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Influence of deposition and spray pattern of nasal powders on insulin bioavailability

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Abstract

The influence of the deposition pattern and spray characteristics of nasal powder formulations on the insulin bioavailability was investigated in rabbits. The formulations were prepared by freeze drying a dispersion containing a physical mixture of drum dried waxy maize starch (DDWM)/Carbopol® 974P (90/10, w/w) or a spray-dried mixture of Amioca® starch/Carbopol® 974P (25/75, w/w). The deposition in the nasal cavity of rabbits and in a silicone human nose model after actuation of three nasal delivery devices (Monopowder, Pfeiffer and experimental system) was compared and related to the insulin bioavailability. Posterior deposition of the powder formulation in the nasal cavity lowered the insulin bioavailability.

To study the spray pattern, the shape and cross-section of the emitted powder cloud were analysed. It was concluded that the powder bulk density of the formulation influenced the spray pattern. Consequently, powders of different bulk density were prepared by changing the solid fraction of the freeze dried dispersion and by changing the freezing rate during freeze drying. After nasal delivery of these powder formulations no influence of the powder bulk density and of the spray pattern on the insulin bioavailability was observed.

Keywords: Powder formulation; Deposition; Spray pattern; Bulk density; Nasal bioavailability; Insulin

1. Introduction

Peptides are not suitable for oral administration due to their degradation in the gastro-intestinal tract by acids or proteolytic enzymes and to their limited membrane permeability through the intestinal mucosa. Furthermore, peptides are exposed to a high first pass metabolism in the liver. This explains why peptides are mainly delivered by parenteral administration. However, due to the disadvantages associated with parenteral administration several alternative routes (pulmonary, nasal, . . .) have been described (Zhou and Po, 1991). As mucociliary clearance, enzymatic activity and the epithelium combined with the mucus layer constitute barriers to nasal absorption of high molecular weight and hydrophilic peptides, the use of absorption enhancers, enzyme inhibitors and powder formulations has been investigated to enhance their nasal bioavailability. Powder formulations have been developed to improve the nasal bioavailability of peptides, based on a decrease in clearance rate of the powder particles from the nasal cavity (Soane et al., 1999; Callens and Remon, 2000). Callens et al. (2000, 2003a) reported an absolute nasal bioavailability of 14 and 18% in rabbits after administration of insulin using a powder formulation (1 IU insulin/mg) based on a physical mixture of drum dried waxy maize starch (DDWM)/Carbopol® 974P (90/10) and on a spray-dried mixture of Amioca® starch/Carbopol® 974P (25/75), respectively. The higher bioavailability of the latter was contributed to the higher viscosity and elasticity after dispersing the powder in the nasal mucus (Callens et al., 2003b).

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Besides the powder characteristics the nasal bioavailability can be influenced by the nasal delivery device and the spray pattern of the powder formulation. In present study three nasal delivery devices were compared based on their insulin bioavailability after nasal delivery of the powder formulations to rabbits. To explain the different bioavailabilities, the deposition pattern and mucociliary clearance of the devices were investigated by gamma scintigraphy. Additionally, the initial deposition area of the different delivery devices in rabbits was compared to the deposition in a silicone human nose model. In a next experiment, the spray pattern of different powder formulations from the delivery device was visualized using an imaging system (shape and cross-section of powder cloud) and these observations were correlated with powder bulk density and nasal bioavailability.

2. Materials and methods

2.1. Materials

Actrapid® HM 100 (100 IU/ml) (human monocomponent insulin) was obtained from Novo-Nordisk (Bagsvaerd, Denmark). The spray-dried mixture of Amioca® starch (an amylopectin corn starch)/Carbopol® 974P (25/75, w/w) was prepared by National Starch and Chemical Company (Bridgewater, NJ, USA). Drum dried waxy maize starch and Carbopol® 974P (C 974P) were supplied by Eridania Béghin-Say Cerestar (Vilvoorde, Belgium) and Noveon Co. (Cleveland, OH, USA), respectively. ^{99m}Technetium (^{99m}Tc), as sodium pertechnate, was received from the Ghent University Hospital (Belgium). All chemicals used were of analytical grade.

2.2. Nasal delivery devices

Beside the Monopowder (Valois, Marly-le-Roi, France) and Pfeiffer system (Pfeiffer, Radolfzell, Germany) an experimental device based on the system developed by Sørensen (1991) was evaluated. This experimental device was composed of a polyethylene tube (inner diameter: 1.5 mm, length: 5.5 cm) (Medisize, Hillegom, The Netherlands) filled with the nasal powder formulation. The powder was sprayed into the nostrils by attaching the tubes to a syringe containing 1 ml compressed air (2.5 bar).

2.3. Preparations of insulin formulations

2.3.1. I.v. formulation

An insulin solution of 0.8 IU/ml was prepared by diluting Actrapid® HM 100 in a phosphate buffered saline solution, pH 7.4, of which 0.5 ml was administered intravenously to rabbits.

2.3.2. Nasal powder formulations

1.0 g physically mixed DDWM/C 974P (90/10, w/w) or spray-dried Amioca® starch/C 974P (25/75, w/w) was dispersed in 10.0 and 30.0 ml distilled water, respectively. After neutralization to pH 7.4 with 0.2 or 2.0 M NaOH, respectively, the insulin solution (Actrapid® HM 100) was added to obtain a final concentration of 1 IU insulin per mg powder. The dispersion was freeze dried in vials using an Amsco-Finn Aqua GT4 freeze dryer (Amsco, Germany). The dispersion was frozen to 228 K within 175 min at 1000 mbar. The primary drying was performed at 258 K and at a pressure varying between 0.8 and 1 mbar during 13 h, followed by the 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 desiccator at 4–8 °C until use.

2.4. Nasal bioavailability study

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 Combistress® (Kela Laboratoria, Hoogstraten, Belgium). The rabbits received 0.4 IU insulin intravenously. Ten milligrams powder formulation (equivalent to 10 IU insulin) was administered in each nostril. The delivery device was 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 samples were centrifuged (700 × *g*, 5 min) and the sera were frozen at −20 °C until 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 individual serum concentration-time profiles were analysed using MW/Pharm Version 3.15 (Medi-ware, Utrecht, The Netherlands) and the maximum serum insulin concentrations (C_{max}) and t_{max} values were determined from the individual serum concentration-time profiles. Statistical significance was calculated according to one-way ANOVA ($P < 0.05$) followed by a Scheffé test.

2.5. In vivo deposition and clearance study in rabbits

The radiolabelling procedure was based on the method of Ridley et al. (1995) and Soane et al. (1999). Fifty milligrams DDWM/C 974P (90/10, w/w) was dispersed in 200 μl of an aqueous stannous chloride solution (10 mg/ml). After addition of ^{99m}Tc (740 MBq ≈ 20 mCi) distilled water was added until a volume of 10 ml was obtained. The dispersion was stirred (10 min) using a magnetic stirrer followed by centrifugation (1420 × *g*, 20 min). The supernatant was removed and the starch/C 974P mixture was washed with 5 ml distilled water and centrifuged (1420 × *g*, 10 min). After removal of the supernatant, the starch/C 974P mixture was freeze dried, sieved and stored as described under Section 2.3.2.

New Zealand white rabbits (3.0 ± 0.5 kg, $n = 3$) were used in this study. The animals were sedated with Rompun® (0.2 ml/kg) and after 30 min anesthesized with ketamine (15 mg/kg). Ten milligrams powder, containing 1–2 MBq ^{99m}Tc, was sprayed in the right nostril with the different delivery devices. A cross-over design was used with a wash-out period of 2 weeks between the treatments. The deposition and the clearance of the different

delivery devices were followed by gamma scintigraphy using PRISM 1500, AXIS and HELIX gamma cameras (Marconi Medical Systems, Cleveland, OH, USA). Dynamic lateral views were recorded every 3 min during 150 min. Circular regions of interest (ROI) of the nasal cavity and the whole body of the rabbit were drawn manually. To quantify the ROI, the background activity and the radioactive decay was taken into consideration. The measured radioactivity was expressed as a percentage of the initial activity measured immediately after administration.

2.6. In vitro deposition study using a silicone human nose model

Two hundred milligrams DDWM/C 974P (90/10, w/w) was dispersed in 4 ml distilled water and 40 mCi ^{99m}Tc was added. This dispersion was freeze dried, sieved and stored as described under Section 2.3.2. The powder formulation was sprayed into the nasal cavity of a silicone human nose model (Pfeiffer, Radolfzell, Germany) using the different delivery devices. Before spraying the nasal powder formulation, liquid paraffine oil was rubbed into the nasal cavity whereby the powder deposition could be evaluated visually. Besides this the powder deposition was also studied by gamma scintigraphy using a PRISM 1500 gamma camera (Marconi Medical Systems, Cleveland, OH, USA). The amount of powder leaving the nasal cavity by the pharynx was collected in a plastic bag.

During 5 min static recordings were made of the nasal delivery system, the nose model and the plastic bag. Circular regions of interest of the device, the nasal cavity, the pharynx and the plastic bag were drawn manually. To quantify the ROI, the background activity was taken into consideration.

2.7. Analysis of spray pattern

The powder formulations containing DDWM/C 974P (90/10, w/w) and spray-dried Amioca®/C 974P (25/75, w/w) were prepared as described under Section 2.3.2, but without the addition of insulin. Digital images of the cross-section and the shape of the powder cloud after spraying the powder formulation using the experimental device were recorded with a SprayVIEW NSx system (Image Therm Engineering, Sudburry, USA). The images were analysed to calculate the angle under which the powder particles leave the delivery system, the spray time (defined as the time needed for a complete depletion of the powder formulation from the polyethylene tube of the nasal delivery system) and the minimum and maximum width of the cross-section.

2.8. Determination of bulk density

Dispersions were prepared containing spray-dried Amioca® starch/C 974P (25/75, w/w) in a concentration of 1.0, 2.5 and 7.0% (w/w). To prepare the 1.0 and 2.5% dispersion, 1.0 g powder was dispersed in 30.0 ml distilled water and to prepare the 7.0% dispersion, 1.0 g powder was hydrated in 1.0 ml distilled water. After neutralization to pH 7.4 with 2.0 M NaOH, the insulin solution (Actrapid® HM 100) was added to obtain a final concentration of 1 IU insulin per mg powder. Finally distilled water was added until the desired concentration was obtained. The dispersions were frozen to 228 K within 175 min at 1000 mbar. Additionally, the dispersion containing 2.5% Amioca®/C 974P (25/75, w/w) was frozen to 228 K within 30 and 350 min. Primary drying, secondary drying, sieving and storage were performed as described under Section 2.3.2.

The apparent powder bulk density (defined as the ratio of the powder weight over the bulk volume) of the freeze dried products was determined in the cylindrical polyethylene tube of the experimental delivery device filled with 10.0 mg of the Amioca®/C 974P (25/75, w/w) mixture. Results are presented as a mean value ± standard deviation ($n = 10$).

Table 1
Absolute bioavailability, C_{max} and t_{max} (mean ± S.D.) after nasal delivery to rabbits of DDWM/C 974P (90/10) (1 IU insulin/mg) using the experimental, Monopowder and Pfeiffer delivery device

Delivery device	Absolute bioavailability (%)	C_{max} (μIU/ml)	t_{max} (min)
Experimental	14.4 ± 3.5	418.6 ± 73.3	28.2 ± 3.7
Monopowder	6.6 ± 2.7[a,**]	286.3 ± 115.4[a,**,b]	24.1 ± 4.9
Pfeiffer	3.6 ± 1.4[a,**]	131.5 ± 65.8[a,*]	28.7 ± 5.5

[a] Significantly lower than the experimental delivery device ([*] $0.05 \geq P > 0.01$; [**] $P \leq 0.001$).
[b] Significantly higher than Pfeiffer delivery device ($0.05 \geq P > 0.01$).

2.9. Determination of particle size

The particle size of the powder formulations was determined by laser diffraction (Mastersizer S, Malvern Instruments, Worcestershire, UK), using Miglyol 812N (Sasol, Witten, Germany) as dispersion medium, and the median volume diameter (VMD) was calculated.

3. Results and discussion

The first part of this study describes the relationship between deposition, mucociliary clearance and absorption. To determine the distribution of a formulation in the nasal cavity two different types of deposition have to be considered: the initial deposition immediately after administration and the secondary deposition due to translocation by the mucociliary clearance. The initial deposition affected the rate of the mucociliary clearance and the secondary deposition the absorption rate by the covered mucosal surface (Kublik and Vidgren, 1998). The relation between deposition and absorption of three different nasal powder devices was evaluated after spraying the powder formulation containing DDWM/C 974P (90/10) into the nostrils of rabbits. After administration using the experimental device, the Monopowder and Pfeiffer system, bioavailabilities of 14.4 ± 3.5, 6.6 ± 2.7 and 3.6 ± 1.4%, respectively, were obtained (Table 1). To explain the significantly higher bioavailability using the experimental device, the deposition of DDWM/C 974P (90/10) in the nasal cavity of rabbits was studied using gamma scintigraphy, which allowed to follow the mucociliary clearance (Perkins and Frier, 1996).

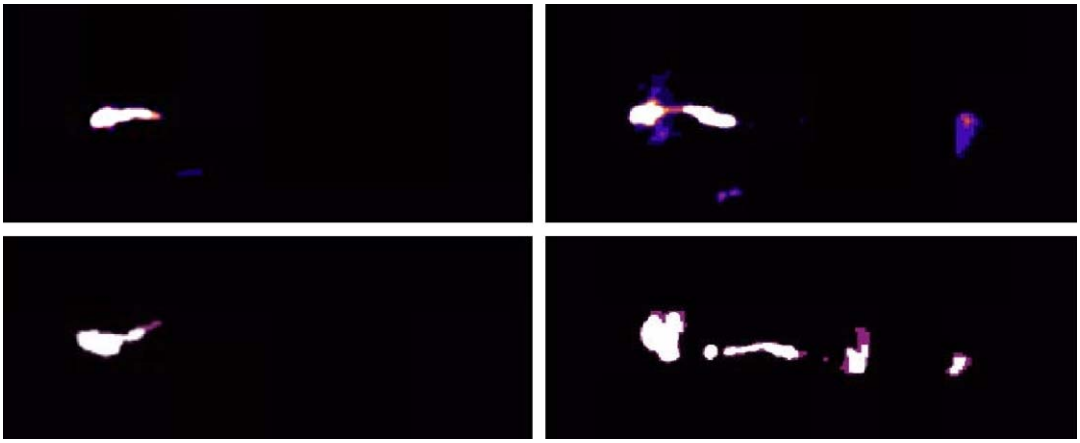

Fig. 1. Initial deposition (left) and deposition 150 min after administration (right) of the DDWM/C 974P (90/10) powder formulation to rabbits using the experimental (top) and Pfeiffer device (bottom).

Immediately after administration of DDWM/C 974P (90/10) using the experimental and Pfeiffer device mostly all powder was deposited into the nasal cavity (Fig. 1). Due to the mucociliary clearance radioactivity was observed in the oesophagus, stomach and bladder 150 min after administration with the Pfeiffer device, while for the experimental delivery system only a minimum of radiolabel was detected in the bladder. The radioactivity in the nasal cavity after 150 min was $81.9 \pm 12.7\%$ for the experimental device while only $64.5 \pm 3.7\%$ for the Pfeiffer device. The faster clearance of the powder from the nasal cavity using the Pfeiffer system can explain the significantly lower bioavailability compared to the experimental system.

In the following experiment, the initial deposition of the three delivery devices was evaluated in a silicone human nose model. The different regions of interest used to quantify the powder in the nasal cavity, pharynx, delivery system and plastic bag (representative for the amount of powder leaving human model via the pharynx) are depicted in Fig. 2. The best depletion (99.2%) of the device was obtained for the Monopowder system. For the experimental and Pfeiffer system about 3.0 and 30%, respectively, of the total dose remained in the device after actuation. On the other hand, 22% powder was recovered in the plastic bag with the Monopowder device compared to only 0.5 and 2% for the experimental and Pfeiffer system, respectively. Using the Monopowder system also the highest percentage of powder (21%) deposited in the pharynx while, only 4.8 and 3.2% when using the experimental and Pfeiffer system, respectively.

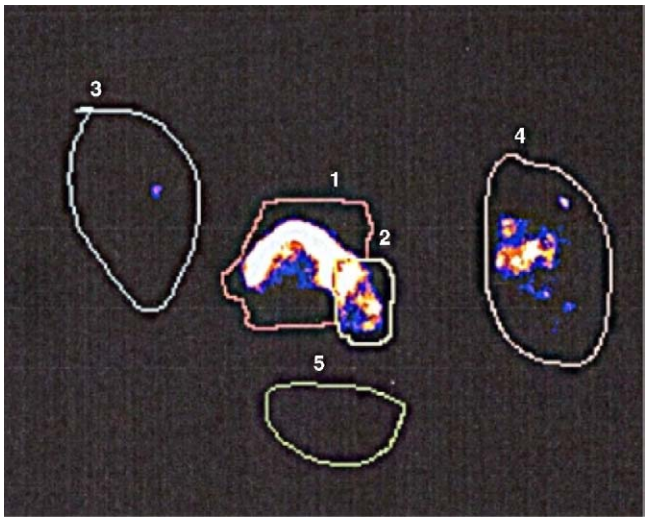

Fig. 2. Regions of interest (ROI) after spraying the DDWM/C 974P (90/10) powder formulation in the silicone human nose model using the Monopowder device: nasal cavity (1), pharynx (2), device (3), plastic bag (4) and background (5).

The deposition in the human nose model was also verified visually. Using the experimental delivery system, the powder was mainly found in the anterior part of the nasal cavity. With the Monopowder device, the powder was mainly situated near the upper turbinate and in the pharynx what might explain the significantly lower bioavailability compared to the experimental system since the deposition in the anterior part contributes to nasal absorption (Harris et al., 1986). The Pfeiffer system spread the DDWM/C 974P (90/10) particles homogeneously over the turbinates and the faster clearance in comparison with the experimental system can be explained by the deposition in the posterior part of the nose. Ciliated cells cover mainly the posterior part of the nasal epithelium providing a faster clearance of particles (Mygind and Dahl, 1998). Ridley et al. (1995) and Illum et al. (1987) reported different clearance half-lifes in humans after administration of starch microspheres using different delivery devices. The Rhinyle catheter used by Ridley et al. (1995) resulted in a shorter half-life of clearance as the powder was deposited in the anterior part of the nose as well as in the turbinates, while the Lomudal nasal insufflator used by Illum et al. (1987) deposited the powder mainly in the anterior part of the nose leading to a slower clearance.

It can be concluded that the delivery device is important in the development of a nasal powder formulation as it has an impact on the deposition pattern and hence the nasal clearance: the faster a formulation is cleared from the nasal cavity, the shorter the drug absorption time (Soane et al., 1999).

Furthermore, a correlation was found between the nasal delivery device of the powder formulation, the nasal clearance rate from the nasal cavity in rabbits and the distribution in the

human nose model. The lower the insulin bioavailability for a nasal delivery device, the faster the clearance rate of the powder formulation from the nasal cavity in rabbits, the more posterior the powder was deposited in the human nose model.

Beside the delivery device, the spray pattern of the powder formulation may also influence the deposition, and consequently contribute to the nasal bioavailability. Therefore, the spray pattern (shape and cross-section) of the powder formulations based on DDWM/C 974P (90/10) and Amioca®/C 974P (25/75) was investigated using the experimental device (Fig. 3). The spray time for delivering 10.0 mg DDWM/C 974P (90/10) and Amioca®/C 974P (25/75) was 20 and 46 ms, respectively. The DDWM/C 974P (90/10) and Amioca®/C 974P (25/75) powder particles were released from the delivery device at an angle of 6.9° and 12.8°, respectively. The mean minimum and maximum width of the cross-section were, respectively, 17.3 and 18.8 mm for DDWM/C 974P (90/10) and 22.1 and 24.3 mm for Amioca®/C 974P (25/75). These numbers can be explained by the differences in powder bulk density and particle size. The bulk density of DDWM/C 974P (90/10) and Amioca®/C 974P (25/75) was 0.071 and 0.165 mg/mm^3, respectively, and their median volume diameter 88.7 and 51.9 μm. The higher bulk density and smaller particle size of Amioca®/C 974P (25/75) resulted in a compact powder having a higher resistance to air flow when spraying the powder, resulting in a slower spray time and larger spray pattern.

Callens et al. (2000, 2003a) determined the insulin bioavailability after nasal delivery of the powder formulations based on Amioca®/C 974P (25/75) and DDWM/C 974P (90/10) in rabbits. The higher bioavailability for Amioca®/C 974P (25/75) (18%) compared to DDWM/C 974P (90/10) (14%) was contributed to a higher viscosity and elasticity after dispersion of Amioca®/C 974P (25/75) into the nasal fluid (Callens et al., 2003b). The aim of the next experiment was to evaluate if the nasal bioavailability was also influenced by the spray pattern of the powder formulations. Bond et al. (1985) and Newman et al. (1987) showed that the cone angle of a nasal adaptor determined the width of the spray pattern and hence the deposition area in the nasal cavity. Changing the cone angle from 60° to 35° or 30° resulted in a more posterior deposition in the nasal cavity and in a faster clearance rate of the formulation because of the higher deposition in the ciliated area. The smaller angle at which the

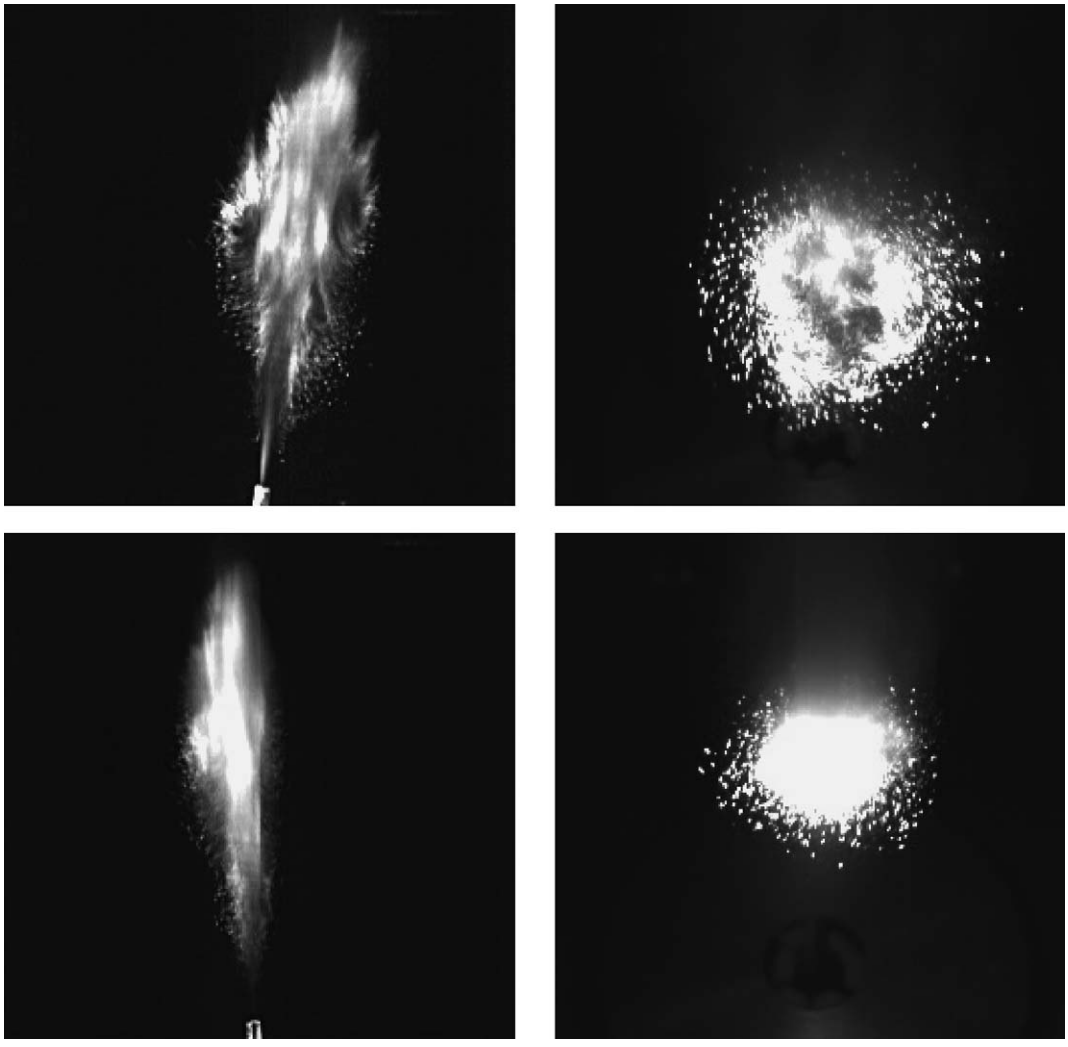

Fig. 3. The spray pattern (shape (left) and cross-section (right)) of powder formulations composed of Amioca®/C 974P (25/75) (top) and DDWM/C 974P (90/10) (bottom) using the experimental device.

DDWM/C 974P (90/10) particles exited from the experimental device resulted in a narrow cross-section of the particle cloud and might contribute to a posterior deposition in the nasal cavity and hence to the lower bioavailability obtained after nasal delivery of this powder formulation. On the other hand, its lower bulk density might result in a higher degree of covering of the nasal epithelium due to the larger powder volume. It need to be emphasized that a more extensive spreading over the nasal epithelium does not guarantee an increase in bioavailability as the distribution of the formulation between anterior and posterior part of the nose remains an important parameter for absorption.

As the density of the formulation influenced the spray pattern from the device, the objective of the following experiment was to investigate the influence of powder bulk density on the nasal bioavailability of insulin using the rabbit as an animal model. The powder bulk density of the Amioca®/C 974P (25/75) powder formulation was modified by changing the solid fraction of the dispersion to be freeze dried and by changing the freezing rate during the freeze drying cycle.

The bulk density, particle size and moisture content of the powder formulations having different solid fractions are shown in Table 2. Significantly higher powder bulk densities ($P \leq 0.01$) were obtained when the solid fraction of the freeze dried dispersion was increased. This increase in bulk density could not be attributed to the median volume diameter as the different powder formulations had a similar particle size. It can be explained by the formation of smaller ice crystals during the freezing phase of the process. When a dispersion with a higher solid fraction is used smaller ice crystals are formed, yielding a freeze dried cake with smaller pores after sublimation of the ice (Pikal, 1991).

The influence of the freezing rate was investigated by freezing the dispersion containing 2.5% Amioca®/C 974P (25/75) to 228 K within 30, 175 and 350 min. Varying the freezing rate had a limited effect on the powder bulk density (Table 2). Freezing the 2.5% Amioca®/C 974P (25/75) dispersion at the highest freezing rate resulted in a powder formulation with a significantly higher bulk density in comparison with dispersions frozen at a lower freezing rate. Fast freezing resulted in the formation of smaller ice crystals due to a higher degree of supercooling (Dawson and Hockley, 1991), resulting in smaller pores of the freeze dried cake after sublimation. The solid fractions of the dispersions and freezing rates used are production limits, hence the powder bulk densities can also be considered as the limits. Although only small changes in bulk density were obtained, they could be essential differences for nasal delivery in the rabbit. The nasal cavity of the rabbit is characterized by a small volume and surface area of 6 ml and 61 cm^2, respectively (Gizurarson, 1990), and consequently small changes in powder bulk density might result in large differences in the degree of covering of the nasal epithelium. A higher degree of covering might occur with low bulk density powders because of their larger volume and can contribute to a higher nasal bioavailability. The results of the absolute bioavailability, C_{max} and t_{max} obtained after nasal delivery of the Amioca®/C 974P (25/75) powder formulations using the experimental device to rabbits are shown in Table 3. Although a significant difference in absolute bioavailability was observed, no influence of the powder bulk density on the absolute bioavailability of insulin after nasal delivery to rabbits was found. When increasing the solid fraction of the dispersion an increase in powder bulk density was seen, but the bioavailability after nasal delivery was similar. Only for the 7.0% dispersion a significant decrease in area under the curve was seen compared to the 2.5% dispersion, although their C_{max} and t_{max} values were not significantly different.

It can be concluded that differences in spray pattern of nasal powder formulations due to the powder bulk density have no influence on the nasal insulin bioavailability in rabbits.

Table 2
Influence of the solid fraction and freezing rate on powder bulk density, median volume diameter (VMD) and residual moisture content of freeze dried Amioca®/C 974P (25/75)

	Bulk density (mg/mm^3)	VMD (μm)	Residual moisture (%)
Solid fraction (w/w) (freezing rate 228 K within 175 min)			
1.0%	0.116 ± 0.009	55.8	9.9 ± 0.9
2.5%	0.165 ± 0.016[a]	51.9	7.5 ± 1.0
7.0%	0.184 ± 0.015[a,b]	41.9	6.8 ± 1.0
Freezing rate to 228 K within (2.5% dispersion)			
30 min	0.202 ± 0.010	61.3	3.55 ± 0.24
175 min	0.165 ± 0.016[c]	51.9	7.54 ± 0.99
350 min	0.160 ± 0.014[c]	48.5	3.72 ± 0.22

[a] Significantly higher than 1.0% (w/w) dispersion ($P \leq 0.001$).
[b] Significantly higher than 2.5% (w/w) dispersion ($0.01 \geq P > 0.001$).
[c] Significantly lower than freezing to 228 K within 30 min ($P \leq 0.001$).

Table 3
Influence of solid fraction and freezing rate on absolute bioavailability, C_{max} and t_{max} (mean ± S.D.) after nasal delivery to rabbits of Amioca®/C 974P (25/75) powder formulations (1 IU insulin/mg) using the experimental delivery device

	Absolute bioavailability (%)	C_{max} (μIU/ml)	t_{max} (min)	n
Solid fraction (freezing rate 228 K within 175 min)				
1.0%	17.4 ± 5.3	588.6 ± 112.5	44.4 ± 13.4	7
2.5%	19.2 ± 5.3	681.4 ± 246.6	50.9 ± 7.4	8
7.0%	11.1 ± 3.0[a]	455.5 ± 99.4	39.9 ± 6.5	7
Freezing to 228 K within (2.5% dispersion)				
30 min	18.2 ± 3.7	708.4 ± 210.2	41.5 ± 9.3	7
175 min	19.2 ± 5.3	681.4 ± 246.6	50.9 ± 7.4	8
350 min	22.4 ± 3.3	852.4 ± 84.0	40.9 ± 11.7	6

[a] Significantly lower than 2.5% (w/w) dispersion ($0.05 \geq P > 0.01$).

4. Conclusions

The nasal delivery device was a critical parameter in the development of a nasal powder formulation: anterior deposition of the formulation in the nasal cavity and the slower mucociliary clearance increased the bioavailability of insulin. The spray pattern from a device is influenced by the bulk density of the

powder formulation, but had no effect on the bioavailability of insulin in rabbits.

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