In-situ-micronization of disodium cromoglycate for pulmonary delivery

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Received 6 September 2002; accepted in revised form 21 November 2002

Abstract

Drug particle properties are critical for the therapeutic efficiency of a drug delivered to the lung. Jet-milling, a commonly used technique for micronization of drugs, has several disadvantages such as a non-homogeneous particle size distribution, and unnatural, thermodynamically activated particle surfaces causing high agglomeration. For pulmonary use in a dry powder inhaler, in addition to a small particle size, good de-agglomeration behaviour is required. In this study disodium cromoglycate is prepared in situ in a respirable particle size by a controlled crystallization technique. First the drug is dissolved in water (4%) and precipitated by a solvent change method in the presence of a cellulose ether (hydroxypropylmethylcellulose) as a stabilizing hydrocolloid. By rapidly pouring isopropyl alcohol into the drug solution in a 1:8 (v/v) ratio, the previously molecularly dispersed drug is associated to small particles and stabilized against crystal growth in the presence of the hydrophilic polymer. This dispersion was spray-dried. The mean particle size of the drug was around 3.5 μm and consequently was in the respirable range. The in-situ-micronized drug powder was tested for its aerodynamic behaviour and compared with jet-milled drug powder and with commercial products using the Spinhaler®, the Cyclohaler®, and the FlowCaps®-Inhaler as model devices. The fine particle fraction (FPF) (<5 μm) was increased from 7% for the jet-milled drug to approximately 75% for the in-situ-micronized drug when the pure drug powder was dispersed without any device. Delivery of the engineered particles via the Spinhaler®, the FlowCaps®-Inhaler and the Cyclohaler® increased the FPF from 11 to 46%, 19 to 51%, and 8 to 40%, respectively.

Keywords: In-situ-micronization; Pulmonary drug delivery; Dry powder inhaler; Controlled crystallization; Disodium cromoglycate

1. Introduction

Disodium cromoglycate (DSCG) is a common drug for the treatment of mild to intermittent bronchial asthma. The drug is available for use in dry powder inhalers (DPI), metered dose inhalers (MDI) and nebulizers. The efficiency of these delivery systems is still problematic as in some cases only about 10% of the inhaled drug substance reaches the lung [1]. Drug delivery from DPIs is affected by the powder formulation, the device, and the inspiratory effort of the patient. Due to different device resistances and different air flow rates through the device, high variability in plasma concentrations has been observed [2].

The common way to reduce the particle size to the respirable range by air-jet-milling provides only a limited opportunity for the control of important product character-
micronized (median size 1.6 μm) and milled (7.2 μm) nedocromil sodium [18]. Due to the association with active sites of a carrier or within the micronized drug, the dispersibility decreases [19].

Due to the fact that milling techniques present several disadvantages, interest has increased in the possibilities afforded by powder processing as well as in the development of dry powder devices which can deliver those particles efficiently. However, the main body of research (represented by the number of patent applications) in the pulmonary drug delivery area concerns the development of devices [20] which are noted for their improved lung delivery. In addition, new techniques which produce the drug directly in the required small particle size are favourable. Micron-sized spherical particles can be prepared by spray-drying of a drug solution. Spray-dried drugs, which are amorphous, show a smaller and more homogeneous particle size and a higher respirable fraction than mechanically micronized drugs [21,22]. However, even spray-dried amorphous DSCG shows only incomplete dispersibility, indicated by a fine particle fraction (FPF) of 15–36% (Rotahaler® and Diskhaler® at 90 l/min, respectively) depending on the particle size, on the air flow and on inhaler design [23]. Compared with mechanically micronized DSCG, the FPF of spray-dried DSCG is increased but still not very high (stage < 3.3 μm: 26% for the spray-dried and 10% for the jet-milled drug, respectively) [21].

In addition, the flowability of drug powders affects metering and delivery from the device [24]. Consequently, the properties of the drug powder need to be optimized. Besides the size of individual particles, the agglomeration behaviour of the powder is important. For a good DPI formulation, drug particles with a low agglomeration tendency, adequate flow properties, and good batch to batch conformity are required [25].

In a recent study a technique for the in-situ-micronization of sparingly water-soluble drug substances for oral [26] and pulmonary [27] delivery was evaluated. The aim of this study was to establish this technique also for water-soluble substances. Micron-sized DSCG was prepared by a solvent change process that precipitates and stabilizes the drug in a small particle size by the use of hydroxypropylmethylcellulose (HPMC). As HPMC shows surface activity [28] it can be adsorbed onto the newly created surface of the precipitated drug in order to lower the interfacial tension. So the precipitated drug is sterically stabilized [29] against crystal growth by adsorbed polymer. Accordingly, the molecularly dispersed drug is associated with particles in the required size range and simultaneously stabilized in the formed dispersion by HPMC. After drying this dispersion, a drug powder with a high drug load is obtained. For aerosol characterization, a device-less application system, and devices with low resistance (Spinhaler®, medium resistance [30] (Cyclohaler®), and high resistance (FlowCaps®-Inhaler) were used.

2. Materials and methods

2.1. Materials

DSCG (Polfa, Poznan, Poland) was supplied jet-milled and used as received in this study for comparison. Further, non-micronized DSCG was supplied by Profarmaco S.r.l. (Milano, Italy). Isopropyl alcohol (Merck KG, Darmstadt, Germany) was of analytical grade. The water used was double-distilled quality. The employed stabilizing agent was HPMC (HPMC type 2910, USP; Metolose® 60 SH 50, Shin Etsu, Tokyo, Japan). Previously, other substances were tested as stabilizers. However, as most of them were not suitable, only data for poloxamer 188 (BASF AG, Ludwigshafen, Germany) are exemplarily shown. Lactose (Pharmatose® P325M, DMV International, Veghel, The Netherlands) was used as a carrier for dry powder formulations. As commercial products, Flutari® DSCG capsules for inhalation (Lot No. 03609, Zambon GmbH, Kerpen, Germany) and Intal® (Lot No. S10135, Fisons GmbH, Cologne, Germany) were used.

2.2. Crystallization procedure

Controlled crystallization was carried out using the solvent change method by instantaneously mixing two liquids in the presence of HPMC as a stabilizing agent as described by Rasenack and Müller [31] (Fig. 1). The process was carried out at room temperature. HPMC was chosen as a stabilizing agent in this study as it has shown the best prevention of particle growth. In the first step the drug (4 g/100 ml) was dissolved in a 1% (w/v) solution of HPMC in water. As non-solvent, which is miscible with water, isopropyl alcohol was used. Concentrations employed and the solvent to non-solvent ratio were previously determined by preparing different dispersions (data not shown). The non-solvent was poured rapidly from a beaker into the drug solution under stirring conditions using a magnetic stirrer. By batch-wise mixing of the two liquids (1:8) a dispersion is formed as the drug is precipitated. The HPMC is still in solution and can be adsorbed onto the newly created particle surfaces. After spray-drying the prepared dispersion under standardized conditions (Büchi 190 Mini Spray-dryer, Büchi Labortechnik AG, Flawil, Switzerland) a micron-sized drug powder was obtained. In this study, spray-drying was not used to form particles as if solutions were to be dried, but to dry the pre-formed particles. As the drug powder micronized using this technique is prepared directly in the micron-sized state during the particle formation without any further size reduction, this technique can be described as ‘in-situ-micronization’.

2.3. Jet-milling

In order to compare physicochemical characteristics of non-micronized and jet-milled powder, DSCG was freshly...
jet-milled for powder X-ray diffraction analysis (PXRD) (two cycles at a pressure of 8 bar, Jet-O-Mizer 00, Fluid Energy Aljet, Plumsteadville, PA).

2.4. Particle characterization

2.4.1. Scanning electron microscopy (SEM)

Scanning electron micrographs were taken using a Philips XL 20 (Philips, Eindhoven, The Netherlands). Samples were fixed on an aluminium stub with conductive double-sided adhesive tape (Leit-Tabs, Plano GmbH, Wetzlar, Germany) and coated with gold in an argon atmosphere (50 Pa) at 50 mA for 50 s (Sputter Coater, Bal-Tec AG, Liechtenstein).

2.4.2. X-Ray diffractometry

PXRD patterns were collected in transmission using an X-ray diffractometer with a rotating anode (Stoe and Cie GmbH, Darmstadt, Germany) with Cu Kα1 radiation (monochromator: graphite) generated at 200 mA and 40 kV. Powder was packed into the rotating sample holder between two polyethyleneterephthalate (PETP) films.

2.4.3. Particle size

The volume particle size distribution was measured using a laser diffractometer (Helos, Sympatec GmbH, Clausthal Zellerfeld, Germany). The dispersions were diluted with isopropyl alcohol and measured in a cuvette. As a second determination method, the dry powder was measured after dispersion by compressed air (2 bar; Helos Rodos).

2.4.4. Aerodynamic particle size analysis

The aerodynamic particle size was evaluated using a Multistage Liquid Impinger (MLI, apparatus C; Ph. Eur.; Erweka, Heusenstamm, Germany). In order to characterize the drug powder without the influence of any device, the pure drug (5 x 2 mg) was delivered to the impinger by using a device-less application system. The powder was weighed on an applicator (Fig. 2) which was connected to the impinger with a rubber gasket. The flow rate was adjusted to a pressure drop of 4 kPa as typical for the inspiration by a patient [32] resulting in an air flow rate of 82 l/min. The powder was delivered into the impinger by rotating the sample cup holder to feed the powder into the air stream.

Further runs were carried out using different single unit devices which all use capsule preparations. However, the devices used represent different functional principles. The Spinhaler®, as a low resistance device [30], aerosolizes the powder by means of a capsule spinning eccentrically around its axis and a propeller. In the case of the Cyclohaler®, the pierced capsule is set to rotate by the air stream and the powder is evacuated through centrifugal force. The FlowCaps®-Inhaler (Fig. 3) uses a ‘dancing cloud’ caused by a powerful cyclone inside the capsule. This in turn is caused by pressure disequilibrium inside the capsule.

The sample preparation was done according to a fixed protocol. After sieving (sieve Ph. Eur. 180) hard gelatine capsules (size 2) were filled with DSCG. For the Cyclohaler® a mixture (1:1) with lactose was prepared according to the corresponding commercial drug preparation (Flui® DSCG) (Turbulamixer, 15 min, 5 ml aluminium drum). The conditions of the MLI tests were as follows: in the case of the Spinhaler® a pressure drop of 1.3 kPa was generated by using a flow of 100 l/min, as indicated by the Ph. Eur. A pressure drop of 4 kPa results in a flow of 97 l/min for the Cyclohaler®, and 33 l/min for the FlowCaps®-Inhaler. In all cases the air flow was kept constant for a certain period of time so that a volume of 4 l of air was used for each actuation. The drug deposition in the throat, the four stages and the filter (stage 5) were determined spectrophotometrically at 238 nm (Lambda40 UV VIS Spectrometer, Perkin-Elmer, Norwalk, CT). All impinger tests were done in triplicate. The drug that was deposited in the different stages was calculated as a percentage of the total amount of the drug. Accordingly, the FPF (fraction of total drug mass <5 µm) was related to the total amount of drug. Consequently, in the case of the commercial drug products, the total amount of drug (25 mg in each capsule) and not the declared amount (20 mg) was used for the calculation.

![Application system for device-less drug application into the MLI.](image)

The powder is dispersed into the air stream by rotating the fill cavity from the ‘fill’ to the ‘feed’ position.
3. Results and discussion

Changes in the crystal surface by jet-milling are well known. In the case of DSCG, differences in PXRD patterns are also detectable when non-micronized and freshly jet-milled drug are compared (Fig. 4). From different relative peak intensities partial defects in the crystal lattice can be concluded. In order to avoid these thermodynamically activated sites, in this study the drug was prepared directly micron-sized without the use of size reduction techniques by milling.

3.1. Characterizing the precipitated dispersion

In this study, DSCG is in-situ-micronized by rapid precipitation in the presence of a stabilizing agent. When a drug substance is precipitated using the solvent change process, the energy of the system increases due to the interfacial tension. Thus, a stabilizing agent, provided that it has at least some affinity for the surface, covers the newly formed surface spontaneously. Thereby, the surface energy and consequently the enthalpy of the system are lowered. The small particles, which normally would aggregate due to their hydrophobic surface in order to lower the surface energy, are stabilized sterically against crystal growth by a layer of protective polymer [29]. In a screening of different potential stabilizers, HPMC was found to allow the production of micronized drugs. After precipitating the drug, a dispersion with a mean particle size of 0.5 μm is obtained (Fig. 5). In the case of the use of other or no stabilizer molecules, crystal growth was observed resulting in a particle size that is outside the respirable range as shown exemplarily for poloxamer which was used in the same concentration as HPMC (Fig. 5). This shows the effect of HPMC in stabilizing the small particles formed by the rapid crystallization.

3.2. Characterizing the drug powder

3.2.1. Physicochemical characterization

Jet-milled DSCG shows non-homogeneous particle shapes and a broad particle size distribution as is typical of jet-milled products [4] (Fig. 6a). In the in-situ-micronized drug powder, two different types of particles can be...
observed (Fig. 6b,c). There are recognizable small particles below approximately 1 \( \mu \text{m} \) (as indicated with the arrow in Fig. 6c) which correlates with the particle size distribution of the dispersion (Fig. 5). Beside these particles, there are larger spherical particles. The small particles are mainly agglomerated by the HPMC during the spray-drying process, and only a few remain un-agglomerated. Thus, the particle size distribution shows fine particles and can be explained as the addition of two homogeneous particle size distributions (Fig. 7). In contrast, the jet-milled drug shows a broad particle size distribution with its typical fines content.

An important property of the drug is its stability during storage and use. Due to the thermodynamic instability of amorphous drugs, crystalline solids are preferable. The PXRD patterns of in-situ-micronized DSCG show crystalline peaks which can be attributed to DSCG (Fig. 8). However, due to the coverage with amorphous HPMC (Fig. 8) a high baseline noise is observed in PXRD patterns of in-situ-micronized DSCG. After storage at 40 °C/75% relative humidity for 3 months, no differences in PXRD patterns occur.

### 3.2.2. Characterizing the aerodynamic behaviour

Besides the particle size of a drug, the de-agglomeration behaviour in an air stream is important for pulmonary drug delivery. The aerodynamic behaviour analyzed in a multi-stage liquid impinger is shown in Table 1. In general, the fraction <5 \( \mu \text{m} \) can be increased by preparing the drug according to the in-situ-micronization technique. These drug powders are less cohesive and adhesive as their surfaces are naturally grown and thus more uniform than in milled products. The jet-milled drugs are agglomerated and electrostatically charged, increasing the aerodynamic particle size. The in-situ-micronized powder is suitable for all investigated devices and shows an increased FPF, both as pure drug and in a lactose blend. If used in different devices, no essential differences in the FPF are detected. The device-less application system shows the most evident difference between jet-milled and in-situ-micronized drug. Here the powder is only de-agglomerated by the air stream without any special powder de-agglomeration by, for example, a propeller (Spinhaler®) or capsule rotation (Cyclohaler®). As no capsule was used, the amount of drug remaining in the application system is low resulting in an extremely high FPF for the in-situ-micronized powder. The powder is well dispersed leading to good aerosolization behaviour and deposits mainly on stages 3, 4 and filter (Fig. 9a). In contrast, jet-milled DSCG shows a higher retention in the application system and mainly deposits in the throat and on stage 1 underlining the bad de-agglomeration behaviour (Fig. 9a).

Fig. 6. SEM photographs of (a) jet-milled and (b,c) in-situ-micronized DSCG.
In a second study, the in vitro deposition of the different drug powders was analyzed with three different inhalation devices, the Spinhaler®, the Cyclohaler® and the FlowCaps®-Inhaler. When using the Spinhaler®, hard gelatine capsules were filled with the pure drug and delivered to the impinger. The jet-milled DSCG and the commercial product, Intal®w, gave nearly the same FPFs (11.4% and 14.1%, respectively). However, the distribution on the different stages of the impinger varied slightly: the jet-milled product emptied very badly and led to a capsule retention of 70% whereas the commercial Intal®powder, which also contains micronized DSCG, still had approximately 50% capsule retention. In addition to poor emission, the FPF for the Intal® product was also low as these drug particles seem to deposit on stage 1. The in-situ-micronized DSCG exhibits better capsule emptying (30% retention) and high deposition on the lower impinger stages leading to a FPF of 45% (Fig. 9b).

For the Cyclohaler® a 1:1 mixture of DSCG with lactose was used for the in vitro deposition tests as commercial products, such as Flui® DSCG, also contain lactose as a diluent. The commercial product shows very high deposition on stages 1 and 2 and has a FPF of 10%. The blend with the jet-milled DSCG and inhalation grade lactose shows a completely different deposition pattern: a high capsule retention (approximately 45%) with very low deposition on stages 1 and 2 is observed. The FPF is nearly double that of the commercial inhalation powder (19% vs. 10% for Flui® DSCG). These different deposition properties can be linked to the different drug and lactose qualities used. The powder blend with the in-situ-microinized DSCG again shows reduced capsule retention and enhanced deposition on the lower impinger stages leading to a FPF of 50% (Fig. 9c).

With the FlowCaps®-Inhaler, a new dry powder inhalation device, HPMC capsules were filled with the pure drug powder and delivered to the impinger. When compared to the other two devices, the FlowCaps®-Inhaler operates at a lower flow rate and it was tested at a flow rate of 33 l/min accordingly. As was observed for the other devices, the retention in the capsule/device is lowered from nearly 50% for the jet-milled drug to 15% for in-situ-microinized DSCG, thereby increasing the FPF from 8% to 40%, respectively (Fig. 9d).

In summary, the in-situ-micronized DSCG shows beneficial dispersion and de-agglomeration properties as compared to the conventionally micronized drug and commercial products. The efficiency of DSCG inhalation could be improved distinctly with the engineered DSCG particles as lower doses of DSCG might be administered to obtain the same effect.

### 4. Conclusions

In-situ-micronized DSCG has advantages compared with the jet-milled drug powder as the FPF is increased. This advantage is visible across different devices and it can be concluded that an optimized dry powder formulation plays a more important role than an optimized device. Thus, development of DPI technologies should focus much more on the powder formulation than on the development of new devices. The in-situ-microinization technique offers a

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**Table 1**

<table>
<thead>
<tr>
<th>Device used (air flow rate)</th>
<th>Fraction $&lt; 5 \ \mu m$ (%) (m/m) ($\pm$ SD)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Device-less application (84 l/min)</td>
<td>–</td>
</tr>
<tr>
<td>Spinhaler® (100 l/min)</td>
<td>14.1 (1.4)¹</td>
</tr>
<tr>
<td>Cyclohaler® (97 l/min)</td>
<td>9.7 (3.2)²</td>
</tr>
<tr>
<td>FlowCaps®-Inhaler (33 l/min)</td>
<td>–</td>
</tr>
</tbody>
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* n = 3; SD, standard deviation (μm). ¹ Pure drug; ² blended with lactose.

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**Fig. 8.** PXRD patterns of in-situ-micronized DSCG and pure HPMC (arrows indicate crystalline peaks of DSCG).
relatively easy method for the production of a micron-sized drug. Critical effects resulting from milling processes are avoided. Preparation can be carried out discontinuously or continuously using a static mixer. The in situ production of a micron-sized drug is possible as a one-step process and only requires common equipment. This technique requires a suitable stabilizer as described above. HPMC was found to show the best stabilizing properties. HPMC is a common excipient for oral, dermal and ophthalmic use. However, as it is not yet used for inhalation, no toxicological data on inhaled HPMC are so far available, but inhalation toxicity is expected to be low.

References


