with the data from the mass loss studies, which indicated that

the integrity of the hydrated gel matrix is an important factor for bioadhesion. Only the HV-inserts showed no mass loss.

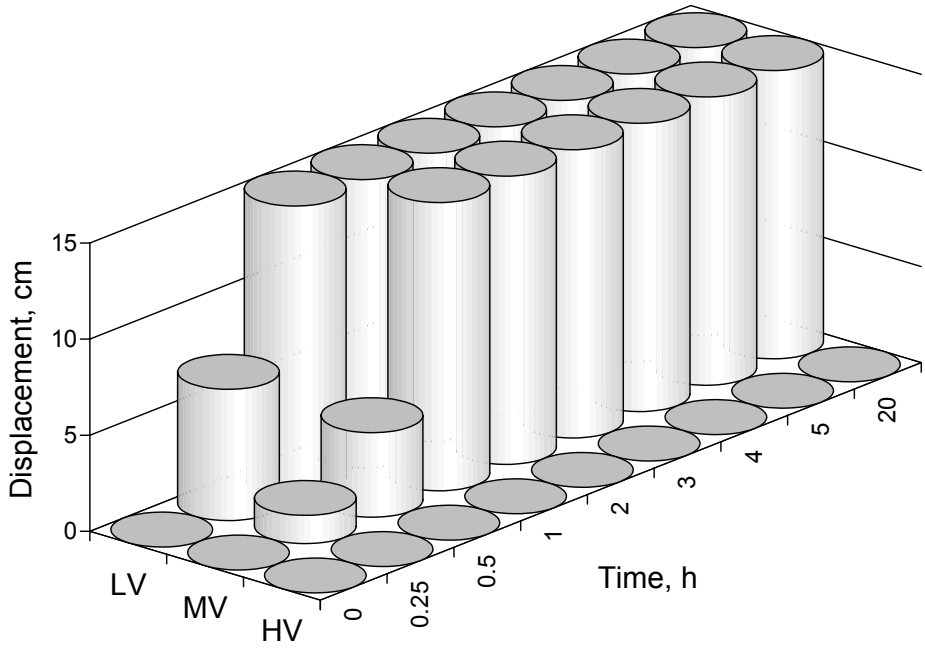


Figure 3. 16 Effect of Na-alginate molecular weight on bioadhesion potential of inserts (polymer 1% w/w, V = 0.1 ml, n = 3, CV = 10.4 ± 4.9%).

Summary. The polymer molecular weight can be used to control the release of drugs from in situ gelling nasal inserts. Inserts prepared from lower molecular weight polymers release the drugs faster than high molecular weight species. However, polymer dissolution and polymer / drug interactions have to be considered when using this approach to control the drug release from nasal inserts. In addition, the bioadhesion as well as the mechanical properties are negatively affected by polymer molecular weight reduction.

3.2.4. Polymer blends

Polymer blends offer the possibility to combine the advantages of two polymers: one giving strength for bioadhesion and mechanical properties and the other contributing low viscosity to enhance the drug release rate. By varying the blend ratio drug release may effectively be controlled. The effect of the blend ratio of carrageenan and a low molecular weight Na-alginate (Protanal) on the drug release from in situ gelling nasal inserts and on other inserts properties such as bioadhesion potential and mechanical properties were investigated.

Solution viscosity. For the investigation of polymer blends, untreated carrageenan was mixed with a low molecular weight Na-alginate to cover a broad range of viscosities. As expected, the solution viscosities of the blends decreased with higher Na-alginate content (Table 3. 14). The differences became minor between the ratios of l:19, l:39, and pure Na-alginate solution (0:l). The described solutions were freeze-dried to form nasal inserts and further investigated with regard to their water uptake behavior, release of model drugs, mechanical properties, and bioadhesion potential.

Table 3. 14 Effect of polymer blend ratio (carrageenan : Na-alginate on the solution viscosity (total polymer 2% w/w, C20/4°, D = 5 s^{-1}, 22°C, n = 3) and on the contact angle (phosphate buffer pH 6.0 on polymer film, l min, n = 3).

Carrageenan : Na-alginate	Viscosity, mPas	Contact angle, °
1:0	803.5 ± 18.9	69.8 ± 2.4
1:1	1541.1 ± 23.1	73.6 ± 4.7
1:3	837.6 ± 4.6	63.9 ± 2.9
1:19	371.4 ± 6.2	49.8 ± 2.0
1:39	331.1 ± 4.8	46.3 ± 0.9
0:1	293.4 ± 3.9	47.7 ± 1.1

Water uptake and mass loss. Nasal inserts prepared from blends of carrageenan and Na-alginate showed an increased water uptake with higher Na-alginate content (Figure 3. 17A) which was attributed to the more hydrophilic nature of Na-alginate as reflected in the contact angle (Table 3. 14). Unexpectedly, pure carrageenan inserts did not show the slowest water uptake but an intermediate one. A possible reason may be more rapid swelling of the Na-alginate compared to carrageenan which would lead to faster formation of the gel under simultaneous loss of the porosity. This would result in a shorter time for water uptake by capillary forces and subsequent reduced initial water uptake as discussed in section 3.1.3. The consequence would be the observed biphasic behavior of carrageenan water uptake as opposed to the gradual uptake of Na-alginate inserts, which was later followed by gel disintegration (Figure 3. 17A). Inserts from blends of carrageenan and Na-alginate showed a reduced initial water uptake compared to pure carrageenan inserts (1:0). The water uptake rate

of the second phase of the profile was similar between pure carrageenan inserts (1:0) and the blend 1:1 and increased with higher Na-alginate content. The biphasic behavior became less pronounced with higher Na-alginate content.

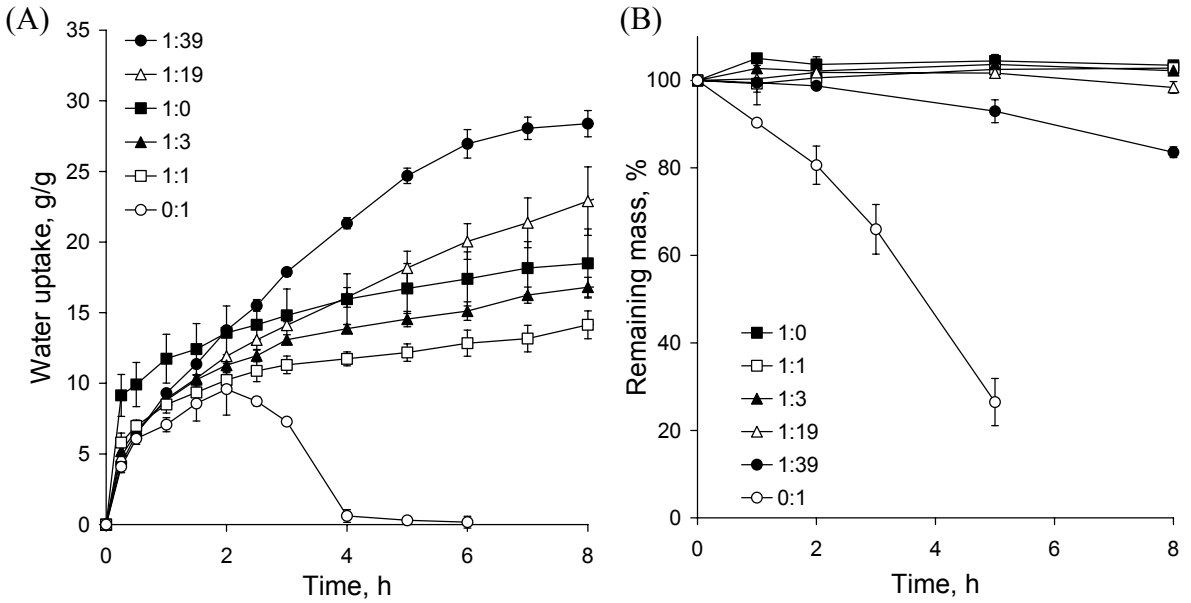


Figure 3. 17 Effect of the polymer blend ratio (carrageenan : Na-alginate) on (A) water uptake and (B) mass loss during water uptake of inserts (total polymer 2% w/w, V = l.5 ml, n = 3).

The low water uptake of pure Na-alginate inserts (0:1) was caused by the continuous mass loss of these inserts during hydration (Figure 3. 17B). None of the blends showed mass loss except the ratio 1:39, starting after more than 2 h and whose water uptake leveled off after 6 h as a result (Figure 3. 17A and B). The missing mass loss of inserts with all other ratios may result from the formation of entanglements / physical cross-links and / or hydrogen bonds between both polymers during the freeze-drying process and / or the rehydration, preventing dissolution of Na-alginate. Similar observations were made by Dürig and Fassihi (2002), who found that mass loss of guar gum based matrix tablets was restricted to drug leaching without erosion of the tablet matrix when microcrystalline cellulose was added compared to pure guar gum tablets, which showed substantial matrix erosion. Physical cross-links were discussed as a possible explanation.

The observed variation in the water uptake behavior and mass loss of the gel matrix during hydration of inserts prepared from blends of carrageenan and Na-alginate was likely to affect the release of the incorporated drug.

Drug release. Aim of this part of the work was to control the drug release from in situ gelling nasal inserts by varying the polymer blend ratio. The oxymetazoline HCl release from inserts prepared from blends of carrageenan and Na-alginate generally increased with increasing amount of Na-alginate (Figure 3. 18A). This could be explained with the increased water uptake and the reduced polymer solution viscosity with increasing Na-alginate content (Figure 3. 17A and Table 3. 14). Polymer gel matrix disintegration further contributed to the fast drug release for the ratio 1:39 and the pure Na-alginate inserts (0:1) (Figure 3. 17B). A blend ratio of carrageenan and Na-alginate of l:l did not change the release rate compared to carrageenan alone, although the viscosity of the 2% (w/w) polymer solution was markedly reduced (Figure 3. 18A and Table 3. 14). This indicated that the lower viscosity of the 1:1 blend was counteracted by the lower water uptake of the insert (Figure 3. 17A), resulting in gels with a similar diffusion barrier for the drug. The release from the pure carrageenan insert and the inserts of the blend 1:1 were the most retarded one and seemed to follow an apparent zero-order-kinetic due to electrostatic interactions (see section 3.1.3). As previously mentioned, also Na-alginate interacted electrostatically with oxymetazoline HCl, but for pure Na-alginate inserts the gel matrix dissolution rate governed the drug release rather than the dissolution of the drug-polymer-salt. The ability of both polymers to form salts with the model drug may account for the apparent zero-order-release achieved for all blend ratios.

The relation between the release rate of the initial, apparent zero-order-kinetic phase of each blend and the blend ratios was empirically described by a linear equation using the logarithms of the release rate and the carrageenan percentage in the polymer blend (subset in Figure 3. 18B). The use of polymer blends covered a broad spectrum of drug release rates. Fine-tuning of the release rate will be possible by adjusting the polymer blend ratio. Further studies were performed to investigate the effect of the carrageenan / Na-alginate blends on the mechanical properties and the bioadhesion potential of nasal inserts.

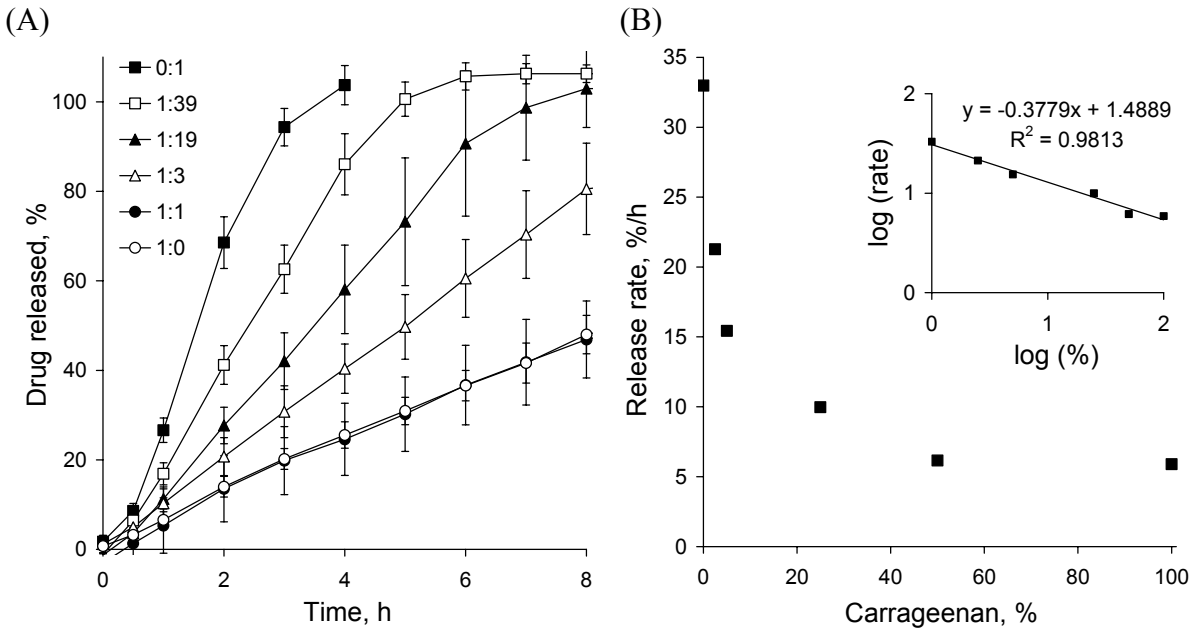


Figure 3. 18 Effect of polymer blend ratio (carrageenan : Na-alginate) on oxymetazoline HCl release from inserts (total polymer 2% w/w, drug 5% w/w based on total polymer, V = 1.5 ml, n = 3).
(A) Release profile, (B) Release rate vs. carrageenan content in the blend.

Mechanical properties. The hardness of inserts prepared from blends of carrageenan and Na-alginate correlated well with the polymer blend solution viscosity, was in excess of the minimum hardness of 2 N, and decreased with increasing Na-alginate content. The elasticity was not significantly affected by the blend ratio (Table 3. 15). Acceptable mechanical properties were obtained with inserts prepared from all blend ratios.

Table 3. 15 Effect of polymer blend ratio on the mechanical properties of inserts (total polymer 2% w/w, n = 3).

Carrageenan : Na-alginate	Hardness, N	Elasticity, %
1:0	5.18 ± 0.84	35.2 ± 4.2
1:1	5.40 ± 0.79	36.9 ± 3.5
1:3	4.18 ± 0.87	28.2 ± 6.4
1:19	3.06 ± 0.85	36.9 ± 6.6
1:39	2.52 ± 0.26	39.0 ± 2.3
0:1	2.06 ± 0.21	39.7 ± 1.9

Bioadhesion potential. Carrageenan is a polymer with very strong bioadhesive properties. The bioadhesion potential of carrageenan inserts (1:0) was beyond the limits of the test, resulting in no displacement of the inserts over the entire test period of 20 h (Figure 3. 19). Interestingly, inserts made from carrageenan / Na-alginate blends did not show the expected gradually decreasing bioadhesion potential with increasing Na-alginate content (Figure 3. 19). Excellent bioadhesion potential (no displacement) was found above a threshold content of carrageenan (blend ratios 1:0 to 1:19), whereas the adhesion potential was dramatically reduced below this threshold (blend ratios l:39 and 0:1). This correlated well with mass loss studies performed during hydration of inserts (Figure 3. 17B). Only inserts prepared from blend ratios of 1:39 and 0:1 showed significant mass loss over the study period of 8 h. The disintegration of the insert / gel therefore limited the bioadhesion potential.

Variations in the blend ratio of carrageenan and Na-alginate affected the bioadhesion potential of the inserts much less than the drug release, which was highly dependent on the blend ratio.

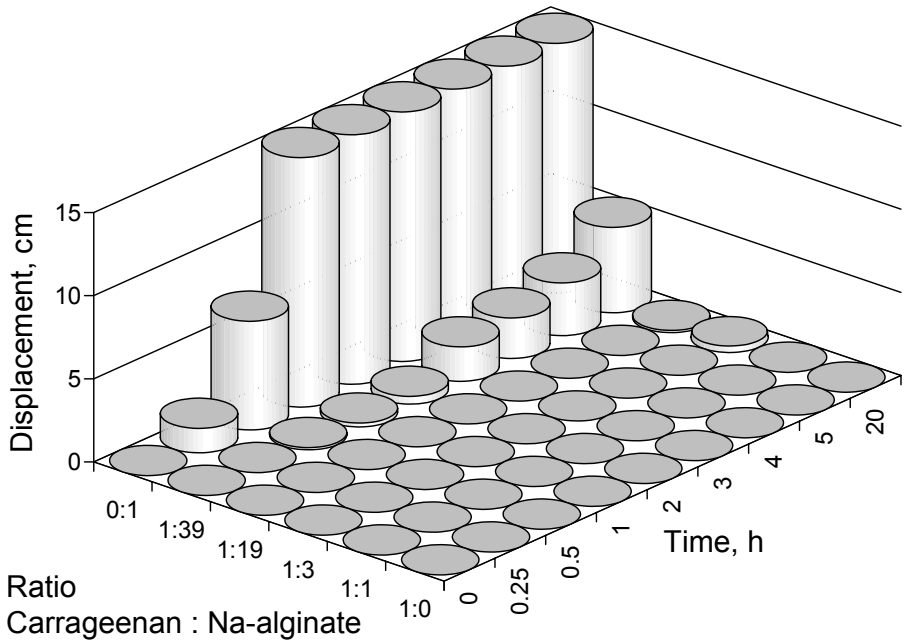


Figure 3. 19 Effect of polymer blend ratio (carrageenan : Na-alginate) on bioadhesion potential of inserts (total polymer 2% w/w, V = 0.1 ml, n = 3, CV = 15.2 ± 11.3%).

Bioadhesion potential of various other polymer blends. The ability of carrageenan to form strongly bioadhesive inserts even in blends with low carrageenan content raised the question whether this can be expected from all blends of a strong and a weak bioadhesive polymer.

Therefore, the bioadhesion potential of blends of carrageenan with the bioadhesive polymer NaCMC 600 and the weakly bioadhesive polymers HPMC E5 and PVP 90 were investigated (Figure 3. 3). Furthermore, inserts from blends of Carbopol® and xanthan gum, two strong bioadhesives (no displacement over 20 h), with Na-alginate were examined. A constant blend ratio of 1:19 between the strong and weak bioadhesive polymer was chosen, because it represents the ratio with the lowest carrageenan content in carrageenan / Na-alginate inserts, which still had a high bioadhesion potential.

Similar to Na-alginate, inserts prepared from carrageenan blends with NaCMC 600, HPMC E5 and PVP 90 had no or only a minor displacement indicating high bioadhesion potential (Figure 3. 20). However, neither Carbopol® nor xanthan gum blended with Na-alginate reached the bioadhesion potential of carrageenan-containing blends.

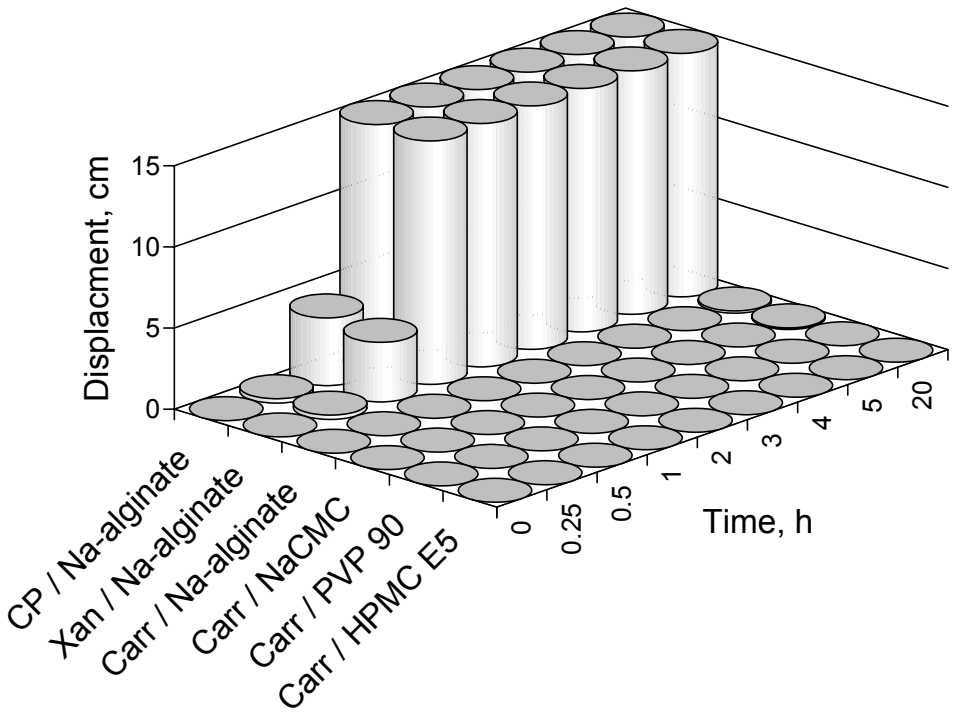


Figure 3. 20 Effect of blended polymer species (blend ratio 1:19) on bioadhesion potential of inserts (total polymer 2% w/w, V = 0.1 ml, n = 3, CV = 15.6 ± 11.8%). (CP…Carbopol®, Xan…xanthan gum, Carr…carrageenan).

Solution viscosity, water uptake, and mass loss were investigated in order to understand the bioadhesion potential of inserts made from different polymer blends. The bioadhesion potential did not correlate with the viscosity of the polymer solutions, which was mainly

determined by the major polymer in the blend, which contributed 95% (blend ratio 1:19) of the total polymer mass (Table 3. 16). The water uptake of the inserts was a result of the contribution of both polymers (Figure 3. 21A). Again, no direct relation to bioadhesion potential was found. The inserts from blends of xanthan gum / Na-alginate and Carbopol® / Na-alginate reached a maximum water uptake after approximately 5 h indicating mass loss during the study. Mass loss determinations showed a significant dissolution of the polymer for both of these blends (Figure 3. 21B). Also carrageenan / HPMC E5 inserts showed mass loss, although to a lower extent. Similar to inserts prepared from carrageenan / Na-alginate blends at different blend ratios, the mass loss during hydration seemed to be the most crucial parameter in determining the bioadhesion potential of inserts. The good bioadhesion performance of carrageenan / HPMC E5 inserts, despite slight mass loss during hydration, indicated that carrageenan, the major bioadhesion potential determining component in the inserts, did not dissolve but retained its integrity and therefore its bioadhesion potential.

The good bioadhesion potential of inserts prepared from carrageenan blends at the ratio 1:19 could not be achieved with blends containing the other highly bioadhesive polymers tested (xanthan gum, Carbopol®). The reason for this behavior of carrageenan is not clear. Polymer molecular weight, the ability to form hydrogen bonds with the second polymer and polymer chain flexibility as a prerequisite for the formation of physical entanglements may play a role.

Table 3. 16 Effect of blended polymer species on viscosity of polymer solutions (C20/4°, D = 5 s^{-1}, 22°C, n = 3).

Polymers, blend ratio 1:19	**Viscosity, mPas**		
	First polymer 2% (w/w)	**Second polymer 2% (w/w)**	**Polymer blend 2% total (w/w)**
Carrageenan : NaCMC	2032.8 ± 183.3	1171.2 ± 136.2	1120.1 ± 13.5
Carrageenan : PVP 90	2032.8 ± 183.3	22.9 ± 2.7	70.6 ± 2.2
Carrageenan : HPMC E5	2032.8 ± 183.3	23.2 ± 6.1	25.6 ± 3.7
Carrageenan : Na-alginate	2032.8 ± 183.3	293.4 ± 3.9	371.4 ± 6.2
Carbopol® : Na-alginate	3875.3 ± 127.6	293.4 ± 3.9	414.0 ± 4.2
Xanthan gum : Na-alginate	2394.6 ± 523.3	293.4 ± 3.9	324.0 ± 5.0

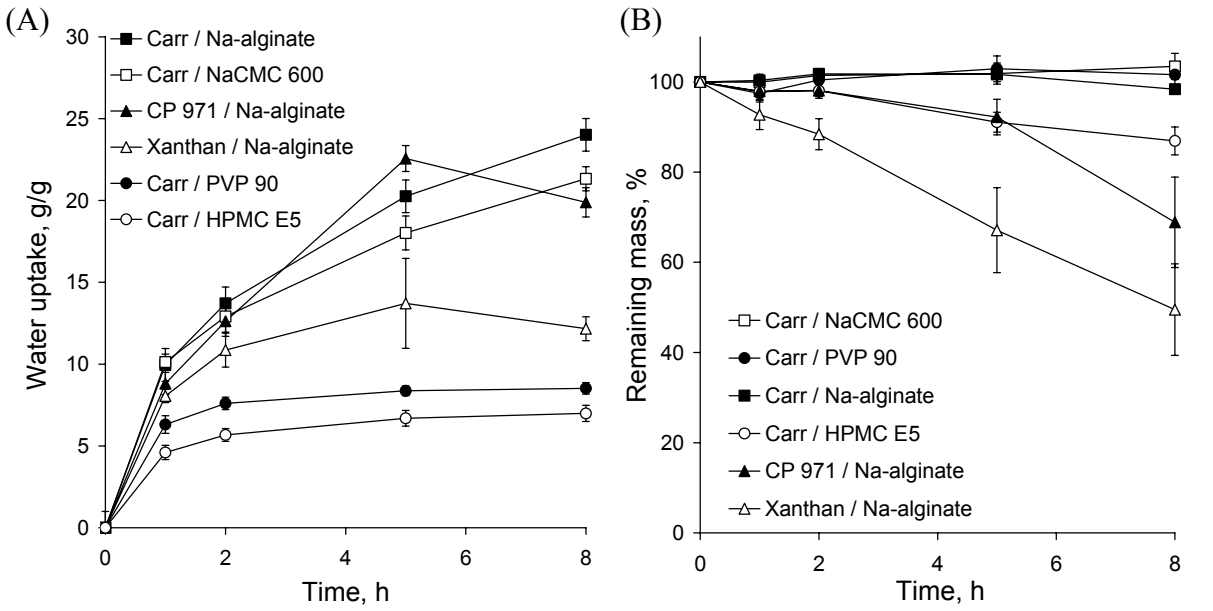


Figure 3. 21 Effect of blended polymer species (blend ratio 1:19) on (A) water uptake and (B) mass loss during water uptake of inserts (total polymer 2% w/w, V = 1.5 ml, n = 3).

Summary. The use of polymer blends (carrageenan and Na-alginate) to prepare in situ gelling nasal inserts allows a broad control of the drug release rate from nasal inserts with the possibility of exact rate adjustment by choosing the appropriate polymer blend ratio. The blend ratio determined also the effect of these blends on other inserts properties, e.g. polymer solution viscosity, water uptake, and mechanical properties. Bioadhesion potential deteriorated above a threshold ratio of 1:19 and correlated with the mass loss. The blend of carrageenan with Na-alginate had a superior bioadhesion potential compared to Carbopol® or xanthan gum blends with Na-alginate, while the Na-alginate was exchangeable, e.g. by PVP 90 or HPMC E5. Acceptable mechanical properties were obtained with insert prepared from all blend ratios. Overall, these findings render the use of polymer blends as drug release control tool for in situ gelling nasal inserts superior to variation of the polymer content, addition of freely water-soluble additives, and polymer molecular weight reduction.

3.3. Effect of drug and release medium on drug release

Ideally, a drug delivery system should be able to perform well with a wide variety of drugs with broad physico-chemical properties. Therefore, in this section, the release of different model drugs from in situ gelling nasal inserts is reported. The effect of the type of drug and drug loading as well as the embedding of the drug in the polymeric structure have been studied and related to the observed release behavior. Three low molecular weight model drugs with different physico-chemical properties were chosen (Table 3. 17). Two polymers, HPMC K15M (neutral) and carrageenan (anionic), were evaluated as carrier materials.

As mentioned previously, the performance of a drug delivery system may not only depend on its composition and structure but also on the physiological conditions at its site of administration (see section 1.2.3). The nasal fluid varies in pH, ion content, and osmolality, which could affect the performance of in situ gelling nasal inserts. Thus, further studies were conducted to investigate the influence of the release medium on the oxymetazoline HCl release and the water uptake of in situ gelling nasal inserts.

Table 3. 17 Physicochemical properties of low molecular weight model drugs.

Drug	**MW**	**Solubility in water, mg/ml**	**pKa**	**Melting point, °C**
Oxymetazoline HCl	297 (262 w/o Cl^-)	149	9.9	300-303 (decomposition)
Diprophyllin	254	330	-	158
APAP	151	14	9.5	169-170.5

3.3.1. Influence of drug species and drug loading

No differences in drug release were observed for the three drugs at 5% loading (w/w based on polymer) with inserts prepared from HPMC K15M, a high molecular weight, uncharged cellulose derivative (Figure 3. 22A). This indicated that the drug release from HPMC K15M inserts is neither solely controlled by drug diffusion, nor electrostatic drug-polymer interaction, nor drug solubility.

In contrast to HPMC K15M, carrageenan is a negatively charged polysaccharide. The neutral drug species diprophyllin and APAP were released at a similar rate from the carrageenan inserts (Figure 3. 22B). Oxymetazoline HCl release, on the other hand, was slower. This drug carries a positive charge at pH 6 and thus was able to interact with the anionic carrageenan. This interaction led to the observed apparent zero-order-release kinetics (see section 3.1.3). Similar observations were described by Puttipipatkhachorn et al. (2001) for chitosan (a cationic polysaccharide) films loaded with the anionic salicylic acid.

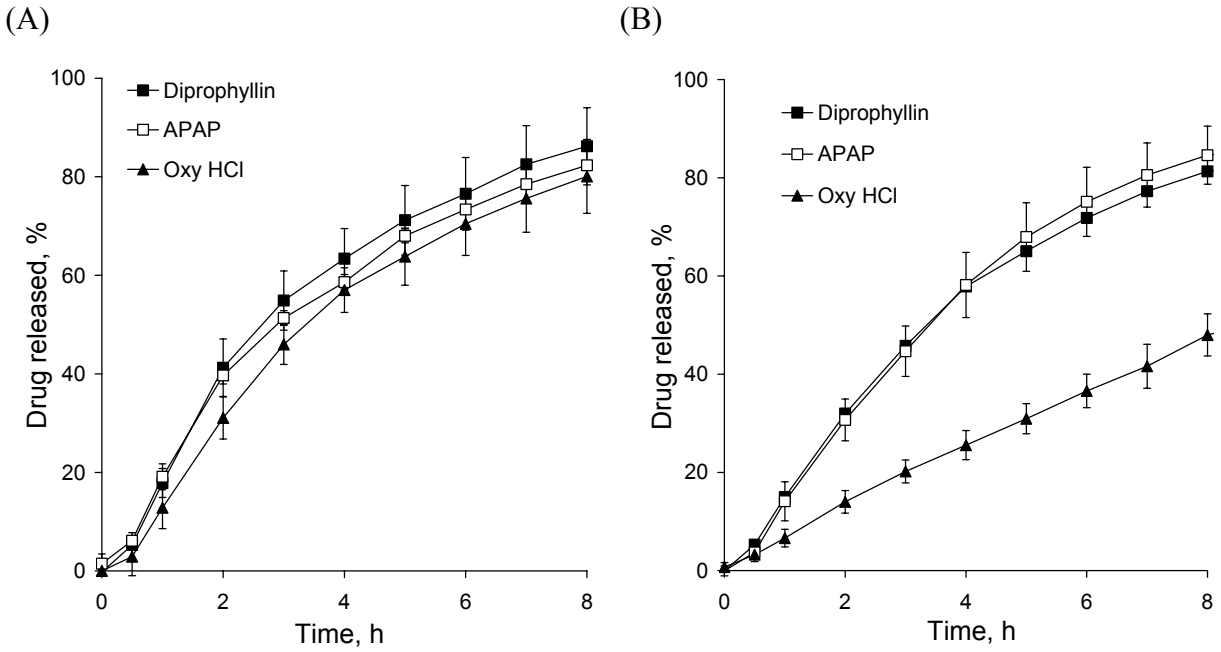


Figure 3. 22 Drug release of diprophyllin, APAP, and oxymetazoline HCl from (A) HPMC K15M and (B) carrageenan inserts (polymer 2% w/w, drug 5% w/w based on polymer, V = 1.5 ml, n = 3).

Precipitation studies showed the formation of an insoluble oxymetazoline / carrageenan salt in concentrated solutions (see section 3.1.3). Oxymetazoline-carrageenan salts were investigated by Raman spectroscopy, which showed a peak shift from 1568 cm^{-1} to 1578 cm^{-1} (region 1490 - 1580 cm^{-1} representative of the secondary amino group of the drug) (Figure 3. 23). In addition, the peak at 1188 cm^{-1} was missing in the spectrum of the drug-loaded inserts (region 1150 - 1260 cm^{-1} representative for the sulfate group of the polymer). This is an indication of a mutual interaction of these functional groups.

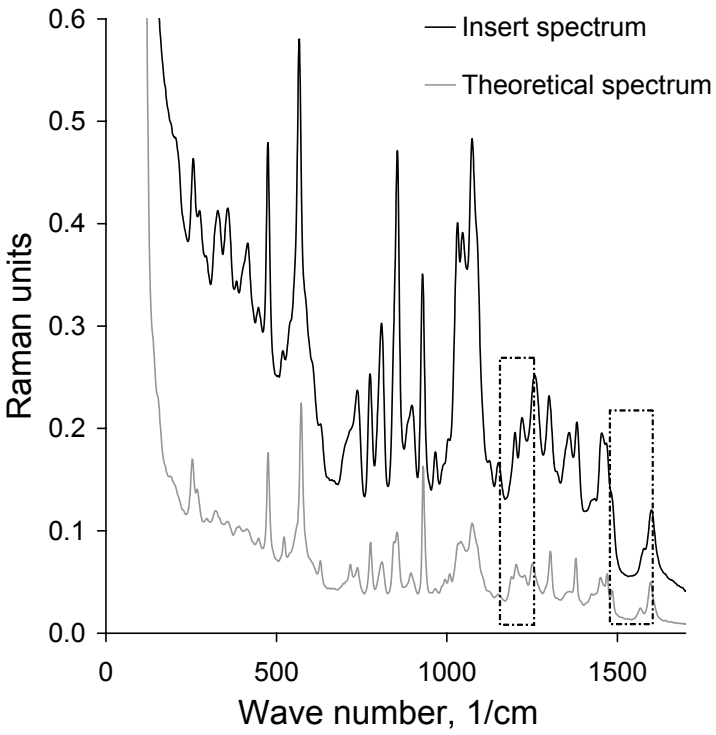


Figure 3. 23 Raman spectrum of carrageenan inserts loaded with oxymetazoline HCl (polymer 2% w/w, drug 20% w/w based on polymer) in comparison to the theoretical spectrum calculated from the single substances.

Next, the effect of drug loading on the drug release and the physical state of the drug in the solid inserts was investigated in order to explain the release mechanism. With carrageenan inserts, the release of the uncharged diprophyllin and APAP was independent of drug loading (5 - 40% w/w based on polymer) (Figure 3. 24A and B). In contrast, different oxymetazoline HCl loadings of carrageenan inserts resulted initially in similar drug release profiles (up to 3 h) and then in a decreased relative release with increasing drug loading in the later time period (Figure 3. 24C). This further supported the statement of electrostatic interactions between polymer and drug. Even at the highest loading investigated (30% drug w/w based on polymer), the theoretical number of negative charges of carrageenan exceeded the number of positives charges of oxymetazoline HCl by a factor of 4 to 5. Similar observations were made by Stockwell et al. (1986) in gel systems based on Na-alginate with the oppositely charged drug chlorpheniramine maleate. An increasing amount of the drug increased the amount of the drug-polymer complex formed. As a result, the increased charge neutralization effect on the polymer caused a reduction in intramolecular repulsion such that the system was able to take up a tighter, more compact conformation and thereby affect diffusional pathways.

(A) Diprophyllin (B) APAP (C) Oxy HCl (1% polymer)

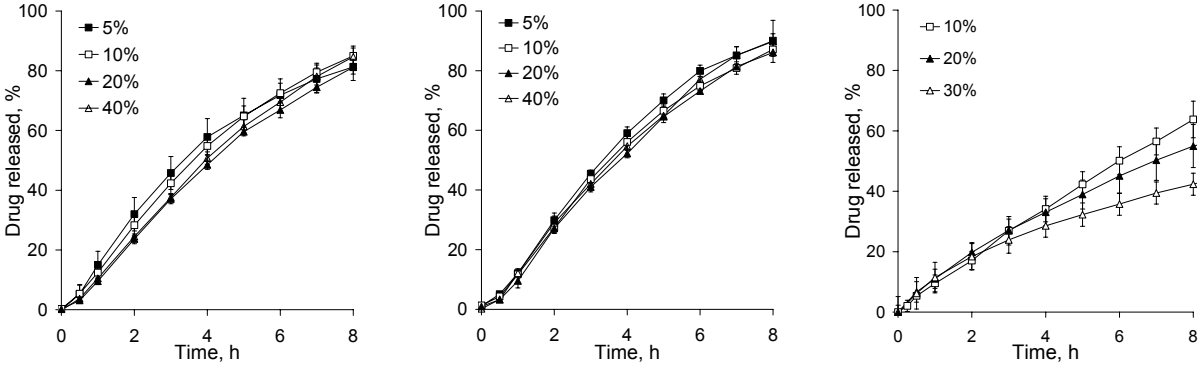


Figure 3. 24 Release of diprophyllin, APAP, and oxymetazoline HCl from carrageenan inserts with different loadings (polymer 2% w/w, drug % w/w based on polymer, V = 1.5 ml, n = 3).

(A) Diprophyllin (B) APAP (C) Oxy HCl

(D) 10% drug loading (E) 20% drug loading (F) 50% drug loading

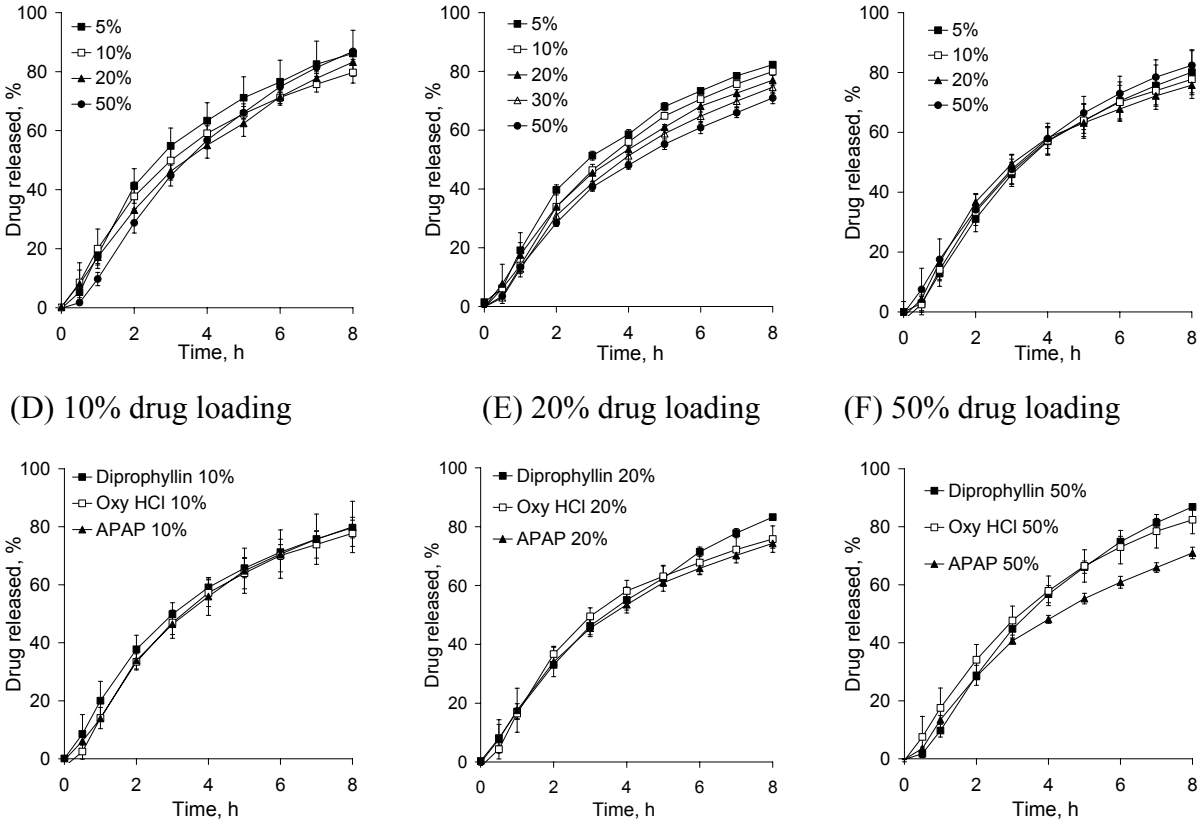


Figure 3. 25 Release of diprophyllin, APAP, and oxymetazoline HCl from HPMC K15M inserts with different loadings (polymer 2% w/w, drug % w/w based on polymer V = 1.5 ml, n = 3).

The release of the three drugs from the HPMC inserts was almost independent of the drug loading (5 - 50% w/w based on polymer) (Figure 3. 25A to C). For APAP, the least soluble, the release decreased slightly with increasing APAP loading (Figure 3. 25B). This slightly reduced release rate of APAP from HPMC K15M inserts at higher loadings was attributed to its lower aqueous solubility compared to the other two drug species (Table 3. 17). Based on water uptake (Figure 3. 4), polymer mass loss during hydration (Table 3. 5) and drug release studies, the concentration of the remaining drug in rehydrated carrageenan and HPMC K15M inserts was calculated (Figure 3. 26). The local APAP concentration in carrageenan inserts was reduced below the solubility of the drug for all loadings investigated within 3 h (Figure 3. 26A) corresponding to approximately 35% drug release. Although in the beginning of the release test the APAP was not completely dissolved in the rehydrated insert, the drug was completely dissolved during the majority of the study and could diffuse freely. This resulted in a drug release from carrageenan inserts independent of the drug loading (Figure 3. 24B).

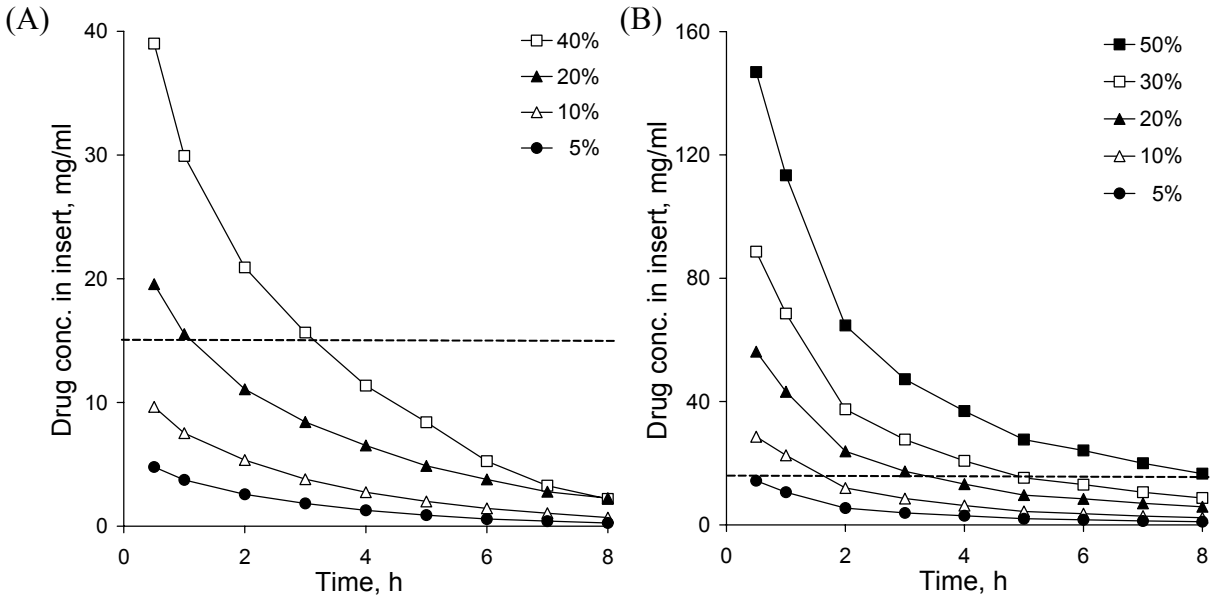


Figure 3. 26 Theoretical drug concentration in the hydrated (A) carrageenan and (B) HPMC K15M inserts with different loadings of APAP) (polymer 2% w/w, drug % w/w based on polymer, V = 1.5 ml, n = 3). The dotted line represents the aqueous solubility of the drug.

HPMC K15M inserts, on the other hand, took up much less water during hydration studies and the local APAP concentration was therefore higher (Figure 3. 4 and Figure 3. 26B). The local APAP concentration dropped below the solubility at < 0.5 h for a drug loading (w/w

based on polymer) of 5%, ~ 1.75 h for 10%, ~ 3.5 h for 20%, ~ 5.0 h for 30%, ≥ 8 h for 50% (Figure 3. 26B). For 50% loading, white spots of undissolved drug in the gel matrix were observed after 8 h of drug release study. The maintenance of a depot of undissolved drug over a long time of the drug release study resulted in the reduced APAP release rate from HPMC K15M inserts (Figure 3. 25B). In direct comparison with the freely water-soluble drugs diprophyllin and oxymetazoline HCl at identical loadings, the reduced APAP release rate from HPMC K15M inserts became clearly visible at 50% drug loading compared to 10% and 20% (w/w based on polymer) (Figure 3. 25D to F).

However, visual observation of HPMC K15M and carrageenan inserts during water uptake studies showed that the absorbed water is, at least initially, not homogeneously distributed in the inserts as it must be assumed for the calculation of the local drug concentration. Also the effect of the polymer, e.g. the surface active properties of HPMC (Persson et al., 1996), on the solubility of the drug as well as the influence of the drug and the drug loading on the water uptake were not considered.

Neither diprophyllin nor oxymetazoline HCl resulted in local drug concentrations in HPMC K15M and carrageenan inserts during the drug release test (first measurement point at 15 min) that exceeded the aqueous solubility of the drugs.

Besides the investigation of the local solubility of the drug in rehydrated nasal inserts, the physical state of the drug in films and inserts was also investigated to explain the observed drug release behavior. When drug-polymer solutions were lyophilized into the solid inserts, the drug could be dissolved or dispersed in amorphous or crystalline form in the solid polymer matrix. Film casting experiments with visual observation, DSC measurements, and scanning electron microscopy were performed to characterize the physical state of the drug in films and inserts (Table 3. 18). Films of the pure polymers were clear. Clear drug-containing films roughly indicated that the drug was dissolved in the matrix, while turbid films indicated the presence of dispersed drug.

As expected from the turbidity of the solutions for film casting, carrageenan / oxymetazoline HCl films were turbid because of the formation of the poorly water-soluble salt (see section 3.1.3). Due to thermal degradation of oxymetazoline HCl, no DSC studies could be performed with this drug. SEM revealed no crystals in carrageenan inserts loaded with up to 40% drug (w/w based on polymer) (Table 3. 18). As previously described, the poorly water-soluble oxymetazoline / carrageenan salt determined the retarded drug release behavior of these inserts.

Table 3. 18 Summarization of the physical state of different model drugs in carrageenan and HPMC K15M films and inserts with different drug loading (% w/w based on polymer) (polymer content of inserts for SEM 2% w/w).

Polymer / Drug	**Film turbidity**	**DSC**	**SEM**
Detectable drug	Undissolved	Crystalline	Crystals
Carr / Oxy HCl	≥ 5%	n.p.	≥ 40%
Carr / APAP	≥ 5%	≥ 5%	n.p.
Carr / Dipro	≥ 10%	≥ 49%	n.p.
HPMC K15M / Oxy HCl	n.d. up to 100%	n.p.	n.d. up to 40%
HPMC K15M / APAP	≥ 40%	≥ 37%	n.p.
HPMC K15M / Dipro	≥ 60%	≥ 48%	n.p.

n.d. = not detectable, n.p. = not performed

Film turbidity at ≥ 10% and crystalline drug (DSC) at ≥ 49% (w/w based on polymer) in carrageenan / diprophyllin films indicated the presence of amorphous drug within this loading range (Table 3. 18). Due to the very high water solubility of diprophyllin the physical state of the drug in nasal inserts may be less important for the drug release because formation of diffusible drug by dissolution of either the solid polymer / drug solution (< 10%) or the solid crystalline drug alone (amorphous < 49% or crystalline ≥ 49%) occurred very rapidly.

Carrageenan / APAP films contained the drug in crystalline form (film turbidity and crystallinity (DSC) at ≥ 5% drug w/w based on polymer) (Table 3. 18). Because the crystalline state of APAP was maintained over the entire loading range tested, an effect of the change of the physical state of APAP on the release from carrageenan inserts could be excluded. Here, the solubility of the APAP crystals determined the release. Studies to the local APAP concentration have already shown that the water taken up by the carrageenan inserts was sufficient to dissolve the APAP after an initial phase (Figure 3. 26A).

In HPMC K15M films, the three drugs were dissolved in the polymer matrix: APAP up to ~ 37% loading (w/w based on polymer), diprophyllin up to ~ 48%, oxymetazoline HCl up to > 40% (Table 3. 18). The dissolved state of all three drugs in HPMC K15M resulted in the similar drug release of all three drugs from HPMC K15M inserts up to 20% loading (Figure 3.

25D and E). The hydration of the polymer matrix led to the release of diffusible drug without the extra step of drug dissolution. At 50% loading (w/w based on polymer), only APAP was significantly present in the crystalline form (Table 3. 18). At this loading, not only matrix hydration but, more significantly, the dissolution of the drug crystals determined the release. This resulted in the lower drug release rate for APAP compared to diprophyllin and oxymetazoline HCl from HPMC K15M inserts (Figure 3. 25).

Summary. The release of different low molecular weight drugs from in situ gelling nasal inserts was compared. Drug-polymer interactions resulted in the slower release of oxymetazoline HCl from carrageenan inserts compared to HPMC K15M inserts. The latter showed no dose effect. Diprophyllin loading did not influence the relative release rate from HPMC K15M and carrageenan inserts. Low drug solubility in case of APAP in hydrating HPMC K15M inserts resulted in reduced relative release rates with higher loadings. Results from film casting, DSC, and SEM studies were used to explain the release data. In conclusion, the results showed that the main physico-chemical drug properties involved in the drug release process are (i) solubility, when its is locally exceeded, (ii) the physical state in the solid inserts, and (iii) electrostatic interactions between drug and polymer.

3.3.2. Influence of release medium

As mentioned previously, the performance of a drug delivery system may not only depend on its composition and structure but also on the physiological conditions at its site of administration. The nasal fluid varies in pH, ion content, and osmolality, which could affect the performance of in situ gelling nasal inserts. Thus, further studies were conducted to investigate the influence of the release medium on the oxymetazoline HCl release from and the water uptake of in situ gelling nasal inserts. The anionic polysaccharide carrageenan and the neutral cellulose derivative HPMC K15M were chosen as model polymers for these investigations.

The osmolality of the release medium was adjusted by sorbitol addition and the ion content in media of different osmolality was kept constant. The osmolality of the medium neither influenced the water uptake nor the drug release from inserts prepared from either polymer (Figure 3. 27 and Figure 3. 28). Effects at higher osmolalities cannot be excluded but were found irrelevant for physiological and pathophysiological conditions.

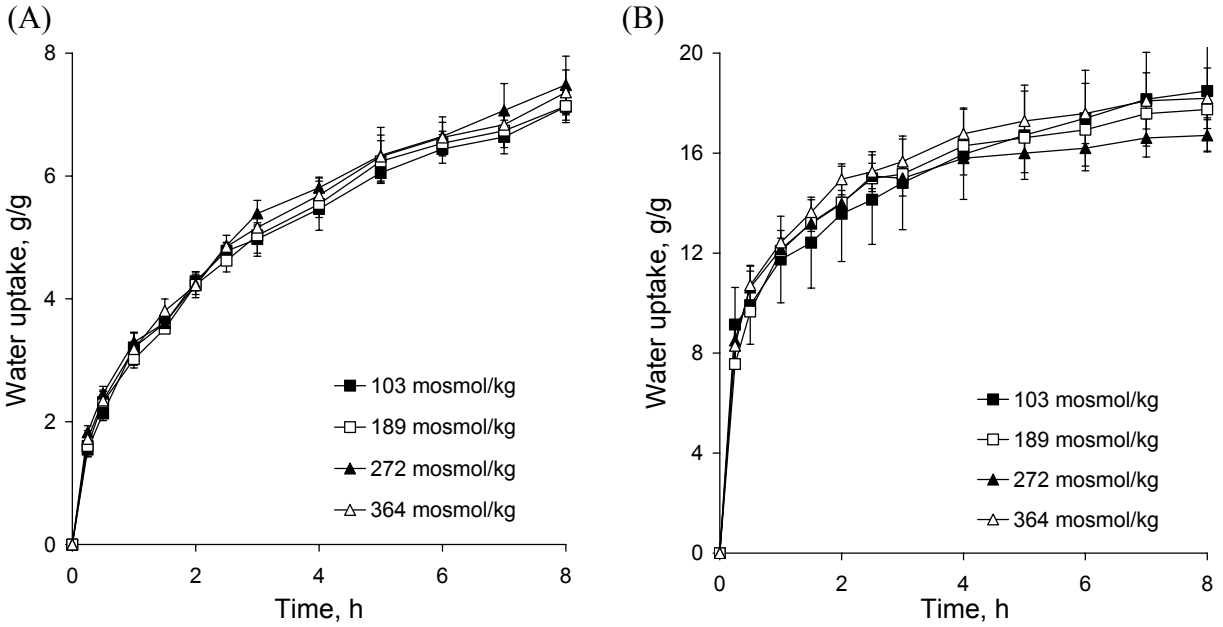


Figure 3. 27 Effect of release medium osmolality on water uptake of (A) HPMC K15M and (B) carrageenan inserts (polymer 2% w/w, V = 1.5 ml, pH 6, $[Na^+]$ = 0.005 mol/l, n = 3).

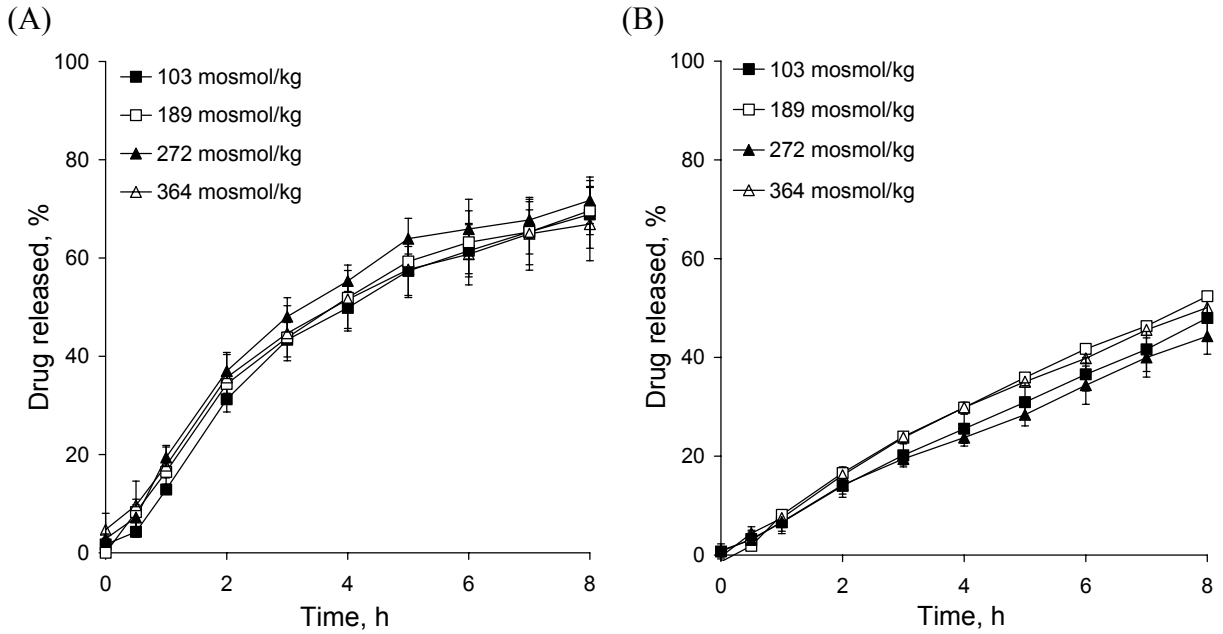


Figure 3. 28 Effect of release medium osmolality on oxymetazoline HCl release from (A) HPMC K15M and (B) carrageenan inserts (polymer 2% w/w, drug 5% w/w based on polymer, V = 1.5 ml, pH 6, $[Na^+]$ = 0.005 mol/l, n = 3).

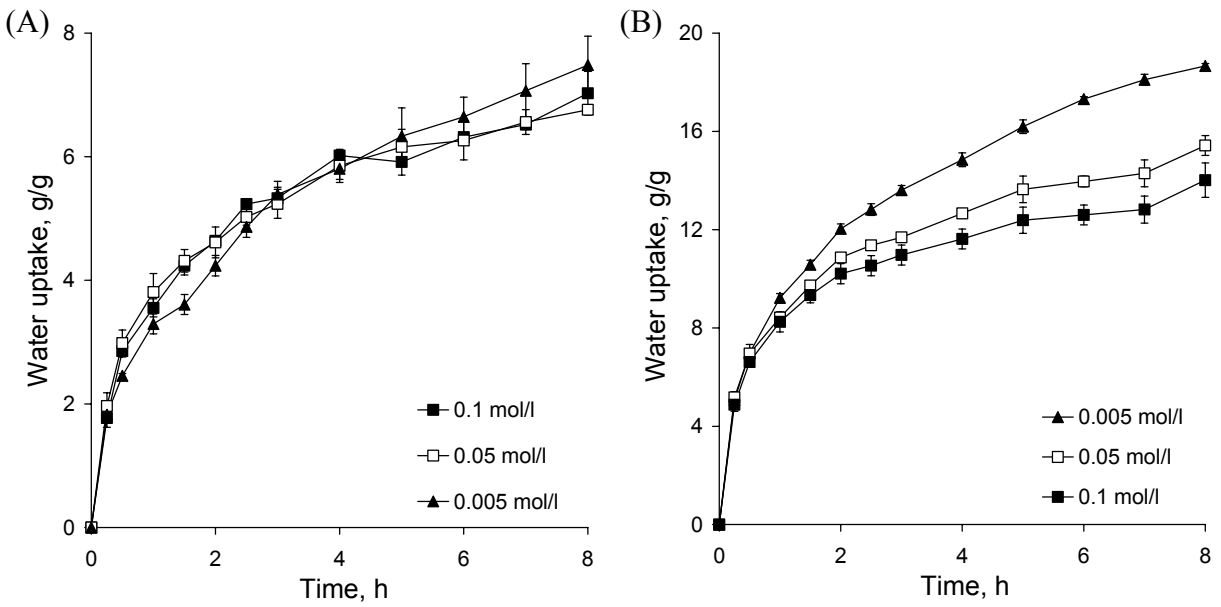


Figure 3. 29 Effect of release medium Na^+-concentration on water uptake of (A) HPMC K15M and (B) carrageenan inserts (polymer 2% w/w, V = 1.5 ml, pH 6, 272 mosmol/kg, n = 3).

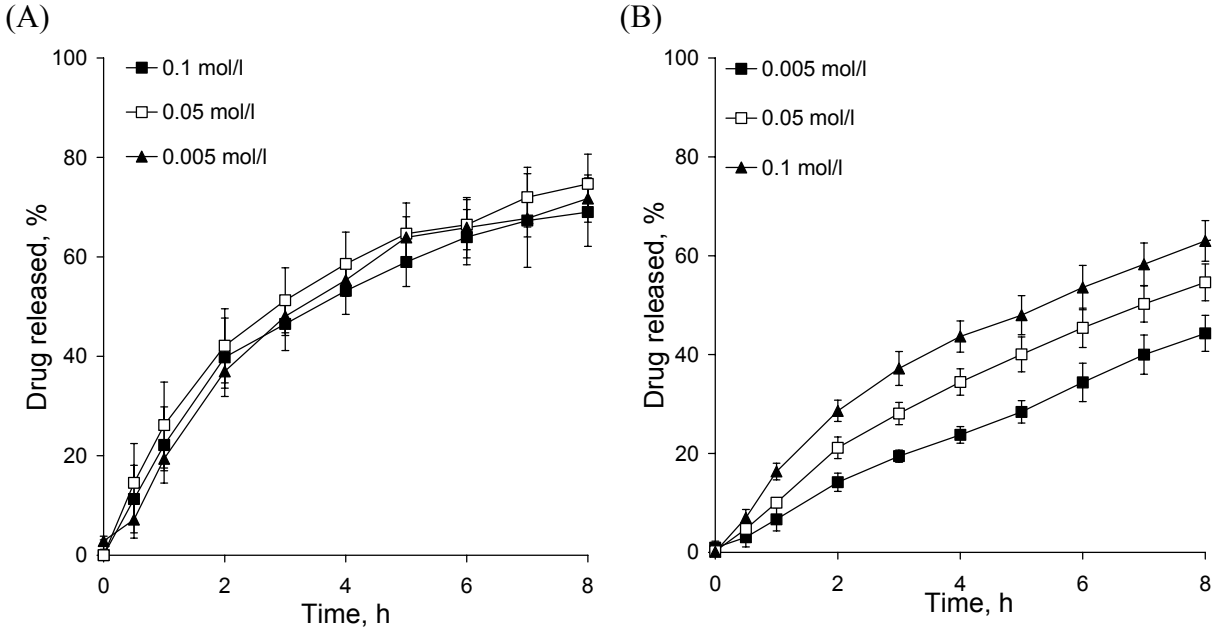


Figure 3. 30 Effect of release medium Na^+-concentration on oxymetazoline HCl release from (A) HPMC K15M and (B) carrageenan inserts (polymer 2% w/w, drug 5% w/w based on polymer, V = 1.5 ml, pH 6, 272 mosmol/kg, n = 3).

The Na^+-concentration of the medium had no effect on water uptake and drug release from HMPC K15M inserts due to the polymer's neutral character (Figure 3. 29A and Figure 3. 30A). Carrageenan inserts, on the other hand, were strongly affected by the Na^+-concentration

of the medium. The water uptake was reduced with increasing Na^+-content of the medium, which was attributed to the formation of a stronger gel with a higher viscosity (Figure 3. 29B, Figure 3. 35A). The enhancing effect of various cations on carrageenan gelling properties has previously been described (Akahane et al., 1982). A gel with increased viscosity can act as a diffusion barrier for water uptake. The reduced water uptake, however, was not mirrored by the oxymetazoline release from carrageenan inserts. In contrast, the drug release was enhanced with increasing presence of sodium ions (Figure 3. 30B). This phenomenon was related to the previously mentioned ionic interaction between oxymetazoline HCl and carrageenan. Na^+ replaced protonated oxymetazoline at the sulfate groups of the polymer. Higher Na^+-concentrations led to a more quantitative replacement of oxymetazoline and thus to a faster drug release. Similar observations had previously been made by Gupta et al. (2001) for chlorpheniramine maleate loaded carrageenan tablets.

The pH of the medium did not affect the water uptake and drug release of HPMC K15M inserts (Figure 3. 31A and Figure 3. 32A). This was again attributed to the absence of charged groups in the polymer. In contrast, the sulfate groups (pKa 1 - 2) of carrageenan were sensible to pH-changes. At pH 2, at least some of the groups are protonated and therefore neutralized. This reduced the osmotic pressure in the rehydrating insert, resulting in a lower water uptake at pH 2 as compared to pH 6 and 10 (Figure 3. 31B). The release of oxymetazoline HCl from carrageenan inserts at different pH was governed by the ability of the insert components to interact electrostatically (Figure 3. 32B). At pH 2, as previously discussed, carrageenan was partly neutralized, while pH 10 led to partial neutralization of oxymetazoline. In both cases the electrostatic interactions between drug and polymer were weakened due to partial neutralization of charges. This resulted in faster drug release. In contrast, at pH 6 both species were fully charged, leading to the slowest release rate due to stronger drug-polymer interactions. The free oxymetazoline base, which is presumably present at pH 10, has a lower aqueous solubility than the hydrochloride and could thus retard the drug release as described previously for the low solubility drug APAP. However, this was not observed, which indicates that the solubility of the base was not exceeded either due to sufficient water uptake by the inserts or due to partial neutralization only (pKa = 9.9 and pH = 10, thus only about 50% of the drug existed as free base). At low pH, acid catalyzed hydrolysis of carrageenan may also contribute to the increased drug release rate. However, this is known to occur only in the dissolved state of iota-carrageenan but not in the gelled state as present during water uptake and drug release studies (Hercules Inc., 1997).

No differences in drug release in different pH-media were found with diprophyllin, a neutral drug under the chosen conditions because the charge of the polymer was not relevant for the release of a neutral drug (Figure 3. 33).

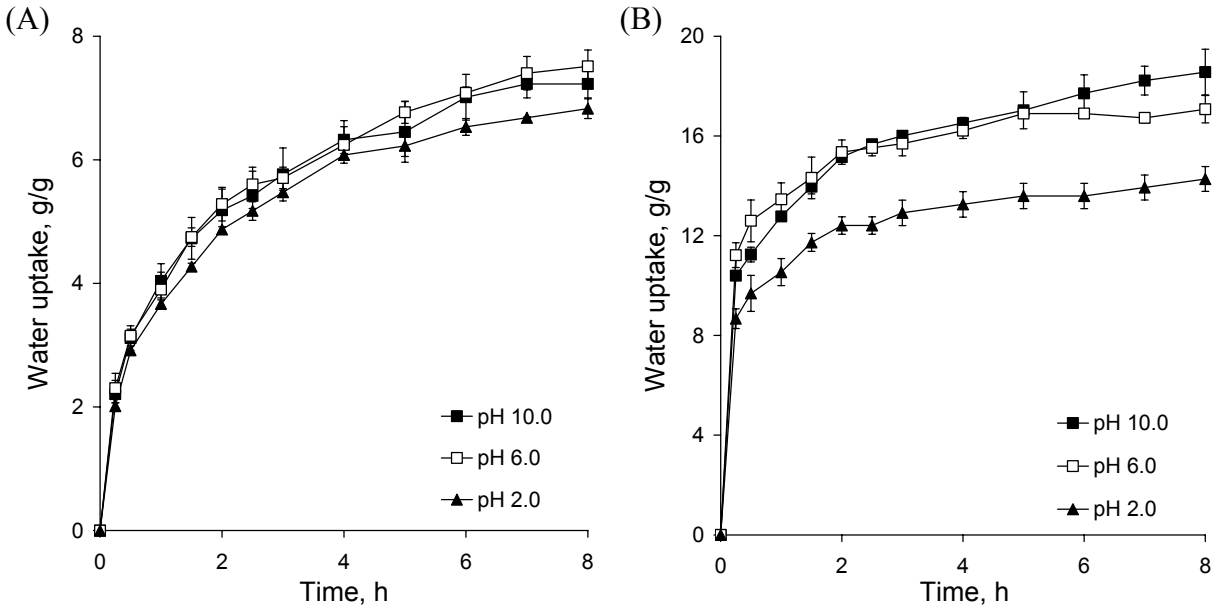


Figure 3. 31 Effect of release medium pH on water uptake of (A) HPMC K15M and (B) carrageenan inserts (polymer 2% w/w, V = 1.5 ml, 272 mosmol/kg, $[Na^+]$ = 0.044 mol/l, n = 3).

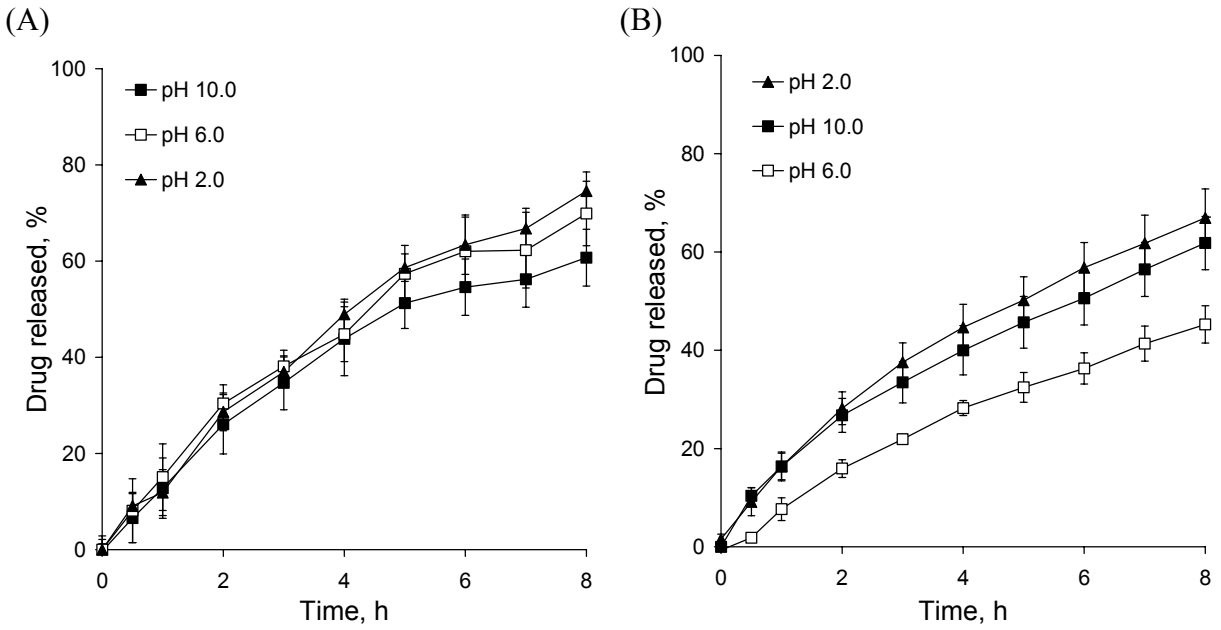


Figure 3. 32 Effect of release medium pH on oxymetazoline HCl release from (A) HPMC K15M and (B) carrageenan inserts (polymer 2% w/w, drug 5% w/w based on polymer, V = 1.5 ml, 272 mosmol/kg, $[Na^+]$ = 0.044 mol/l n = 3).

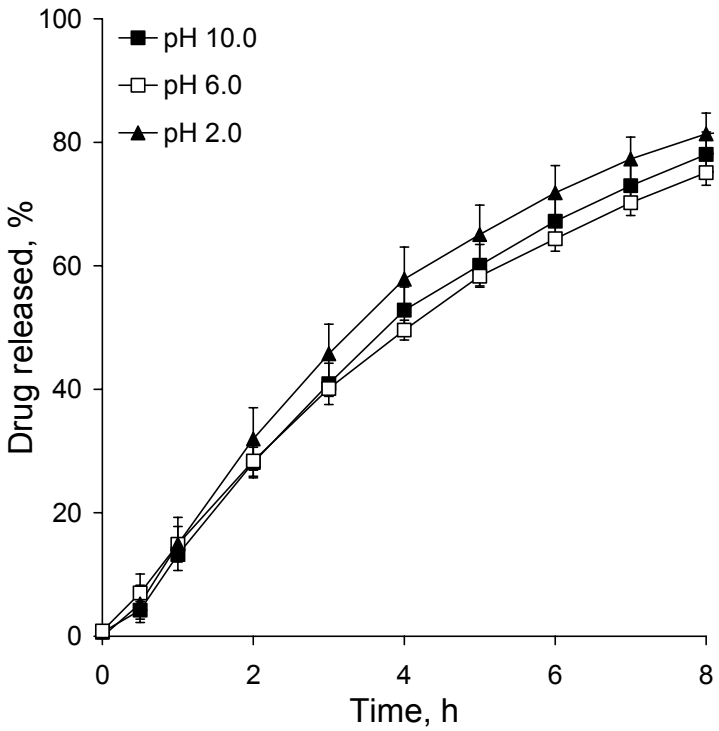


Figure 3. 33 Effect of release medium pH on diprophyllin release from carrageenan inserts (polymer 2% w/w, drug 5% w/w based on polymer, V = 1.5 ml, 272 mosmol/kg, $[Na^+]$ = 0.044 mol/l, n = 3).

Summary. Due to the neutral character of HPMC K15M and the absence of electrostatic interactions between HPMC K15M and the model drug oxymetazoline HCl, water uptake and release were not influenced by pH, Na-ion content or osmolality of the medium. Water uptake and oxymetazoline HCl release of carrageenan inserts were also independent of the osmolality. Effects of the Na-ion content on water uptake and release of oxymetazoline HCl from carrageenan inserts were attributed to the swelling behavior of carrageenan and ion exchange phenomena. Water uptake and drug release of carrageenan inserts were also susceptible to pH changes of the medium. This was associated with neutralization of charges and corresponding lower interaction. Overall, changes in the composition of the medium for water uptake and drug release can vary the behavior of nasal inserts with a possible impact on the in vivo performance.

3.4. Estradiol delivery

So far only water-soluble drugs (oxymetazoline HCl, diprophyllin, APAP) have been incorporated into the nasal inserts and investigated (see sections 3.1, 3.2, and 3.3) because of their sufficient solubility in the aqueous polymer solution prior to freeze-drying. Hydrophobic / poorly water-soluble drugs pose a challenge because the water required for the hydration of the inserts and thus for the dissolution of the drug is limited by the nasal fluid volume and the water uptake behavior of the polymer forming the inserts.

Cyclodextrins (CDs) are cyclic oligosaccharides with a hydrophilic outer surface and a relatively lipophilic cavity. CDs are able to form water-soluble inclusion complexes with water-insoluble drugs. Especially highly water-soluble CD-derivatives, such as methyl-, hydroxypropyl- or sulfobutylether-β-cyclodextrin, have been used as solubilizers for lipophilic drugs (Brewster, et al., 1988; Fridriksdottir et al., 1996; Okimoto et al., 1999). In addition to their solubilizing effect, several CDs act as mucosal membrane permeation enhancers (Irie et al., 1992; Irie and Uekama, 1999; Vermehren et al., 1996), although the penetration of CDs themselves is negligible due to their size and hydrophilicity. The increase in drug concentration at the biological barrier surface, protection of protein drugs against chemical and enzymatic degradation as well as the increase of the membrane permeability by solubilization of membrane lipid components have been discussed as the absorption enhancement mechanisms of CDs (Irie and Uekama, 1999; Loftsson and Masson, 2001). CDs are safe excipients when used in concentrations needed for solubilization and penetration enhancement, causing no damage to the mucosal tissue and maintaining the ciliary beat frequency (Asai et al., 2002; Marttin et al., 1995).

A nasal spray based on an aqueous solution of a complex of the practically water-insoluble drug estradiol (solubility = 5 µg/ml in water at 25°C [Kabasakalian et al., 1966]) and randomly methylated β-cyclodextrin (MβCD) is commercially available under the trade name Aerodiol® for the treatment of postmenopausal estrogen deficiency symptoms (Pelissier et al., 2001).

The objective of the present study was to prepare solid, in situ gelling nasal inserts loaded with estradiol and to investigate the effect of the excipient MβCD on polymer solution viscosity and on bioadhesion, mechanical properties, water uptake, and moisture sorption of the estradiol-containing nasal inserts. Additionally, the influence of the insert-forming polymer, the estradiol dose, and the estradiol:MβCD molar ratio on the drug release from

nasal inserts was studied and compared to other common nasal dosage forms such as solutions, films, and microparticles. Finally, the in vivo performance of the nasal inserts was evaluated and compared to the commercial product (Aerodiol®) and microparticles.

Viscosity. Surprisingly, the addition of MβCD had a strong effect on the viscosity of carrageenan solutions. The viscosity of a 40% (w/w) MβCD solution in pure water at 22°C was very low (5.0 ± 0.4 mPas; D = 30 s^{-1}, 22°C). Increasing concentrations of MβCD (2 - 20% w/w based on total solution) in a 2% (w/w) carrageenan solution led to a steady increase in solution viscosity (Figure 3. 34A), while the viscosity of a 0.5% (w/w) carrageenan solution was not affected by MβCD concentrations up to 20% (w/w) (Figure 3. 34B). However, 40% (w/w) MβCD increased the viscosity significantly. The viscosity enhancing effect of MβCD was therefore dependent on the concentration of both carrageenan and MβCD (Figure 3. 34).

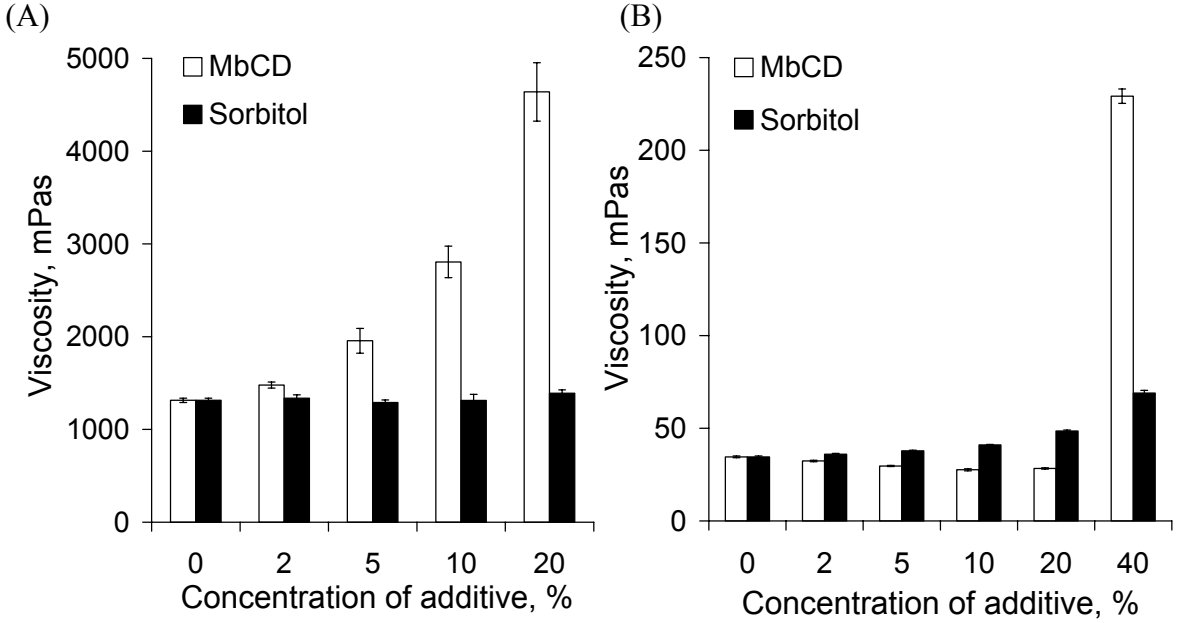


Figure 3. 34 Effect of MβCD and sorbitol concentration on the viscosity of carrageenan solutions of different concentrations (A) 2% (w/w) and (B) 0.5% (w/w) (D = 30 s^{-1}, 22°C, n = 3).

MβCD is a freely water-soluble additive (solubility: 80 g / 100 ml water at 25°C). Binding of free water by MβCD could lead to enhanced coiling, folding or interaction between carrageenan chains resulting in an increased viscosity. No precipitation was observed in the concentration range tested. If the viscosity enhancing effect of MβCD was related to its ability to dehydrate the polymer, similar trends should be observed with other freely water-soluble

additives, such as sorbitol (solubility: 83g / 100 ml water). However, no effect of the sorbitol concentration was observed in a 2% (w/w) carrageenan solution (Figure 3. 34A). Although sorbitol led to a steady increase of the viscosity of a 0.5% (w/w) carrageenan solution, it was by far exceeded by MβCD at 40% (w/w) additive concentration (Figure 3. 34B).

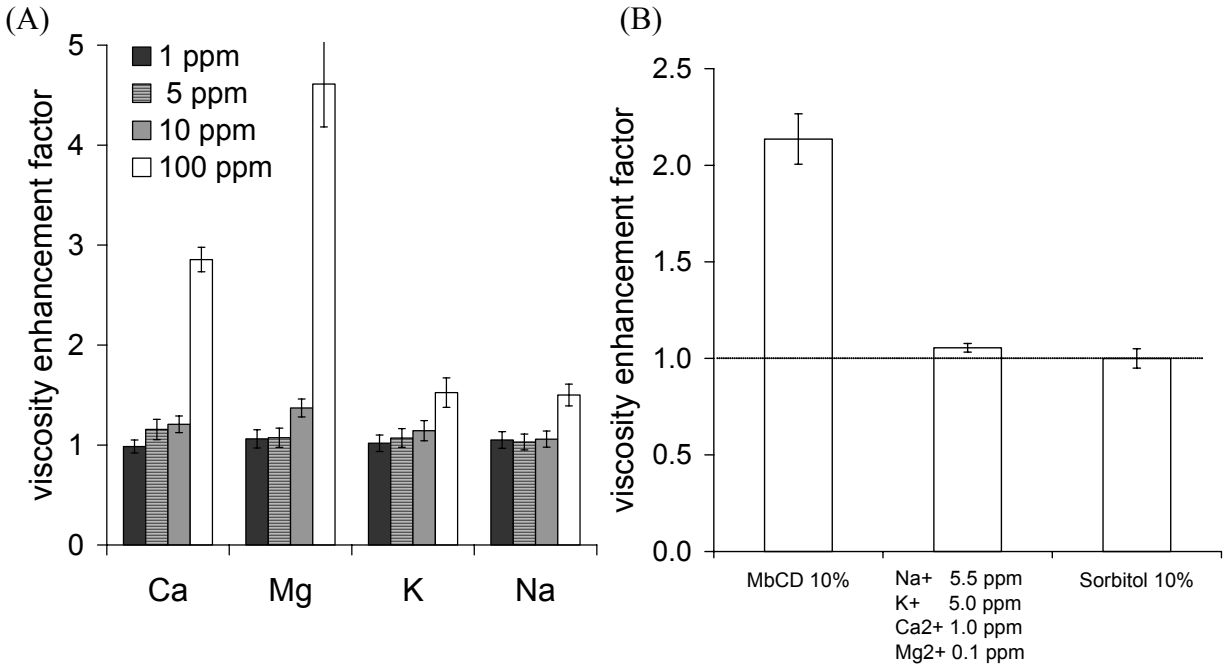
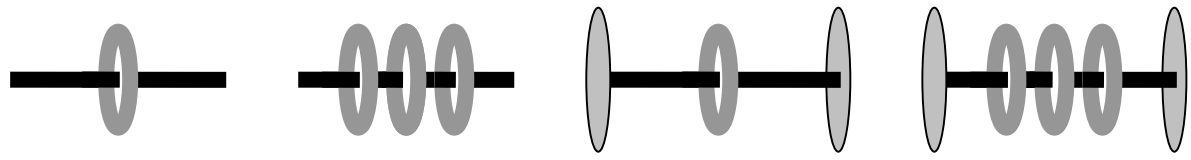


Figure 3. 35 Effect of (A) cations and (B) various additives on the viscosity of carrageenan solutions (polymer 2% w/w, D = 30 s^{-1}, 22°C, n = 3).

The carrageenan viscosity is susceptible to the presence of cations, such as potassium and calcium (Akahane et al., 1982). MβCD was therefore analyzed for impurities of Na^+, K^+, Ca^{2+}, and Mg^{2+} by flame absorption and flame emission spectroscopy. All four ion species increased the viscosity of a 2% (w/w) carrageenan solution, but to varying extent (viscosity enhancement factor = viscosity of carrageenan solution with additives / viscosity of pure carrageenan solution) (Figure 3. 35A). Monovalent cations can induce formation of ordered helix structures of carrageenan polymer chains by electrostatic binding and thus increase the solution viscosity (Zhang et al., 1994). This effect was found to be specific for certain cations. Divalent ions such as Ca^{2+} and Mg^{2+} form bridges between adjacent double helices through electrostatic binding thus further stabilizing and strengthening the network (Hercules Inc., 1997). The concentration of Ca^{2+} and Mg^{2+} in a 10% (w/V) MβCD solution was below the lowest standard (Ca^{2+}: 1 ppm and Mg^{2+}: 0.1 ppm) and below the detection limit (Ca^{2+}: approx. 0.5 ppm and Mg^{2+}: approx. 0.02 ppm). The Na^+- and K^+-content of a 10% (w/V) MβCD

solution was 5.5 ± 0.4 ppm and 5.0 ± 0.4 ppm, respectively. A 10% (w/w) MβCD solution thus contained approximately 5.5 ppm Na^+, 5.0 ppm K^+, < 1 ppm Ca^{2+}, and < 0.1 ppm Mg^{2+}. Addition of the ions at these concentrations increased the viscosity of carrageenan by a factor of only 1.1, compared to 2.1 for 10% (w/w) MβCD (Figure 3. 35B). Thus, the observed viscosity increase of carrageenan solutions by the addition of MβCD was not attributable to traces of cations in the additive, which are known to affect the carrageenan viscosity.

Interactions of cyclodextrins and their derivatives with polymers have been observed by various research groups (Harada, 1997; Kawaguchi et al., 2000; Lo Nostro et al., 2002). Linear polymer chains can slip through the cavity of the ring-shaped cyclodextrins resulting in a pearl-string-like supramolecular structure called pseudorotaxane (Figure 3. 36). Spacey head groups may be used to fix the cyclodextrin on the polymer (rotaxanes). Without fixation they are in equilibrium with the free cyclodextrin. The phenomenon of pseudorotaxane formation is driven by hydrophobic interactions and hydrogen bond formation (Lo Nostro et al., 2002). The increase of the polymer chain molecular weight and its hydrodynamic volume as well as the reduction of its flexibility result in a viscosity increase (Gibson et al., 1997, Gong et al., 1998). Precipitation and formation of a crystalline structure due to the ordering of the cyclodextrins are often observed. To our knowledge, polymers with small monomers, such as polyethylene glycol and polypropylene glycol, or the side chains of e.g. poly(4-sodium styrenesulfonate) have been reported to form these structures. In the latter case, even a charged group was found to slip through the hydrophobic cyclodextrin cavity (Leclercq et al., 1998). In principle, it may be possible for a single carrageenan chain to slip through the cavity of MβCD, although carrageenan is reported to form a double helical structure (Janaswamy et al., 2001; Viebke et at., 1995). However, also single helix structures as well as random coils of carrageenan have been reported (Le Questel et al., 1994; Zhang et al., 1994).



Pseudorotaxane Polypseudorotaxane Rotaxane Polyrotaxane

Figure 3. 36 Supramolecular structures composed of cyclodextrins and linear polymers (adapted from Harada, 1997).

Carrageenan and MβCD powder as well as films of carrageenan and MβCD, which were prepared by drying a 2% (w/w) carrageenan solution with 5% and 40% (w/w) MβCD, were amorphous, as determined by X-ray analysis (Figure 3. 37A). ^{13}C-CP/MAS-NMR measurements failed to show the formation of polypseudorotaxanes (Figure 3. 37B). Already pure MβCD did not show resolved C-1, C-4, and C-6 resonances which were expected from the less symmetric conformation of the cyclodextrin in a crystal when its cavity is not filled by a guest molecule (Harada, 1997; Kawaguchi et al., 2000) due to the missing crystallinity of MβCD. Changes in the carrageenan spectrum were not observable due to the extreme band widths in the spectrum.

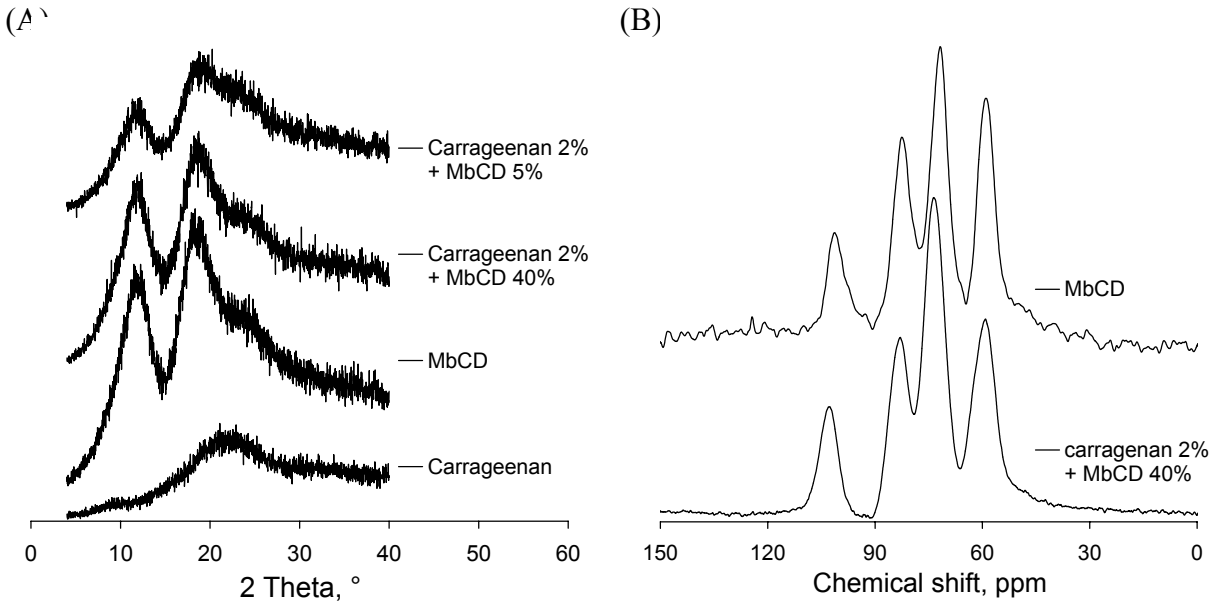


Figure 3. 37 (A) X-ray spectra and (B) ^{13}C CP/MAS NMR spectra of MβCD, carrageenan and (ground) films of carrageenan with MβCD.

Other interactions between polymers and CDs besides polypseudorotaxane formation have been reported, e.g. between β-CD and aqueous solutions of sodium carboxymethylcellulose or methylcellulose (Hladon and Cwiertnia, 1994). Addition of β-CD to the polymer solutions led to the formation of suspensions due to the low aqueous solubility of β-CD. The concomitantly increased viscosity was attributed partly to the solid phase content of the solution. In addition, formation of molecular associates of the investigated polymers was shown by rheological analysis and confirmed by laser light scattering spectrometry. These associates were also hold responsible for the viscosity increase.

Although the reason for the viscosity enhancing effect of MβCD on carrageenan solutions remained unclear, the consequences of this phenomenon on the properties of nasal inserts were investigated in further studies.

Bioadhesion. Bioadhesion guarantees a sufficient nasal residence time of the inserts for prolonged drug absorption. Carrageenan showed no displacement in the adhesion potential test over a 20 h period and therefore had a high adhesion potential (Figure 3. 3). Inserts prepared from 2% (w/w) carrageenan solutions with up to 9.4% (w/w) MβCD gave also no displacement over the entire test period (data not shown). Carrageenan was therefore not a good polymer to examine the effect of the MβCD concentration on the bioadhesion potential and HPMC K15M, a polymer of intermediate bioadhesion potential (Figure 3. 3), was studied instead. HPMC K15M inserts showed a trend towards larger displacements (lower bioadhesion) with higher MβCD content (Figure 3. 38). This could likely be attributed to reduced polymer chain flexibility and thus reduced entanglements with the agar / mucin gel. Similar to carrageenan, the viscosity of a 5% (w/w) HPMC K15M solution was slightly increased by a factor 1.2 by addition of 10% (w/w) MβCD.

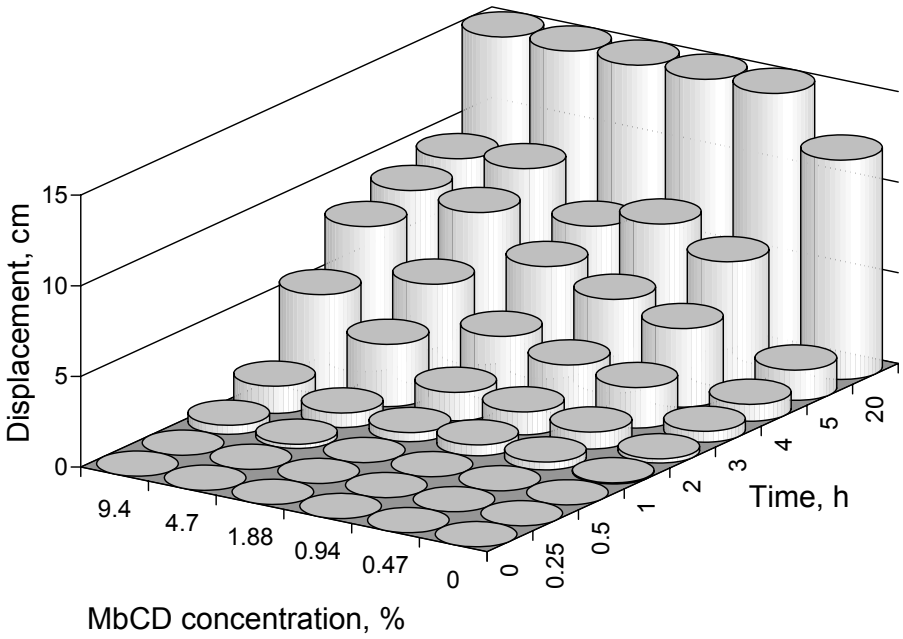


Figure 3. 38 Effect of MβCD concentration on the adhesion potential of HPMC K15M inserts (polymer 2% w/w, V = 0.1 ml, n = 3, CV = 4.9 ± 4.3%).

Mechanical properties. The mechanical properties of nasal inserts, such as hardness and elasticity, are a measure of the stability of inserts during handling and administration and their ability to reform the sponge-structure after compression. The hardness of nasal inserts based on carrageenan increased with higher MβCD-content, while the elasticity was reduced by high amounts of the additive (Table 3. 19), indicating that the inserts were more brittle at higher MβCD-content. Possible reasons could be a reduced polymer chain flexibility (dehydration, rotaxane formation) or an increased polymer chain molecular weight (rotaxane formation).

Table 3. 19 Effect of MβCD concentration on the mechanical properties of carrageenan inserts (polymer 2% w/w, n = 6).

MβCD, % (w/w)	Hardness, N	Elasticity, %
0.00	5.2 ± 0.8	35.2 ± 4.2
0.24	6.3 ± 0.6	38.3 ± 7.2
0.47	7.2 ± 0.4	41.4 ± 8.2
0.94	6.7 ± 0.4	30.2 ± 6.9
1.88	7.2 ± 1.3	22.6 ± 7.1
4.70	8.5 ± 1.3	15.7 ± 2.9

Water uptake and moisture sorption. The in situ gelling nasal inserts are designed to rapidly take up fluid from the nasal mucosa to form a gel. This gel then acts as a diffusion barrier for the drug resulting in extended drug release. The in vitro measurements of water uptake showed similar weight increase of nasal inserts independent of the MβCD content (weight increase normalized to the initial dry polymer weight, which was constant during the hydration study for all inserts irrespective of the MβCD content) (Figure 3. 39A). After a rapid initial phase the water uptake of all inserts slowed down and reached approximately 20 g/g polymer after 8 h. The biphasic water uptake behavior of carrageenan inserts was previously discussed and could likely be attributed to rapid initial water imbibition due to capillary forces resulting from the highly porous structure of the inserts and delayed formation of a gel barrier, which reduced the diffusivity of water and thus its uptake rate (see section 3.1.3). Contrary to other studies, no wicking effect of MβCD, which would lead to

faster imbibition of water into the entire insert matrix with increasing MβCD content and increased hydration (Bibby et al., 2000), was observed for nasal inserts. This could have resulted from the highly porous structure of the inserts, which already allowed a very fast water uptake.

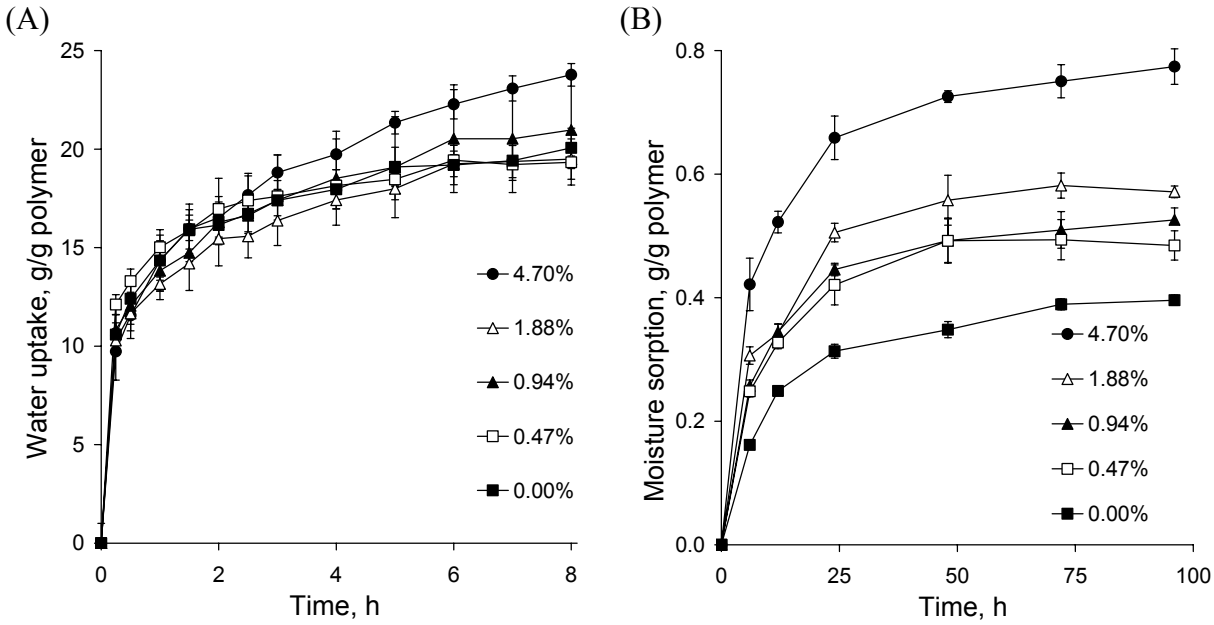


Figure 3. 39 Effect of MβCD concentration on (A) water uptake and (B) moisture sorption of carrageenan inserts (both normalized to the initial dry polymer weight, polymer 2% w/w, V = 1.5 ml, n = 3).

Since MβCD is a slightly hygroscopic substance, the moisture sorption of inserts was investigated. As expected, moisture sorption was enhanced by increasing amounts of MβCD (Figure 3. 39B). Within the study time of 4 days, all inserts were nearly saturated with moisture, reaching 140 to 180% of their initial polymer weight. Similar to the water uptake studies, the decrease in the moisture sorption rate may be related to the formation of a gel barrier. However, simple reduction of the sorption rate due to reaching saturation is more likely. This moisture sorption will influence the shelf-life of and drug stability in the inserts. Appropriate conclusions with regard to packaging have to be drawn.

Drug release. Estradiol was released from the inserts either as the complex or as free drug due to the dynamic equilibrium of the complex formation. In the release medium free drug

was complexed again in the released MβCD. The estradiol detection method did not discriminate between free and complexed drug.

The estradiol release was highly influenced by the type of polymer forming the nasal inserts as previously shown for oxymetazoline HCl (see section 3.1.3). The release of estradiol was in the order HPMC E5 ≥ PVP 90 > Na-alginate > xanthan gum > carrageenan (Figure 3. 40). This order was related to the viscosity of the polymer solutions (2% w/w solution: HPMC E5 = 4.1 ± 0.2 mPas, PVP 90 = 6.1 ± 0.5 mPas, Na-alginate = 345.2 ± 8.6 mPas, xanthan gum = 835.2 ± 5.6 mPas, carrageenan = 1177.7 ± 20.9 mPas, respectively) (Table 3. 2), with the polymers resulting in more viscous gels releasing at a slower rate.

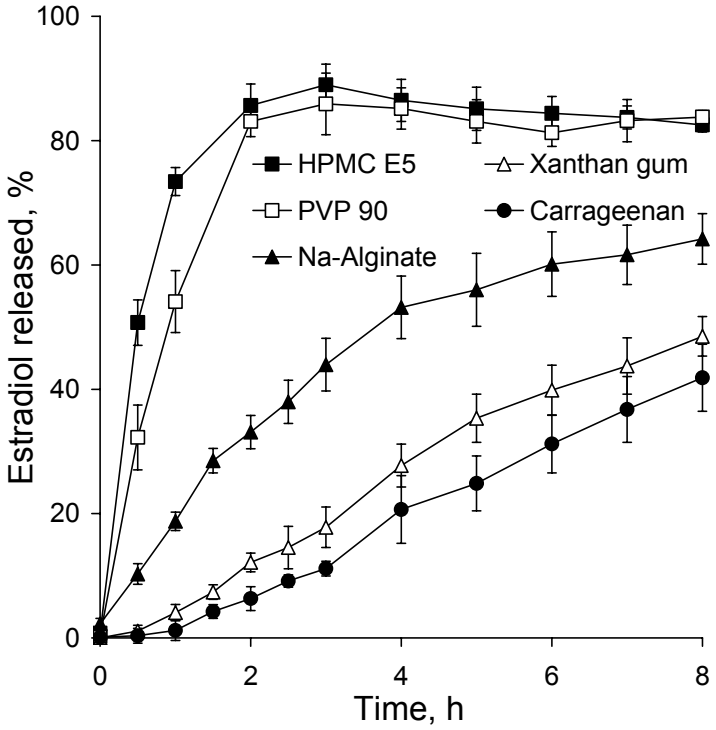


Figure 3. 40 Estradiol release from inserts of different polymers (polymer 2% w/w, estradiol dose 100 µg, estradiol:MβCD molar ratio 1:2, V = 0.2 ml, Sartorius membrane, n = 3).

The release of estradiol from nasal inserts was compared to other nasal dosage forms: aqueous solutions with / without polymer (2% w/w), polymer films (216 ± 31 µm film thickness), and microparticles prepared by film grinding (sieve fraction 50 - 100 µm) (El Kharraz et al., 2003) (Figure 3. 41). The aqueous, polymer-free solution released the estradiol rapidly within 1.5 h. Addition of carrageenan (2% w/w) to the estradiol / MβCD solution led to a significant reduction due to the increased solution viscosity. Microparticles and films released the

complex similar to the 2% (w/w) polymer solution, although a larger reduction of the release rate when compared the polymer solution was expected. Films and microparticles formed a gel after contact with the release medium. The higher viscosity of these gels compared to the simple polymer solution was probably counteracted by the smaller diffusional distances, because films and microparticles gelled to a smaller volume than that of the polymer solution (200 µl). Carrageenan inserts rehydrated quickly while approximately maintaining their volume (200 µl) by incorporation of air bubbles in the rather stiff gel. Together with the higher viscosity, this increased diffusional distance contributed to the more extended release of the estradiol from carrageenan inserts compared to microparticles and films. In addition, the drug release test design offered only a defined and constant contact area between the dosage form and the release medium (diameter of the filter membrane = 8 mm). In vivo, solutions or microparticles have the potential to reach a larger mucosal surface area when compared to films or inserts. This might increase the drug release from solutions or microparticles in vivo, when compared to in vitro release (Kublik and Vidgren, 1998).

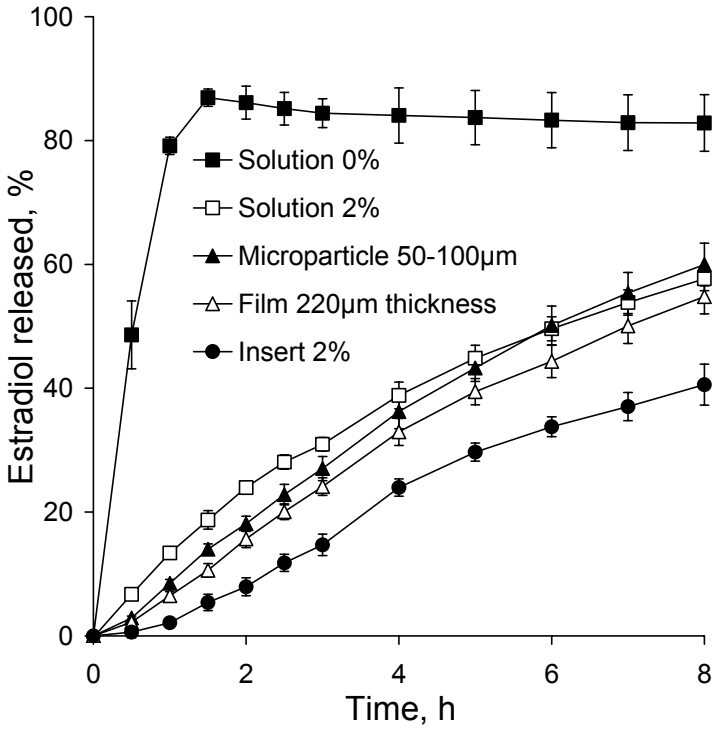


Figure 3. 41 Estradiol release from inserts (V = 0.2 ml), microparticles (50 - 100µm), films (thickness 216 ± 31 µm) and solutions (V = 0.2 ml) (carrageenan % w/w, estradiol dose 100 µg, estradiol:MβCD molar ratio 1:2, Sartorius membrane, n = 3).

The estradiol dose (estradiol:MβCD molar ratio = 1:2) had no effect on the drug release despite the concomitantly increasing viscosity of the rehydrated nasal insert due to the higher MβCD content. The same fraction of drug was released independently of the drug content (Figure 3. 42). However, the estradiol dose range investigated corresponded to a MβCD content of 0.47% to 4.7% (w/w), which had shown only an intermediate viscosity increase from 1314 ± 24 mPas to 1956 ± 134 mPas in a 2% (w/w) carrageenan solution (Figure 3. 34A). Thus, the viscosity effect may not be sufficient to influence the estradiol release. This assumption was strengthened by the MβCD release rate from nasal inserts with increasing MβCD content. Also the fractional release of MβCD from inserts was independent of the loading and thus the viscosity of the gel matrix (Figure 3. 43). The faster release of MβCD (Figure 3. 43) as compared to estradiol with increasing dose (Figure 3. 42) stemmed from the higher diffusivity of the empty MβCD compared to the estradiol loaded one due to a lower molecular weight and / or from the use of different filter membranes in the release device. The adsorption of MβCD to the filter was neglectable (recovery 100%) compared to estradiol (recovery 86 ± 3%) (see section 2.2.10).

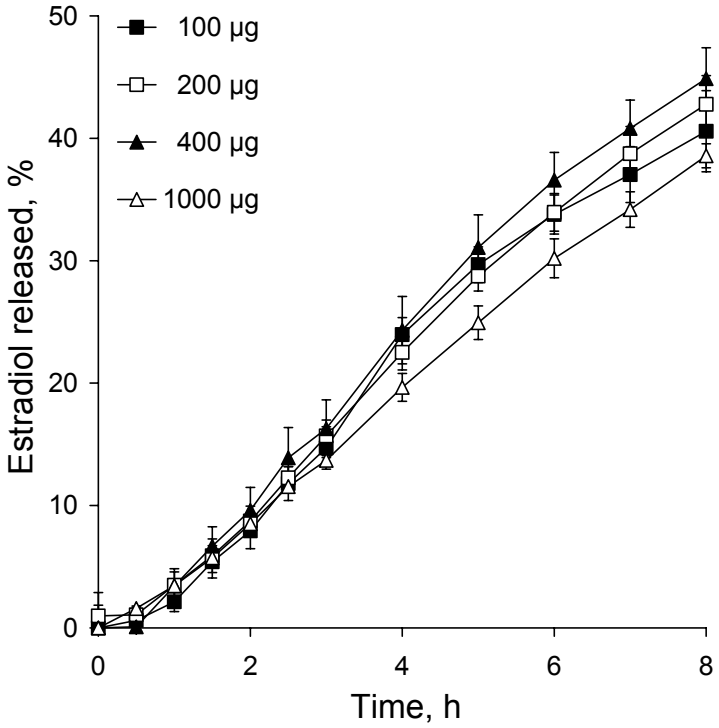


Figure 3. 42 Effect of estradiol dose on the estradiol release from carrageenan inserts (polymer 2% w/w, estradiol:MβCD molar ratio = 1:2, V = 0.2 ml, Sartorius membrane, n = 3).

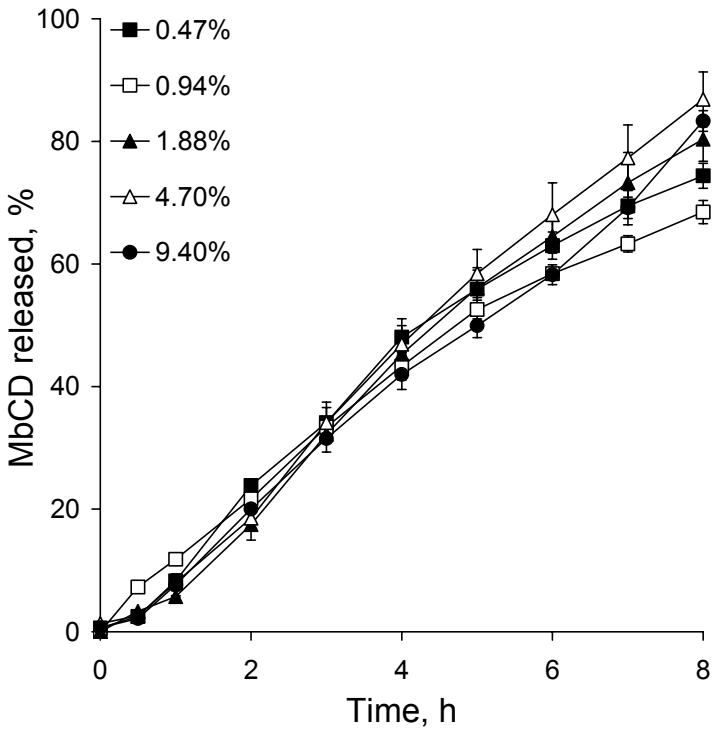


Figure 3. 43 Effect of MβCD content on MβCD release from carrageenan inserts (polymer 2% w/w, V = 0.2 ml, no drug, Schleicher&Schuell membrane, n = 3).

Carrageenan inserts with increasing loadings of estradiol and constant MβCD content (estradiol:MβCD molar ratio 1:20 to 1:2) released the drug independently of the estradiol loading and complex molar ratio (Figure 3. 44A). This was expected, because a complex molar ratio of 1:2 was already sufficient to completely solubilize the estradiol in the formulations. Excess MβCD had no further effect on the solubility of the drug. Also the viscosity of the carrageenan solutions was constant due to the identical MβCD content of the formulations.

Keeping the estradiol content in the nasal inserts constant and increasing the MβCD amount in the inserts (estradiol:MβCD molar ratio from 1:2 up to 1:40) resulted in a significant reduction of the drug release rate from carrageenan (Figure 3. 44B). A possible explanation would be the increased viscosity of carrageenan solutions at higher MβCD contents but this was disproved by the independence of the estradiol release with increasing dose (Figure 3. 42) and MβCD with increasing MβCD content (Figure 3. 43). Frömming and Szejtli (1994) state that addition of an excess amount of a soluble cyclodextrin shifts the dissociation equilibrium towards the complexation, which can be used to obtain prolonged release. The shift towards formation of the drug-cyclodextrin complex in systems, where no solid drug is present, results

in a reduction of the concentration of the free dissolved drug. Bibby et al. (2000) then argue that due to the differences in the diffusion coefficient between free (molecular weight of estradiol 272 g/mol) and complexed drug (molecular weight of estradiol / MβCD complex approximately 1580 g/mol) the drug release rate is reduced with increasing cyclodextrin content as it was also observed in the present study. Sufficient solubility of estradiol in the release medium was maintained by the released free MβCD, which was able to complex the drug again when the complex association constant was exceeded. Similar observations were made with cross-linked chitosan microparticles, which released a complex of the poorly water-soluble drug nifedipine with hydroxypropyl β-cyclodextrin (HPβCD) slower than the pure drug (Filipovic-Grcic et al., 1996). The release of the HPβCD from the microparticles was not analyzed.

(A) (B)

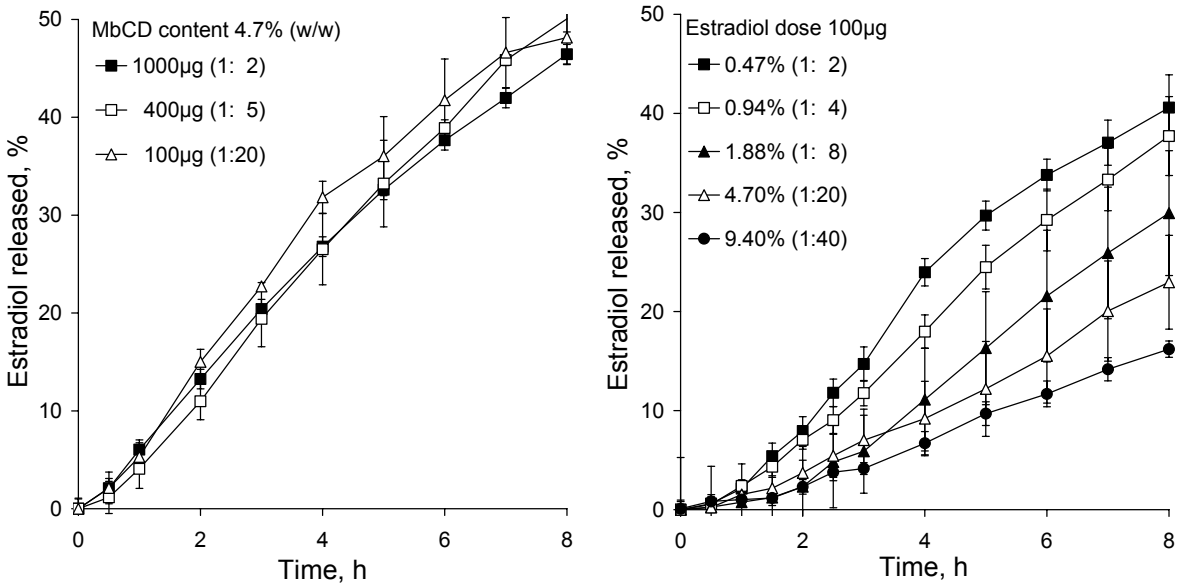


Figure 3. 44 Effect of (A) estradiol dose (molar ratio) and (B) MβCD content (molar ratio) on estradiol release from carrageenan inserts with (A) constant MβCD content (4.7% w/w, Schleicher&Schuell membrane) and (B) constant estradiol dose (100 µg, Sartorius membrane) (polymer 2% w/w, V = 0.2 ml, n = 3).

It is important to note that Figure 3. 44A and B are not directly comparable because the data was obtained with different membranes in the release device. The membranes in the release devices absorbed estradiol to a different extent (see section 2.2.10) resulting in higher amounts of released drug in Figure 3. 44A compared to B. The reason for the different influence of the estradiol:MβCD molar ratio on the estradiol release from carrageenan inserts

depending on which component of the complex is kept constant is so far not entirely clear and further investigations of this phenomenon will be necessary. Similar observations were also made with estradiol / MβCD complexes in HPMC K15M inserts and diprophyllin / MβCD complexes in carrageenan inserts (data not shown).

In vivo studies. Animal studies with male Wistar rats were conducted to evaluate the in vivo performance of nasal inserts in comparison with microparticles (sieve fraction 50 - 100 µm) and a commercially available solution of an estradiol / MβCD complex (Aerodiol®, $\eta = 1.6 \pm 0.4$ mPas; $D = 30\ s^{-1}$, 22°C). The inserts and microparticles were prepared from the Aerodiol® solution for better comparison. Inserts and microparticles were prepared from carrageenan and PVP 90. Additionally, three formulation based on estradiol absorbed to Eudragit® RL nanoparticles (mean diameter 100 nm) were investigated: an aqueous dispersion (Eudragit® RL 30D; $\eta = 1.9 \pm 0.2$ mPas; $D = 30\ s^{-1}$, 22°C), an aqueous dispersion containing 1% (w/w) HPMC K15M ($\eta = 331.5 \pm 1.4$ mPas, $D = 30\ s^{-1}$, 22°C), and inserts prepared from a 2% (w/w) PVP 90 solution containing 4% (w/w) Eudragit® RL nanoparticles to which estradiol was previously adsorbed (5% w/w estradiol based on Eudragit® RL). The quaternary ammonium groups and therefore the permanent positive charge of this polymer could potentially enhance bioadhesion and absorption (Bucolo et al., 2002). The Eudragit® RL solid content in all three formulation was 4% (w/w). The estradiol loading was 5% (w/w) based on the nanoparticle mass.

The estradiol serum levels were normalized to rat body weight and the actually administered estradiol amount in order to exclude variations in the animal body weight and in the delivered dose (40 µg estradiol / animal) (Figure 3. 45). The maximum serum concentration (C_{max}), the time until C_{max} (T_{max}), the area under the estradiol serum curve from zero to 360 min (AUC_{0-360}), and the relative bioavailability (F) compared to the Aerodiol® solution were calculated (Table 3. 20). All formulations raised the serum estradiol concentration of male rats above the saline control level of 1 - 3 pg/ml and exceeded the female physiological levels of 50 - 110 pg/ml (bodyweight 264 - 339 g) (Sokol et al., 1999). Most formulations followed a biphasic behavior, which may have resulted from the clearance of the formulation from the nasal cavity to the throat / GI-tract and the consequent changes in absorption and elimination rates. This biphasic behavior was not found for carrageenan inserts. The inserts formed a gel, which was still present in the nasal cavity after 360 min, in contrast to all other formulations,

which had been cleared from the nose. This demonstrated the superior residence time of the inserts.

The Aerodiol® solution was very rapidly absorbed from the nasal cavity, resulting in a short T_{max} (first data point) and a high peak concentration. The microparticle formulations gave similar serum estradiol profiles as the Aerodiol® solution. This could be explained by the large surface, which the microparticles could reach by being sprayed into the nasal cavity. A slightly retarding effect of carrageenan compared to PVP 90 microparticles was detectable in T_{max}. The relative bioavailability of both microparticle formulations reached about 80 - 90% of the Aerodiol® solution. Thus, the microparticle formulations would be a good replacement of an aqueous solution in case of drugs, which are susceptible to degradation in aqueous medium, and to avoid preservatives, which are necessary for water-based formulations but known to impair the ciliary movement (Schipper et al., 1991). Although high AUC-values were reached for Aerodiol® solution as well as PVP 90 and carrageenan microparticles, the percentage of the C_{max} remaining after 6 h was very low (7%, 3%, and 5%, respectively) indicating a high drug loss from the blood stream.

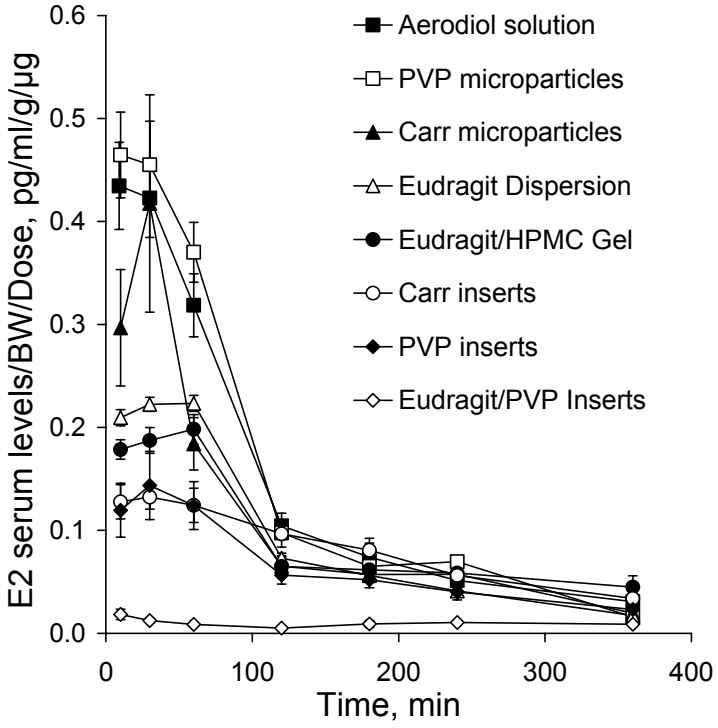


Figure 3. 45 Estradiol (E2) serum levels normalized to rat body weight and estradiol dose after application of nasal formulations (estradiol dose 40 µg, estradiol:MβCD molar ratio 1:2 or bound 5% (w/w) to Eudragit® RL, Error bars = standard error of the mean, n = 6).

Inserts based on PVP 90 and carrageenan retained 17% and 26%, respectively, of their C_{max} after 6 h. The detection of the carrageenan gel in the nasal cavity after 6 h allowed the conclusion that the estradiol release was not yet finished after the study time and elevated serum estradiol levels could be achieved over a period of more than 6 h. This would explain the relatively low AUC and bioavailability but proofs the extended systemic nasal drug delivery with nasal inserts. Fragments of the PVP 90 inserts, on the other hand, were not detectable after 6 h. PVP 90 is a fast dissolving polymer without significant bioadhesion potential (Figure 3. 3). The lower bioavailability of the PVP 90 insert compared to the PVP 90 microparticles and the Aerodiol® solution probably resulted from the delayed initial absorption due to the presence of the polymer, relatively small contact area with the nasal mucosa, and rapid clearance from the nasal cavity.

Table 3. 20 Pharmacokinetic parameters of estradiol after nasal administration in various formulations (n = 6).

Formulation (% w/w)	**T_{max}, min**	**C_{max}, pg/ml/g/µg**	**AUC_{0-360}, pg/ml/g/µg·min**	**$F_{relative}$, %**
Aerodiol solution	10	0.43 ± 0.10	58.1 ± 8.1	100.0 ± 14.0
Carrageenan insert 2%	30	0.13 ± 0.03	28.6 ± 8.2	49.2 ± 14.2
PVP 90 insert 2%	30	0.14 ± 0.08	23.0 ± 7.7	39.6 ± 13.2
Carrageenan microparticles	30	0.42 ± 0.26	46.5 ± 8.9	80.1 ± 15.3
PVP 90 microparticles	10	0.47 ± 0.10	51.5 ± 9.1	88.7 ± 15.7
Eudragit® RL / PVP insert 2%	10	0.18 ± 0.01	3.3 ± 1.2	5.7 ± 2.1
Eudragit® RL dispersion	60	0.22 ± 0.02	31.2 ± 2.7	53.8 ± 4.7
Eudragit® RL / HPMC gel 1%	60	0.20 ± 0.04	31.8 ± 6.7	54.8 ± 11.5

The PVP 90 inserts containing estradiol bound to Eudragit® RL showed the lowest estradiol serum levels of all formulations tested. Although redispersion in vitro was possible by vortexing (2000 rpm, 0.5 ml water), this seemed not to occur sufficiently in vivo, resulting in large particle aggregates which were rapidly cleared from the nasal cavity. The Eudragit® RL aqueous dispersion and the HPMC K15M based gel, on the other hand, raised the serum

levels of estradiol resulting in 54 ± 5% and 55 ± 12% relative bioavailability, respectively. However, the gel retained 22% of its C_{max} after 6 h, whereas the aqueous dispersion had serum levels decreased to 8% of its C_{max} after 6 h, indicating a higher potential of the gel for sustained nasal estradiol delivery.

Summary. Solubilization of the poorly water-soluble drug estradiol by inclusion into water-soluble cyclodextrins (MβCD) allowed the incorporation of this drug into in situ gelling nasal inserts. In that way the applicability of inserts as nasal drug delivery system is enlarged to another group of drugs. The cyclodextrin additive increased the viscosity of carrageenan solutions, reduced the bioadhesion potential of HPMC K15M nasal inserts, increased the moisture sorption of inserts and affected the mechanical properties of dry nasal inserts. The estradiol release was independent of the dose but partially influenced by the estradiol:MβCD ratio. In vivo studies showed the potential of nasal inserts based on carrageenan to deliver estradiol over a period of at least 6 h without initial high estradiol serum peaks.

3.5. Nasal influenza vaccination

Mucosal routes of immunization are attractive alternatives to parenteral immunization because they do not require trained personnel for administration and lead to a potentially higher patient compliance. It is widely accepted that the majority of the invading pathogens enter the body via mucosal surfaces (Mestecky et al., 1997). An overview over nasal immunology and vaccine delivery systems is given in section 1.4.

Influenza viruses are capable of causing recurrent annual epidemics and more serious pandemics, which spread rapidly. The severity of the spreading and the resulting pharmaco-economic impact are influenced by the high antigenetic variability of the virus, the emergence of new strains, and the degree of pre-existing protective immunity in the exposed population (Glueck, 2001). Currently, annual intramuscular vaccinations with formaldehyde inactivated virus particles, split or subunit vaccines are applied to control the morbidity and mortality associated with influenza outbreaks.

Despite intensive research and approval of the first nasal vaccine preparations for human use, current nasal vaccine delivery systems still suffer a number of disadvantages, such as low immunogenicity, danger of toxicity (especially with adjuvants of viral and bacterial origin), complex production processes, and vaccine protein instabilities, which hinder broad application to a wide variety of vaccines.

The aim of this study was to investigate the potential of in situ gelling nasal inserts as nasal vaccine delivery system in vitro as well as in vivo. The sponge-like inserts were prepared by freeze-drying solutions of the vaccine and a hydrophilic polymer, which forms the carrier matrix of the insert. Prior to the actual study with the inserts, it was necessary to identify vaccine-compatible polymers and absorption enhancers / adjuvants. Thus, the effect of additives, i.e. the polymers to form nasal inserts and absorption enhancers to increase the mucosal uptake of the vaccine proteins, on the solution properties and on the immune response to nasally applied influenza vaccine solution was tested.

3.5.1. In vitro properties of polymer solutions

The vaccine solution in buffer pH 7.4 is a colloidal solution due to the high molecular weight of the proteins and is therefore slightly turbid. The major protein hemagglutinin (approximately 1500 amino acid residues and molecular weight of 240 kDa) is a trimer composed of identical monomers, which each consist of two chains linked by disulfide bonds

(Sinyakow et al., 1980; Weis et al., 1988). It is negatively charged at neutral pH-values. The vaccine contains also an equivalent amount of nucleoprotein (75 kDa, positively charged at neutral pH-values) and traces of matrix proteins.

The solutions of vaccine and hydrophilic polymers were prepared by directly dissolving the polymer in the vaccine solution in order to avoid further dilution of the vaccine. The dissolution of hydrophilic polymers in the vaccine solution was not problematic for negatively charged (Carbopol®, carrageenan, xanthan gum) or neutral polymers (Lutrol® F68, PVP 90, HPMC K15M). An exception was the positively charged chitosan, with which the vaccine solution became more turbid. Chitosan is also known to act as an absorption enhancer for proteins (Fernández-Urrusuno et al., 1999, McNeela et al., 2000).

Table 3. 21 Properties of various absorption enhancers and visual appearance of vaccine solutions with and without enhancers (vaccine protein concentration 45 µg / 200 µl) at 22C°. Turbidity: + = slight, ++ = medium, +++ = strong, ++++ = slight precipitation.

Enhancer (0.5% w/w)	**Charge**	**Charge density, 10^{-3}/da**	**MW**	**Comments**	**Visual appearance**
No enhancer	-	-	-	-	+
SDS	Negative	3.47	288	Surfactant	+
Na-Glycocholate	Negative	2.05	488	Surfactant	+
DC-cholesterol	Positive	2.00	537	Liposomes	++++
Poly-L-arginine	Positive	6.41	95,000	Peptide	+++
Chitosan	Positive	5.28	460,000	86% DA[1]	+++
Protamin sulfate	Positive	5.05	4 x 4800	Protein	++
Eudragit® RL	Positive	3.88	150,000	Nanoparticles	+++

[1] Deacetylation

Commonly used negatively charged absorption enhancers (SDS, Na-glycocholate) did also not lead to any visual change of the vaccine protein solution (Table 3. 21). All positively charged enhancers, including chitosan, increased the visually observed turbidity of the

vaccine solution, indicating the formation of complexes, presumably with the negatively charged hemagglutinin. This shielding of the protein's negative charge and thus an increase in its hydrophobicity could be a mechanism for potential absorption enhancement, because lipophilic membranes are usually impermeable for highly charged molecules. Observations supporting this theory have already been made for the gastrointestinal uptake of the highly charged anticoagulant heparin, which was successfully delivered bound to polycationic polymeric nanoparticles (Jiao et al., 2002). Although the exact mechanism of absorption was not identified, the importance of the polycationic nature of the polymer was stressed. Condensation of larger, highly charged drugs by electrostatic complexation for improved drug absorption was also discussed in the literature (MacLaughlin et al., 1998; Bally et al., 1999).

For large molecules, such as the influenza vaccine antigens, aggregation and precipitation of the formed complexes on the mucosal surface could enhance the immune response by particulate uptake via M-cells of the nose-associated lymphoid tissue (Davis, 2001).

The neutral polymer HPMC K15M was chosen as carrier for the liquid formulations containing different absorption enhancers (except chitosan which by itself can form inserts) because of its good potential to form nasal inserts, its relatively high viscosity compared to other polymers (0.5% w/w polymer solution: chitosan 5.6 ± 4.1 mPas, PVP 90 10.9 ± 3.6 mPas, carrageenan 31.2 ± 5.7 mPas, Lutrol® F68 (25% w/w) 33.1 ± 4.3 mPas, HPMC K15M 55.9 ± 2.6 mPas, xanthan gum 498.8 ± 9.5 mPas, Carbopol® 730.7 ± 10.5 mPas; D = 5 s^{-1}, 22°C) and due to its compatibility with the charged absorption enhancers (negatively charged polymers were not compatible with positively charged enhancers). In addition, the higher viscosity of the HPMC K15M solutions could result in a longer nasal residence.

Viscosity measurements showed that the addition of the enhancers Na-glycocholate, protamine sulfate, and Eudragit® RL 30D did not affect the viscosity of the HPMC K15M solution (Table 3. 22). However, SDS, DC-cholesterol, and poly-L-arginine increased the HPMC K15M solution viscosity approximately by a factor of two. The viscosity enhancing effect of SDS on HPMC K15M was previously described and related to absorption of the polymer onto SDS-micelles (Nielsson, 1995). The DC-cholesterol is known to form liposomes in aqueous solution. Positively charged liposomes were shown to increase the viscosity of a Carbopol® 974P NF gel likely due to electrostatic interactions (Boulmedarat et al., 2003). HPMC was also shown to absorb onto the surface of liposomes (Gutierrez de Rubalcava, 2000). Thus, a viscosity increase with a similar mechanism as for SDS could be

possible. Poly-L-arginine is a polymer with a molecular weight of 95 kDa and may thus increase the HPMC K15M solution viscosity by itself and due to increased polymer chain entanglements.

Table 3. 22 Viscosity of HPMC K15M solutions 0.5% (w/w) containing different absorption enhancers 0.5% (w/w) (22°C, D = 5 s^{-1}, 22°C, n = 6).

Enhancer	Viscosity, mPas
None	55.9 ± 2.6
Na-glycocholate	52.8 ± 5.3
Protamine sulfate	54.2 ± 2.3
Eudragit® RL 30D	58.3 ± 4.7
SDS	102.1 ± 3.1
DC-cholesterol	107.4 ± 4.0
Poly-L-Arginine	111.2 ± 7.2

After the compatibility studies, the vaccine release was investigated first from polymer solutions. Polymer solutions containing influenza vaccine are an intermediate product during the manufacture of in situ gelling nasal inserts by freeze-drying and were used for the in vitro and in vivo screening of potential polymers and absorption enhancers / adjuvants.

The protein was released from different polymer solutions (Figure 3. 46). The protein detection method was universally applicable to all proteins and did not differentiate between hemagglutinin, nucleoprotein and the matrix protein traces. Due to absorption to the filter membrane, only a maximum of approximately 70% of the vaccine proteins was released into the medium. Most of the formulations did not reach 70% but leveled off earlier. This indicated incomplete drug release from the formulations. Mass balance studies showed that the missing protein remained above the filter membrane. The amount of protein absorbed to the filter, released, and recovered above the filter added up to 100%.

The release from Lutrol® F68 solutions was rapid and reached a plateau after 2.5 h and was similar to polymer-free vaccine solutions (Figure 3. 46). PVP 90 showed a slightly retarded release with a slightly lower plateau. This retardation effect was even stronger for HPMC

K15M due to its higher viscosity. These three polymers (Lutrol® F68, PVP 90, HPMC K15M) were of neutral character so that no electrostatic interaction with the major vaccine proteins (hemagglutinin negatively charged, nucleoprotein positively charged) would be expected. As a result, the total amount of protein released from these solutions were similar to the pure vaccine solution. Carbopol®, carrageenan and xanthan gum showed similar protein release profiles (Figure 3. 46). All three leveled off at approximately 35 - 38% protein released. This low value may be related to the negative charge of these polymers. The missing amount of protein corresponded to the nucleoprotein fraction of the vaccine. Incomplete release of this fraction due to electrostatic binding to the polymers may have occurred. The similar slow protein release from chitosan solutions with a plateau at 38%, on the other hand, was attributed to electrostatic interactions between the polymer and the hemagglutinin, thus avoiding complete protein release.

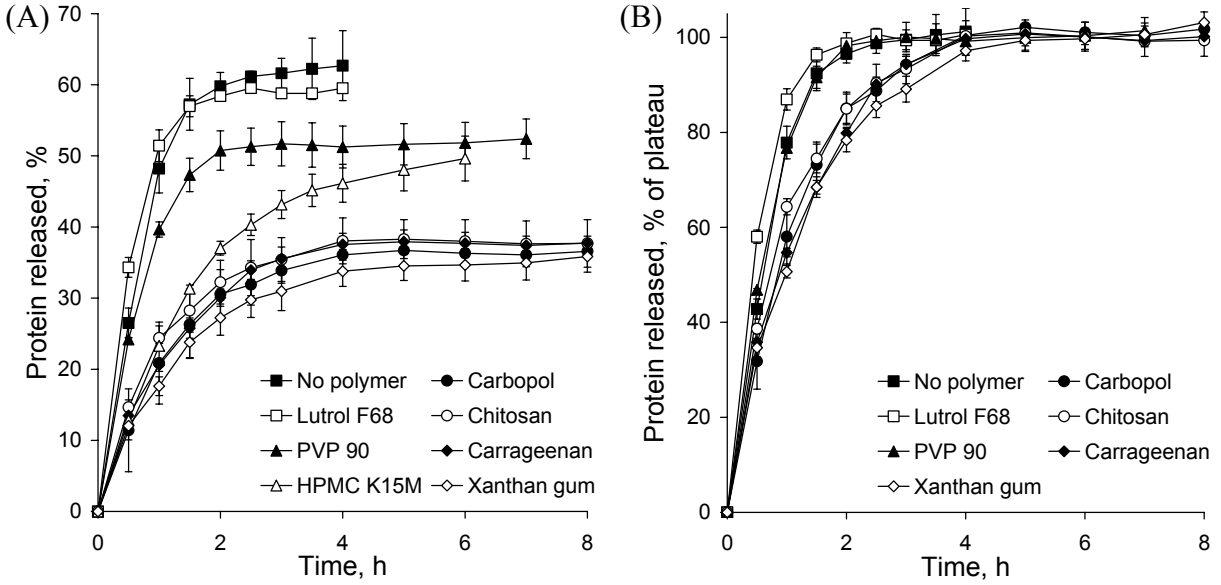


Figure 3. 46 In vitro protein release of nasal solutions (polymer 0.5% w/V [25% Lutrol® F68], vaccine dose 45 µg hemagglutinin, V = 0.2 ml, n = 3). (A) 100% = total protein content of inserts, (B) 100% = protein released at plateau.

The protein release data from different polymer solutions was replotted by taking the protein amount released at the plateau as the 100%-value to compare the release rates from the various solutions (Figure 3. 46B). HPMC K15M was not replotted, because it did not reach a plateau within the investigated 8 h. Two groups of polymers were identified. Solutions of Lutrol® F68 and PVP 90 reached 100% release (= plateau) within 2 h, similar to the polymer-free vaccine solutions. The slightly enhanced release from Lutrol® F68 solutions

may be attributed to the surfactant properties of the polymer, thus solubilizing the protein. The second group of polymers (carrageenan, xanthan gum, chitosan, Carbopol®) reached the plateau (100%) within 4 h. Also HPMC K15M belonged into this group. The prolonged release is related to the viscosities of the solutions and / or electrostatic interactions between proteins and polymers.

3.5.2. In vivo performance of polymer solutions

The seric immune response to influenza vaccine solutions with different polymeric additives at day 35 post primary application on day 1 and boost on day 22 was measured as specific IgG serum titers (Figure 3. 47A). All polymers except Carbopol® led to increased IgG titers. With the exception of xanthan gum, the average titers (including responders and non-responders) of the polymers chitosan, PVP 90, carrageenan, Lutrol® F68 and HPMC K15M were below that of the pure vaccine. However, the pure vaccine control was not prepared and shipped together with the other formulations, so that direct comparison may be inappropriate.

Comparison of the different polymers revealed the following order of IgG immune response: xanthan gum > chitosan > carrageenan = PVP 90 = Lutrol® F68 > HPMC K15m > Carbopol®. Solutions applied to the nasal cavity are usually very rapidly removed by the ciliary clearance (Marttin et al., 1998). The solutions are transported to the back of the throat, from where they are swallowed. Swallowing of the influenza vaccine would inactivate the proteins and result in no immune response. Bioadhesive formulations can prolong the nasal residence time. This could be the reason for the superior IgG serum response for xanthan gum and chitosan solutions compared to the other polymers (see section 3.1.3). Bacon et al. (2000) observed a similar increased local and serum immune response after nasal application of an influenza virus vaccine in solution with gellan gum, an extracellular anionic polysaccharide, and chitosan. The comparatively low IgG response for HPMC K15M may result from the clearance of the formulation from the nasal cavity prior to complete release of the vaccine due to less pronounced bioadhesive properties of the polymer (see section 3.1.3) and slow drug release compared to PVP 90 and Lutrol® F68. No correlation was found between the in vitro drug release results and the vivo behavior.

The mucosal specific IgA response, determined in nasal washes, showed similar results as the seric IgG titers (Figure 3. 47B). Xanthan gum was again the most promising polymer. PVP 90 showed also a raised IgA immune response compared to the pure vaccine solution.

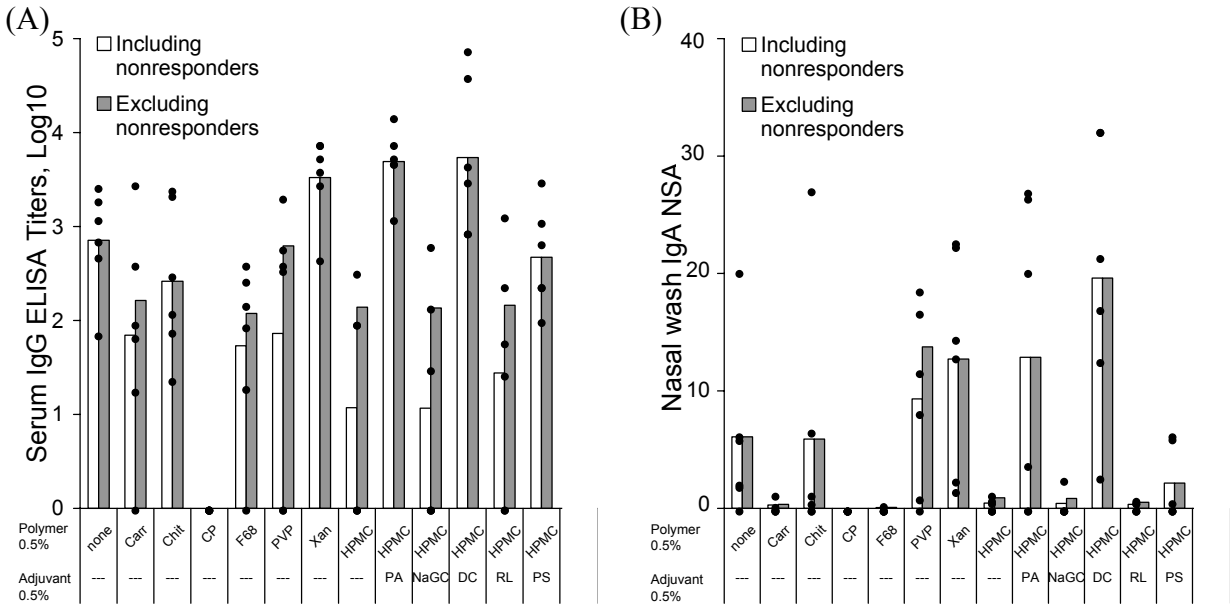


Figure 3. 47 (A) Serum immune response (IgG ELISA titer) and (B) local immune response (normalized specific activity [NSA] of IgA in nasal washes) to vaccine solutions with various polymers (0.5% w/V) and absorption enhancers (0.5% w/V) in mice (vaccine dose 4.5 µg hemagglutinin, V = 20 µl, n = 6, points represent the data of individual animals).

(Abbreviations: carr - carrageenan, Chit - chitosan, CP - Carbopol®, F68 - Lutrol® F68, PVP - PVP 90, Xan - xanthan gum, HPMC - HPMC K15M, PA - poly-L-arginine, NaGC - sodium glycocholate, DC - DC-cholesterol, RL - Eudragit® RL 30D, PS - protamine sulfate).

Next, the effect of various absorption enhancers was investigated with HPMC K15M solutions because of their good compatibility (no precipitation) with this uncharged polymer (Figure 3. 47). The absorption enhancers Na-glycocholate and insoluble Eudragit® RL nanoparticles formulated with HPMC K15M revealed no enhancing effect (enhancement factor f = titer HPMC K15M with enhancer / titer HPMC K15M without enhancer = 1.0 and 2.3, respectively) (Figure 3. 47A). The formulation containing SDS could not be administered quantitatively due to its high viscosity and was therefore not measured. Protamin sulfate, poly-L-arginine, and DC-cholesterol given with HPMC K15M increased the serum titers by a factor f = 39.8, 417 and 457, respectively, compared to vaccine solutions containing HPMC K15M alone. Best enhancement of the mucosal specific IgA response was also observed for poly-L-arginine and DC-cholesterol (Figure 3. 47B).

The enhancing effect of bile salts described in the literature is often explained by their ability to damage the mucosal membrane, thus destroying its barrier function (Hersey and Jackson,

1987). Additional inhibition of proteolytic enzymes (e.g. leucin aminopeptidase) has been discussed for Na-glycocholate (Donovan et al., 1990). The poor performance of Na-glycocholate in the present study may be explained as follows: the vaccine proteins were no substrate for the inhibited protease or the large size of the vaccine proteins (colloidal size range) still prevented the uptake even though the membrane had become more porous.

The cationic absorption enhancers, on the other hand, may aggregate and condense the colloidally dispersed, high molecular weight antigens as previously discussed, and thus allow uptake by M-cells. The adjuvant effect of the cationic enhancers tested correlated roughly with their charge density, except for DC-cholesterol (Table 3. 21). Hamman et al. (2000) found that the absorption enhancing effect of N-trimethylchitosan for nasally administered ^{14}C-mannitol in rats depended on its degree of quarternization as a measure of charge density. A minimum of 22% quaternization was required for effective enhancement. The superior effect of DC-cholesterol despite lower charge density may be attributable to its ability to form liposomes and thus to interact with membranes and / or to trigger numerous pathways of immune response (Guy et al., 2001). The ineffectiveness of Eudragit® RL nanoparticles may be related to its low charge density and / or to its water-insoluble nature.

As mentioned above, vaccine solutions containing Carbopol® did not provoke an immune response (Figure 3. 47A and B). This led to the assumption that the vaccine was not released from the solution or at least not in the active form. A 0.5% (w/w) Carbopol® solution in water had a pH of 3.3, whereas all other polymers were in the pH range of 6.4 - 7.5 (chitosan pH 4.8). Conformational changes of the hemagglutinin around pH 5 have previously been described and might be responsible for the inability of the Carbopol® solutions to elicit an immune response (Skehel et al., 1982). The Carbopol®-vaccine solution was more turbid than the pure vaccine solution and also less viscous than the pure Carbopol® solution, which indicated electrostatic interactions. To substantiate these assumptions, vaccine was released from identical Carbopol® solutions using as release medium phosphate buffers of pH 6.0 and pH 3.3 which were both adjusted to the same osmolality (0.82 osmol/kg). The lower pH resulted in a much slower vaccine release compared to pH 6.0, thus explaining the missing immune response for Carbopol® containing vaccine solutions (Figure 3. 48).

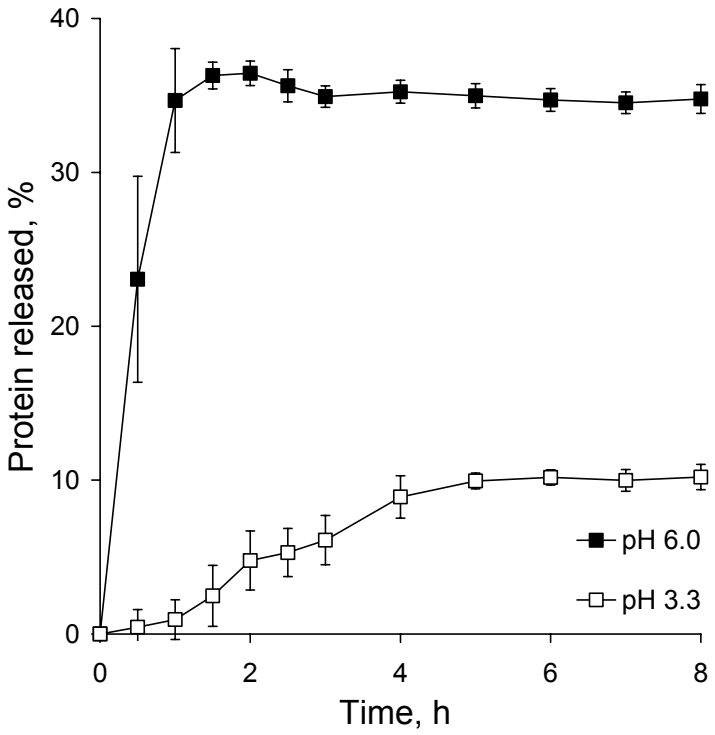


Figure 3. 48 In vitro protein release of Carbopol® solutions at different pH of the release medium (polymer 0.5% w/V, vaccine dose 45 µg hemagglutinin, V = 0.2 ml, n = 3).

3.5.3. Formulation and in vitro properties of nasal inserts

Xanthan gum was chosen as polymer and DC-cholesterol as adjuvant for further studies because of their good performance in the in vivo studies using nasal solutions. A simultaneous dissolution of xanthan gum and DC-cholesterol prior to freeze-drying for the preparation of in situ gelling nasal inserts resulted in precipitation because of their opposite charge. pH-adjustment was not suitable to avoid precipitation, since precipitation of the xanthan gum / DC-cholesterol was only prevented at pH < 1 and not up to pH 12, where the DC-cholesterol itself precipitated. Salts (low molecular weight electrolytes) have been shown to disrupt interpolyelectrolyte complexes of DNA and poly(N-alkyl-4-vinylpyridinium) cations due to shielding of the charges (Izumrudov and Zhiryakova, 1999). Thus, salt addition was investigated for the stabilization of mutual solutions of xanthan gum and DC-cholesterol. Turbidity of a solution of xanthan gum and DC-cholesterol could not be prevented, but sufficiently high concentrations of NaCl (0.4 mol/l) or $CaCl_2$ (0.2 mol/l) could avoid precipitation (Table 3. 23). No precipitation occurred during 10 min centrifugation at 3000 rpm at room temperature and after 2 days storage at 4°C when dissolving both

DC-cholesterol and xanthan gum in the vaccine solution in the presence of 0.2 mol/l $CaCl_2$ or 0.4 mol/l NaCl.

Table 3. 23 Stabilization of solutions of DC-cholesterol (0.25% w/V) and xanthan gum (0.25% w/V) by salt addition (pH of salt solutions: NaCl pH 6.6, $CaCl_2$ pH 6.0).

C_{Na+}, mol/l	**C_{Ca2+}, mol/l**	**Visual appearance (precipitation)**	**Centrifugation (3000 rpm, 10 min)**	
			Sediment	**Supernatant**
–	–	Strong	Strong	Clear
0.1	–	Medium	Medium	Turbid
0.2	–	Low	Low	Turbid
0.4	–	None	None	Turbid
–	0.1	Low	Low	Turbid
–	0.2	None	None	Turbid
–	0.4	None	None	Turbid

In situ gelling nasal inserts were prepared by freeze-drying the salt-stabilized vaccine solutions containing xanthan gum and DC-cholesterol. Due to the relatively high salt content, the susceptibility of the nasal inserts to moisture sorption at elevated humidity was investigated (Figure 3. 49). The weight increase due to moisture uptake increased substantially with $CaCl_2$ due to the hygroscopic nature of this salt (Figure 3. 49). The weight increase with $MgCl_2$ was lower, but still high. NaCl, a non-hygroscopic salt, did not enhance the moisture sorption. The lowest moisture sorption was observed for inserts prepared in purified water. The presence of the DC-cholesterol had no effect on the moisture sorption.

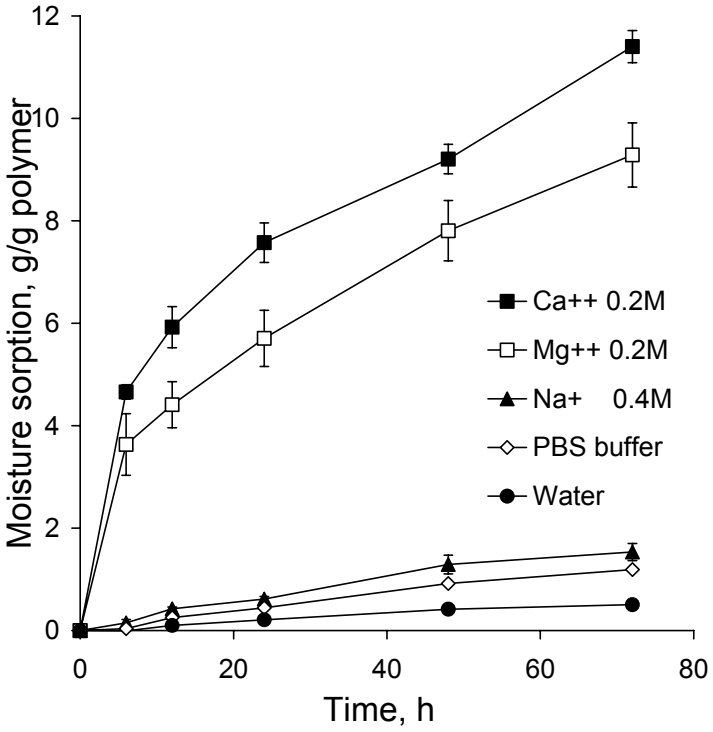


Figure 3. 49 Moisture sorption of xanthan gum inserts with stabilizing chloride salts prepared in PBS buffer or demineralized water during storage in humid air (polymer 0.5% w/V, V = 1.5 ml, n = 3).

Solid nasal inserts were formed by freeze-drying vaccine-xanthan gum (0.5 and 2% w/w) solutions. In contrast to vaccine-polymer solutions, protein release from inserts required water uptake and hydration of the polymer matrix prior to the diffusion of the proteins. The release was therefore slightly lower for inserts when compared to the corresponding solution (Figure 3. 50). However, the hydration was rapid, so that the time until the inserts were completely moistened was only 4.7 ± 0.4 min for inserts containing xanthan gum 0.5% + DC-cholesterol 0.5% + NaCl 0.4 M. The delay in drug release due to transformation from the dry to the wet was thus very short. This is one of the advantages of these rapidly gelling insert. The final polymer concentration in the rehydrated insert was higher than that of the solutions. Thus the vaccine proteins were exposed to a higher concentration of negative charges and subsequent more efficient binding of protein fractions (hemagglutinin) to the oppositely charged xanthan gum. This led to the lower plateau of the inserts with increasing polymer content and compared to the xanthan gum solution. Increasing the polymer content of the solution prior to freeze-drying and adding the DC-cholesterol stabilized with $CaCl_2$ further reduced the protein release rate (Figure 3. 50).

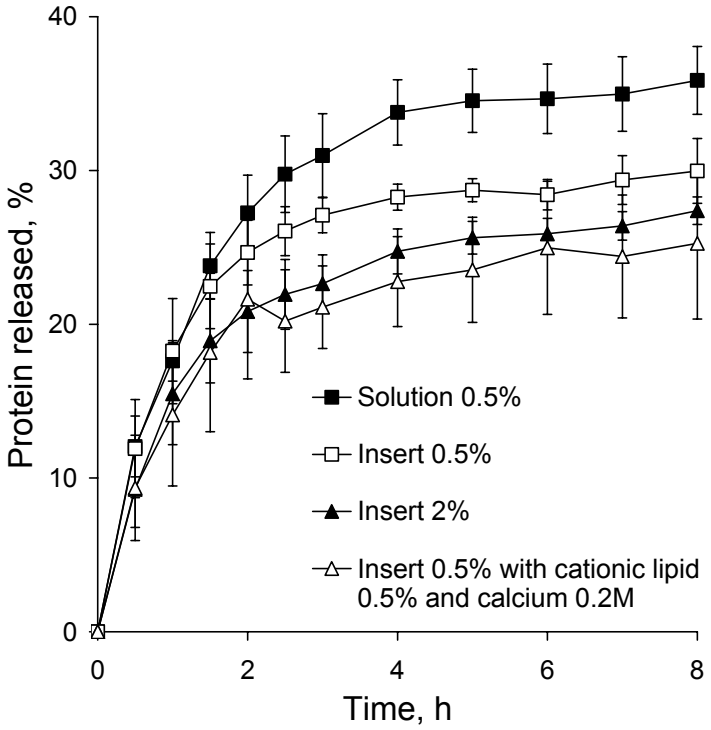


Figure 3. 50 In vitro protein release of xanthan gum solutions and inserts with varying polymer content (polymer % w/V, vaccine dose 45 µg hemagglutinin, V = 0.2 ml, n = 3).

3.5.4. Hemagglutination activity in solutions and inserts

It is important to maintain the specific activity of a protein during its incorporation into dosage forms and during storage in order to deliver a therapeutically / immunologically active protein. The agglutination of erythrocytes (hemagglutination = HA) is a measure of the specific activity of hemagglutinin, the major influenza vaccine protein. The success of an influenza vaccination is often measured in a serum hemagglutination inhibition test using the fact that anti-hemagglutinin antibodies prevent erythrocyte clotting in the presence of hemagglutinin.

None of the additives alone (polymers, absorption enhancers, stabilizing salts) exhibited a hemagglutination activity. The vaccine solution had a HA titer of $\log_2$ 6.3 (Table 3. 24). Boiling the vaccine solution for 1 min resulted in a total loss of the activity due to heat denaturation of the hemagglutinin, while freeze-drying of the vaccine without additives preserved its activity. Inserts prepared by freeze-drying of vaccine-xanthan gum or -HPMC

K15M solutions generally maintained the hemagglutination activity or led only to minor reductions (xanthan gum). Preparation of nasal inserts by freeze-drying is a mild preparation method and did not affect the specific activity of the incorporated vaccine. Storage of inserts over 2 months at 4°C had also no effect on the hemagglutination activity.

Table 3. 24 Influence of boiling and freeze-drying of the vaccine solution and storage time of inserts (polymer 0.5% w/V) (over dried silica gel in a desiccator at room temperature) on the hemagglutination activity of the vaccine (protein concentration 45 µg / 200 µl).

Composition	**Sample type**	**Storage time, months**	**HA Titer Log_2**
Untreated	Solution	0	6.3 (6,6,7)
Boiled	Solution	0	0.0 (0,0,0)
Freeze-dried	Powder	0	6.0 (6,6,6)
Xanthan gum	Insert	0	5.7 (5,6,6)
Xanthan gum	Insert	2	6.0 (6,6,6)
HPMC K15M	Insert	0	6.3 (6,6,7)
HPMC K15M	Insert	2	6.0 (6,6,6)

Addition of NaCl or $CaCl_2$ to the vaccine led to an increased hemagglutination activity, especially for the NaCl (Table 3. 25). The reason for this effect remained unclear. Disruption of loose hemagglutinin agglomerates due to the high salt concentration and thus a higher effective concentration of available erythrocyte binding sites may be speculated. Addition of the DC-cholesterol reduced the hemagglutination titer strongly because of the previously observed electrostatic interaction. The lipid probably blocked the active center of hemagglutinin and thus prevented binding of the erythrocytes to the protein. The hemagglutination activity of the vaccine was further reduced by simultaneous addition of DC-cholesterol and calcium chloride and disappeared completely when xanthan gum was also added for both solution and insert. In contrast, NaCl in combination with DC-cholesterol and xanthan gum increased the hemagglutination activity compared to the DC-cholesterol alone for both solution and insert. 0.4 M NaCl was chosen as stabilizer for xanthan gum and

DC-cholesterol solutions based on results obtained during storage and handling under ambient conditions and with the hemagglutination assay.

Table 3. 25 Influence of different chloride salts, DC-cholesterol, and xanthan gum on the hemagglutination activity of the vaccine in solutions and inserts (protein concentration 45 µg / 200 µl).

Composition (% w/V)				HA-Titer Log_2
Formulation	**Ion, mol/l**	**DC-cholesterol, %**	**Xanthan gum, %**	
Solution	-	-	-	6.3 (6,6,7)
Solution	Ca^{2+}, 0.2	-	-	6.7 (6,7,7)
Solution	Na^{+}, 0.4	-	-	9.0 (9,9,9)
Solution	-	0.5	-	2.7 (2,3,3)
Solution	Ca^{2+}, 0.2	0.5	-	2.0 (2,2,2)
Solution	Ca^{2+}, 0.2	0.5	0.5	0.0 (0,0,0)
Insert	Ca^{2+}, 0.2	0.5	0.5	0.0 (0,0,0)
Solution	Na^{+}, 0.4	0.5	0.5	5.0 (5,5,5)
Insert	Na^{+}, 0.4	0.5	0.5	4.7 (4,5,5)

3.5.5. In vivo performance of nasal inserts

In vivo vaccination studies with nasal inserts were performed in rats. Seric IgG response was measured on day 37 post primary immunization on day 1 and boost on day 22. Nasal inserts based on xanthan gum, HPMC K15M and chitosan without additives resulted in a reduced immune response compared to the pure vaccine solution (Figure 3. 51). However, again both controls, pure vaccine and vaccine with DC-cholesterol (200 µg = double amount compared to inserts), were not prepared and shipped together with the other formulations, so that direct comparison may be inappropriate. Also the volume of the control solutions (V = 100 µl) did not correspond to that of the inserts (V = 20 µl) so that the controls reached a larger area than the inserts. In case of xanthan gum, the immune response was mainly reduced because of one non-responding rat in the group (n = 5). As already seen with nasal polymer solutions (Figure

3. 47), xanthan gum inserts elicited higher IgG levels than HPMC K15M. Chitosan is often described to enhance the immune response (Bacon et al., 2000; McNeela et al., 2000). Its lower IgG immune response compared to xanthan gum and especially HPMC K15M remains unclear. Addition of DC-cholesterol as adjuvant increased the immune response to xanthan gum as well as HPMC K15M inserts (Figure 3. 51). The differences between xanthan gum inserts with / without DC-cholesterol became small when the non-responding animals were excluded. A part of the DC-cholesterol could have interacted with the oppositely charged xanthan gum rather than with the vaccine proteins. Poly-L-arginine did not show a significant enhancing effect in nasal HPMC K15M inserts although it was expected from the in vivo solution studies in mice. The missing enhancement may be related to the low stability of poly-L-arginine during storage of the inserts (supplier suggests storage temperature < 0°C).

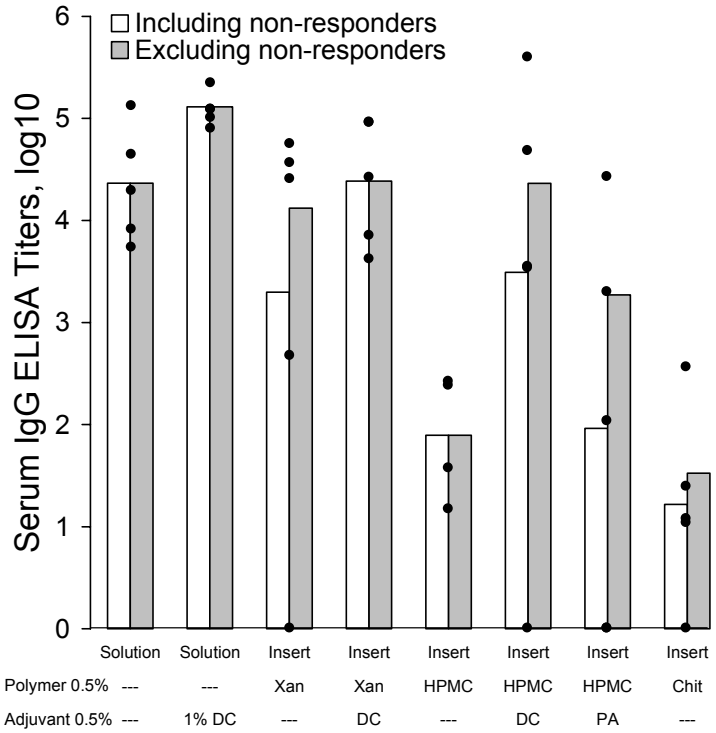


Figure 3. 51 Serum immune response (IgG ELISA titer, arbitrary units) to vaccine solutions and inserts (polymer and adjuvant each 0.5% w/V) in rats (vaccine dose 4.5 µg hemagglutinin, V = 20 µl, n = 5, points represent the data of individual animals) (Abbreviations as in Figure 3. 47).

Taken together, in situ gelling nasal inserts seemed suitable for the delivery of influenza vaccine. Although a direct comparison to pure nasal vaccine was not possible, xanthan gum inserts with DC-cholesterol as adjuvant provoked a similar immune response. This may not be

an advantage for influenza vaccine, whose proteins are relatively stable in aqueous solutions. However, incorporation into xanthan gum inserts with adjuvant may allow to increase the storage stability of antigens which are unstable in solution while simultaneously eliciting a similar immune response as the antigen solution.

Summary. Influenza split vaccine was successfully incorporated into nasal inserts without loss of the hemagglutinin specific activity. The in vitro protein release from polymer solutions containing vaccine was incomplete, likely due to electrostatic binding of fractions of the vaccine proteins (hemagglutinin positively charged, nucleoprotein negatively charged) to the gelled polymer. In vivo solution studies revealed seric and local mucosal immune response in mice. Xanthan gum had immune response enhancing properties. Out of the adjuvants tested, DC-cholesterol and poly-L-arginine showed the highest potential. Further in vivo immunization studies revealed that also vaccine-loaded nasal inserts provoked an immune response. Nasal inserts have therefore a potential as nasal vaccine delivery system although more investigations are necessary to elucidate the different performance of various polymers and enhancers and to further optimize the choice of materials.