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Influence of heat treatment on spray-dried mixtures of Amioca® starch and Carbopol® 974P used as carriers for nasal drug delivery

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ABSTRACT

A mucoadhesive spray-dried starch/poly(acrylic acid) powder underwent different heat treatments in order to induce cross-linking between the functional groups of starch (Amioca®) and poly(acrylic acid) (Carbopol® 974P). After heat treatment the water-absorbing capacity, viscosity and elasticity of the mucoadhesive powder increased. NMR analysis in combination with FT-IR indicated that heat treatment induced a low degree of cross-linking between the polymers. Nasal administration of Amioca®/Carbopol® 974P powders without heat treatment resulted in an absolute bioavailability in rabbits of 8.2 ± 3.0% for insulin. Due to the difference in water-absorbing capacity (which opened the tight junctions of the nasal mucosa), elasticity and plasticity (which reduced mucociliairy clearance and prolonged residence time) heat treatment at 120 °C improved the bioavailability: 26.4 ± 21.9, 36.5 ± 11.0 and 19.3 ± 17.3% after heat treatment during 30 min, 1 h and 4 h, respectively. Heat treatment at 60 °C was less efficient. This study demonstrated that the nasal insulin absorption improved via heat treatment of the Amioca®/Carbopol® 974P powder (prior to the addition of insulin). The bioavailability-enhancing effect of a 1 h heat treatment at 120 °C was confirmed using the same polymer matrix in combination with different drugs (salmon calcitonin, human growth hormone and metoprolol tartrate).

1. Introduction

Replacement of parenteral administration of proteins and peptides by administration via the nasal route offers great opportunities as it eliminates several of the problems associated with parenteral drug delivery (poor patient compliance, pain during administration, high cost, trained personnel required) (Ugwoke et al., 2005). The large surface area of the thin, highly vascularized nasal epithelium is ideal for the absorption of peptides and can mimic the pulsatile delivery pattern of some drugs like insulin. Absorbed peptides pass directly into the systemic circulation thereby avoiding hepato-gastrointestinal first-pass metabolism (Chang and Chien, 1984; Chien and Chang, 1985). In spite of the potential of nasal drug delivery, some limitations have to be overcome: (a) only a limited amount of peptides can be administered via the nose, (b) enzyme activity in the nasal cavity, (c) residence time of the formulation is limited by the mucociliairy clearance mechanism and (d) the mucus layers and nasal epithelium are barriers that obstruct peptide absorption (Ugwoke et al., 2005). To avoid fast clearance of the formulation from the nasal cavity, viscosity-enhancing or mucoadhesive polymers can be used to reduce ciliairy beat frequency (Nagai et al., 1984; Critchley et al., 1994; Illum et al., 2001; Callens et al., 2003). In this study, a spray-dried Amioca®/Carbopol® 974P powder was used for nasal administration of the model peptide insulin. This spray-dried combination of a maize starch (Amioca®, mainly consisting of amylopectine) and a cross-linked acrylic acid-based polymer (Carbopol® 974P) amplifies the mucoadhesive capacity of the formulation. The viscosity-enhancing capacity of the powder after hydration in contact with the nasal mucosa allows insulin delivery without additional absorption enhancers as bile salts, surfactants, chelating agents or fatty acids (Hinchcliffe and Illum, 1999).

In order to cross-link the network of Amioca® and Carbopol® 974P (ratio 25/75), the spray-dried powder underwent different heat treatments (temperature and duration as variables). After physico-chemical analysis (liquid uptake, rheological properties, NMR) these heat-treated mixtures of starch and Carbopol® 974P were evaluated as platform for the nasal delivery of different peptides (insulin, salmon calcitonin and somatropin) in rabbits. The bioavailability-enhancing effect of the carrier after heating was also tested following nasal delivery of a conventional drug (metoprolol tartrate) via the powder formulation.

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2. Materials and methods

2.1. Materials

Actrapid® HM 100 (100 IU/ml) (human monocomponent insulin) was obtained from Novo-Nordisk (Bagsvaerd, Denmark). Miacalcic® syringe (100 IU/ml) (salmon calcitonin) was obtained from Novartis Pharma (Brussels, Belgium). Genotonorm® (12 mg/ml) (somatropin) was obtained from Pfizer (Puurs, Belgium). Metoprolol tartrate was kindly donated by EQ Esteve (Barcelona, Spain). The spray-dried mixture of Amioca® starch and Carbopol® 974P (ratio: 25/75, w/w) (SD 25/75) (batch: 13724-4A) was prepared by National Starch and Chemical Company (Bridgewater, NJ, USA). All other chemicals were of analytical grade.

2.2. Heat treatment of Amioca®/Carbopol® 974P 25/75

SD 25/75 powder was packed in sealed Alu-bags and stored for 30 min, 1 h and 4 h in an oven (Memmert, type U50, Schwabach, Germany) at 60 and 120 °C.

2.3. Liquid uptake rate

The liquid uptake rate was studied hydrodynamically. 50 mg powder formulation was placed on the upper side of a filter connected to a reservoir filled with simulated nasal fluid (SNF) (7.45 mg/ml NaCl, 1.29 mg/ml KCl and 0.32 mg/ml $CaCl_2$; Melon, 1967). The measurements were performed at a temperature of 32 ± 0.5 °C, simulating the temperature of the nasal cavity. The amount of absorbed SNF was determined volumetrically in function of time.

2.4. Rheological properties

The elasticity (G') and viscosity (G'') of the powder formulations were determined by means of a TA Instruments AR 1000-N Rheometer (Zellik, Belgium). Different powders were dispersed in SNF (10%, w/w) before measuring G' and G''. The measurements were performed at 32 ± 0.5 °C using a cone of 4 cm with an angle of 1° and applying an oscillation stress of 30 Pa and a frequency of 0.1 Hz.

2.5. Physical analysis

2.5.1. ^1H liquid-state NMR

The ^1H NMR spectra were recorded from a solution in D_2O (or gel for the heat-treated specimen) at room temperature on a 300 MHz Varian Inova spectrometer. Spectra were acquired with a 90° pulse of 5 μs, a spectral width of 4500 Hz, an acquisition time of 3.5 s, a preparation delay of 6 s and 300 accumulations.

2.5.2. ^1H wide-line solid-state NMR

Solid echo (Powles and Strange, 1963) proton wide-line NMR measurements were carried out on a Varian Inova 400 spectrometer in a dedicated wide-line probe equipped with a 5 mm coil. Hereto, on-resonance Free Induction Decays (FIDs) were acquired by applying the solid echo technique ($90^\circ_{x'} - t_{se} - 90^\circ_{y'} - t_{se} -$ acquire), in an effort to overcome the effect of the dead-time of the receiver (Powles and Strange, 1963). The 90° pulse length was set to 1.2 μs and spectra were recorded with a spectral width of 2 MHz (0.5 μs dwell time) allowing an accurate determination of the echo-maximum (the data point with maximum intensity). The solid echo delay is formed with a maximum at $\tau = (3t_{90}/2 + 2t_{se}) = 6$ μs, where t_{90} is the duration of the 90° pulse. The samples were placed in 5 mm glass tubes, which were closed tightly with Teflon stoppers. A preparation delay of 5 times the T_1H relaxation decay time was always respected between successive accumulations to obtain quantitative results.

To obtain the T_2H (spin–spin relaxation) decay times, on-resonance FID curves were acquired with 64 accumulations and the time point of the echo-maximum has been calibrated to time zero. These FID curves were converted into ASCII files and analysed bi-exponentially by means of Origin Version 6.0 software as a function of the acquisition time t (non-linear least-squares fit according Levenberg–Marquardt algorithm) with the following equation:

$$I(t) = I_0^S \exp\left(-0.5\left(-\frac{t}{T_2H^S}\right)^2\right) + I_0^L \exp\left(\frac{t}{T_2H^L}\right)$$

in which the superscripts S (short decay time) and L (long decay time) refer to the fast (Gaussian shape function) and slow (Lorentzian shape function) decaying proton fractions, respectively.

The T_1H relaxation decay times (spin-lattice relaxation in the lab frame) were measured by placing an inversion recovery filter in front of the solid echo part ($180^\circ_{x'} - t - 90^\circ_{x'} - t_{se} - 90^\circ_{y'} - t_{se} -$ acquire). The integrated proton signal intensity was analysed mono-exponentially (non-linear least-squares fit by Levenberg–Marquardt algorithm) as a function of the variable inversion time t according to:

$$I(t) = I_o\left(1 - 2\exp\left(-\frac{t}{T_1H}\right)\right)$$

in which I_o is the intensity of the resonance at equilibrium.

The average 95% confidence limit for the T_1H and T_2H decay times and proton fractions is about 1–2%.

2.5.3. ^{13}C solid-state NMR

The ^{13}C CP/MAS NMR spectra were recorded at room temperature on a Unity 200 Varian spectrometer operating at a static magnetic field of 4.7 T. Magic angle spinning was performed at 7 kHz, making use of ceramic Si_3N_4 rotors. The aromatic signal of hexamethylbenzene was used to determine the Hartmann–Hahn condition ($\omega_{1H} = \gamma_H B_{1H} = \gamma_C B_{1C} = \omega_{1C}$) for cross-polarisation and to calibrate the carbon chemical shift scale (132.1 ppm). Other spectral parameters used were a 90° pulse length of 8.4 μs, a spectral width of 50 kHz, an acquisition time of 20 ms, a preparation delay of 2.5 s and 10,000 accumulations. High power decoupling was set to 65 kHz during the acquisition time.

2.5.4. FT-IR

FT-IR spectra of the reference formulation and the heat-treated (120 °C, 1 h) powder were obtained using KBr pellets on a Bruker Vertex 70 FT-IR spectrometer with a DTGS detector at a resolution 4 cm^{-1}. 32 scans were acquired.

2.6. Preparation of formulations

2.6.1. Solutions for intravenous administration

Insulin (0.8 IU/ml), salmon calcitonin (20 IU/ml) and somatropin (0.145 mg/ml) solutions were prepared by diluting Actrapid® HM 100 (100 IU/ml), Miacalcic® (100 IU/ml) and Genotonorm® (12 mg/ml), respectively, in a phosphate buffered saline solution (PBS, pH 7.4), of which respectively 500 μl (0.4 IU), 500 μl (10 IU) and 300 μl (43.5 μg) were administered intravenously to rabbits (3.0 ± 0.5 kg, $n = 2$). A metoprolol tartrate solution of 2 mg/ml was prepared in a phosphate buffered saline solution (PBS, pH 7.4) (2.38 g $Na_2HPO_4 \cdot 2H_2O$, 0.19 g KH_2PO_4 and 8.0 g NaCl per liter distilled water), of which 300 μl was intravenously administered to rabbits ($n = 6$).

2.6.2. Nasal powder formulations

After different heat treatment procedures of the powders 500 mg powder (spray-dried mixture of starch and Carbopol® 974P, ratio 25/75) was dispersed in 15 ml distilled water, followed by a neutralisation step to pH 7.4 using NaOH 2 M. Afterwards the insulin solution (Actrapid® HM 100) was added to obtain a final concentration of 1 IU insulin per mg powder.

The aqueous dispersion was freeze-dried using an Amsco-Finn Aqua GT4 freeze-dryer (Amsco, Germany). The dispersion was frozen to 228 K within 175 min at 1000 mbar. Primary drying was performed at 258 K and at a pressure varying between 0.8 and 1 mbar during 13 h, followed by secondary drying at elevated temperature (283 K) and reduced pressure (0.1–0.2 mbar) for 7 h. After freeze-drying the powder was sieved (63 μm) at low relative humidity (20%) and ambient temperature. The fraction below 63 μm was stored in a desiccator at 4–8 °C until use.

The same procedure was used to obtain powders containing 2 IU salmon calcitonin, 0.045 mg somatropin and 0.24 mg metoprolol tartrate per mg powder.

2.7. Nasal bioavailability study

The protocol of the animal experiments was approved by the Ethics Committee of the Institute for Agricultural and Fisheries Research (ILVO) (Merelbeke, Belgium). New Zealand white rabbits (3.0 ± 0.5 kg) were fasted 16 h prior to the experiment. Water was available ad libitum. They were sedated with an intramuscular injection of 0.05 ml/kg Placivet® (Codifar, FL, USA) immediately after administration. A group of rabbits received 0.4 IU insulin, 10 IU salmon calcitonin, 0.9 mg somatropin or 0.6 mg metoprolol tartrate intravenously. Ten micrograms powder formulation (equivalent to 10 IU insulin, 20 IU salmon calcitonin, 0.45 mg somatropin or 2.4 mg metoprolol tartrate) was administered in each nostril of a second group of rabbits using polyethylene tubes (Medisize, Hillegom, The Netherlands). The powder was released from the tubes using a syringe containing 1 ml compressed air (2.5 bar). This device was based on a system developed by Sørensen (1991).

The tubes were filled under conditions of low relative humidity (20%) and ambient temperature. Blood samples were collected from the ear veins at −5, 1, 5, 10, 15, 20, 30, 40, 50 and 60 min after intravenous administration and at −5, 2, 5, 10, 15, 20, 25, 30, 35, 45, 60, 90, 120, 150 and 180 min after nasal delivery of the powder formulations. The sera were separated by centrifugation (700 × *g*, 5 min) and samples were stored frozen at −20 °C until analysis. After nasal administration of metoprolol tartrate, heparin (LEO Pharma, Wilrijk, Belgium) was added to the test tubes before centrifugation in order to obtain plasma samples.

2.8. Analysis of the blood samples

Insulin serum samples were evaluated using RIA-analysis (Coat-A-Count® kit, DPC, Humbeek, Belgium). The radioactivity of the samples was quantified using a Cobra gamma counter (Canberra Packard Benelux, Zellik, Belgium).

The samples containing salmon calcitonin and somatropin were analysed using an Enzyme-Linked Immunosorbent kit (Active® Ultra-sensitive salmon calcitonin and DSL-10-1900, Diagnostic Systems Laboratories, TX, USA). The fluorescence measurements were carried out on a fluorometer (Wallac 1420 multilabel counter, PerkinElmer, Turku, Finland) at 450 nm.

Metoprolol tartrate plasma concentrations were determined by a validated HPLC-fluorescence method. All chemicals were of analytical grade. Samples were prepared by adding 20 μl of an internal standard solution (5.625 μg/ml of alprenolol in water for analysis of blood samples after nasal administration and 1.925 μg/ml alprenolol in water for analysis of the intravenous samples) and 680 μl PBS to 300 μl plasma sample. The drug was extracted using a solid phase extraction (SPE) method. The SPE columns were conditioned consecutively with 1 ml methanol, 1 ml water and 1 ml PBS. Next, the plasma samples were spiked on the SPE columns (OASIS HLB (1 ml, 30 mg) cartridges (Waters, Brussels, Belgium) in combination with a 16-port vacuum manifold (Alltech Europe, Laarne, Belgium)). Columns were rinsed with 1 ml water and metoprolol tartrate was eluted with 1 ml methanol. The eluates were evaporated to dryness under a nitrogen flow, the residue was dissolved in 150 μl water and 20 μl was injected. The plasma concentrations were determined via a calibration curve. The standards for the calibration curve (20 μl of internal standard solution, 20 μl of a standard solution with known metoprolol tartrate concentration in water, 280 μl blank plasma and 680 μl PBS) underwent the same treatment as the plasma samples. The concentrations of the standard solutions were 0.375; 0.5625; 0.75; 2.25; 5.625; 7.5; 15.0; 22.5 μg/ml metoprolol tartrate in water for analysing the samples after nasal administration and 0.275; 0.550; 0.875; 1.1; 1.925; 2.75; 4.4 μg/ml metoprolol tartrate in water for analysing the intravenous samples.

The HPLC equipment (Hitachi, Darmstadt, Germany) consisted of a solvent pump (L-7110 pump) set at a constant flow rate of 0.800 ml/min, a fluorescence detector (L-7480) set at 275 nm as excitation wavelength and 300 nm as emission wavelength, a LiChrosper® 100 CN column (5 μm, 250 mm × 4 mm) and pre-column LiChrosper® (5 μm, 4 mm × 4 mm), an autosampler and injector (Gilson 234 autoinjector, WI, USA) with an injection loop of 50 μl (Valco Instruments Corporation, Houston, TX, USA). The area under the curve was calculated with the software pack D-7000 Multi-Manager (Merck, Darmstadt, Germany). The eluens had the following composition: 5 ml of a 2 M NaH_2PO_4 buffer solution, 50 ml acetonitrile and 945 ml water, adjusted to pH 3 with 150 μl fosforic acid.

2.9. Statistical analysis

The individual serum concentration–time profiles were analysed by MW/Pharm version 3.15 (Medi-ware, Utrecht, The Netherlands) and the maximum serum concentrations (C_{max}) and t_{max} values were determined from the individual serum concentration–time profiles. The influence of the powder formulations on the absolute bioavailability, C_{max} and t_{max} of the peptides and metoprolol tartrate was analysed using one-way ANOVA. Normal distribution of the data was tested using the Kolmogorov–Smirnov test and the homogeneity of variances was tested using the Levene's test. If the distribution of the data was not normal or the variances were not homogeneous, the data were logarithmically transformed. The statistical analysis of the somatropin data was performed with an independent samples *T*-test. The software program SPSS version 15.0 was used for statistical analysis.

3. Results and discussion

In this study, heat treatment was investigated as a method to induce cross-links between the functional groups of pre-gelatinized amylopectin (Amioca®) and poly(acrylic acid) (Carbopol® 974P) in order to improve the mucoadhesive properties of the powder and increase the bioavailability after nasal administration. Whereas Yu and Hui-min (2006) created a novel superabsorbing polymer by chemical graft polymerization of acrylic acid onto a carboxymethyl-chitosan chain and subsequent cross-linking, heat treatment was selected in the present study as a soft and widely available technique to possibly create a polymer with improved liquid absorbing capacity.

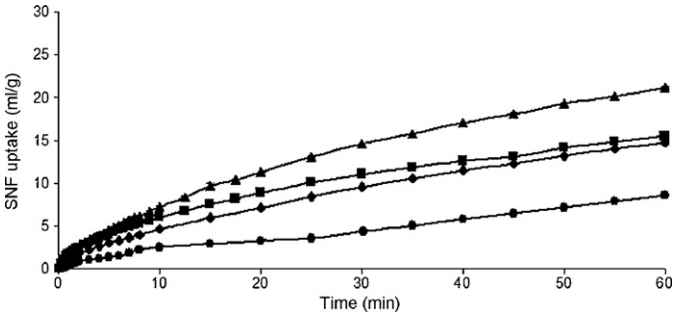

Fig. 1. Influence of heat treatment of Amioca/Carbopol powder (ratio: 25/75) (SD 25/75) at 60 °C during 0 min (♦), 30 min (■), 1 h (▲) and 4 h (●) on simulated nasal fluid (SNF) uptake profiles of SD 25/75 powder formulation ($n = 3$, mean ± SD).

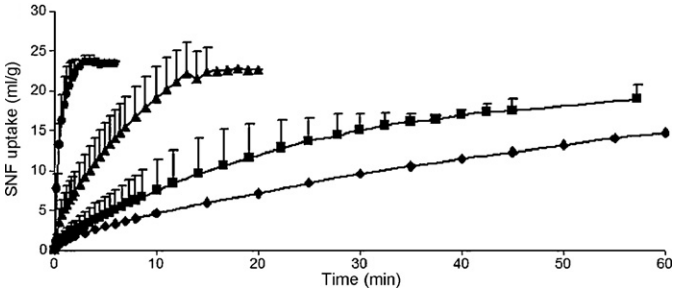

Fig. 2. Influence of heat treatment of Amioca/Carbopol powder (ratio: 25/75) (SD 25/75) at 120 °C during 0 min (♦), 30 min (■), 1 h (▲) and 4 h (●) on simulated nasal fluid (SNF) uptake profiles of SD 25/75 powder formulation ($n = 3$, mean ± SD).

The uptake of simulated nasal fluid (SNF) after heat treatment of the mucoadhesive powders (SD 25/75) at 60 and 120 °C is presented in Figs. 1 and 2, respectively. After 60 min the SD 25/75 powder heated for 1 h at 60 °C had absorbed 20.1 ml/g powder, while the liquid uptake of the reference formulation was 14.7 ml/g over the same period. Heat treatment at 60 °C during 30 min and 4 h resulted in a slower SNF uptake. In contrast, heating the SD 25/75 powder at 120 °C for 4 h induced a fast hydration rate: hydration of the polymer chains (water uptake: 23.3 ml/g) was complete within 3 min, versus 2.2 ml/g for the reference formulation (Fig. 2).

The viscosity and elasticity after dispersion of heat-treated SD 25/75 powders in SNF are shown in Table 1. The storage modulus (G') reflects the solid-like component of a visco-elastic material and will be large if a sample is predominantly elastic or highly structured, the loss modulus (G'') is a measure of the liquid-like component which will be large when a sample is predominantly viscous (Ceulemans and Ludwig, 2002). In case of a cross-linked system G' (elastic) $\gg G''$ (viscous), while $G' > G''$ indicates a network consisting of secondary bonds and $G' < G''$ a physically entangled polymer solution (Fery, 1970). The elasticity or storage (G') modulus of the SD 25/75 powder heated at 60 °C during 1 h was slightly higher compared with the reference formulation. Its G'' value was similar to the reference formulation. G' and G'' values increased in function of heat treatment at 120 °C. Since G' is larger than G'', it indicated that a cross-linked gel is formed after hydration of the Amioca®/Carbopol® powder (Madsen et al., 1998). The viscosity of the heat-treated samples resulted not only from the mechanical entanglement of the hydrated polymers and the high density of hydrogen bonding groups in Amioca starch and Carbopol®, but also cross-linking of the polymers during heat treatment was a contributing factor.

Table 1
Influence of heat treatment of Amioca/Carbopol powder (ratio: 25/75) (SD 25/75) on elasticity (G') and viscosity (G'') after dispersion into simulated nasal fluid (mean ± SD, $n = 3$).

Heat treatment procedure	G' (Pa)	G'' (Pa)
Without heat treatment	1886 ± 26	196 ± 27
60 °C – 30 min	1983 ± 154	162 ± 28
60 °C – 1 u	2477 ± 63	155 ± 14
60 °C – 4 u	2297 ± 11	158 ± 5
120 °C – 30 min	3879 ± 54	256 ± 24
120 °C – l u	7168 ± 558	553 ± 73
120 °C – 4 u	18,753 ± 714	1263 ± 211

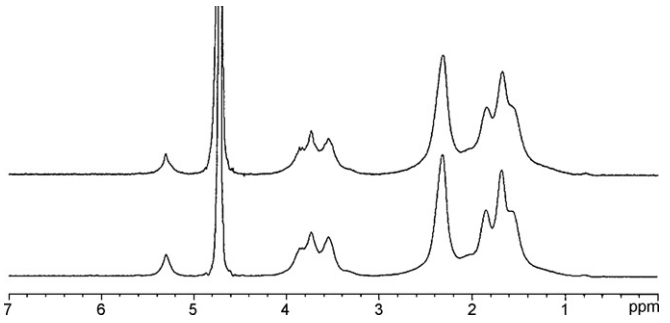

Fig. 3. Liquid-state ^{1}H NMR spectrum of Amioca/Carbopol 974P powder (ratio: 25/75) (concentration: 1 mg/ml) without heat treatment (upper profile) and after heat treatment at 120 °C during 1 h (lower profile).

Based on the rheological properties and the liquid uptake rate, the reference formulation (without heat treatment) and the SD 25/75 powder after 1 h heat treatment at 120 °C were evaluated via NMR to identify any structural changes in the powder after heat treatment.

A first indication of cross-linking resulted from liquid-state ^{1}H NMR measurements. Whereas the native specimen (reference formulation without heat treatment) dissolved easily in D_2O, the heat-treated specimen formed a gel (even at half the concentration). In the ^{1}H-spectra, the cross-linking of the heat-treated specimen resulted in an increase of the line-widths. This is demonstrated in Fig. 3 which compares the spectra of the native and heat-treated specimens: the increase in line-width was specifically prominent in the spectral region 1–4 ppm.

One of the very few techniques available to confirm cross-linking (and to determine the relative degree of cross-linking) can be found in solid-state proton wide-line-NMR relaxometry measurements. By this technique, the proton relaxation decay times, which are sensitive to the freedom of molecular motions, can be studied. In the solid-state, the T_2H (spin–spin) decay times are extremely short, typically between 10 and 100 μs, and represent the strength of the dipolar interactions. For the specimens studied here, the T_2H relaxation had to be analysed bi-exponentially with a short (T_2H^S) and long (T_2H^L) decay time, corresponding to a very rigid and a more mobile proton fraction (Table 2). This table shows that specifically the long decay time dropped upon heat treatment. This was a direct indication of a reduced molecular mobility. Measuring at 40 °C, which speeds up the molecular motions, resulted in a longer T_2H^L decay time and larger mobile proton fraction (Table 2).

Table 2
Proton relaxation decay times T_1H (s) and T_2H (μs) of Amioca®/Carbopol® 974P powder (ratio: 25/75) without heat treatment and after heat treatment at 120 °C during 1 h. The average 95% confidence limit for the T_1H and T_2H decay times and proton fractions is about 1–2%. The proton fractions of the T_2H analysis are given between brackets.

	Decay time		
	T_1H (s)	T_2H^S (μs)	T_2H^L (μs)
Without heat treatment	2.31	9.90 (85.7)	65 (14.3)
120 °C – 1 h	2.49	9.84 (85.8)	61 (14.2)
120 °C – 1 h – measured at 40 °C	2.00	9.89 (81.1)	82 (18.9)

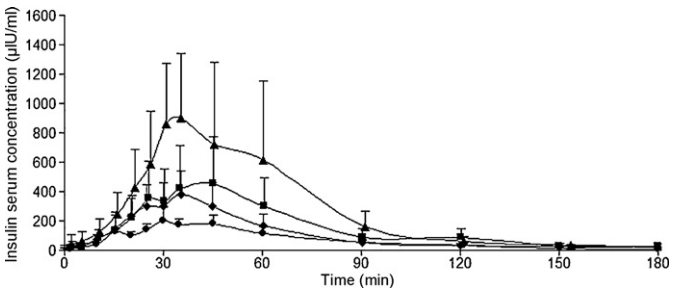

Fig. 4. Influence of heat treatment of Amioca/Carbopol powder (ratio: 25/75) (SD 25/75) at 60 °C during 0 min (♦), 30 min (■), 1 h (▲) and 4 h (●) on insulin serum concentration profiles after nasal delivery to rabbits of SD 25/75 powder formulation (1 IU insulin/mg).

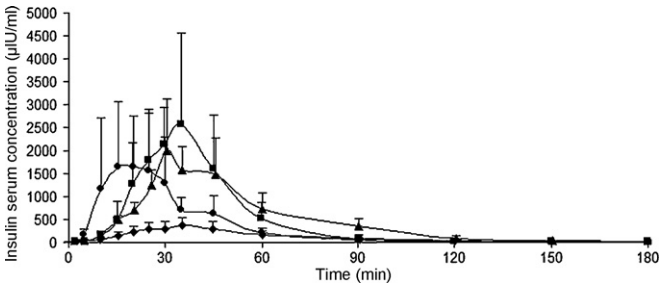

Fig. 5. Influence of heat treatment of Amioca/Carbopol powder (ratio: 25/75) (SD 25/75) at 120 °C during 0 min (♦), 30 min (■), 1 h (▲) and 4 h (●) on insulin serum concentration profiles after nasal delivery to rabbits of SD 25/75 powder formulation (1 IU insulin/mg).

The T_1H relaxation (in the order of seconds) on the other hand was dominated by the spectral density of segmental chain motions in the MHz region. The T_1H relaxation behaved mono-exponentially and increased upon heat treatment. Since a correlation diagram (a plot of log T_1H versus the correlation time of segmental chain motion) showed a distinct minimum, a T_1H measurement at 40 °C (so for a shorter correlation time) demonstrated that the T_1H relaxation was situated at the right site of the minimum of the correlation curve. This allowed to confirm the T_2H results, i.e. that the longer T_1H decay time measured for the heat-treated specimen pointed to a reduced molecular mobility due to cross-linking.

Since both FT-IR and solid-state ^{13}C-CP/MAS NMR spectroscopy (results not shown) did not show any sign of ester (cross-linking) functionalities, it was concluded that the degree of cross-linking introduced by the heat treatment was low.

A similar thermal ageing phenomenon with the formation of cross-links was already described by Bodek and Bak (1999) for a chitosan–diclofenac sodium system: thermal ageing of chitosan pellets at temperatures between 20 and 80 °C reduced the polarisation and dielectric properties due to cross-linking (resulting in additional confinement of molecular movements). This phenomenon was confirmed by IR spectroscopy and the influence on molecular weight distribution.

Fig. 4 and Table 3 show the pharmacokinetic parameters of insulin after intranasal administration of SD 25/75 powder heated at 60 °C. Using SD 25/75 heat treated at 60 °C during 1 h resulted in a significantly higher C_{max} compared to the other formulations heated at 60 °C. The highest bioavailability (18.5%) obtained after heat treatment at 60 °C during 1 h corresponded with the highest uptake of SNF (Fig. 1). After heat treatment at 60 °C powders with similar G' and G'' values resulted in a similar bioavailability, except for the powder heated at 60 °C during 4 h which had a significantly lower value ($0.05 > P > 0.01$) compared to the powder heated during 1 h.

Fig. 5 and Table 3 show the serum profile and the pharmacokinetic parameters of insulin after intranasal administration of SD 25/75 powder heated at 120 °C. Heat treatment at 120 °C during 1 h resulted in the highest bioavailability (36.5%), whereas heating the SD 25/75 powder at 120 °C during 4 h gave a significantly lower bioavailability and t_{max} ($0.05 \geq P > 0.01$). The fast liquid saturation of the powder heated during 4 h (Fig. 2) correlated with a significantly lower t_{max} of 22.5 min (Table 3). The high bioavailability obtained with the SD 25/75 powder heated at 120 °C during 1 h was correlated with a high water-absorbing capacity (Fig. 2).

The reduced mucociliairy clearance and prolonged residence time of the formulation stimulated insulin uptake as the formation of a gelled system after contact of the SD powder with the nasal mucosa provided a high drug concentration at the epithelial absorptive surface (Illum et al., 2001). Although the highest G' and G'' values were obtained after heat treatment at 120 °C during 4 h (in combination with the fastest liquid uptake), the nasal insulin bioavailability was lower and highly variable (19.3 ± 17.3%). A possible explanation for this phenomenon is that insulin is entrapped in the highly viscous and elastic polymeric network and is not able to diffuse from this dense structure towards the nasal capillaries.

The higher nasal absorption of insulin observed with this mucoadhesive powder is not only due to the prolonged residence time in the nasal cavity (Pereswetoff-Morath, 1998). An important role is also played by the fast hydration rate of the powder upon contact with the nasal mucosa, thereby shrinking the epithelial cells and probably opening the tight junctions (Edman and Bjork, 1992; Illum et al., 1994). Since this paracellular process (opening of the tight junctions) is reversible, a faster absorption of drugs transported via this pathway will mainly occur during the short period when tight junctions are opened (Pereswetoff-Morath, 1998) after hydration of the polymers. This explained the low t_{max} values measured after nasal insulin administration in SD 25/75 powders.

The improvement of bioavailability via thermal treatment was reproducible as repeating the entire procedure (i.e. heat treatment at 120 °C for 1 h, drug loading, freeze-drying, bioavailability testing, etc.) did not result in significant differences for water uptake, rheological properties and pharmacokinetic parameters of the insulin-containing replicates.

Since the highest bioavailability of insulin (5808 Da) was obtained using the Amioca/Carbopol 974P powder heated at 120 °C during 1 h, this carrier for nasal drug delivery was also combined with smaller (salmon calcitonin, 3432 Da) and larger (somatropin, 22 kDa) peptides as well as conventional drugs (metoprolol tartrate) to investigate the universal applicability of this procedure to improve bioavailability.

Table 3
Influence of heat treatment of Amioca/Carbopol powder (ratio: 25/75) (SD 25/75) on the pharmacokinetic parameters of insulin after nasal delivery to rabbits (mean ± SD).

Heat treatment procedure	*BA* (%)	C_{max} (µIU/ml)	t_{max} (min)	*n*
Without heat treatment (reference)	8.2 ± 3.0	352.3 ± 150.7*	33.6 ± 9.0	7
60 °C – 30 min	12.4 ± 5.1	513.0 ± 318.3*	37.2 ± 7.7	7
60 °C – 1 h	18.5 ± 13.8	1092.9 ± 378.7	37.9 ± 10.0	7
60 °C – 4 h	5.4 ± 1.8*	227.5 ± 81.0***	36.9 ± 8.0	7
120 °C – 30 min	26.4 ± 21.9[b]	3288.0 ± 1345.1[b]	33.8 ± 6.8	6
120 °C – 1 h	36.5 ± 11.0[c]	1178.3 ± 494.6[a]	36.4 ± 6.3	4
120 °C – 4 u	19.3 ± 17.3[d]	1967.5 ± 1294.0[a]	22.5 ± 12.2[d]	6

* Significantly lower than SD 25/75 treated at 60 °C during 1 h ($0.05 \geq P > 0.01$).
*** Significantly lower than SD 25/75 treated at 60 °C during 1 h ($P \leq 0.001$).
[a] Significantly higher than reference formulation ($0.05 \geq P > 0.01$).
[b] Significantly higher than reference formulation ($0.01 \geq P > 0.001$).
[c] Significantly higher than reference formulation ($P \leq 0.001$).
[d] Significantly lower than SD 25/75 treated at 120 °C during 1 h ($0.05 \geq P > 0.01$).

Table 4
Absolute bioavailability, C_{max} and t_{max} (mean ± SD) of salmon calcitonin, somatropin and metoprolol tartrate after nasal administration to rabbits. The drugs were formulated in Amioca®/Carbopol® (SD 25/75) without heat treatment (reference formulation) and after 1 h heat treatment at 120 °C.

Formulation	*BA* (%)	C_{max} (pg/ml)	t_{max} (min)	*n*
Salmon calcitonin				
Without heat treatment	5.1 ± 2.1	55.4 ± 22.5	16.8 ± 6.8	8
1 h heat treatment at 120 °C	9.6 ± 5.6	112.2 ± 67.6*	10.5 ± 6.6	8
Somatropin				
Without heat treatment	7.4 ± 4.0	15.9 ± 7.7	54.6 ± 12.2	8
1 h heat treatment at 120 °C	27.9 ± 8.0***	61.7 ± 13.0***	57.0 ± 16.2	8
Metoprolol tartrate				
Without heat treatment	73.6 ± 24.9	13.3 ± 4.9	20.8 ± 9.2	6
1 h heat treatment at 120 °C	99.2 ± 44.5	19.7 ± 3.5	15.3 ± 9.5	5

* Significantly higher than SD 25/75 reference formulation ($0.05 \geq P > 0.01$).
*** Significantly higher than SD 25/75 reference formulation ($P \leq 0.001$).

Table 4 shows the pharmacokinetic parameters after nasal delivery of Amioca®/Carbopol® 974P powder heated at 120 °C during 1 h in combination with salmon calcitonin, somatropin and metoprolol tartrate. For each molecule, the parameters obtained with the heat-treated formulation were compared to a reference formulation (i.e. powder without heat treatment). Although *BA* of salmon calcitonin did not significantly increase after heat treatment, C_{max} of salmon calcitonin after heat treatment of SD 25/75 was significantly higher ($0.05 \geq P > 0.01$) than the non-heat-treated SD 25/75 powder. In case of human growth hormone, the bioavailability was 28% and C_{max} 62 ng/ml after heat treatment which was significantly higher than the reference formulation ($P \leq 0.001$). After nasal delivery of metoprolol tartrate via the heat-treated carrier, bioavailability (99.2 ± 44.5%) and C_{max} (19.7 ± 3.5 μIU/ml) increased in comparison with the reference formulation (73.6 ± 24.9% and 13.3 ± 4.9 μIU/ml, respectively).

4. Conclusion

The present study demonstrated that nasal absorption of peptides (insulin, salmon calcitonin and somatropin) as well as conventional drugs (metoprolol tartrate) could be improved via heat treatment of an Amioca®/Carbopol® 974P powder prior to the addition of active ingredient. Heat treatment of the powder at 120 °C during 1 h resulted in low levels of cross-linking which are responsible for a fast liquid uptake rate and higher G' (elasticity) and G'' (viscosity) values. After nasal administration of an insulin-containing powder to rabbits a reproducible absolute bioavailability of 30% was obtained. These heat-treated mucoadhesive powder formulation are a promising platform for nasal drug delivery since the procedure allowed to improve the bioavailability of different drugs (insulin, salmon calcitonin, somatropin, metoprolol tartrate).

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