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The influence of relative humidity on the carrier particle surface characteristics used in dry powder inhalation formulation

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ABSTRACT

Purpose: Investigation of the effect of carrier storage at elevated relative humidities on the carrier surface fines. The resulted effects of this storage conditions on the carrier surface characteristics when mixed with micronized salbutamol sulphate and the subsequent *in vitro* deposition were investigated.
Methods: Lactose (InhaLac120) and mannitol (Pearlitol160) carrier particles (112 µm – 140 µm) were subjected to different relative humidities for 6 weeks period of time in order to get a smoother surface. *In vitro* deposition carried out for those modified carriers after mixing with salbutamol sulphate using a Next Generation Impactor (NGI). The modified carriers were characterized by scanning electron microscope, Brunauer Emmet and Teller specific surface area determination, differential scanning calorimetry, X-ray powder diffraction and water vapour sorption in comparison with the treated carrier without added fines.
Results: The modified lactose and mannitol carriers stored at different relative humidities starting from 35% RH-75% RH show no change in fine particle fraction (FPFs). Subsequently, the carrier storage at 95% RH shows a significant decrease in the in vitro drug release. This probably by increasing drug-carrier adhesion and show a difficult detachment of the drug particles from the carrier surface smoothed at 95% RH.
Conclusions: Storage of lactose and mannitol carriers at different relative humidities up to 75% result in un-significant change in drug fine particle fraction using the NGI. Additionally, storage of those carriers at high relative humidity up to 95% RH found to decrease significantly the dispersion of the drug and subsequently the FPF in the *in vitro* deposition test.

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1. Introduction

Dry powder inhalation formulations (DPIs) consist of an active pharmaceutical ingredient (API) of suitable aerodynamic size (usually 1-5 µm) for inhalation (Bérard *et al*., 2002, Hoppentocht *et al*., 2012) mixed with carrier material contained within a device, which, upon inhalation, provides sufficient deagglomeration of drug particles to deliver a therapeutic dose to the lungs. Recently, a DPI containing carrier particles as well as drug has been developed to overcome the drug particle agglomeration and provide better drug detachment from the carrier surface during the inhalation process (Hoppentocht *et al*., 2012; Kaialy *et al*., 2012; Podczeck 1999; Peng *et al*., 2016). It was also reported that carrier surface properties (e.g., surface area, morphology and roughness) play a significant role in determining interparticulate interactions, stability, ease of dispersion, de-agglomeration and efficient drug release from the inhaler used (Kaialy *et al*., 2012, Lee *et al*., 2009, Young *et al*., 2009). For adhesive mixtures (API mixed with carrier), many variables exist which vary from the type and size distribution of the carrier and the drug content in the mixture to the blending conditions during the preparation of the mixture. All these variables influence the flow properties and dispersion performance of the formulation during inhalation and by that, the (consistency of) delivered dose and fine particle dose (Dunbar *et al*., 1998, Kawashima *et al*., 1998, Steckel *et al*., 2004) Storage of carriers at elevated relative humidities may allow water vapour to condensate in the interparticulate capillaries. This condensation expected to result in smoother carrier surfaces, which result in easier separation of drug particles from carrier surfaces and higher drug deposition in the lungs. Highly soluble materials such as lactose monohydrate and mannitol may undergo limited dissolution at interparticulate contact points with subsequent solidification, thus resulting in solid bridge formation between particles. Especially the carrier fines present on the coarse carrier surfaces are prone to undergo partial dissolution due to their enhanced solubility caused by their small particle size and possibly due to their amorphous content. This leads to particulate fusion between the carrier fines and the coarse particles which is expected to increase the carrier surface smoothness. "Ostwald ripening" is the phenomenon of smaller carrier particles, which expected to dissolve in a saturated solution and being impeded into the larger particles, which have a lower interfacial energy (Ostwald, 1896). When the smaller particles have dissolved in such way the solution becomes supersaturated, which then promotes the precipitation of the solid (Cleaver *et al*., 2004) and result in more smoothed carrier surfaces. The carrier powders were placed in Petri dishes at different relative humidities in desiccators for 6 weeks. Then they were stored over silica gel for another week at room temperature before the preparation of interactive mixtures.

A comprehensive investigation was undertaken to evaluate the effect of humidity on the lactose and mannitol carrier surface characteristics used in dry powder inhalation formulations. Lactose (InhaLac120) and mannitol (Pearlitol160) carrier particles (112 µm – 140 µm) were subjected to different relative humidities for 6 weeks period of time in order to get a smoother carrier surface. Those smoother carrier surfaces were expected to ease the drug particle release from the smoothed carrier surface, which subsequently would result in more drug deposition in the deep lung parts. Additionally, rough carrier surface contains microstructures, which increases the contact points for drug particles and hence decreasing the drug deposition (Scherliess, 2016).

2. Materials and methods:

2.1 Materials:

Mannitol was kindly supplied by Roquette Freres (Lestrem, France) as Pearlitol160 C, Lactose was used as InhaLac120 (α-lactose monohydrate) supplied kindly by Meggle GmbH (Wasserburg, Germany). Salbutamol sulphate was supplied kindly by Lindopharm (Hilden, Germany). Methanol and acetonitril (HPLC degree) were purchased from VWR international GmbH, Darmstadt, Germany. Acetic acid was purchased from Mallinckrodt Baker B. V., Deventer, Holland.

2.2 Methods

2.2.1 Particle crystallinity

2.2.1.1 Determination of the extent of crystallinity by differential scanning calorimetry

Thermal stability and changes in crystallinity were investigated using a differential scanning calorimeter (DSC30, Mettler-Toledo GmbH, Schwerzenbach, Switzerland) calibrated with indium. A small amount (≈3mg) of carrier or drug was crimp-sealed in an aluminium pan with pierced lid. The pan was then placed in the sample chamber and an empty matched aluminium pan was used as the reference for all measurements. The experiments were performed in the range from 0°C-300°C under a nitrogen flow of 50 ml/min. The scanning rate was adjusted to 10°C/min. The onset and/or peak temperatures and heat of enthalpy (ΔH) for each peak were determined from the normalized DSC thermogram. Each experiment was carried out twice. The second of the two determinations is shown.

2.2.1.2 Determination of the extent of crystallinity by X-ray diffractometry

Powder X-ray diffraction patterns of samples were obtained using the Miniflex powder diffractometer (Rigaku Corporation, Tokyo, Japan) with a Cu-Kαradiation (λ=1.5406 Å) as the source of radiation. This diffractometer was operated at the voltage of 30 kV and the current of 10 mA. Each sample was placed in the cavity of an aluminium sample holder flattened with a glass slide to present a good surface texture and inserted into the sample holder. In order to measure the powder pattern, the sample holder and detector were moved in a circular path to determine the angles of scattered radiation and to reduce preferred sample orientation. All samples were measured in the 2θ angle range between 5° and 40° with the scan rate of 0.02° for 2 s and a step size of 0.02°. All samples were analyzed in triplicate. The diffractometer was operated at room temperature and humidity. The second of the three samples are shown in the figures.

2.2.1.3 Water vapour sorption

Plots of water, either adsorbed or desorbed as a function of relative humidity at a constant temperature, are commonly known as sorption isotherms. The sorption/desorption profiles were determined gravimetrically. The gravimetric studies were undertaken in a humidity controlled microbalance system (Projekt Messtechnik, Ulm, Germany). The construction of the water vapour sorption system SPS11 is based on a microbalance capable of measuring changes in sample mass lower than 1 part per million. The system is housed in an incubator to control temperature and surrounding humidity. The apparatus is computer controlled, allowing a pre-programming of sorption and desorption isotherms. Approximately 3gm of samples were loaded; the relative humidity was first set to 0%, and then raised in nine steps of 10% to 90% and one step more to 95%. Subsequently, the relative humidity was decreased from 95% to 90% then to 0% by the same way. This cycle was repeated once more. The equilibrium condition was set to 0.01% mass change per 60 minutes, which had to be reached before the program moved to the next humidity step. The temperature was set at 25°C. Samples were weighed in time intervals of 6 minutes, the whole measurement was run for only one time due to the time consuming of this type of experiments.

2.2.1.4 Particle size distribution measurement by laser diffractometry

The particle size distributions of the micronized drug and the excipients were determined with a Sympatec HELOS laser diffraction spectrometer equipped with a RODOS dry powder dispersing system (Helos H1402/Kf-magic and dry dispenser Rodos, Sympatec GmbH, Clausthal-Zellerfeld, Germany). The powder samples were fed to the dispersing air stream using a funnel connected to the injector of the dry disperser. Depending on the pressure and speed of the passing air stream, the dispersing force could be adjusted. The carrier and active ingredient powders were dispersed by compressed air at 0.3 bar–4.0 bar. The pressure at which only agglomerates are dispersed without destroying single particles was evaluated to be 2.5bar. The size distributions of the samples were obtained using the dispersion pressure at 2.5bar. All calculations were made with the Fraunhofer theory. All data given represent the average values of at least three determinations at dispersing pressure of 2.5bar. Volume median diameter and span (50% undersize and 90% undersize - 10% undersize) were calculated.

2.2.2 Preparation of coarse carrier

The sieved fractions (112 µm-140 µm) of lactose (InhaLac 120) and mannitol (Pearlitol 160 C) were obtained by sieving the sugar particles sequentially through test sieves with an aperture width of 112 µm and 140 µm, using a sieve shaker (Retsch, Haan, Germany). The sieved carriers were placed over silica gel in a desiccator until further required.

2.2.3 Preparation of carriers with reduced fines by storage at different relative humidities

This step of this research work started with storage of the sieve fraction 112 µm-140 µm of lactose (InhaLac 120) and mannitol (Pearlitol 160 C) carriers at different relative humidities for 6 weeks in order to get smoother surfaces. Saturated salt solutions were used to maintain constant relative humidity levels inside small desiccators during the storage of carrier particles A saturated salt solution was manufactured by dissolving as much salt as possible into water, and then adding extra salt to generate a layer of undissolved solid on the bottom of the container (Martin 1962). The desiccators were incubated at 25°C. The following series of saturated salt solutions were used: magnesium chloride (35%), magnesium nitrate (55%), sodium chloride (75%) and potassium nitrate (95%). Relative humidity was measured within the desiccator using a thermo-hygrometer (Testo, Lenzkirch, Germany) with relative variability being <3%.The carrier powders were placed in Petri dishes over these different relative humidities for 6 weeks, then stored over silica gel before the preparation of interactive mixtures.

2.2.4 Micronization of salbutamol sulphate

Salbutamol sulphate particle was milled by using an air jet mill (50AS, Hosokawa Alpine AG, Augsburg, Germany) injection pressure was set to 3bar, milling pressure to 2bar and feeding rate was adjusted to approximately 1 g/min, to prevent a variable particle size distribution, a uniform feed rate was maintained, by using mechanical controller feed part. Micronized salbutamol sulphate was placed over silica gel in desiccators until further required.

2.2.5 Preparation of interactive mixtures

Blends of salbutamol sulphate with coarse lactose or coarse mannitol (112 µm-140 µm) were prepared in a ratio of 1:99 w/w in 8g batch in a stainless steel mixing container using a Tumbling mixer (Turbula T2C, WA Bachofen AG, Switzerland) for 90min, 42 rotations per minute. Micronized salbutamol sulphate was blended with lactose or mannitol in a sandwich method to prepare a binary mixture. Briefly, an amount of a carrier, equivalent to about half the

total mass of the carrier was used to 'sandwich' the drug in the blend. i.e. the half part of the carrier material was put firstly in the mixing container followed by the drug followed by the other half of the carrier material. To minimize the effects of tribocharge, a stainless steel container was used, of 5.2 cm diameter and 3.2 cm height. The sample was then stored in vacuum desiccators over silica gel minimum at least 24 hours to allow electrostatic charge decay.

2.2.6 Content uniformity test

The blend uniformity was determined by taking 12 samples with a special sample taker, which were individually weighed. Each dose was dissolved in 50 ml acetate buffer of pH=3 in a graduated cylinder and the amount of salbutamol sulphate in each sample was analyzed using high performance liquid chromatography (HPLC) at the wavelength of 276 nm.

2.2.7 Particle morphology

The particle morphology of lactose, mannitol before and after storage at elevated relative humidities was examined by using scanning electron microscopy (LEO VP 1430, LEO Electron Microscopy Ltd, Cambridge, England). It is operated by using an electron beam at the acceleration voltage of 19 kV and a working distance of approximately 18 mm. Samples (≈0.5 mg) were mounted via a graphite tape to an aluminum stub. After stripping off the upper side of the adhesive, a small amount of particles was scattered on the stub and dispersed by tapping lightly on the edge of the stub with a spatula to break agglomerates or by using an air stream. The particles were then coated with ~15 nm–20 nm of gold with an Agar manual sputter coater (Agar Scientific Ltd., Stansted, Essex, England), using an electrical potential of 1.5kV and the current of 20 mA. Photomicrographs were taken randomly of several different areas of the powder on each stub. Representative areas were photographed with different magnification power.

2.2.8 Surface area measurement

The carriers' surface area was measured by nitrogen adsorption for carrier and drug particles. Prior to surface area measurement, known masses of the samples were accurately weighed into sample tubes and out gassed for 24 h at 40°C and vacuum for mannitol and at 50°C and N_2 current for lactose to remove any adsorbed gases from the surfaces of the particles. This difference of preparation temperatures between the two carriers was due to the sensitivity of each carrier and depending on preliminary experiments before. After outgassing, the sample tubes were connected to a surface area apparatus connected to a computer. The carrier specific surface area was obtained by BET nitrogen adsorption measurement from a Micromeritics Tristar 3000 (Micromeritics GmbH, Moenchengladbach, Germany). Each sample was measured in triplicate and the mean with the standard deviation was calculated. The specific surface area was calculated according to (Deutsches Institut for Normung, 1973).

2.2.9 *In vitro* deposition determined using the Next Generation Impactor (NGI)

The aerodynamic particle size distribution of salbutamol sulphate was carried out using the Next Generation Impactor (Copley Scientific, Nottingham, UK). Methodology followed that of the British Pharmacopoeia (2007). The NGI was connected to a vacuum pump with adjustable flow rate between 60 and 100 l/min. The flow manoeuver through the inhaler was controlled with a previously adjusted corresponding pressure drop across the device with a flow controller (Copley, Nottingham, UK). The inspiration time was controlled with a time controlled solenoid valve and was set to 3 seconds. The adhesive mixtures were filled into Novolizer® cartridges (Viatris GmbH & Co. KG, Frankfurt, Germany) and dosing was performed with the built-in metering system. The aerosolization was done at 79.3 L/min.

The impactor plates were coated with a viscous solution of emulgator (Brij 580.25%) in glycerol anhydride (4.75%) and isopropanol HPLC degree (95.00%). A specified volume (2 ml for stages 2-7 and 4ml for stage 1 and the micro-orifice collector) of this solution was distributed on each collection plate to provide a film. The plates were left to dry under ambient room conditions for at least 2 hours prior to each analysis. The NGI plates were covered with this viscous solution to eliminate the bouncing of the powder particles off the plates, which can give incorrect size distributions. Prior to each measurement, the temperature and relative humidity of the surrounding environment were measured using a thermo-hygrometer, in order to measure the influence of surrounding environment on the FPF produced.

The impactor was assembled and a Novolizer® was then fitted into the moulded rubber mouthpiece attached to the throat of the impinger. The TPK Copley pump (Copley Scientific, Nottingham, UK) which was connected to the outlet of the apparatus was switched on and allowed to run for 3 seconds prior to the release of the dose. The pump was then allowed to run for another three seconds at 79.3 L/min. This process was repeated 49 times. The impactor was dismantled and the individual plates, as well as the micro orifice collector (MOC) were carefully washed with acetate buffer pH 3. The inhaler mouthpiece, the throat, and preseparator were washed into volumetric flasks of 100 ml, and the washing solution was made up to a set volume with the same solvent (acetate buffer, pH 3). The concentration of salbutamol sulphate in each of the samples was analyzed by high-performance liquid chromatography (HPLC). The particles of less than 5 µm were expected to be deposited in the lung after inhalation, which used to describe the inhalation properties of DPIs. The fine particle fraction (FPF) is calculated as the dose of the drug exhibiting an aerodynamic diameter <5µm by addition of the doses of drug on stages 3–7 plus the part of drug <5µm on stage 2 obtained by interpolation at 79.3l/min. Experiments were run in triplicate, each time on a new mixture of salbutamol sulphate with the carrier at the same proportion (3 batches of each formulation (8 g) were prepared).

2.2.10 HPLC analysis of salbutamol sulphate

Salbutamol sulphate was analyzed by high performance liquid chromatography (HPLC) employing a mixture of 50% acetonitrile and 50% acetate buffer (2.5 gm glacial acetic acid (100%) in 1000 ml distilled water). The pH was adjusted to pH 3.0. The flow rate of 0.52 ml/min was used. The HPLC system consisted of a pump (LC 6A Shimdazu, D-Duisburg), a multiple wavelength detector (SPD-6AV, Shimdazu) operating at 276 nm. The system was equipped with an auto sampler (SIL-6B, Shmidazu) and a 15cm x 4.6 mm internal diameter reversed phase column packed with 5µm C-18 Nucleosil HD RP 18 MN 250/4 (Machery u. Nagel, Dueren, Germany). The retention times for salbutamol sulphate and the standards were 2.71min and 5.49min, respectively. Standard solutions were made to contain drug concentrations between 0.100 µg/50 ml-0.700 µg/50 ml. These standard solutions were employed to construct a calibration curve of peak height against drug concentration. The calibration was prepared on a daily basis, and a calibration curve with R^2>0.99 was considered acceptable. The injection volume was 10µl. Each sample was measured in duplicate. The content of salbutamol sulphate from the impactor experiment of each cup and the preseparator was calculated from the peak heights of the chromatograms. The total amount of the delivered drug (or mass balance) was also calculated, the amount of salbutamol sulphate was calculated in µg/dose and as the percentage of the total amount of drug delivered.

2.2.11 Statistical tests

The *in vitro* deposition data were examined for statistically significant differences by the ANOVA single variance test. A P-value of <0.05 was considered significant.

3. Results and discussion

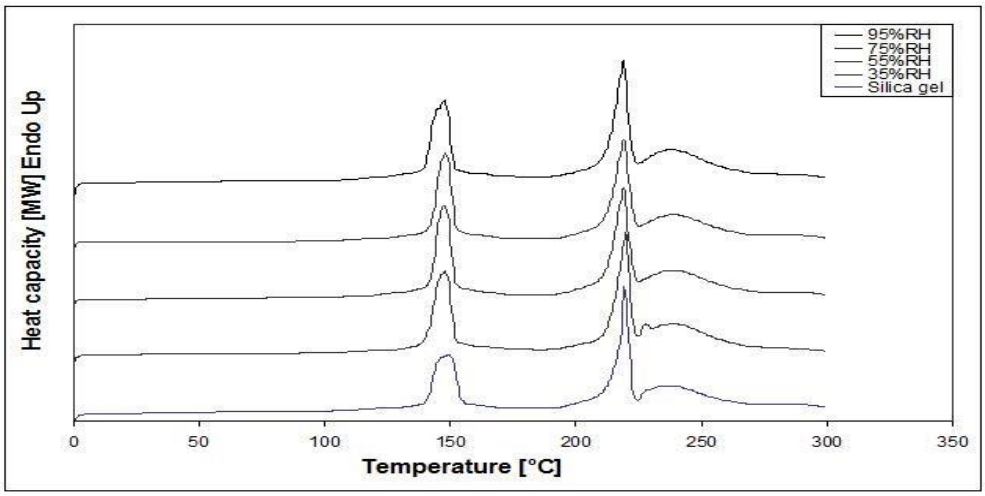
2.3 Determination of the extent of crystallinity

2.3.1 Differential scanning calorimetry

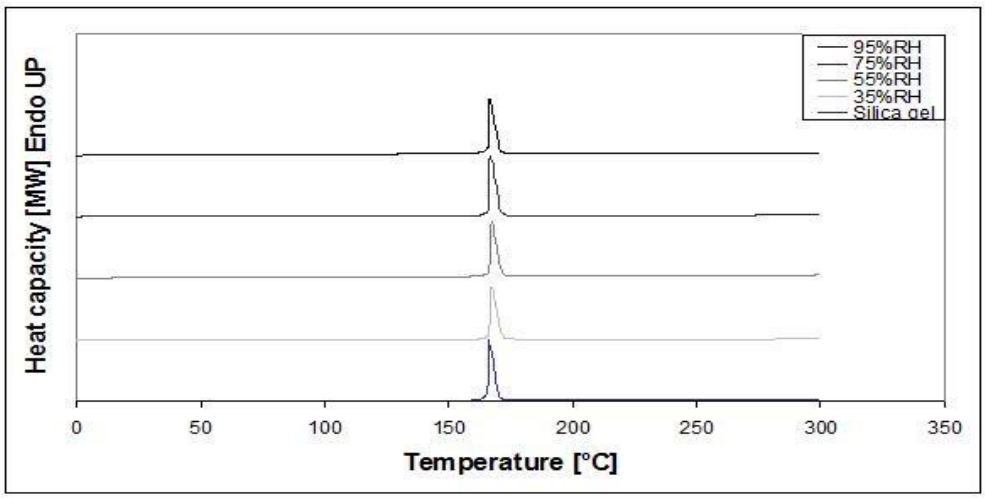
The extent of crystallinity was examined using a differential scanning calorimeter (DSC). The DSC was performed on lactose and mannitol in order to provide an indication of the variation of the crystalline/amorphous content and polymorphism, which might be induced by the storage at elevated relative humidities. DSC thermograms of mannitol (Fig. 1b) show a single peak at approximately 166.19°C with a total enthalpy of 293.16 J/g while lactose may exhibit complex thermo-analytical transitions because of its several crystalline, as well as amorphous forms. α-lactose monohydrate becomes anhydrous at 120°C and has a melting point of 146°C–147°C, whereas the endothermic peak at approximately 217°C is the melting endotherm of β-lactose dehydrate (Fig. 1a). Thermograms and enthalpies of fusion (Table 1) of both carrier materials after storage at low (silica gel) and different elevated relative humidity reveal no differences in the crystalline behaviour irrespective of the storage conditions. This illustrates the stability of lactose and mannitol carrier materials even when they were stored for a long time at elevated relative humidity. Additionally, the detection limits for amorphous contents with such technique have a lower cutoff of 5-10%, this detection limit is due to the fact that this technique measures the entire sample (Buckton *et al.*, 1999).

Table 1
DSC parameters of the carriers after 6 weeks storage at different relative humidities (n=2), mean ± range (the range indicates the maximal and minimal values).

SAMPLE NAME	ENTHALPY [J/G]	ONSET [°C]	PEAK [°C]
Lactose stored in silica gel	153.51±1.19	141.17±4.06	147.40±1.86
Lactose stored at 35% RH	153.60±0.73	140.51±0.39	147.73±0.08
Lactose stored at 55% RH	159.80±1.69	143.43±1.04	146.61±1.16
Lactose stored at 75% RH	158.77±2.99	142.08±0.21	147.77±0.18
Lactose stored at 95% RH	164.72±0.58	137.47±2.26	147.74±0.10
Mannitol stored at silica gel	293.16±7.26	165.49±0.14	166.19±0.04
Mannitol stored at 35% RH	294.66±0.26	166.25±0.11	167.03±0.07
Mannitol stored at 55% RH	293.37±1.25	166.32±0.14	167.18±0.28
Mannitol stored at 75% RH	297.89±9.94	166.03±0.01	166.63±0.01
Mannitol stored at 95% RH	292.59±1.69	165.95±0.08	166.77±0.12



a



b

Fig. 1. DSC traces of lactose (***a***) and mannitol (***b***) after storage at different relative humidities for 6 weeks, the second of two measurements is shown.

2.3.2 X-ray powder diffraction

The X-ray diffractograms of stored carriers irrespective of the storage conditions show characteristic sharp peaks as indication of crystallinity and absence of a broad, amorphous halo peak as shown in Fig. 2. The diffractograms of both, lactose and mannitol carrier materials, show no humidity induced changes occurring in the crystallinity after storage at elevated relative humidity, suggesting that the presence of water vapour has no effect on the crystal lattice of these carrier particles within the range of the investigation. If those carrier samples had contained an amorphous part before storage it would be expected to recrystallize during storage at elevated relative humidities, resulting in differences of the diffractograms of samples stored under silica gel conditions and at elevated relative humidity. The results obtained by X-ray diffraction come in agreement with the results obtained from the DSC thermograms, where also no storage-induced differences in the crystallinity were observed. Furthermore, DSC and X-ray diffraction techniques will measure the properties of the sample as a whole. The detection limits for amorphous content with such techniques can vary, but will generally have a lower cutoff of 5-10% (Buckton *et al.*, 1999).

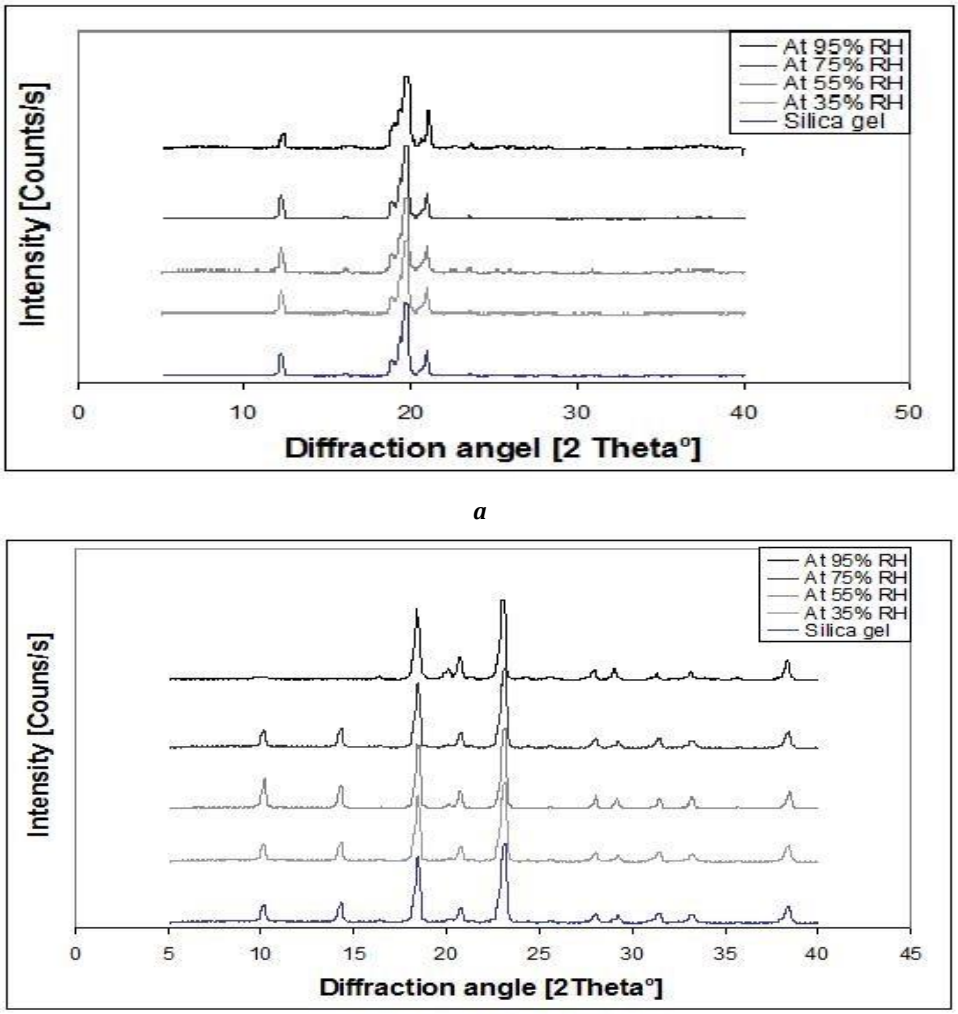
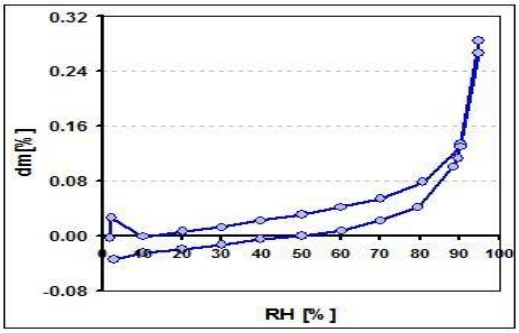


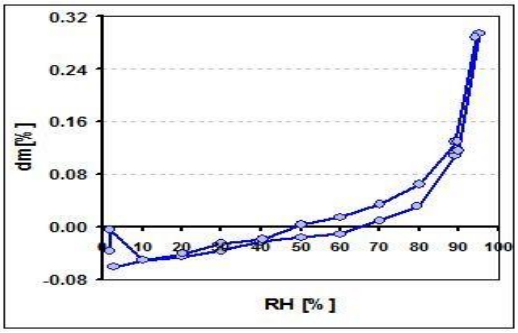
Fig. 2. X-ray diffraction of lactose (**a**) and mannitol (**b**) after storage at different relative humidities for 6 weeks, the second of two measurements is shown.

2.3.3 Investigation of stored carrier surface using water vapour sorption

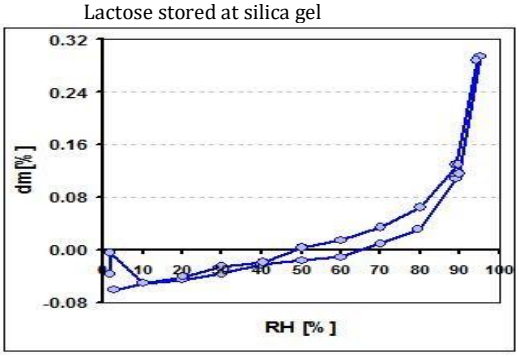
Water vapour sorption is used to investigate the relative moisture sorption of carrier samples. Six weeks storage of lactose at 35% and 55% RH showed an unchanged water uptake with respect to the sample stored under silica gel conditions. This suggests no change can be predicted in the lactose carrier water uptake at those two percent of relative humidities with respect to the sample stored under silica gel conditions. Storage of lactose at 75% and 95% RH (Fig. 3) showed a decrease of water uptake with respect to 35% and 55% RH respectively, which indicates the merging of the carrier fines with the coarse one, which subsequently results in a decrease of the carrier surface area exposed to those relative humidities. This decrease in the carrier surface area results in less water sorption by lactose carrier at 75% and 95% RH. The storage of mannitol carrier at 35%, 55% and 75% RH showed no change of the water uptake with respect to the sample stored under silica gel conditions. This suggests no detectable change in the mannitol carrier water uptake at those relative humidities after 6 weeks storage. However, the storage at 95% (Fig. 3) showed an increase of water uptake, which might be explained that the dissolution of carrier fines into the coarser particles and the aimed smoothing of the mannitol carrier surface needs more quantity of water than lactose carrier fines. Additionally, mannitol is not affected by the elevated relative humidity unless they reach a high level of increase (95% RH). Those results come in agreement with the results obtained from DSC and X-ray diffraction before. Since the water vapour sorption indicates no change in the carrier's crystallinity and proofs the merging of carrier fines to the coarse lactose, which results in smoothing of lactose particle surfaces.



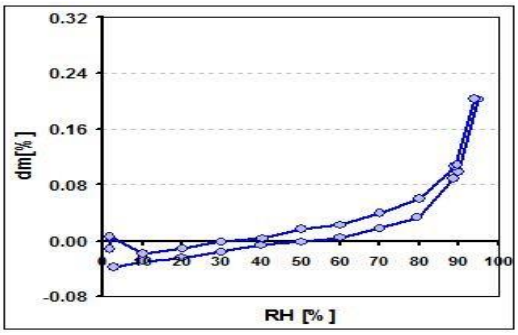
Lactose stored at silica gel



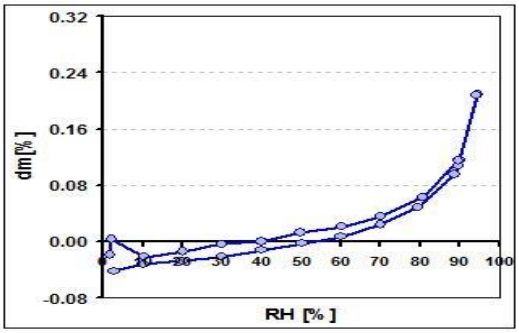
Lactose stored at 35% RH



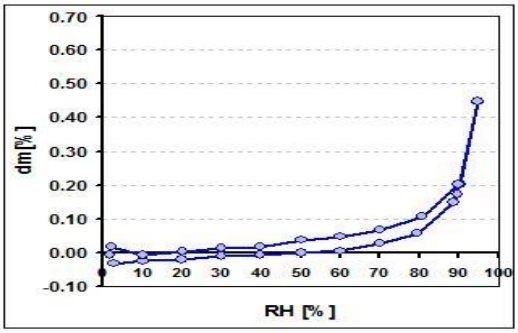
Lactose stored at 55% RH



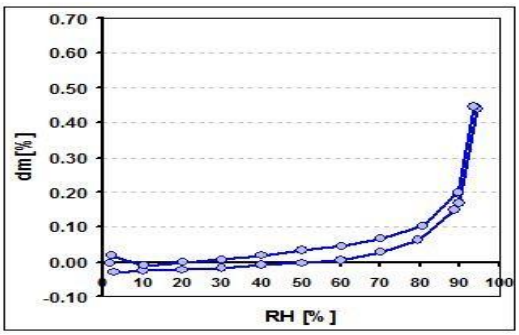
Lactose stored at 75% RH



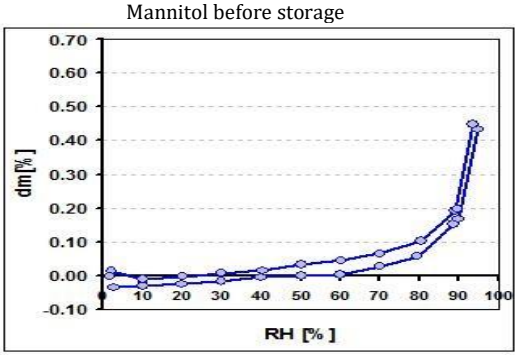
Lactose stored at 95% RH



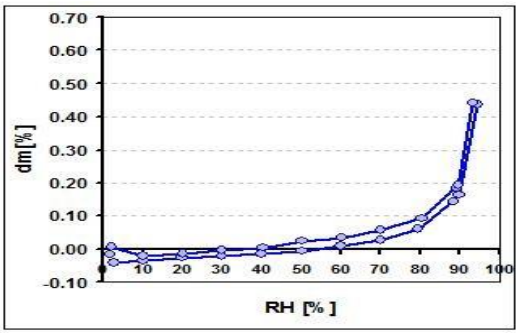
Mannitol before storage



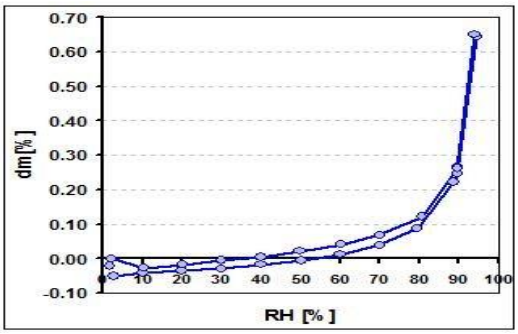
Mannitol stored at 35% RH



Mannitol stored at 55% RH



Mannitol stored at 75% RH



Mannitol stored at 95% RH

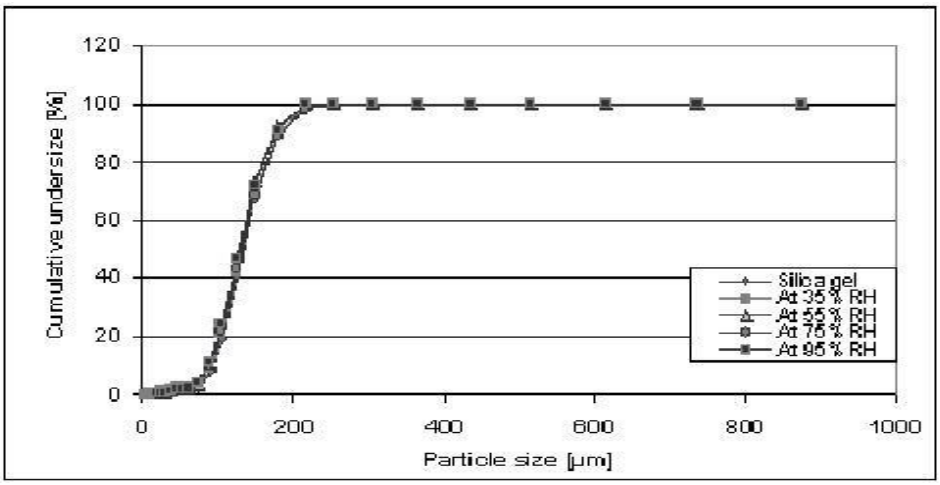
Fig. 3. Water vapour sorption isotherms of lactose and mannitol carrier materials after storage at different relative humidities for 6 weeks.

2.4 Determination of particle size distribution by laser diffraction

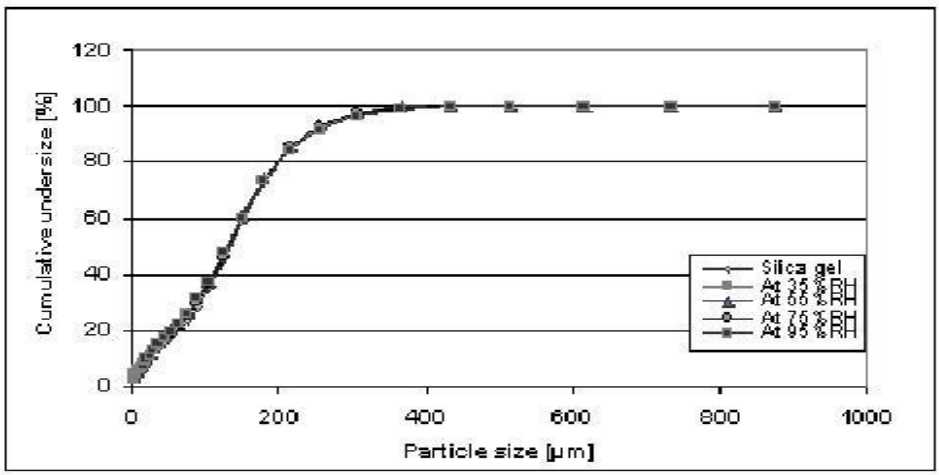
As water uptake, partial dissolution and subsequent interparticulate fusion is not limited to the carrier fines but may also happen to the coarse carrier particles, substantial particle size increase may occur (Maggi *et al.*, 1999). Table 2 and Fig. 4 suggest that no change occurred after storing both carrier materials (lactose and mannitol) at different relative humidities for 6 weeks with respect to the carriers stored at silica gel. However, particle size can influence solubility: smaller particles have a greater curvature, and consequently a higher surface area and energy, which can promote dissolution in a saturated solution (Cleaver and Wong, 2004).

Table 2
Particle size distribution of lactose and mannitol after storage for 6 weeks at elevated relative humidities using laser diffraction (n=3, mean ± SD)

Substance	X_{10}[µm]	X_{50}[µm]	X_{90}[µm]
Lactose stored in silica gel	91.98±1.71	133.48±0.69	184.56±0.71
Lactose stored at 35% RH	91.95 ±0.61	132.96±0.48	183.27±1.04
Lactose stored at 55% RH	88.63±1.23	128.32±1.07	177.49±1.02
Lactose stored at 75% RH	90.14±1.01	130.62±1.03	80.63±3.75
Lactose stored at 95% RH	87.62±1.83	128.06±1.16	177.16±0.95
Mannitol stored at silica gel	23.33±1.38	132.43±2.43	240.04±5.32
Mannitol stored at 35% RH	22.47±2.17	131.35±1.65	238.09±5.12
Mannitol stored at 55% RH	20.81±0.49	130.09±0.42	239.69±2.02
Mannitol stored at 75% RH	22.14±0.55	130.34±1.67	236.77±1.91
Mannitol stored at 95% RH	23.79±2.21	131.16±3.61	246.28±5.07



a



b

Fig. 4. Particle size distribution of lactose (**a**) and mannitol (**b**) after storage for 6 weeks at different relative humidities, the second of three measurements is shown.

This unchanged particle size of both lactose and mannitol carrier material after 6 weeks storage time at different relative humidities (Fig. 4) indicates that interparticle fusion between coarse particles does not take place at least.

2.5 Characterization of particle shape and surface by scanning electron microscopy

Moisture uptake and loss due to changes in relative humidity can result in local dissolution and recrystallization, leading to irreversible aggregation through solid bridge formation (Dunbar *et al.*, 1998), which might be detected by scanning electron microscopy (SEM). Therefore, the morphology and surface characteristics of lactose and mannitol were investigated using SEM. The scanning electron micrographs show, that there is no significant difference of the surface of carriers stored under silica gel conditions and those stored at different elevated relative humidities (Fig. 5). Although the interparticulate fusion of the carrier surface fines with the coarse carrier surface following a water uptake. Partial dissolution and subsequent solid bridge formation, induced by storage at elevated relative humidities and subsequent decrease of the relative humidity has been expected, obviously, the extent to which this phenomenon occurs is too small to be reliably detected by SEM if there is any effect at all.

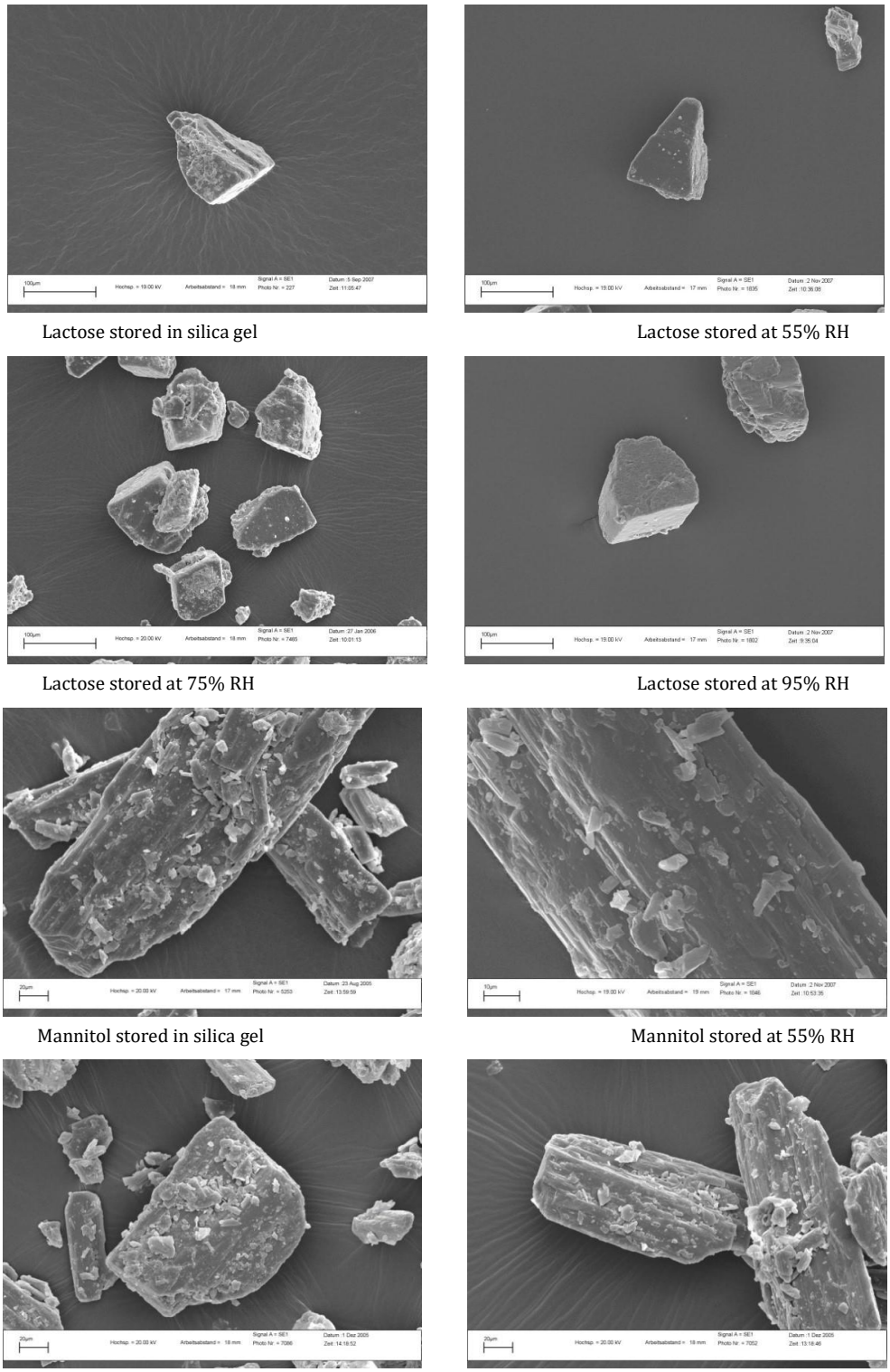


Fig. 5. Scanning electron micrographs of lactose and mannitol after storage for 6 weeks at different relative humidities.

2.6 Determination of surface area by BET measurement

The specific surface area of the lactose and mannitol carriers was measured by multi-point Brunauer-Emmett-Teller (BET) nitrogen adsorption. It is expected, that humidity induced merging of the carrier surface fines with the coarse carrier surface will result in a decrease of the specific surface area.

Fig. 6 shows a significant decrease of the lactose carrier surface area after 6 weeks storage at 95% RH with respect to the sample stored under silica gel conditions, which is an indication for an increased smoothness of the lactose carrier material stored at this relative humidity. The surface area of mannitol shows a significant decrease at 55% RH and above after 6 weeks storage. This significant decrease in the carrier surface area of lactose and mannitol after 6 weeks indicates enhanced smoothness of the carrier surface due to merging of carrier fines with the coarse carrier. Those results come in agreement with the results obtained by the water vapour sorption technique in case of lactose carrier material, while the mannitol carrier exhibited a decrease of the specific surface area with BET technique which was not so predictable with the water vapour sorption technique.

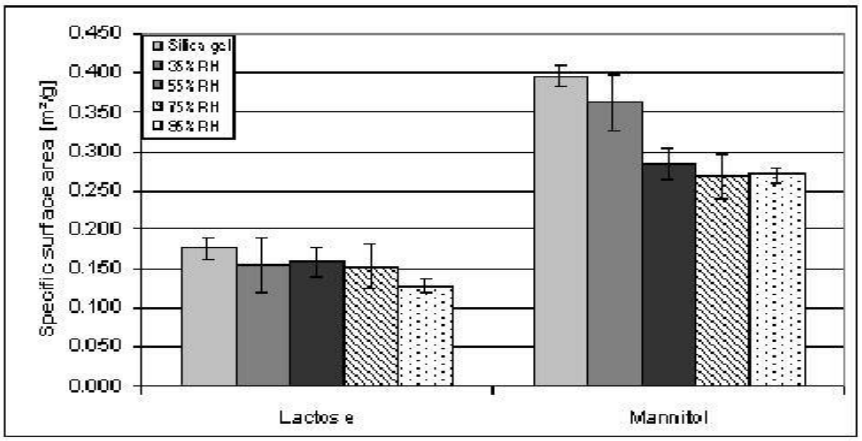


Fig. 6. Specific surface area of lactose and mannitol carriers after storage for 6 weeks at different relative humidities determined by nitrogen adsorption, n=3, mean ±SD.

2.7 *In vitro* deposition test

The results obtained from the *in vitro* deposition tests are shown in Fig. 7 and are represented as fine particle fraction. Fig. 8 depicts the mass of the delivered dose. A plot of the fine particle fraction depending on 6 weeks storage time at different relative humidities is presented in Fig. 7. Those plots suggest that a change occurred due to storage at higher relative humidities in comparison to the sample stored under silica gel conditions. However, the delivered dose shown in Fig. 8 has not been markedly changed after storage at different relative humidities. The relationship between the increased relative humidity and the unchanged performance of both carrier materials up to 75% RH could be attributed to non-hygroscopic nature of those two carrier powders. This property of the lactose and mannitol carriers can be considered a useful character of both carriers to the ensure stability of dry powder formulation against the storage at elevated humidity. On the other hand, storage at 95% RH of both carrier materials shows a significant decrease in the fine particle fraction with respect to the samples stored under silica gel conditions, which indicates the serious role of this high relative humidity in reducing the *in vitro* deposition after 6 weeks storage time. This reduction suggests that particle smoothness is achieved by storage of lactose and mannitol carrier materials at 95% RH. This carrier surface smoothness was also predicted with the BET surface area measurement for lactose and mannitol carrier substances. Additionally, the water vapour sorption has indicated the smoothness of lactose carrier surface, while mannitol carrier still takes a high quantity of water at this high relative humidity (95% RH) to achieve the aimed smoothness. Storage of carrier materials at elevated relative humidities may allow water vapour to condensate in the capillaries that exist between individual particles. Furthermore, highly soluble materials such as lactose and mannitol may undergo limited dissolution at interparticulate contact points with subsequent solidification, thus resulting in solid bridge formation between particles, leading to particulate fusion. This fusion results in carrier particles with smoother surface. The enhanced smoothness provides a higher contact area with the drug particles yielding stronger adhesion forces and finally reducing the FPF. This reduction in the FPF might be related to the formation of capillary forces between salbutamol sulphate and the modified carrier surfaces in comparison to the unmodified carrier surfaces (Peng *et al.*, 2016).

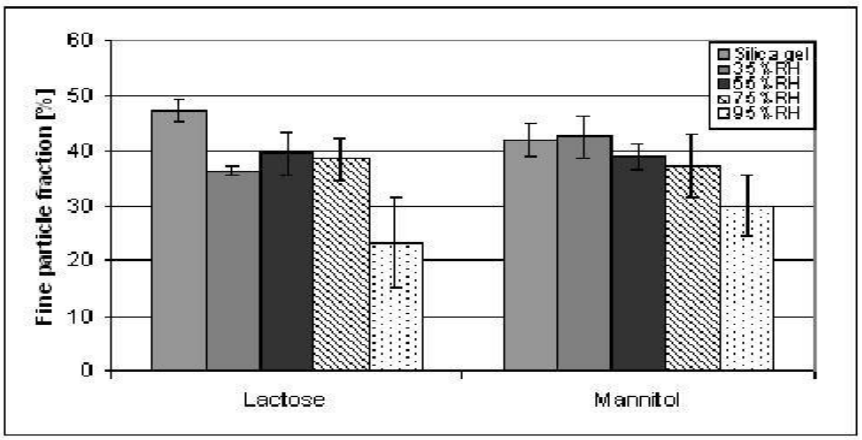


Fig. 6. Fine particle fraction of mixtures of salbutamol sulphate mixed interactively with lactose and mannitol carriers after 6 weeks storage at different relative humidities, n=3, mean ±SD.

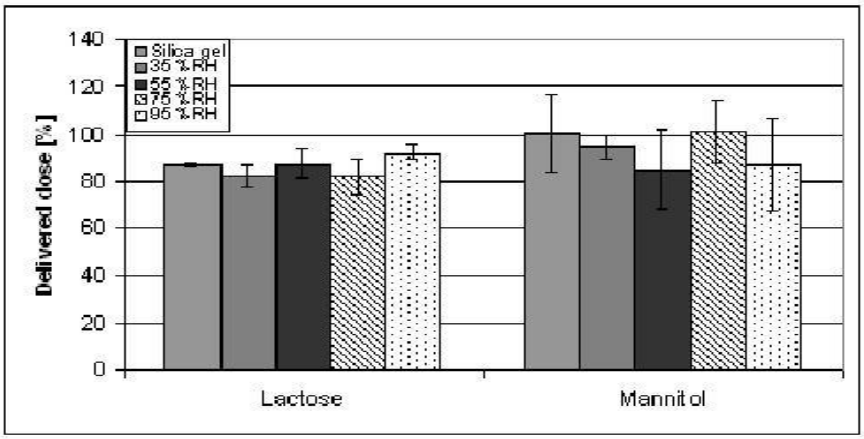


Fig. 7. Effect of humidity on the delivered dose of salbutamol sulphate mixed interactively with lactose and mannitol carriers before and after 6 weeks storage time n=3, mean ±SD.

3. Conclusion

It can be concluded that the storage of lactose and mannitol carrier materials at different relative humidity up to 75% RH has not improved the *in vitro* deposition of the carrier used in DPI based formulations. Additionally, storage of those carrier materials at 95% RH reveals a significant decrease in the fine particle fraction. This might be explained by the assumption that during storage the fine particles of the carrier itself will fuse with the coarse carrier surface leading to a smoother surface, which provides a higher contact area with the drug particles yielding stronger adhesion forces and finally reducing the FPF. Lactose and mannitol carrier materials showed a smoothed surface and no change in the crystallinity after this storage of the carrier materials before the formulation with the active constituent. This storage conditions might resemble what happened to both carriers when lifted under different environmental humidities before blending with the drug in the ordered mixture. Further investigation for a longer time of storage at elevated relative humidities must be carried out to predict to which extent the surface smoothness would affect the drug *in vitro* deposition.

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