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Physicochemical stability and aerosolization performance of mannitol-based microcomposites

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The aim of this work was to test the stability of mannitol-based co-spray-dried microcomposites containing meloxicam for use as dry powder inhalers. The effects of temperature and relative humidity (RH) on the physicochemical properties and aerosolization performance were investigated and the effects of polyvinylpyrrolidone (PVP) and polyvinyl alcohol (PVA) were analysed. Accelerated stability tests performed at 40 ± 2 ºC and 75 ± 5 % RH during 6 months demonstrated that the mannitol-based microcomposites containing meloxicam, PVA and L-leucine were more stable than those containing PVP against RH and temperature. As concerns water uptake, the number of hydrogen-bonds was lower after storage in the case of the samples containing PVP, and the fine particle fraction, which determines the aerosolization parameter, therefore decreased below 50 %. Such a composition may serve as an innovative drug delivery system for local lung treatment in the anti-inflammatory and mono- and combination therapy of cancer, pulmonary fibrosis and pain.

Key words: Microcomposites – Co-spray-dried – Accelerated stability test – Meloxicam – Aerodynamic performance – DPI form.

Systems involving inhalation through a dry powder inhaler (DPI) are extensively used for both local [1, 2] and systemic drug delivery [3]. A drug in DPI form may be applied alone (without a carrier) or with different carriers (e.g. lactose monohydrate, mannitol, sorbitol, cyclodextrin, xylitol, glucose, raffinose, trehalose, etc.) to ensure the distribution of the active agent. In some cases, industrial products contain the drug and the carrier (e.g. lactose monohydrate) in the form of an interactive physical mixture. A new tendency in the development of DPIs is the design of carrier-based microcomposites (particle size 3-5 μm) as pulmonary drug delivery systems involving different carriers and adjuvants. The adjuvants are applied in small amounts in the microcomposites in order to promote physicochemical stability, wettability, dispersibility and aerodynamic properties.

The carriers and the adjuvants significantly influence the physicochemical properties of DPIs during storage and application. Changes in the dispersibility of DPIs often occur during storage at high relative humidity (RH) [4, 5]. Investigations have been performed of the physical properties of carrier particles, such as the particle shape and the particle size [6-9]. There is currently considerable interest in studies of the influence of temperature and RH during storage on the aerodynamic parameters [10]. In most of the reported studies, formulations were exposed to storage under fixed RH for only short periods of time (a maximum of 7 days).

The US Food and Drug Administration (FDA) and the European Inhalanda Group have published their agreements on the tests required for the approval of new DPIs [11-14]. The FDA requires the stability testing of DPI powders, with determination of the appropriate storage conditions and the effects of storage on the particle size distribution, including the effects of moisture. The influence of the effect of the storage RH on dispersibility has been reported in many published papers [15-21].

The storage conditions during stability testing are determined on the basis of the ICH (International Conference on Harmonization) harmonized Guideline of Stability Testing of New Drug Substances and Products Q1A (R2) [22]. The ICH Guideline specifies the following storage conditions for accelerated tests: 40 ± 2 ºC with 75 ± 5 % RH. Samples are stored in hard gelatine capsules in open containers; the duration of storage is 6 months. The long-term storage conditions: 25 ± 2 ºC with 60 ± 5 % RH, for a minimum period of 12 months. The results of long-term stability testing have not been described in recent studies.

In previous work, we formulated co-spray-dried microcomposites of meloxicam (MX) and identified specific process parameters [23]. β-D-Mannitol (M) was primarily chosen as a carrier in our study because its particle size and form can be readily controlled by spray-drying [24] and spray-dried M has a crystalline state [25]. M is a highly water-soluble compound with low toxicity and low hygroscopicity, and which gives an obvious sweet aftertaste. Furthermore, M is a suitable carrier for the aerosol delivery of proteins and demonstrates significant stability in DPI formulations [1, 26-31].

After process parameter optimization, we earlier preformulated M-based co-spray-dried microcomposites with the application of polyvinypyrrolidone (PVP and polyvinyl alcohol (PVA) as stabilizer agents, L-leucine (LEU) as dispersibility enhancer and Tween as wetting agent. Excipients such as polymers (PVP or PVA) [32-34], amino acids (LEU) [35] and Tween [32] have been tested for their efficacy in improving pulmonary administration and safety for usage. The polymers modify the surface of the microcomposites as "coating material" and induce individual particles [36]. The polymer coating of particles plays a significant role by decreasing adhesion to the wall of the capsule or the medical device and increases the aerosol efficiency [33].

MX has also been investigated via the inhalation route. As a non-steroidal anti-inflammatory drug, MX, is not toxic to lung epithelial cells [37]. It has been proposed for pulmonary therapy [38, 39]. A 1:1 mixture of MX and M has been found to be non-toxic on monolayers of Calu-3 cells [40]. Many *in vitro* investigations have been reported concerning the applicability of MX for local anti-inflammatory lung treatment and it can be useful for pulmonary mono- and combination therapy [41-44].

The aim of the present work was the accelerated stability testing of M-based co-spray-dried products containing MX as DPI forms. The samples that were investigated were chosen in a preformulation study [36]. In this study, the physicochemical parameters revealed that the M-based microcompositions with PVP and PVA as stabilizer agents and with LEU as dispersibility enhancer were suitable for the development of an MX-containing DPI form.

We examined the influence of the RH and temperature on the physicochemical properties and aerosolization parameters of the co-spray-dried microcomposites during storage. The effects of PVA and PVP were additionally investigated in terms of particle size, shape, physicochemical stability and aerosolization of the DPI form by using

the Andersen Cascade Impactor (ACI) Model. Our overall aim, based on the results of the stability tests, was to suggest an MX-containing composition as a novel DPI form for pulmonary application for local anti-inflammatory lung treatment and for the mono- and combination therapy of cancer, pulmonary fibrosis and pain. At present, MX-containing DPI products for pulmonary therapy are not marketed.

I. MATERIALS AND METHODS

1. Materials

MX (4-hydroxy-2-methyl-N-(5-methyl-2-thiazolyl)-2H-benzothiazine-3-carboxamide-1,1-dioxide) (EGIS Ltd., Budapest, Hungary) was used as a DPI active pharmaceutical ingredient. PVP-K25 and PVA 3-88 were purchased from ISP Customer Service GmBH, Cologne, Germany, and M and L-LEU were from Hungaropharma, Budapest, Hungary.

2. Methods

2.1. Preparation of co-spray-dried products containing MX

The preparation parameters and compositions were optimized in earlier studies [23, 36]. Two samples were chosen for stability testing, i.e. mixtures of MX-M-PVP-LEU and of MX-M-PVA-LEU, with appropriate properties for local drug delivery to the lungs. The components of the microcomposites are presented in *Table I*. The microsuspension of MX was spray-dried from a solution of M, LEU and PVP or PVA (in the case of PVA, pre-stirring was necessary at 25 °C to achieve dissolution without hydrolysis). Each presuspension was made up to 100 g with water, using an Ultraturrax (T-25, IKA-Werke, Germany) operated at 24 000 rpm for 10 min. The particle size of the MX in the presuspension was decreased by cavitation with a high-pressure homogenizator (Avestin Emulsiflex C3, Canada) at 1 500 bar for 10 cycles. The process resulted in a microsuspension of MX which contained M, PVP or PVA and LEU in dissolved form. Such microsuspensions were spray-dried with a Büchi Mini Dryer B-191 (Switzerland). The suspensions were homogenized with a magnetic stirrer during a drying. The parameters of the spray-drying procedures are presented in *Table II*. These parameters ensured optimal drying efficiency in the case of co-spray-dried samples. The spray-drying efficiency was in all cases 75-80 %. During co-spray-drying, solid products were obtained, comprising microcomposites containing MX crystals in micronized form.

2.2. Investigation of stability of products

Stability tests were performed as recommended by the international guidelines specified in ICH Q1A (R2) - Stability Testing of New Drug Substances and Products. The stability testing was carried out in a Binder KBF 240 (Binder GmbH Tuttlingen, Germany) with constant-climate chamber. An electronically controlled APT.line preheating chamber and refrigerating system ensured temperature accuracy and reproducible results in the temperature range between 10 and 70 °C and the RH range between 10 and 80 %. Accelerated testing was performed at 40 ± 2 ºC with 75 ± 5 % RH. Samples were stored in hard gelatine capsules (size 3) (Capsugel, Belgium) in open containers; the duration of storage was 6 months. Sampling was carried out after 0 and 10 days, and 1, 2, 3 and 6 months.

Table I - Components of the microcomposites (each pre-suspension was prepared from these components and made up to 100 % with water).

Products	Substances	Mass (%)
MX-M-PVP-LEU	Meloxicam Mannitol Polyvinylpyrrolidone K-25 L-leucine	5.00 5.00 0.025 0.2
MX-M-PVA-LEU	Meloxicam Mannitol Polyvinyl alcohol 3-88 L-leucine	5.00 5.00 0.1 0.2

Table II - Spray drying conditions for co-spray drying powders.

Samples	Inlet (°C)	Outlet (°C)	Feed rate (mL/min)	Aspiration air (1 h^{-1})	Aspiration rate (m^3 min^{-1})
MX-M-PVP-LEU	130	77	4.0	600	0.065
MX-M-PVA-LEU	131	80	4.0	650	0.065

2.3. Thermogravimetry (TG)

Residual water content was analysed by TG-DTA with a Mettler Toledo TG 821e thermal analysis system with the STARe thermal analysis program V9.1 (Mettler Inc., Schwerzenbach, Switzerland) under a constant flow of dry nitrogen gas flow of 100 mL min^{-1}. One hundred-microlitres alumina crucibles were used for the samples and the reference. Scans were recorded at constant heating rate (5 °C min^{-1}) up to 300 °C. The TG-DTA oven was pre-equilibrated at room temperature and each sample (ranging between 12 and 20 mg) was weighed as fast as possible in order to minimize moisture uptake or release from the sample. The mass losses were recorded, and the moisture contents [% wet basis] were estimated from the normalized scans, the actual mass being divided by the initial mass. The loss of water basically occurred between 5 and 110 °C, and the higher temperature was used for the determination of bound water.

2.4. Particle size analysis

The particle size distribution of the microcomposites from the dry dispersion unit was estimated by laser diffraction (Malvern Mastersizer Scirocco 2000, Malvern Instruments Ltd., Worcestershire, United Kingdom). Approximately 1 g of product was loaded into a feeding tray. The dispersion air pressure was adjusted to 2.0 bar, in order to determine whether particle attrition had occurred. At least three repeat measurements were performed on each sample and the mean value was calculated. The particle size distribution was characterized by the D(0.1), D(0.5) and D(0.9) values and the Span values were calculated according to *Equation 1*. A high Span value (above unity) denotes a broad particle size distribution. The higher the Span value, the broader the particle size distribution [45]:

$$Span = [D(0.9) - D(0.1)]/D(0.5) \quad \textbf{Eq. 1}$$

2.5. Scanning electron microscopy (SEM)

The morphology of the microcomposites was examined by SEM (Hitachi S4700, Hitachi Scientific Ltd., Tokyo, Japan). A sputter-coating apparatus (Bio-Rad SC 502, VG Microtech, Uckfield, United Kingdom) was applied to induce electric conductivity on the surface of the samples. The air pressure was 1.3-13.0 mPa. Briefly, the samples were sputter-coated with gold-palladium under an argon atmosphere, using a gold sputter module in a high vacuum evaporator, and the samples were examined with the SEM instrument set at 10-15 kV.

2.6. Structural analysis by X-ray powder diffraction (XRPD)

XRPD spectra were recorded with a Bruker D8 Advance X-ray diffractometer (Bruker AXS GmbH, Karlsruhe, Germany) system with Cu Kα1 radiation (λ = 1.5406 Å) over the interval 5-30°/2. The measurement conditions were as follows: target, Cu; filter, Ni; voltage, 40 kV; current, 40 mA; time constant, 0.1 s; angular step 0.010°. In the determination of the degree of crystallinity, the total area of the three peaks with largest intensity was examined, after smoothing and background removal.

2.7. FT-IR analysis

For study of the interaction between the components in the mi-