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Optimization of a Dry Powder Inhaler Formulation of Nacystelyn, a New Mucoactive Agent

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Abstract

The aim of this study was to optimize a dry powder inhaler formulation containing a new mucoactive drug, nacystelyn. Formulations were made using three types of lactose, crystalline α-lactose, spray-dried lactose and a roller-dried anhydrous β-lactose. The roller-dried anhydrous β-lactose possessed the most adequate surface properties, resulting in a significantly higher ($P < 0{\cdot}05$) in-vitro lung deposition of nacystelyn than the conventional crystalline α-lactose and spray-dried lactose. The particle size distribution of roller-dried β-lactose was optimized also. Within the size ranges tested (63–100, 90–125 and 100–160 μm), the coarser the lactose, the higher the in-vitro deposition of the drug (up to 40%). In contrast, the in-vitro lung deposition of 100–160 μm roller-dried β-lactose was very low (< 0·5%), so limiting the potential risk of lung irritation due to the carrier. The influence of the ratio of active ingredient/excipient (w/w) was also investigated. No difference was observed for mixtures from 1 : 2 to 1 : 4 while higher dilutions (1 : 5 and 1 : 6) showed significantly ($P < 0{\cdot}005$) lower deposition results. Finally, the influence of the airflow rate was assessed. No dependence of the fine particle dose was observed between 40 and 80 L min^{-1} while significantly higher results were obtained at 100 L min^{-1}.

The dry powder inhaler formulation of nacystelyn using the unusual roller-dried anhydrous β-lactose resulted in very high and reproducible in-vitro deposition results. However, the latter needs to be confirmed by in-vivo studies.

In the treatment of lung disease aerosol inhalation is a well established method of drug delivery to the respiratory tract. This route of administration has several advantages. It has a rapid and predictable onset of action, it requires a lower dose than that used by the oral route with consequently less side effects, and it avoids drug interaction and gastrointestinal or hepatic degradation (Timsina et al 1994). Three delivery systems are commonly available, nebulizers, metered dose inhalers (MDI) and dry powder inhalers (DPI). MDIs containing chlorofluorocarbon (CFC) gases have been the inhalation systems most prescribed for some years, despite a number of associated inconveniences (Daly 1992). There is the need for good co-ordination between the patient's inspiratory effort and the actuation of the aerosol, which makes their correct use difficult in a large proportion of patients (Crompton 1982). The dose emitted from MDIs is limited to a few milligrams and the respirable fraction is low (10% of the nominal dose). Furthermore, the propellants may provoke bronchoconstriction, and it has been shown that their accumulation in the atmosphere destroys the ozone layer and they act as greenhouse gases. This last factor has led to a progressive but definitive reduction of CFC-containing aerosols. Consequently, three possibilities are offered to formulators for replacing them. Firstly, there are the CFC-free MDIs that are in effect limited to the use of hydrofluoroalkane gases, and still have the problem of coordination as mentioned above, together with other potential ecological problems (the greenhouse effect). Secondly, there are nebulizers, which have undergone considerable technological improvement (Weston et al 1991), but are cumbersome and uncomfortable for the ambulatory patient's use. There has therefore been a growing interest by pharmaceutical companies in the third possibility, the development of DPI formulations. With DPIs, the problem of coordination does not

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occur since the delivery of the drug is driven by patient inhalation Clark 1995). Consequently, the lung deposition obtained may depend on patient inspiratory flow, which is a problem in severely ill asthmatics, elderly people or young children. There are two main types of DPIs available, predispensed or single dose systems which use capsules or blisters to contain one dose, and reservoir or multidose systems which remove a dose by some means from a powder mass equivalent to many doses and present this to the patient (EMEA 1998).

Lung deposition after a DPI inhalation is mainly governed by three parameters: the patient, the device and the formulation (Ganderton & Kassem 1992). The contribution of the patient and of the device, although of great importance, will not be discussed here. However, it must be remembered that the choice of the device and the formulation are inextricably linked. The DPI formulation composition is generally simple in that it involves the mixture of the active ingredient with only one or, at most, two excipients. In some cases the active ingredient is formulated alone, for instance sodium cromoglycate is "pelletized" in weak and redispersible agglomerates without the need of any excipient (Edwards & Chambers 1989). But most often the micronized active ingredient is mixed with a coarse and inert ingredient in order to increase the flowability of the composition. This design corresponds to the theory of ordered mixtures (Hersey 1970). The choice of the carrier excipients is very limited because of the lack of toxicological data. Until now, mono- or disaccharides (and most often lactose) are the only inactive ingredients accepted in marketed DPI drugs. The main parameters influencing the drug deposition are: size and surface roughness of the active ingredient particles and nature (α or β isomeric form), size and surface roughness of lactose particles, ratio of active ingredient to lactose and the manufacturing process of the drug (allowing the control of relative humidity and also for limiting creation of electrostatic charges).

L-Lysine-*N*-acetylcysteinate or nacystelyn is a novel mucoactive agent possessing mucolytic, antioxidant and anti-inflammatory properties (Tomkiewicz et al 1994; Vanderbist et al 1996; Nagy et al 1997). It is currently being tested in phase 3 clinical trials for the treatment of cystic fibrosis. Chemically, it is a salt of L-lysine and *N*-acetylcysteine. This drug appears to present an activity superior to its parent molecule *N*-acetyl cysteine because of a synergistic mucolytic activity of L-lysine and *N*-acetylcysteine. Furthermore, because of its almost neutral pH (6·2) in solution, it can be administered to the lungs as an inhalation powder form with a very low incidence of bronchospasm, unlike the acidic *N*-acetylcysteine (pH 2·2) (Tomkiewicz et al 1996). Nacystelyn is very difficult to formulate as a DPI because the micronized drug is very hygroscopic and sticky, and the optimal pulmonary dose is relatively high (2 mg). The aim of this study was to optimize the formulation of nacystelyn as a powder for inhalation to enable a large category of patients to inhale the therapeutic dose of the drug in a reproducible way.

Materials and Methods

Materials

Nacystelyn ($C_{11}H_{23}N_3O_5S$; Freedom Chemical Diamalt, München, Germany) was micronized using a Coball-Mill MS-12 (Fryma, Switzerland) crushing machine. The particle size distribution of the powder obtained was checked by laser diffraction (Mastersizer X, Malvern, Meyvis, The Netherlands). Nacystelyn was delivered from a monodose DPI (Miat monodose inhaler) (Cocozza 1976). Different sorts of lactose (Pharmatose DCL 21, Pharmatose DCL 11 and Pharmatose 325 M) were purchased from DMV International (Veghel, The Netherlands). When necessary, the lactose samples were sieved using a vibrational sieve shaker (Fritsch, Analysette, Germany). Scanning electron photographs were obtained using either an Electroscan ESEM 2020 (Electroscan, USA) (Figure 1) or a GSMB 840 Jeol microscope (Jeol, Japan) (Figure 2). The powder was scattered on a thin film of a two-component epoxy resin and coated with a layer of gold ($\pm$ 300 Å). The acceleration voltage during observation was 15 kV. The mapping was obtained by retrieving the nacystelyn sulphur atom from the picture. The flowability of lactose powder samples was assessed using a tap density tester (Jolting Volumeter Model SVM 2/UZ, Erweka). A graduated cylinder was filled slowly with approximately 100 g powder. The cylinder was first tapped 10 times and the volume occupied by the powder was determined (V_{10}). The cylinder was then tapped 500 times and the volume recorded (V_{500}). Hausner's ratio, given by the relation V_{10}/V_{500}, was calculated. The lower Hausner's ratio, the better the flowability of the powder.

Assay

Nacystelyn was assayed using a validated titrimetric method. Mercury (II) nitrate 5.10^{-5} M

(Merck, Darmstadt, Germany) was the titrating agent (redox reaction) used with an automatic titrator (Mettler DL 67) equipped with an automatic sample changer (Mettler ST 20A). Lactose was assayed using a liquid chromatographic method coupled with mass spectrometric detection (LC-MS-MS). Briefly, the liquid chromatograph (HP 1100, Hewlett-Packard) was coupled to a mass detector (API 300, PE, Sciex). The stationary phase was a Nucleosil $5\,NH_2$, $5\,\mu m$, $125\,mm \times 4\,mm$ (Macherey-Nagel) and the mobile phase was methanol/ammonium acetate (50 mM), 7 : 3.

Preparation of nacystelyn capsules

Nacystelyn was mixed with the excipient manually in a mortar without crushing. For the tests involving a mixture of coarse and fine lactose, the lactose was mixed before adding the active ingredient. The fine lactose used was a crystalline monohydrate α-lactose (Pharmatose 325 M, DMV Internationals, The Netherlands), sieved so that the particles were $< 38\,\mu m$. The homogeneity of each mix was assessed using the titrimetric method described above before the deposition tests. Only mixtures presenting a homogeneous nacystelyn content between 90 and 110% were accepted. N°3 hard gelatin capsules (Snap-Fit, Capsugel, Bornem, Belgium) were then filled manually with $30 \pm 1\,mg$ powder mixtures using analytical scales (Mettler Toledo).

In-vitro deposition tests

A glass impinger and a multistage liquid impinger (both Copley Instruments, Nottingham, UK) were used for the in-vitro deposition tests. The test was generally performed at $60\,L\,min^{-1}$ and the suction time was 4 s unless otherwise indicated. The main parameters resulting from the deposition tests were for the glass impinger, the fine particle fraction (FPF), i.e. the fraction (%) of nacystelyn retrieved from the lower stage of the apparatus (cut-off diameter $= 6{\cdot}4\,\mu m$) expressed as a percentage of the nominal dose, and for the multistage liquid impinger, the fine particle dose (FPD), i.e. the dose (mg) of nacystelyn per capsule having a diameter less than $5{\cdot}0\,\mu m$ (extrapolated from the curve of cumulative mass vs cut-off diameter).

Statistical analysis

Student's *t*-test ($P = 0{\cdot}05$) was performed to compare the means while the analysis of variance test ($P = 0{\cdot}05$) was performed to assess the dependence on the airflow rate.

Results and Discussion

Influence of the nature of the carrier

Four types of lactose were compared for the in-vitro deposition of nacystelyn in the glass impinger i.e. crystalline α-lactose 325 mesh, crystalline α-lactose monohydrate (63–100 μm), spray-dried α-lactose monohydrate (63–100 μm), and roller-dried anhydrous β-lactose (63–100 μm). These last three sugars differ in their manufacturing process resulting in different surface properties (roughness), shape and flowability of particles (Figure 1). The spray-dried and roller-dried lactose are conventionally used for direct compression of tablets or wet granulation. The purpose in sieving the lactose samples in the range 63–100 μm was first to increase the flowability of powder mixtures and thus the output of the drug from the device, and secondly to remove the influence of the carrier size on deposition. Results of the in-vitro deposition studies are shown in Table 1. The crystalline α-lactose 325 mesh was found to give the lowest deposition results compared with the three other types. The particle size distribution of lactose is apparently important since the FPF was nearly twice as high for the crystalline α-lactose (63–100 μm) than for the crystalline α-lactose 325 mesh ($P < 0{\cdot}005$). The roller-dried anhydrous β-lactose gave the best deposition, significantly higher than

Table 1. In-vitro deposition results (glass impinger, $60\,L\,min^{-1}$) of 1 : 4 (w/w) of nacystelyn/lactose mixtures (30 mg nacystelyn/capsule).

	Crystalline α-lactose 325 mesh	Crystalline α-lactose (63–100 μm)	Spray-dried α-lactose (63–100 μm)	Roller-dried β-lactose (63–100 μm)
Device (mg)	$6{\cdot}3 \pm 1{\cdot}4$	$5{\cdot}1 \pm 1{\cdot}2$	$4{\cdot}9 \pm 0{\cdot}9$	$5{\cdot}6 \pm 1{\cdot}2$
Upper stage (mg)	$4{\cdot}6 \pm 1{\cdot}2$	$5{\cdot}8 \pm 1{\cdot}6$	$6{\cdot}2 \pm 1{\cdot}4$	$5{\cdot}8 \pm 1{\cdot}4$
Lower stage (mg)	$3{\cdot}2 \pm 0{\cdot}6$	$5{\cdot}2 \pm 1{\cdot}1$	$5{\cdot}5 \pm 0{\cdot}8$	$5{\cdot}9 \pm 0{\cdot}7$
FPF (%)	$17 \pm 1{\cdot}7$	29 ± 4	31 ± 6	$33 \pm 1{\cdot}8$

Three capsules were used per test. The values are mean ± s.d., n = 5.

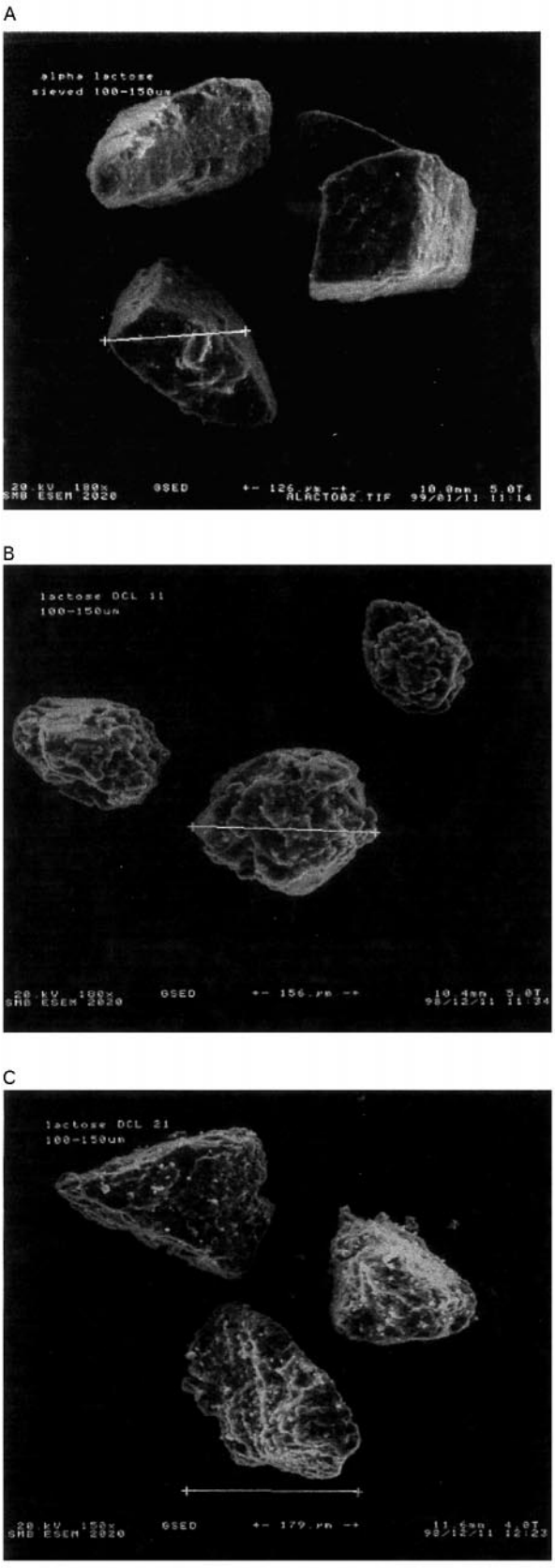

Figure 1. SEM photographs of lactose powder samples of (A) crystalline α-lactose monohydrate (100–160 μm), (B) spray-dried α-lactose (100–160 μm) and (C) roller-dried anhydrous β-lactose (100–160 μm).

those obtained with spray-dried lactose ($P = 0{\cdot}049$) and crystalline α-lactose ($P = 0{\cdot}002$). Those differences may be explained by the surface properties of the lactose as shown in Figure 1. Indeed, ideally the active ingredient must bind to the carrier sufficiently strongly to allow the production of a stable homogeneous powder mixture but it must also be weak enough to permit the redispersion of "respirable particles" during inhalation (Moren 1987). The crystalline α-lactose monohydrate particles present a smoother surface than the roller-dried anhydrous lactose (Figure 1). It is thought that the lower deposition observed with the crystalline β-lactose monohydrate particles is due to too weak bonds with nacystelyn resulting in the formation of free drug agglomerates which are not redispersible during inhalation. Spray-dried lactose shows a rougher surface than roller-dried anhydrous β-lactose which may lead to non-reversible bonds and consequently to a lower FPF. Figure 2 shows a particle of lactose from a mixture of nacystelyn with roller-dried anhydrous β-lactose (1 : 4) and the localization of micronized particles of nacystelyn on the carrier particle. It can be seen that the attachment of nacystelyn on the lactose particles is not homogeneous which presumably means that the surface of the particle carrier presents a range of sites of different affinity. This may be explained by the theory of Staniforth (1996) that lactose particles possess high energy adhesion sites (HA) which are able to bind strongly to the active ingredient, and low energy adhesion sites (LA) which allow the formation of more reversible bonds.

Influence of carrier particle size

Since the particle size distribution of lactose seems to be very important for the in-vitro deposition of nacystelyn, three particle size ranges of roller-dried anhydrous β-lactose (63–100, 90–125 and 100–160 μm) were tested (Table 2). Clearly, deposition in the lower stage of the glass impinger was increased when the carrier particle size was increased. Significant differences ($P < 0{\cdot}05$) were observed between each size fraction. This is in part explained by the analysis of the Hausner's ratios calculated from the different samples of roller-dried anhydrous β-lactose. Hausner's ratio for roller-dried anhydrous β-lactose 100–160 μm was $1{\cdot}14 \pm 0{\cdot}02$, for 90–125 μm it was $1{\cdot}16 \pm 0{\cdot}01$, and for 63–90 μm the ratio was $1{\cdot}35 \pm 0{\cdot}02$ ($n = 5$). The flowability of the various samples was clearly related to their particle size distribution. This is in accordance with the results of flowability obtained by Staniforth (1995). The improvement of the

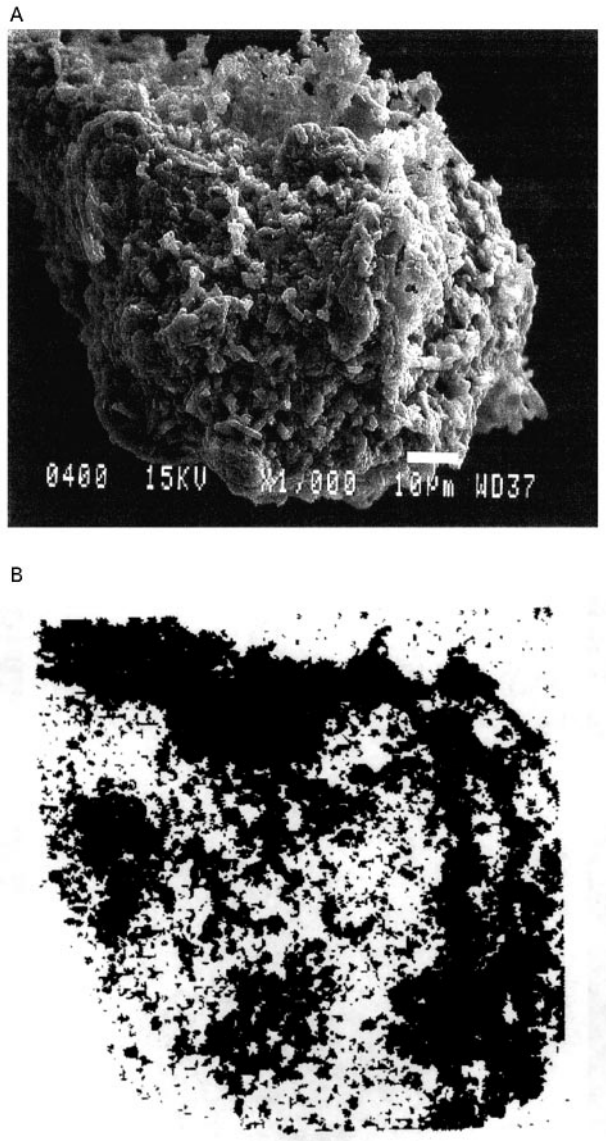

Figure 2. SEM photographs of a 1 : 4 (w/w) nacystelyn/lactose powder mixture. (A) Electronic photograph of a roller-dried anhydrous β-lactose particle (100–160 μm) recovered by micronized particles of nacystelyn and (B) mapping of nacystelyn molecule on the lactose particle.

Table 2. In-vitro deposition results (glass impinger, 60 L min^{-1}) of 1 : 4 (w/w) nacystelyn/roller-dried β-lactose mixtures (30 mg nacystelyn/capsule).

	Roller-dried β-lactose		
	63–100 μm	90–125 μm	100–160 μm
Device (mg)	5·6 ± 1·2	5·1 ± 1·1	4·8 ± 1·2
Upper stage (mg)	5·8 ± 1·4	4·8 ± 0·9	4·6 ± 1·1
Lower stage (mg)	5·9 ± 0·7	7·0 ± 1·5	7·6 ± 1·0
FPF (%)	33 ± 1·8	38·7 ± 2·1	42·0 ± 2·3

Three capsules were used per test. The values are mean ± s.d., n = 5.

flowability properties of the powder may give higher in-vitro lung deposition results by improving the output of the drug from the device during inhalation. Nevertheless, the influence of the particle size of the carrier depends on the active ingredient considered and can not be generalized to other molecules. For nacystelyn, the use of a coarse roller-dried anhydrous lactose seems to be the best choice for the nacystelyn formulation. Such a choice may have another advantage for the patient since large particles of lactose are thought to be unable to reach the lungs. This may help to avoid any allergenic or irritant effect.

A multistage liquid impinger test was performed on the coarse roller-dried anhydrous β-lactose to assess which fraction of the inactive ingredient was retrieved on the lower stages of the apparatus. This result was compared with the conventional crystalline lactose 325 mesh. As predicted, the deposition of lactose in the lower stages of the multistage liquid impinger was very low. The FPF representing less than 0·6% of the nominal dose. The results obtained with the crystalline 325 mesh lactose (1·0% of the nominal dose) was statistically superior ($P < 0{\cdot}05$) but this difference had no consequence with regard to safety as the absolute amount deposited on the respirable stages was still very low.

Influence of the nacystelyn : lactose ratio on the deposition

Nacystelyn : roller-dried anhydrous β-lactose ratios from 1 : 2 to 1 : 6 (w/w) were assessed to examine the influence of this parameter on deposition. The glass impinger was used. The FPF (%) (mean ± s.d., n = 5) were as follows; 39·2 ± 1·2 for 1 : 2, 39·6 ± 1·1 for 1 : 3, 40·4 ± 2·4 for 1 : 4, 24·5 ± 2·2 for 1 : 5, and 21·3 ± 21·2 for 1 : 6. Ratios 1 : 2 to 1 : 4 gave quite similar results while for the lower ratios (from 1 : 5) the FPF were very significantly lower ($P < 0{\cdot}005$). Again the hypothesis of Staniforth (1996) helps to explain these data. When the proportion of nacystelyn decreases in the mixture, the HA sites are occupied first. Consequently, the proportion of irreversible bonds is increased resulting in a lower FPF. To test this hypothesis, Staniforth proposed that the HA sites should be saturated with smaller lactose particles before the mixing with the active ingredient, which can then bind only to the LA sites. Zeng et al (1996) obtained significantly higher deposition results with salbutamol by using a mixture of coarse and fine lactose. We used a lactose monohydrate > 38 μm to saturate the HA sites of the roller-dried anhydrous β-lactose (100–160 μm). Two lactose ratios were assessed 1 : 9 and 1 : 5 (w/w). For nacystelyn, the FPF was not sig-

nificantly modified by this procedure. Nevertheless, some preliminary results obtained on other drugs, acting at low doses (e.g. corticosteroids, β_2-agonists), demonstrated that it was possible to increase the FPF twofold using fine lactose particles (results not shown). For nacystelyn, the ratio 1 : 4 was the best choice for avoiding scaling-up problems. Indeed higher ratios produced a very cohesive mix, due to the high content of micronized powder, making the automatic filling of capsules unreliable and unreproducible.

Influence of the airflow on the deposition

As cystic fibrosis is a hereditary disease, treatment must be available for the widest possible population including young children. Therefore, the influence of the airflow rate on deposition was assessed. As seen in Table 3, there was no significant influence of the airflow rate on the deposition between 40 and 80 L min^{-1} but a significant influence ($P < 0{\cdot}005$) was observed when the airflow was increased to 100 L min^{-1}, resulting in a higher FPF. This dependence on the airflow may be considered to be unimportant when compared with the results obtained with high or medium resistance devices (Zanen et al 1992). This is in part due to the characteristics of the Miat monodose inhaler, which is a low resistance device (Clark & Hollingworth 1993), and to its aerodynamic properties which produce high turbulence during inhalation, so favouring the separation between the drug and the excipient particles. However, the surface characteristics of the carrier particles are also of importance. This disaggregation must occur at low airflows to ensure that even children and severely ill patients are able to take the therapeutic dose when inhaling in the correct manner.

The roller-dried anhydrous β-lactose chosen produced a high and reproducible in-vitro deposition of nacystelyn, which needs to be confirmed by in-vivo studies. It also needs to be established whether this novel approach of formulation can be applied to other hygroscopic drugs.

Table 3. Influence of the airflow rate on fine particle dose (mg) and fine particle fraction (% of the nominal dose) of a 1 : 4 (w/w) nacystelyn : roller-dried lactose (100–160 μm) mix on the multistage liquid impinger (4 L of air inhaled). Each capsule contained 30 mg powder.

Stage	Airflow rate (L min^{-1})			
	40	60	80	100
Device (mg)	2·82 ± 0·85	3·08 ± 0·74	2·52 ± 1·20	2·46 ± 0·75
Throat (mg)	1·75 ± 0·36	2·72 ± 0·26	2·58 ± 0·33	3·20 ± 0·50
Stage 1 (mg)	5·91 ± 0·44	5·84 ± 0·38	5·64 ± 0·68	5·66 ± 0·73
Stage 2 (mg)	2·12 ± 0·23	2·97 ± 0·21	3·27 ± 0·37	4·00 ± 0·51
Stage 3 (mg)	5·05 ± 0·34	4·32 ± 0·37	4·27 ± 0·40	4·69 ± 0·39
Stage 4 (mg)	1·70 ± 0·15	1·66 ± 0·32	1·21 ± 0·15	1·31 ± 0·33
Filter (mg)	0·10 ± 0·02	0·11 ± 0·05	0·03 ± 0·01	0·04 ± 0·01
FPD (mg)	1·81 ± 0·09	1·88 ± 0·15	1·96 ± 0·17	2·42 ± 0·26

Values are mean ± s.d., n = 5.

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