Flowability Evaluation of Dry Powder Inhalation Formulations Intended for Nasal Delivery of Betahistine Dihydrochloride

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Abstract
For optimum deposition in the nasal cavity drug particles’ size should be in the range 1-25 μm. However, such particles are not free flowing due to cohesion and static charge phenomena. Excipients can improve flowability and reduce particles aggregation. The aim of this study was to develop interactive mixtures of betahistine-loaded microspheres and different carriers for inhalation delivery with optimum flow properties. Forty eight sample mixtures were prepared to study the influence of microspheres/carriers ratio on powder mixtures’ flowability. All the mixtures were studied for angle of repose and Hausner ratio.
Blending the cohesive microspheres with the carriers resulted in lower angle of repose and HR which indicates improved flowability. With high microsphere/carrier ratio improved flow properties were observed.
The results show that good flowability depends on carrier properties and microspheres characteristics and confirm the thesis that choosing the proper carrier excipient is of great
importance for effective nasal drug delivery.

**Key words:** dry powder inhalation, nasal drug delivery, adhesion mixtures, powder flow.

**Introduction**

The nasal route has been recently widely explored as a feasible route of drug administration either for local and systemic therapy. The wide spread interest toward nasal drug delivery arises from its undeniable advantages such as quick access to circulation, avoidance of hepatic “first pass“ effect, enhanced drug stability and improved patient’s comfort and compliance. However, the development of effective and reliable nasal formulation is undoubtedly a serious challenge and numerous issues should be taken into mind and different aspects to be considered.

Inhaled drug delivery systems can be classified into three principal categories: pressurized metered-dose inhalers (pMDIs), dry powder inhalers (DPIs), and nebulizers, each class with its unique strengths and weaknesses. Among all, the most acceptable form for nasal drug delivery is DPI formulation due to environmental sustainability, propellant-free design, little or no requirement of patient coordination and formulation stability. DPIs are complex systems and their performance depends on several key distinctions: device design and performance, dry powder formulation properties and actuation mode (energy sources for generation of airflow). In this study we focused on optimization of dry powder formulation properties. The composition of DPIs has a direct impact on formulation stability and dosing performance. Inhalation powders are formulated either as pure drug or blended with an inert excipient. Powder blends contain micronized drug particles with an excipient, which may be micronized as well, but more often would comprise larger carrier particles.

For optimum deposition in the nasal cavity drug particles’ size should be in the range 1-25 μm. However, particles of that size are not free flowing due to cohesion and static charge phenomena which interfere with handling during manufacture and could aggravate dose uniformity and cause retention within the device. The use of excipients can help to improve powder flowability, reduce particles aggregation and achieve dose uniformity, partly because a carrier used as a bulking agent would enhance reproducible dose metering. The choice of a proper carrier is based on its chemical and physical characteristics which could directly
impact DPIs performance. Hygroscopic excipients should be avoided since uptake of moisture could occur which may influence drug stability and powder flowability, and hence the delivered dose.

Three different formulation types exist: spherical pellets, interactive (adhesive) mixtures and nucleous agglomerates. Nucleous agglomerates are formed as a result of drug particles common distribution around a carrier particle in a multi-layered structure. Spherical pellets consist of micronized drug or blends of micronized both drug and excipient. They are highly porous and mechanically unstable. In contrast, in adhesive mixtures drug particles are homogeneously distributed over the surface area of much larger carrier particles and the attachment is achieved primarily by Van-der-Waals forces. During inhalation, airflow through the device creates shear forces and turbulence, the static powder mixture is fluidized and enters the nasal cavity. Drug particles overcome the adhesion forces and separate from the larger carrier particles, which are deposited into the nasal vestibule and eliminated with mucociliary clearance. The smaller drug particles move forward towards the absorption area.

There are certain limitations in choosing potential carrier excipients for inhalation drug delivery systems due to toxicological and other considerations. Currently, lactose is probably the most preferred excipient for DPIs. It has an established safety and stability profile, and satisfactory flowability necessary for DPI carrier particle. Lactose is widely available and affordable, it is offered in diverse grades in regard to particle size and morphology. Other sugars, such as mannitol and glucose have been studied as an acceptable alternative to lactose. Several other materials, such as phosphatidyl choline and cholesterol have been evaluated as potential carriers in experimental DPI formulations, with different objectives and variant success.

When mixing powders with different properties, particle sizes, and ratios, as is the case with DPI formulations, inadequate mixing can cause poor flowability and thus dose uniformity. Different powders may have different mixing requirements, depending on the forces present between the various particles. Studying the powder flow properties of interactive (adhesion) mixtures will play an essential role in the development of optimum DPI formulation.

The aim of this study was to develop interactive (adhesion) powder formulations of betahistine loaded polymeric microspheres and different carriers for inhalation delivery with optimum flow properties.
Materials and Methods

Betahistine dihydrochloride, chitosan (from shrimp shells, low viscosity, degree of deacetylation >75%), sorbitan monooleate 80 (Span 80) and petroleum ether were purchased from Sigma-Aldrich Chemie GmbH (Taufkirchen, Germany). Carrier excipients were provided as gift samples: Lactohale® 100 and Respitose® SV003 (inhalation grade lactose) were supplied by DFI Pharma, Germany; non-pareil seeds (mesh 60 - 80) and Vivapur® MCC spheres 100 were supplied by JRS Pharma, Germany; Mannogem® EZ (mannitol) and Sorbitab® SD 250 (sorbitol) were kindly provided by SPI Pharma, USA. All other reagents and solvents were of analytical grade and were used as provided.

Preparation of betahistine loaded chitosan microspheres

Chitosan microspheres were prepared by single emulsion/solvent evaporation technique using liquid paraffine as external phase. The applied technique was described in detail in our previous experiments.10 Four batches of microsphere formulations, labeled M1 - M4 were prepared by varying BET and chitosan concentrations and drug/polymer ratio.

Preparation of interactive (adhesive) mixtures

Powder mixtures of microspheres from the different batches and carrier excipients listed above were prepared by trituration in a glass mortar using the geometric dilution method. To study the influence of microspheres/carriers ratio on powder mixtures’ flowability, forty eight sample mixtures were formulated.

Optical microscopy

Size and shape of carrier excipients and formulated interactive mixtures were studied using optical microscope (Leica DM 2000 LED, Wetzlar, Germany) equipped with a camera (Leica DMC 2900) and computer controlled image analysis software (Leica LAS EZ).

Scanning electron microscopy

Scanning electron microscopy (Philips SEM 515, Eindhoven, The Netherlands) was used to examine the shape and surface morphology of the microsphere formulations. The samples
were loaded on a copper sample holder and sputter coated with carbon followed by gold using vacuum evaporator (BH30). The images were recorded at 25 kV acceleration voltage using x2000 magnification.

**Angle of repose (θ)**
The angle of repose of microspheres, carrier excipients and formulated powder mixtures as an indicator of flowability was measured using fixed funnel method at five different measurements. The angle was calculated according the equation:

\[ \theta = \tan^{-1} \frac{h}{r} \]

where \( \theta \) is the angle of repose, \( h \) – the height of the formed cone, \( r \) – the radius of the cone’s base.

**Hausner’s ratio (HR)**
The Hausner ratio was determined by measuring both the bulk density and the tapped density of the powder. A 10 mL volumetric cylinder was used to measure the bulk volume \( V_0 \) and the tapped volume \( V_s \) of the powder by tapping the powder 500 times using SVM tapped density tester (Erweka GmBH, Germany). Hausner ratio was calculated according the following equation:

\[ HR = \frac{\rho_s}{\rho_0} \]

**Statistical analysis**
All experiments were performed at least three times. The obtained results were analysed with Microsoft Excel for Windows 2010 and are expressed as means ± standard deviation (SD).

**Results and Discussions**
Four batches of microsphere formulations, labeled M1 - M4 were prepared by varying BET and chitosan concentrations and drug/polymer ratio. Scanning electron microscopy of betahistine loaded microparticles revealed spherical geometry (Figure 1) with few aggregates formed in all the preparations. Microspheres appeared to have crumpled surface with many
wrinkles and gaps between them.

The particle size of each microsphere formulation is reported in Table 1. Mean sizes of the formulations ranged from 3.82 to 7.69 μm which is considered to be appropriate for optimum deposition in the nasal cavity. However, free flowing properties are not very common for particles of this size range and our results confirm this fact. The established angle of repose of the formulated microspheres was in the range from 40 to 61° and corresponds to passable/very poor flow properties (Table 1).

Figure 1. Scanning electron micrographs of the formulated microspheres from batches M1 (A), M2 (B), M3 (C) and M4 (D) at magnification x 2000.

<table>
<thead>
<tr>
<th>Formulation code</th>
<th>Particle size (μm) ± SD</th>
<th>Angle of repose (°) ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>M1</td>
<td>3.82±0.14</td>
<td>60.95±2.24</td>
</tr>
<tr>
<td>M2</td>
<td>4.49±0.24</td>
<td>39.47±1.88</td>
</tr>
<tr>
<td>M3</td>
<td>4.52±0.33</td>
<td>40.10±2.12</td>
</tr>
<tr>
<td>M4</td>
<td>7.69±0.33</td>
<td>41.19±1.56</td>
</tr>
</tbody>
</table>

Table 1. Flow properties of microsphere batches M1, M2, M3 and M4 (n=5).
Figure 2. Photomicrographs of the excipient carriers Lactohale® 100 (A), Respitose® SV003 (B), Sorbitab® SD 250 (C), Mannogem® EZ (D), MCC spheres 100 (E) and Non-pareil seeds (F) at magnification x 50.
Six different carrier excipients were evaluated for their shape, size and flow properties. Photomicrographs of the samples are depicted on Figure 2 and the content and flow properties are given in Table 2.

To study the effect of these carriers on microspheres flowability interactive (adhesion) mixtures at two different microsphere/carrier ratios were formulated and their flow properties were evaluated. The obtained results are presented on Figures 3 and 4. Photomicrographs of some of the formulated adhesion mixtures are presented on Figure 5.

All the carrier excipients that were studied had an angle of repose in the range of 21 – 31° and Hausner ratio lower than 1.3, indicator for their free flowing nature. Blending the highly cohesive microspheres with the carriers resulted in lower values of the angle of repose and HR of the formulated mixtures which indicates improved flowability. With high microsphere/carrier ratio a tendency towards improvement of flow properties was observed in all the experiments.

Formulated adhesion mixtures showed values for the angle of repose within a broad range – from 23.86° to 52.53°, and for HR - from 1.09 to 1.95 which comes to notify the exceeded variability in flow properties according to both carrier type and microspheres characteristics.

In view of the results is not possible to select a uniform carrier excipient for formulation of adhesion mixtures with microspheres. Considering the fact that the angle of repose of free flowing powders is in the range 25 – 30° and HR <1.11, it is evident that such improvement of flowability was achieved with only two of all 48 formulated adhesion mixtures. In both

<table>
<thead>
<tr>
<th>Carrier excipient</th>
<th>Content</th>
<th>Particle size* (μm)</th>
<th>Angle of repose (°) ± SD</th>
<th>HR ±SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vivapur® MCC spheres 100</td>
<td>Microcrystalline cellulose</td>
<td>100 - 200</td>
<td>21.60±0.59</td>
<td>1.09±0.02</td>
</tr>
<tr>
<td>Non-pareil seeds</td>
<td>Sacharose</td>
<td>180 - 250</td>
<td>25.51±1.89</td>
<td>1.12±0.01</td>
</tr>
<tr>
<td>Lactohale® 100</td>
<td>Inhalation grade crystalline lactose</td>
<td>100 - 200</td>
<td>26.37±1.00</td>
<td>1.21±0.02</td>
</tr>
<tr>
<td>Respitose® SV003</td>
<td>Inhalation grade crystalline lactose</td>
<td>50 - 100</td>
<td>31.03±0.77</td>
<td>1.29±0.03</td>
</tr>
<tr>
<td>Sorbitab® SD 250</td>
<td>Spray dried sorbitol</td>
<td>100 - 250</td>
<td>27.91±1.65</td>
<td>1.18±0.01</td>
</tr>
<tr>
<td>Mannogem® EZ</td>
<td>Spray dried mannitol</td>
<td>75 - 150</td>
<td>27.78±0.87</td>
<td>1.18±0.02</td>
</tr>
</tbody>
</table>

* particles size range according to product specification.
Figure 3. Angle of repose of the formulated adhesion mixtures of microsphere batches M1 (A), M2 (B), M3 (C) and M4 (D) with various carriers for inhalation delivery at 1:5 and 1:10 microspheres/carriers ratio. Dot line represents angle of repose 35° corresponding to free flowing nature.
Figure 4. Hausner ratio of the formulated adhesion mixtures of microsphere batches M1 (A), M2 (B), M3 (C) and M4 (D) with various carriers for inhalation delivery at 1:5 and 1:10 microspheres/carriers ratio. Dot line represents HR 1.18 corresponding to free flowing nature.
cases Sorbitab® SD 250 was used as a carrier in 10:1 ratio with microspheres from batches M2 and M3. However, the same carrier did not result in any satisfactory change with the other batches which confirms the thesis that good flowability depends not only on the carrier properties but also on microspheres characteristics.

For instance, Vivapur® MCC spheres 100 enhance flowability of both batches M2 and M3 in each ratio while fair results were achieved for M1 and M4 only when lower amount of microspheres was used.

The same subordinations were observed with Lactohale®100 and that was expected since both excipients have similar particle size range (100-200 µm). Respitose®SV003 (50-100 µm) improved M1 and M4 powder flow but with batches M2 and M3 the results were not satisfactory. Both Sorbitab® SD 250 and Mannogem® EZ helped in achieving excellent flowability with M1, M2 and M3 when used in high concentration but the effect was insignificant with M4. Sorbitab SD 250 is a spray dried sorbitol with spherical, uniform particle morphology which provides additional formulation benefits of being a good carrier. Its unique porous structure has pockets where actives or other excipients become trapped to provide good content uniformity.\textsuperscript{11} Likewise, Mannogem’s high porosity and narrow particle size make it an ideal choice for triturating with low dose micronized powders such as formulation samples M1, M2 and M3.\textsuperscript{12} M4, being of larger size is unable to adhere to the carriers binding sites in a good manner and form free flowing adhesion mixtures. Ignorable influence with batches M3 and M4 was noticed when sugar spheres (non-pareil seeds) in 1:10 ratio were used; in the other formulations optimum results were not accomplished.

According the obtained powder flow characteristics the formulated adhesion mixtures were classified as such with good flow properties (angle of repose 31-35°, HR 1.12-1.18), fair flowability (angle of repose 36-40°, HR 1.19-1.25) and passable flowability (angle of repose 41-45°, HR 1.26-1.34).\textsuperscript{13} The rest of the formulated adhesion mixtures showed poor to very poor flowability (angle of repose >46°, FH >1.35) and were not of interest for our further study.

The results from the current research show that choosing the proper carrier excipient among the variety available is of a great importance for effective nasal drug delivery.

Based on the obtained data concerning powder flow properties, optimum adhesion mixtures of
Figure 5. Photomicrographs of adhesion mixtures M2/ Lactohale®100 1/10 (A), M3/ Sorbitab® SD 250 1/10 (B) and M4/ Respitone®SV003 1:10 (C) at magnification x 200.
model M1 were obtained with the carrier Mannogem® EZ and to a less extent the carriers Respitose®SV003, Lactohale®100, Vivapur® MCC spheres 100 and Sorbitab® SD 250 at 1:10 ratio. The most felicitous carrier for M2 and M3 occurred to be Sorbitab® SD 250, but the use of Vivapur® MCC spheres 100 and Lactohale®100 regardless the ratio. For M4 undoubtedly with the highest relevance are Respitose®SV003, Lactohale®100 and Vivapur® MCC spheres 100, at 1:10 ratio.

Conclusions
Forty eight sample powder mixtures of microspheres from four different batches and six different types of carrier excipients were prepared to study the influence of microspheres/carriers ratio on powder mixtures’ flowability. All the mixtures were studied for angle of repose and Hausner ratio. Blending the highly cohesive microspheres with the carriers resulted in lower values of the angle of repose and HR of the formulated mixtures which indicates improved flowability. With high microsphere/carrier ratio a tendency towards improvement of flow properties was observed in all the experiments. According to the obtained powder flow characteristics the formulated adhesion mixtures were classified as good flowing (angle of repose 31-35°, HR 1.12-1.18), fair flowing (angle of repose 36-40°, HR 1.19-1.25) and passable flowing (angle of repose 41-45°, HR 1.26-1.34). The results from the current research show that good flowability depends not only on the carrier properties but also on microspheres characteristics and confirm the thesis that choosing the proper carrier excipient among the variety available is of a great importance for effective nasal drug delivery.

References

Authors Column

Bissera Pilicheva is presently doing PhD at the Department of Pharmaceutical sciences, Medical University Plovdiv, Bulgaria. She has expertise in the field of Pharmaceutical technology and biopharmacy. Her research is focused on alternative routes for drug administration (nasal, buccal), development of microparticulate drug delivery systems and studying bioadhesion. She has published eight research papers in national and international journals and attended nine conferences. She also completed two university research projects and is currently involved in one national project.