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Conception, preparation and properties of functional carrier particles for pulmonary drug delivery

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ABSTRACT

Background: The effectiveness of aerosol therapy is significantly reduced by the mucus layer covering the airways of the tracheobronchial tree. According to the present concept, drug particles are delivered to the lung together with the functional carrier particle that facilitates both the drug transport into the lungs and the penetration of deposited particles through the mucus. The approach of manufacturing multi-component powders with mucoactive compounds and anti-asthmatic medicines (DSCG) bound together in a single particle is additionally considered.
Methods: Powders were produced with the spray-drying technique from aqueous precursor solutions containing pure low molecular weight dextran, pure mannitol and dextran/mannitol–N-acetyl cysteine (NAC) mixtures (4:1 and 1:1). NAC has been selected for this purpose as a compound, which is known to be mucolytic. Dextran and mannitol are potentially applicable in the field of inhalation drug delivery. They have been used as stabilizers of functional carrier particles. Powders were characterized for their yield and physicochemical properties including: morphology (SEM), moisture content and thermal properties (DSC). Aerosol performance was determined with NGI impactor after standardized aerosolization of the produced powders in a commercial DPI.
Results: Particle size distributions of dextran–NAC powders were characterized by high fine particle fraction (45–62%), which assures good particle deposition in the lower airways. The thermodynamic properties of the powders based on the temperature of the glass transition T_g (50–63 °C) suggest the required stability during storage at moderate humidity.
Conclusions: Preliminary examination of the required properties of these particles confirms their potential as functional carriers for pulmonary drug delivery.

1. Introduction

The famous sentence by Hippocrates "*primum non nocere*" when related to the procedure of drug delivery could be interpreted as the necessity of minimizing the drug dose for the achievement of an expected result of treatment.

Aerosol therapy is devoted to the delivery of drugs into the body via the respiratory system. The airway and alveolar epithelia constitute the main barrier that restricts the transport of drugs and solutes from the airway lumen into the blood circulation (Patton, 1996; Schneeberger, 1978; Schneeberger and McCormack, 1984). However, in the treatment of respiratory diseases many drugs may act on the receptors, which are localized on the surface of epithelium cells (Barnes, 2004). In this case, the main barrier which limits effectiveness of the treatment is the natural self-defense mechanism of the organism against the presence of strange matter. The main purpose of the mucociliary system is to protect the epithelium and lung tissue in the body from foreign particles. The system is built up essentially of ciliated cells, goblet cells and basal cells. The mucus is synthesized in the goblet cells, temporarily stored there and then secreted to the surface. The cilia, which are beating in a metachronal wave with effective and passive strokes, are transmitting momentum to the mucus which is moving and takes out deposited particulate matter, removing it from the site of deposition (Cone, 2009).

Mucociliary clearance causes the removal of the part of the deposited drug from the targeted region before it becomes pharmacologically active and reaches the receptors at the epithelial surface. Increased effectiveness of an inhalation treatment may be achieved by quick dissolution of powder particles in the airway surface liquid (mucus) and quick diffusion to the destination tissue (Khanvilkar et al., 2001).

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The dissolution phase is not limiting for many drugs. In many cases, the pharmacokinetics of drugs delivered as solid particles and in solution was the same (Newhouse et al., 2003; Sakagami, 2004). This suggests that the limiting step, at least for drug molecules with a certain size and polarity, is related to the transport of such molecules through the mucus layer. The thickness of a mucus layer may be different in various regions of the respiratory tract, although the available data also show the dependence on the measuring methodology. However, in a healthy respiratory tract of a human the mucus thickness is estimated to be 5–10 μm and it is gradually decreasing along the bronchial tree towards the alveoli, where the thickness is estimated to be about 0.05–0.08 μm (Boucher, 1994; Patton et al., 2010; Patton, 1996; Patton and Byron, 2007). In certain lung diseases the depth of mucus layer is significantly increased due to hypersecretion and this effect substantially reduces the effectiveness of drug delivery by inhalation (Kim et al., 1988; King et al., 1985). The rheological properties of the mucus are also changing. Mucus has a very complex chemical and structural composition. It is fairly well established that there is one glycoprotein species, which gives mucus its special character. Characteristically mucociliary glycoproteins – mucins are made up of a protein backbone with sugar side chains linked to serine and threonine (Gilboa and Silberberg, 1976; Meyer, 1977). The molecular entity of mucus glycoprotein block system is composed of several glycoproteins held together by disulphide bridges (King, 2005). It can interact with other mucus components, especially with DNA (Bansil and Turner, 2006). In diseased lungs, changes in mucus rheological properties are related mainly to mucins overproduction. However, in cystic fibrosis (CF) the extremely high viscosity of mucus is associated with the dehydration and elevated DNA content that results from expended neutrophils. Interaction between mucins and DNA results in a higher crosslinking density. The negative influence of these effects on the transport rate was reported (Bhat et al., 1996).

The viscoelastic properties of the mucus can be modified by changing the structure, e.g. by disulphide bond breakage. Several mucolytic substances can be used for such purposes. Some theoretical studies of our collaborators (Gniewek and Kolinski, 2010) show the predictions of changes in the rheological properties of mucus. When mucus loses its viscoelastic properties it may become more transparent for particles penetrating below the mucus blanket. Efficacy of treatment in such a situation is significantly improved, which has also been observed in experimental research, e.g. in the case of inhalation of a mucolytic agent prior to the inhalation of an active pharmaceutical ingredient (Ferrari et al., 2001; Stern et al., 1998). Simultaneous delivery of both the active pharmaceutical ingredient and the mucolytic agent would probably amplify this effect.

1.1. Conception of functional carrier particles for pulmonary drug delivery

Aerosols for inhalation therapy are produced by pneumatic or ultrasonic nebulizers, pressurized metered dose inhalers (pMDI) and dry powder inhalers (DPI). There are advantages and disadvantages with all these systems. A particulate system is chosen based on the ease of use for the patient and the intent to provide the required therapeutic dose with minimum adverse effects. The variety of morphological possibilities of solid, pharmaceutically active, particle formation causes the DPIs to become a more and more popular method of inhalation drug delivery.

In many cases, the inhalation route allows delivering relatively small doses of a drug directly to the airways to achieve high local concentration (Lipworth, 1996). Small doses usually cause a problem with drug fractionation during product preparation. Thus the majority of dry powder inhaler systems require the use of a carrier substance mixed into the drug. The amount of the carrier substance is substantially greater than the drug, and it can represent more than 90% of the weight per inhaled dose in some blends. The main role of such carriers is to assure the uniform distribution of drug particles in the powder blend to provide an appropriate dose of the pharmaceutical delivered into the lungs, but the carriers are also used to improve drug powder dispersion during inhalation (Dolovich, 1997; Granderton and Kassem, 1992).

Sugars, such as lactose or glucose, are typically used as such carriers. Typically, the mean size of lactose carriers for inhalation ranges from approximately 50 to 100 μm (Hickey, 2004; Pilcer et al., 2012). However, several recent studies indicate that formulations with a higher content of fine lactose particles (smaller than 15 μm) result in improved powder aerosolisation performance (Islam et al., 2005; Karhu et al., 2000; Louey et al., 2003; Steckel et al., 2006; Tee et al., 2000; Young et al., 2007a; Zeng et al., 1999). In all cases, the problem of drug–lactose aggregate deposition inside an inhaler and in the upper airways (oropharynx) still exists. As a consequence, the efficiency of drug delivery is reduced and in the case of regular use may induce side effects (Daniher and Zhu, 2008). Another important drawback of lactose is that it is a reducing sugar, which makes it incompatible with drugs that have primary amine moieties (Patton and Platz, 1992).

All these circumstances cause problems with the effectiveness of DPI. They may be overcome by a "particle engineering" approach. "Particle engineering" issues are very important in the development of modern therapeutic strategies. For example, the novel concept of preparation of low-adhesive pharmaceutical nanocarrier particles has been presented recently by Torvela et al. (2011), who demonstrated the flow reactor method of surface modification in order to minimize inter-particle interactions. Similar effects may be obtained using multicomponent precursors in spray-drying powder manufacturing.

Let us imagine some extension in the role of carrier particles (Fig. 1). They can posses all the advantages of a classic carrier regarding the blend formation and, in addition, they can be transported together with the drug particles directly to the targeting side of the deposition. High interaction forces between submicron particles of drugs and functional carrier particles guarantee that such aggregates will be deposited together in the same part of the tracheobronchial tree (Gradoń, 2009). The chemical composition of the carrier particle can facilitate the transport of the drug into the epithelial receptors.

This effect is important in the case of the required tracheobronchial deposition of drug particles, where the mucus blanket shields the effectiveness of the drug transport. The functional carrier of this concept should have the size within the fine particle range (i.e., below 5 μm) and to be composed of substances, which after interaction with the mucus destroy its consistency. Such combination therapy might significantly enhance the effectiveness of inhalations by lowering the required dose of active substances and, consequently, minimizing toxic side effects (Sosnowski and Gradoń, 2010).

1.2. Physicochemical aspects limiting particle formation

The practical accomplishment of the proposed conception of functional carriers for inhalation drug delivery requires a suitable method of their production. Spray-drying was selected as a convenient technique for the preparation of multi-component particles with an aerodynamic diameter below 5 μm. Spray-drying is a single-step method of powder production which allows precise control particle composition and their physicochemical properties (Iskandar et al., 2009; Young et al., 2010). During the contact of atomised liquid droplets with hot air inside a drying chamber, the moisture is evaporated and solid material is formed. As

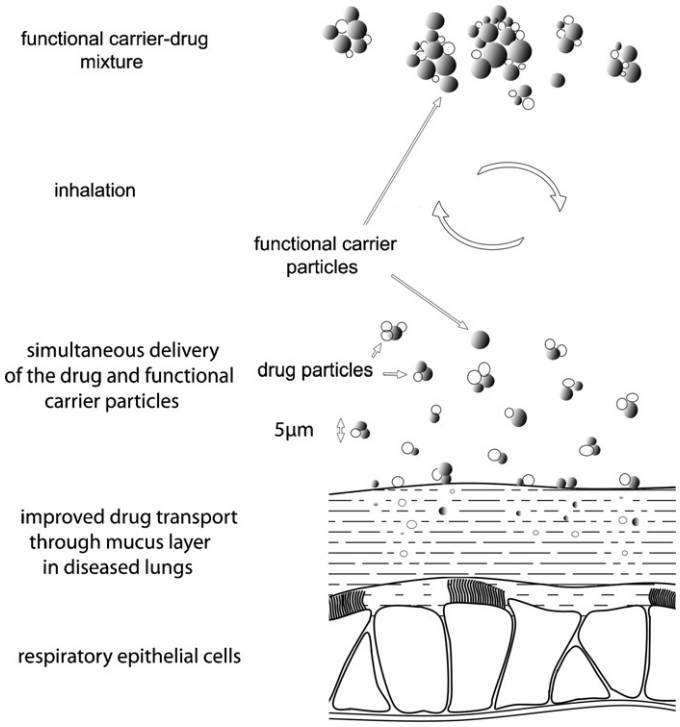

Fig. 1. Schematic illustration of the conception of functional carriers for pulmonary drug delivery. Aggregates made of functional carrier particles and drug particles with overall size below 5 μm deposit on the surface of the mucus layer in the respiratory tract. After dissolution in the mucus, the next step is diffusion through mucus layer. Mucolytic agent contained in functional carrier particles destroys structure of mucus layer increasing transport of the drug to the epithelial cells receptors. Cross-linking density of the mucus layer is symbolized by horizontal strokes.

a result of simultaneous heat and mass transfer, the resulting particles of powder may have different properties depending on feed composition and drying process parameters. As the process is associated with fast solidification, it may result in the formation of amorphous products. Substances in this meta-stable state easily undergo gradual re-crystallization during storage. On the other hand, amorphous compounds have several advantageous properties with respect to drug delivery when compared to crystalline products, for example a faster dissolution and a better absorption (Chow et al., 2007). This leads to the conclusion that the best form of inhalation powder should be amorphous with an increased stability. The stability can be improved by tuning some thermodynamic parameters, the glass transition temperature, T_g, being the most essential.

For solids characterized by a low T_g it is difficult to obtain a good yield of powder production by spray-drying at typical conditions because the sticky powder is collected predominantly on the walls of the device. This effect is well known in the food industry where spray-drying is used very often (Islam and Langrish, 2009). Powders characterized by a low T_g are also cohesive at ambient conditions and this results in the formation of aggregates, which are unsuitable for effective drug delivery by inhalation. The decrease of the glass transition temperature is related to a higher amount of water adsorbed by amorphous powders, which are often hygroscopic. If T_g of amorphous medicinal products is below room temperature, a very poor stability during shelf storage may be expected. As the glass transition temperature depends on solids composition (Hancock and Zografi, 1993), it can be increased by admixing a component with high T_g. Non-hygroscopic adjuvants should be preferred to reduce water vapor uptake by a powder from the environment. Therefore, the concept presented within this work relies on the production of the multi-component (composite) amorphous powders that are thermodynamically stable and might be used as functional carrier particles in inhalation drug delivery.

Crystalline products may be produced by spray-drying as well. It is limited by the nature of the used components, solidification mechanisms and the values of the spray-drying process parameters (Chow et al., 2007; Islam and Langrish, 2009; Vehring, 2008). Crystalline products are thermodynamically stable as their surface energy is low, but they lack the advantages of the amorphous products mentioned earlier. Unfortunately, there are not many compounds which can be used in aerosol therapy and simultaneously meet these criteria (Bosquillon et al., 2001a, 2001b; Smyth and Hickey, 2005).

The limitations regarding the composition of powders used in drug delivery by inhalation come from the acceptance criteria of the FDA or other national regulators. Only lactose is widely accepted to be used as a carrier of inhaled drugs (Hooton et al., 2006; Steckel and Bolzen, 2004; Tee et al., 2000).

Considering the innovative functional carriers for pulmonary drug delivery, one requirement includes bio-compatible compounds with functional properties which are already approved for use by inhalation or are expected to be so in the near future. In this work, we decided to focus on N-acetylcysteine (NAC) as the active mucolytic agent, which efficiently reduces mucus viscosity by breakage of disulphide covalent bonds between polymeric mucin molecules.

In some cases, NAC may enhance the absorption of drugs through the mucus blanket to the bronchial epithelium as demonstrated in cystic fibrosis treatment (Ferrari et al., 2001) and in other comprehensive studies (Matsuyama et al., 2006; Takatsuka et al., 2008). Similarly, Iiboshi (1996) found that the intestinal permeability of dextran (70,000 g/mol) that had been inhaled by rats was improved in the presence of N-acetylcysteine. Moreover, the NAC is an FDA approved mucolytic for inhalation and it is available as a solution for nebulization (Mucomyst).

Unfortunately, pure NAC cannot be used as a powder for inhalation since it is very hygroscopic at room temperature and easily agglomerates. Moreover, its melting temperature, T_m, is too low (104–110 °C) for obtaining good product by spray-drying. Therefore, we propose using two sugars: low molecular weight dextran (4 kDa) and mannitol for the preparation of composite particles with NAC, hoping to get an increased production yield and good stability of the obtained powders that can be used as functional carriers for inhaled medicines.

Dextran is water-soluble and easily cleared both from the lungs (Barrowcliffe et al., 1990) and the whole body (Arturson and Wallenius, 1964). This polysaccharide showed no evidence of toxicity when inhaled by humans (Dubick and Wade, 1994). Clinical tests allow for considering this compound as a component of inhalable powders (Vodak et al., 2011). In the study by Feng et al. (1998), low molecular weight dextran (4000) treatment in vitro significantly reduced the viscoelasticity and spinnability of CF sputum and increased the predicted mucociliary and cough clearability in a dose-dependent fashion. In another study, dextran administered by aerosol to living dogs increased tracheal mucus velocity. (Feng et al., 1999).

There are potentially two mechanisms, which could be considered for the mucokinetic effects of saccharide compounds like dextran (King and Rubin, 2002). One of them is an osmotic mechanism, attracting fluid into the airway milieu. This effect would presumably be maximal for monosaccharides, and its importance decreases with increasing molecular size.

The mechanism for the improvement in mucus fluidity with dextran administration has also been attributed to disruption of the network crosslinks (due to hydrogen bonds) between neighboring mucin macromolecules. Dextran probably competes for hydrogen

bonding sites with mucus glycoproteins resulting in the substitution of oligosaccharide moieties linked to the high molecular weight mucin peptides that make up the mucus gel. It has been observed that the fluidifying effect of dextran on mucus is primarily confined to the lower molecular weight fractions of dextran (Feng et al., 1997). As shown in the work of Hirji et al. (1998) the optimal length for saccharide units is 3–15. Probably, for dextran with lower molecular weight (preferably in the range of 4000 or less) these new hydrogen bonds are structurally and rheologically ineffective, so the overall crosslink density is reduced.

Dextran has also been shown to exhibit anti-adhesive properties in airway epithelial cells, which may make it of value as an antimicrobial agent, e.g., in preventing the *Pseudomonas* infection in CF patients (Barghouthi et al., 1996).

Improvement of the mucociliary clearance was observed also for mannitol which may be used as an alternative to inhaled hypertonic saline (HS) in promoting bronchial mucus clearance in CF patients (Robinson et al., 1999). Mannitol creates an osmotic gradient that increases water content in the airway lumen and it was demonstrated that application of inhaled mannitol powder results in improved lung function in CF (Jaques et al., 2008). Mannitol is already approved for inhalation by FDA (Bosquillon et al., 2001b; Frijlink and De Boer, 2004; Vodak et al., 2011), and as a powder at high concentrations it is used in airway reactivity testing challenge, with a high specificity for the assessment of asthma (Anderson et al., 1997; Brannan et al., 2005). Nevertheless, mannitol is proposed as a promising candidate for being an effective carrier of aerosolized medicines (Kaialy et al., 2010).

This compound should be of interest in multi-component powder technology due to its high melting temperature ($T_m \approx\approx 170\,^{\circ}C$), which should result in the formation of crystalline products in all typical spray-drying conditions where the outflow temperature is much less than T_m. It is presumed therefore that NAC–mannitol mixtures might also be tested as the right candidates to prepare stable powders suitable for inhalation. Similar concepts have been already tested regarding the use of mannitol mixed with other types of drugs (Adi et al., 2010; Kumon et al., 2010). The approach of manufacturing multi-component powders with mucoactive compounds and anti-asthmatic medicines (DSCG) bound together in a single particle is additionally considered within this work.

2. Materials and methods

2.1. Powder production and basic characterization

Powders were produced with the spray-drying method from aqueous precursor solutions with a total solid content 50 mg/ml. Detailed compositions of the precursors are listed in Table 1.

N-acetylcysteine (NAC), dextran (4000 Da), and D-mannitol were purchased from Sigma–Aldrich (USA). Disodium cromoglycate (DSCG) was donated by GSK Pharmaceuticals SA (Poland).

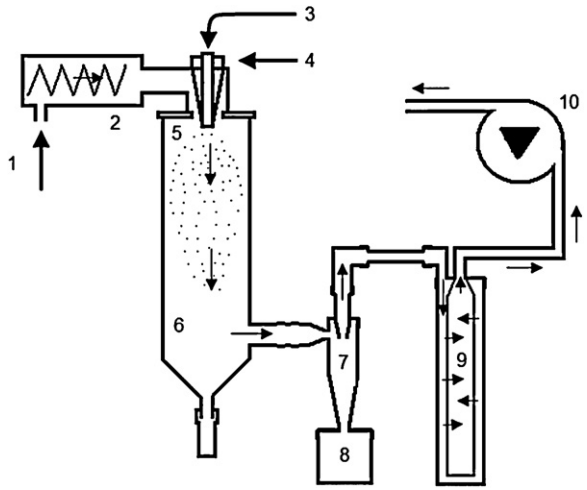

Fig. 2. Schematic diagram showing functional principle of the spray drying process. (1) Air inlet, (2) electric heater, (3) feed solution inlet, (4) compressed air inlet, (5) concentric inlet of the hot air around the spray nozzle, (6) drying chamber, (7) cyclone to separate product particles from gas stream, (8) product collection vessel, (9) outlet filter, and (10) aspirator to pump air through system.

Water used for preparing the precursor solutions and for other analytical purposes was purified by reverse osmosis – RO (Puricom, USA). Rhodamine B (POCH, Poland), a fluorescent maker, was added to all precursor solutions at an extremely low load (0.05 mg/ml) in order to allow for the later quantification of the aerosol size distribution with the Next Generation Impactor (Bosquillon et al., 2001a,b; Codrons et al., 2003). Powders were produced using a laboratory-scale spray dryer, model B-290 (Büchi Labortechnik AG, Switzerland) equipped with the standard two-fluid nozzle (cap-orifice diameter of 0.7 mm) and the high performance glass cyclone (Fig. 2). The optimized process parameters for production of fine particles were determined and used as follows: drying air temperature 120 °C, solution feed 3 ml/min (10%), aspirator rate 37 m^3/h (100%), airflow 0.475 m^3/h, and outflow temperature 55 °C. Collected powders were stored at room temperature (25 °C) and in the closed vessel (the relative air humidity below 50%) until further powder characterization was done.

The efficiency of spray-drying was calculated by dividing the mass of powder in the product collection vessel by the mass of materials dissoluted in the feeding liquid.

The moisture content in all produced powders was measured gravimetrically by determining the weight loss in a sample after drying in a laboratory dryer for 24 h at 70 °C.

The morphology of the spray-dried powder particles was investigated using a scanning electron microscopy SEM, model TM-1000 (Hitachi, Japan), after coating the samples with 25 nm layer of gold (K550x Sputter Coater – Quorum Technologies).

Table 1
Composition of the precursor solutions and the designations of powders.

Powder type	Precursor composition [mg/ml]				Powder designation
	Dextran	Mannitol	NAC	DSCG	
Single component	50	–	–	–	D
	–	50	–	–	M
Bi-component	40	–	10	–	DA-1
	25	–	25	–	DA-2
	–	40	10	–	MA-1
	–	25	25	–	MA-2
Tri-component	37.5	–	2.5	10	DAC
	–	37.5	2.5	10	MAC

Abbreviations: D, dextran; M, mannitol; A, N-acetylceysteine (NAC); C, disodium cromoglycate (DSCG).

2.2. Powder thermal properties

The thermal properties of each powder, which allowed determining T_g and T_m were investigated using a differential scanning calorimeter DSC8500 (PerkinElmer, USA). Powder aliquots (8–15 mg) were placed in DSC sample pans and exposed to a 10 °C/min temperature ramp between −50 and 250 °C. The powders were heated twice around the T_g to eliminate enthalpic relaxation.

2.3. Aerosol particle size distribution measurement

Particle size distribution of the aerosols obtained from the resuspended powders was determined with the Next Generation Impactor – NGI (Copley Scientific, UK) in combination with fluorescence analysis (due to rhodamine B content in the powder). All deposition surfaces in the NGI were coated with a thin layer of Tween 20 solution in glycerine (0.5%) to eliminate particle rebounce.

Approximately 20 mg of each powder was filled into HPMC capsules immediately prior to the experiment and dispersed from the cyclohaler DPI operated at 100 dm^3/min, which is the flow rate recommended by pharmacopeia for such DPI. Five capsules were used in each experiment. After washing the impactor stages with RO water (20 ml for each stage, 30 ml for the preseparator and the induction port), the fluorescence of each collected solution was determined using a Lumina Fluorescence Spectrometer (Thermo Scientific, USA) at the excitation and emission wavelength values equal to 555 nm and 580 nm, respectively. All experiments were run in triplicate and their results were expressed as mean ± standard deviation.

The emitted aerosol dose (ED) was determined as the total powder mass collected in the impactor (after emptying of 5 capsules), including the mass deposited inside the NGI and in the inlet and the pre-separator (non sizing components). The dose of, so-called, fine particles (with aerodynamic diameters smaller than 5.0 μm) was calculated by the interpolation of data showing the cumulative mass vs. cut-off aerodynamic diameter. Then, the dose of fine particles was expressed as a percentage of the emitted dose, resulting in the value of the fine particle fraction (FPF). In addition, the mass median aerodynamic diameter (MMAD) of aerosol particles and geometric standard deviation (GSD) of the distribution was determined based on the cumulative particle size distribution functions obtained from the NGI data.

3. Results and discussion

The summarized data regarding spray-drying yields, powder properties and the characteristics of aerosols obtained by powder aerosolization in the DPI are listed in Table 2. The glass transition temperatures T_g and melting temperatures T_m (not listed in the table) were obtained from DSC thermographs, the examples of which are depicted in Fig. 3.

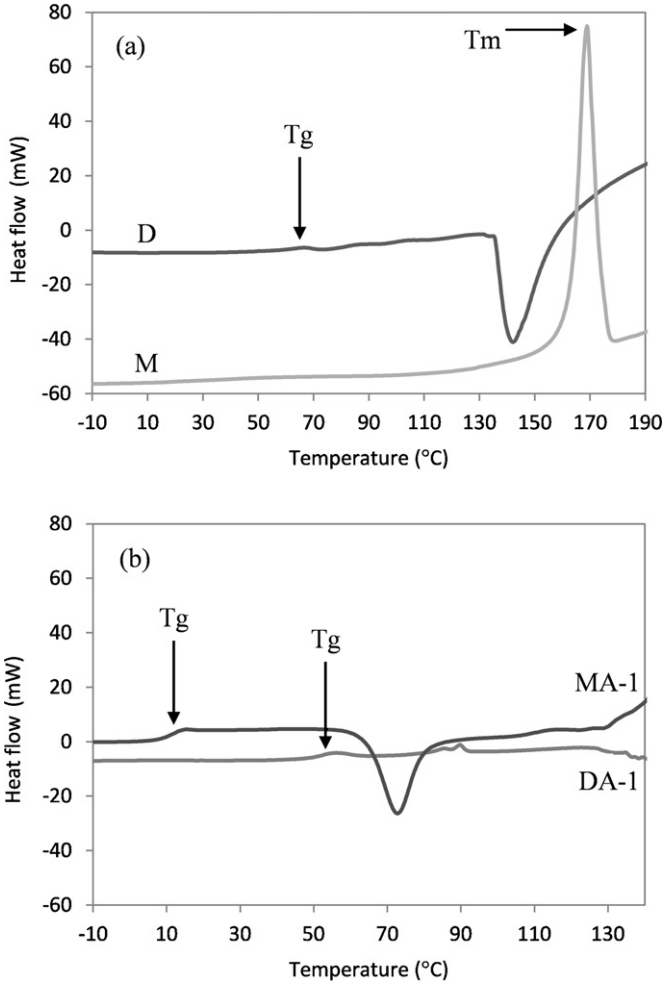

Fig. 3. Sample thermographs of (a) dextran: D and mannitol: M; (b) dextran–NAC: DA-1 and mannitol–NAC: MA-1.

Results of MMAD and FPF listed in Table 2 indicate that it is possible to obtain powders with high yields (87%) by spray-drying of solutions of pure dextran (D) or pure mannitol (M). In both cases, the products are easily dispersed to aerosols characterized by high FPF.

It should be noted that good resuspension of dextran occurs at relatively high powder moisture (11%) which suggests very good properties of the powder regarding inhalation applications. Similar conclusions were reported recently by Vodak et al. (2011). Good resuspension of dextran powder can be attributed to the

Table 2
Powder and aerosol characteristics (average ± SD, number of replicates = 3).

Powder characteristics				Aerosol characteristics			
Designation (according to Table 1)	Yield [%]	T_g [°C]	Moisture content [%]	ED [mg]	FPF [%]	MMAD [μm]	GSD
D	79.31 ± 7.68	63.0	10.9 ± 1.2	80.83 ± 6.93	57.2 ± 3.3	1.65 ± 0.10	2.63 ± 0.07
DA-1	18.35 ± 4.14	51.4	11.2 ± 1.1	66.82 ± 5.79	51.2 ± 2.4	2.28 ± 0.03	2.58 ± 0.18
DA-2	61.43 ± 6.96	60.9	12.6 ± 1.3	84.97 ± 4.85	45.8 ± 2.1	2.43 ± 0.03	2.71 ± 0.06
M	86.82 ± 2.10	–	0.8 ± 0.1	94.23 ± 0.84	80.5 ± 3.8	1.07 ± 0.13	3.27 ± 0.20
MA-1	11.10 ± 3.51	11.1	3.1 ± 0.4	77.87 ± 7.04	8.4 ± 0.4	3.32 ± 0.08	2.81 ± 0.70
MA-2	17.70 ± 2.32	8.0	5.8 ± 0.6	70.86 ± 5.13	9.7 ± 0.8	3.02 ± 0.38	4.35 ± 0.61
DAC	74.67 ± 10.60	59.4	8.8 ± 0.8	63.58 ± 7.52	61.3 ± 2.1	1.72 ± 0.03	2.45 ± 0.07
MAC	13.20 ± 4.70	49.8	3.5 ± 0.4	90.59 ± 13.41	13.8 ± 1.5	3.30 ± 0.01	2.18 ± 0.22

T_g: glass transition temperature; ED: emitted dose; FPF: fine particle fraction; MMAD: mass median aerodynamic diameter; GSD: geometric standard deviation.

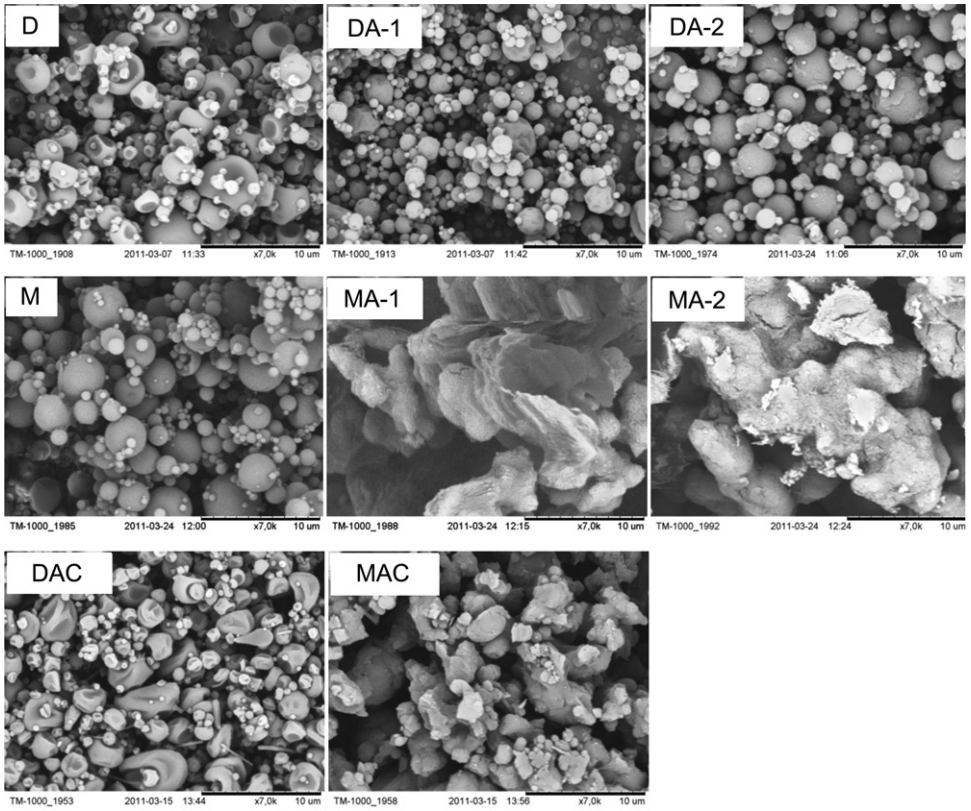

Fig. 4. SEM images of prepared powders. Magnification 7000×. Designations according to Table 1.

high value of glass transition temperature T_g (63 °C) that is significantly above the typical storage temperature. This value is also higher than the outflow temperature in the dryer. This fact explains the high yield and suggests good stability of the powders. Particles have a low tendency to agglomerate as demonstrated by SEM photographs (Fig. 4). Similar properties are observed for two types of dextran–NAC mixtures (DA-1 and DA-2), although particles of bi-component powders are more regular in shape than particles of pure dextran.

The MMAD is increased at a higher NAC content, and this is also related to the rise of powder moisture (up to 12.5%). The gradual reduction of T_g of the composite products was noted at the same time, and this observation confirms the relationship between the glass transition temperature and powder properties (this issue will be discussed later). It should be noticed that FPF of all DA powders is high (48% at minimum) which suggests that these powders can be useful for effective inhalations.

The thermograph of mannitol powder (M) (Fig. 3a) indicates that the product is crystalline. T_g cannot be detected but the endothermic peak of melting is observed at T_m = 169 °C what is similar to the findings of other researchers, e.g. (Adi et al., 2010; Hulse et al., 2009). Other studies showed that more than 89% of spray-dried mannitol could be obtained in crystalline form (Elversson and Millqvist-Fureby, 2005; Kumon et al., 2010; Tang et al., 2009). The crystalline mannitol particles show low adhesion and low water vapor adsorption (1% content). By contrast, the mixtures of mannitol and NAC are not crystalline and their T_g is in the range of 7–11 °C that is below the typical storage temperature. It is also much lower than the temperature at the dryer outflow and this explains the poor yield of the spray-drying process. Such powders adsorb water vapor easily and tend to agglomerate. In effect, it is not possible to use them to obtain the aerosol with the required quality – the FPF is below 10% (Table 2).

A generalized relationship between the glass transition temperature and the MMAD of aerosolized single component powders (M and D) and two-component powders (DA and MA) are shown in Fig. 5 and it confirms that high T_g is associated with a more efficient resuspension. Similarly, high T_g is required to obtain a good yield of the spray-drying process as shown in Fig. 6.

The last two rows of data in Table 2 show the properties of tri-component powders, where DSCG was introduced to the mixture of mucoactive compounds. Similarly to bi-component powders, only the mixture based on dextran may be considered as suitable for inhalation drug delivery due to a good production yield (75%) and high FPF (61%). These properties again are related to high T_g (60 °C), which explains good stability of the product and its low tendency to agglomerate even at relatively high moisture content within the

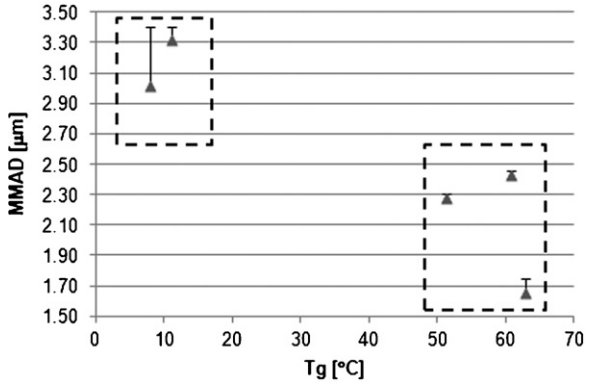

Fig. 5. The relationship between T_g and MMAD of aerosolized single- and bi-component powders indicating two distinct groups of data. Average ± SD, number of replicates = 3.

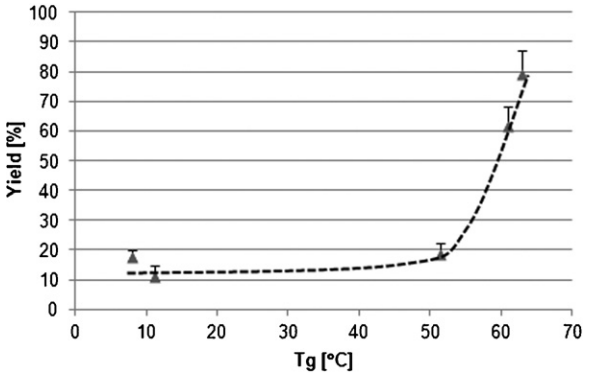

Fig. 6. The relationship between T_g and the yield of spray-drying process for single- and bi-component powders. Average ± SD, number of replicates = 3.

powder (8.8%). Unsatisfactory data have been obtained for mixtures with mannitol, and the reasons are the same as for bi-component powders based on mannitol (i.e., low T_g).

Production of multi-component particles, which contain drug and mucoactive compounds has more limitations than the process elaborated for the functional carrier. In the case of multi-component particles, there is the need to prepare a precursor solution, in which all components are dissolved. While sugars are water-soluble, many inhalation medicines may be not. Another limitation comes from the fact that dissolved components may react or associate in solution before and during the drying process resulting in unpredictable changes in product quality and even in drug inactivation. This problem was also encountered in the case of the NAC–DSCG mixture described in this work. Due to precipitation of the DSCG in the presence of dissolved NAC (Bradberry, 1991), the composition of the precursor solution had to be carefully adjusted. It is therefore concluded that the conception of the functional carrier particles is more versatile as it allows combining the powders of a mucoactive compound with the variety of drug particles physically attached to the carrier.

4. Conclusions

The main goal of our paper was the elaboration of the technology of production of functional carrier particles with all required functions of such particles, i.e. having the dimensions within the respirable fraction, being thermodynamically stable and revealing mucolytic properties. NAC, mannitol and dextran have been selected for this purpose as the compounds which are known to be mucoactive and which are potentially applicable in the field of inhalation drug delivery. It has been shown that it is possible to use spray-drying for the efficient production of powders containing pure dextran, pure mannitol and dextran–NAC mixtures (1:4 and 1:1 w/w) which have properties that make them suitable for application as the inhalable functional carriers. Particle size distributions after standardized aerosolization of these powders using commercial DPI are characterized by sufficiently high fine particle fraction (45–80%), which assures good particle deposition in the lower airways. Thermodynamic properties of these powders, which can be assessed, based on the temperature of glass transition T_g, suggest the required stability during storage at moderate humidity.

Composite mannitol–NAC powders have, by contrast, inadequate quality for inhalation due to a low FPF and insufficient thermodynamic stability as expected from low T_g value. They are also characterized by poor production yield.

In addition to the proposed functional carrier particles, it was also shown that different composite powders could be obtained by incorporation of a model drug (DSCG) into the mixture of mucoactive agents using the same spray drying technology. The obtained tri-component dextran–NAC–DSCG particles have good aerosolization properties (FPF = 49%) and are expected to be thermodynamically stable because their T_g is remarkably above typical storage conditions. On the contrary, incorporation of the cromoglycate into mannitol–NAC mixture was shown to be ineffective because of the insufficient production yield, low product stability and low aerosolization efficiency of the resulting powder. The concept of multi-component particles has also several limitations due to potential interactions between substances dissolved in the precursor solutions. It is therefore concluded that the main presented concept of functional carrier particles is more useful.

This basic research deals with a complex subject. It opens the possibility of the continuation of bringing additional data regarding the quantitative description of composite particle formation and the evaluation of the efficiency of functional carrier particles to fluidize the mucus and enhance the diffusion of the drug. The recent theoretical analysis of the kinetics of the formation of multi-component particles during the spray-drying process shows that there is no stratification of the particular components during the solidification of the dried droplet indicating that the thermophoretic effects are negligible for the considered case (Gac and Gradoń, 2011). Some novel data regarding the optimization of the process of multicomponent powders preparation by spray-drying have also been demonstrated very recently (Kramek-Romanowska et al., 2011). The studies, which are currently underway, are focused on the demonstration of the activity of the functional carrier particles in a model mucus system and on their theoretical explanation utilizing the results of thermodynamic analysis and molecular modeling (Gniewek and Kolinski, 2010). The preliminary results of these investigations are very promising.

Conflicts of interest

No conflicts of interest exist.

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