International Journal of Pharmaceutics, 101 (1994) 81–87

IJP 03318

Development and use of an in vitro system to evaluate inhaler devices

Ralph W. Niven [a,b], Fred D. Lott [a], Anna Y. Ip [a], Kanchana D. Somaratne [a] and Malcolm Kearney [b]

[a] *Amgen Inc., 8-1-A 215, 1840 Dehavilland Dr., Thousand Oaks, CA 91320 (USA) and* [b] *Techmedica, 1380 Flynn Rd, Camarillo, CA 93012 (USA)*

(Received 7 January 1993)
(Modified version received 8 April 1993)
(Accepted 24 May 1993)

Key words: Aerosol; Artificial throat; Deposition; Dry powder inhaler; Lung; Metered dose inhaler

Summary

An in vitro system was developed to better emulate particle deposition in the respiratory tract. Inhalers were connected to a glass throat (BP 1988 Appendix XVII C) or to a silicone throat that exactly duplicated the surface geometry of the oral and pharyngeal cavity. This throat was created from a direct impression of the mouth and CAT scans of a patient's head and neck and could be separated into three parts. This allowed deposition patterns to be observed. Adapters were fabricated so that the outlet of either throat could be connected to a collection filter unit or any one of several sizing instruments. A mass flow meter enabled airflow to be monitored. Airflow was produced from a vacuum pump or by human inhalation. A Rotahaler was tested using capsules containing 20 mg of spray dried mannitol:sorbitol:carboxyfluorescein (CF) 10:1:0.01 of 3.4 μm MMAD. The vacuum pump was set at 30, 60 or 120 l/min air flow for 4 s. The surface of the glass or silicone throat was left 'dry' or was coated with a polyethylene glycol mixture to better represent the 'wet' surface of the oral cavity. Powder in the device, filter unit and throat(s) was quantified by assay of the CF using spectrofluorimetry. The recovery of the weighed dose was 98.6 ± 8.9% (n = 83). The dose emerging from the inhaler was dependent on the flow rate (Anova, $p < 0.01$) and was 1.0 ± 0.2 mg (30 l/min, n = 25), 5.1 ± 0.5 mg (60 l/min, n = 30) and 6.2 ± 0.6 mg (120 l/min, n = 28). However, the percentage of this dispensed dose recovered from the filter unit (lung) was independent of the flow rate and only varied with the type and condition of the throat used (Anova, $p < 0.05$). The mass deposition in the throats was ranked: glass-dry < silicone-dry < glass-wet < silicone-wet. The results indicate that the use of a wet artificial throat, modeled on human anatomy, will provide a more conservative estimate of lung deposition compared to a glass throat when used with a dry powder inhaler.

Introduction

A high percentage of the dispensed dose from propellant based metered dose inhalers and powder inhalers deposits in the oral cavity. It is important to minimize this deposition so that the potential dose reaching the lung can be increased and discomfort to the patient, such as an unpleasant taste and the potential for coughing or gagging, can be minimized. Furthermore, any reduction in deposition will allow a reduction in dosing requirements which will benefit patient and manufacturer in terms of cost.

Correspondence to: R.W. Niven Amgen Inc., 8-1-A 215, 1840 Dehavilland Dr., Thousand Oaks, CA 91320, U.S.A.

The use of artificial throats to estimate particle losses in the oropharynx after administering doses from inhaler devices, is not new. However, they are generally of primitive design and may simply be constructed of a right angled bend in a pipe (Martin et al., 1988; Vidgren et al., 1988) or of a glass bulb with inlet and outlet positioned at right angles to each other (British Pharmacopoeia (BP), 1988). Even 'anatomically' correct models are generally blown from glass (Sciarra and Cutie, 1978), where the nature of the surface as well as its geometry provide a poor representation of a human throat. Consequently, the fraction of an inhaled dose impacted on the surface of these artificial throats may be very different to that found in patient use for different inhaler devices.

As a premise to optimization of the performance of inhaler devices and to relate in vitro deposition data to what might be expected in vivo, an artificial throat model was created from silicone elastomer that duplicated the surface geometry of a human throat. In this study the model was compared against a 'standard' glass throat (BP 1988 apparatus 'A', Appendix XVII C) using the Rotahaler as a test inhaler device.

Materials and Methods

Artificial throats

A silicone elastomer throat was created from a fusion of two people's airways. The 'neck' is that of a 55 year old female. The details were obtained from computer aided tomography scans of the hard palate to the middle of the chest. Unfortunately, the protocol for standard neck scans requires the patient breathe through their nose. This put the patient's tongue on their palate. In addition, the scan was shot in 5 mm thicknesses with 5 mm intervals causing exaggerated image diffusion through metal dental work. It was therefore decided to attach an image of a different patients mouth to the neck scan data of the woman. The mouth was that of an adult male. For this 'second' mouth the tongue was oriented in a position that would be typical during inhalation of an aerosol product. A direct impression was taken of the mouth allowing excellent detail of the oral cavity to be obtained. A plug was inserted into the throat and the mouth was then filled with alginate. Molds were taken of the alginate casting and a mock-up of the 'positive' inverse mouth was poured in polyurethane resin. This mock-up was attached to the neck scan model in an anatomically correct fashion. The neck scan model was fared and clay was added to smooth the transition between the two castings. Sufficient detail from the scans was not available to yield a true representation of the epiglottis region. A mock-up of the area was created based upon the available scan data and the anatomical literature. The mock-up was fabricated in wax and then pressed into clay, leaving a finished impression. The trachea and pharynx were expanded slightly to more closely match the scale of the mouth. In addition, since alginate extruded beyond the plug while the mouth impression was being taken, some reached into the nasopharynx which allowed an impression of a nasopharynx 'leg' to be incorporated into the model. The junction with the oropharynx was anatomically correct. A silicone block mold was prepared from the final hard cast. This was then carefully segmented into 3 individual blocks, which allowed the model to be readily broken apart and the surfaces visually and chemically analyzed for deposition of 'inhaled' aerosols. The approximate cuts of each block are shown in Fig. 1A and are arbitrarily described as the 'upper palate' (A), the 'lower palate' (B) and the 'back of the oropharynx' (C). The flexibility of the silicone, and the placement of insertion points in each component of the throat, allowed the model to be rebuilt correctly without junctures being noticeable between the components when clamped tightly together. The entrance to the mouth was fitted with an adapter so that metered dose inhalers could be directly connected to the model. Adapters were also created for the outlet in the upper trachea such that it could be directly connected to one of several sizing and collection devices. An example of a system set up is shown in Fig. 1B where the throat was connected to a 47 mm air sampling filter unit (BGI Inc. Waltham, MA) containing a 47 mm binderless, glass fiber filter (Type A/E, Gelman Sci., Ann Arbor, MI).

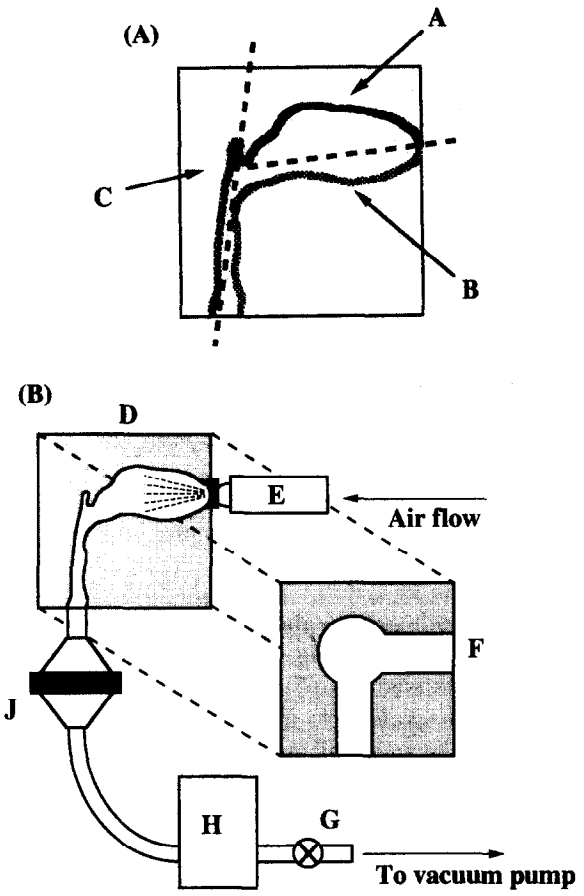

Fig. 1. (A) An illustration of the silicone 'real' throat showing the approximate divisions of the segments where A is described as the 'upper palate', B is the 'lower palate' and C is the 'back of the oro-pharynx'. (B) A representation of the basic test rig used to perform the experiments. D is the silicone throat with an inhaler, E, connected. F is the glass throat which can be substituted for the silicone throat where necessary. Airflow is generated from a vacuum pump and is controlled by a '14 turn' valve, G, and monitored by a digital mass flow meter, H. Powder emerging from either throat is collected in a filter unit, J.

A glass throat was built to the specifications described in the British Pharmacopeia 1988, Appendix XVII C. This consists of a modified 50 ml bulb with a ground-glass inlet and outlet cone of 29/32 and 24/29 mm standardized dimensions, respectively. An adapter was also built to accommodate the outlet cone so that the throat could be connected to the filter unit as described above.

Powder preparation and characterization

A 2.2% w/v mannitol : sorbitol : carboxyfluorescein 10:1:0.01 solution was spray dried (Buchi 190 mini spray drier, Buchi Ltd, Flawil, Switzerland) under the following conditions: 140°C inlet and 88°C outlet temperature; 3 ml/min liquid feed rate and 12 l/min atomization rate. Particles of 1–5 μm mass median diameter, a size range suitable for inhalation, were generated. The powder was sized by 'time of flight' spectrometry (Aerosizer Mach II, API, Amherst, MA) and, secondly, by centrifugal photosedimentation analysis (Horiba CAPA-300, Irvine, CA). For the latter technique the powder was first dispersed in a nonaqueous hydrocarbon medium (Sedisperse A12, Micromeritics Instrument Co., Norcross, GA). To obtain scanning electron micrographs (SEM), powder was placed on 10×10 mm aluminum stubs and sputter coated with palladium (Hummer VII, Anatech Ltd, Alexandria, VA). SEM were then obtained using a Jeol JSM 5200 (Jeol Inc., Peabody, MA) set at 20 kV and 2000 × magnification. The quantity of carboxyfluorescein associated with the particles was determined by dissolving a known weight of powder in 1 mM NaOH and then analyzing the solution by spectrofluorimetry at 494 nm excitation and 514 nm emission wavelengths (Alphascan, PTI Inc., South Brunswick, NJ). The moisture content of the powder was determined by Karl-Fischer titration (Aquastar 2000, EM Science, Gibbstown, NJ). Bulk powder was stored under vacuum and over desiccant at room temperature.

Capsule and throat preparation

Elanco HC no. 3 gelatin capsules were filled with ≈ 20 mg of accurately weighed powder prior to use. The capsules were placed in a dry powder inhaler (DPI) 'Rotahaler' (Glaxo Inc., Research Triangle Park, NC) which was weighed and sealed to the appropriate throat model. The throats used were: (a) silicone model – 'dry' (no surface modification), (b) silicone model – 'wet', (c) the 'standard' glass throat – 'dry', and (d) the glass throat – 'wet'. The wet surface was achieved by coating the surfaces with a water soluble polyethylene glycol (PEG) 1:1 mixture of 600:1000 Mol. Wt (Polysciences Inc., Warrington, PA). This bet-

ter mimics the wet surface of the human throat. The hydrophilic nature of PEG caused it to bead on both the glass and silicone surfaces. However, good surface coverage could be achieved, without visible beading, with the 600:1000 Mol. Wt PEG combination which has a 'waxy' solid consistency and a light coating could be applied effectively. The advantage of PEG, compared to silicone oils which are occasionally used to coat impaction surfaces (Boundy et al., 1990), is its high water solubility and lack of interference with fluorescence.

System operation

Capsules were placed in a Rotahaler and connected to a throat as shown in Fig. 1(B) so that an air tight seal was obtained between throat and mouthpiece. A 47 mm collection filter unit containing a binderless glass fiber filter was connected to the outlet. The vacuum pump was used to establish the steady state air flow through the system. This was fixed at 30, 60 or 120 l air/min. The flow rates were monitored by a digital mass flow meter (Model 820, Sierra Instruments, Carmel Valley, CA) connected downstream of the collection filter. It was linked to a chart recorder to document the 'inhalation' profile. Next, the capsule was pierced by the device and a timer was activated that operated the vacuum pump for 4 s. On completion of experiments the inhaler was reweighed to determine the mass loss. The device and individual components of the system assembly were then washed with fixed quantities of 1 mM NaOH. The wash from the upper trachea of the silicone model was included with the filter unit. Solutions were then analyzed by spectrofluorimetry as described above. The mass deposition on each component was quantified. A minimum of six experiments were completed at each of the three flow rates and for each throat configuration.

Results and Discussion

Powder characteristics

Examination of the electron micrographs illustrate that the spray dried particles are spherical,

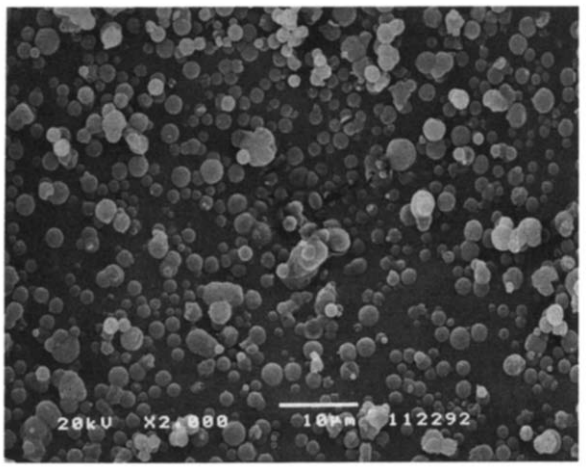

Fig. 2. A scanning electron micrograph of the test powder dispersed onto an aluminum stub. The magnification is 2000× at an accelerating voltage of 20 kV. Bar = 10 µm.

have a smooth surface and generally are less than 5 µm in diameter (Fig. 2). The particles were readily dispersed on to the SEM stub and no large agglomerates are apparent. The mass median aerodynamic diameter estimated by the Aerosizer is 3.4 µm and the volume median diameter estimated by the Horiba CAPA-300 is 4.7 µm. Macroscopically, the powder is densely packed and flow properties are relatively poor. The powder contained 1.1% moisture at the date of preparation and 0.11% w/w of CF.

Dispensing and deposition of dose

The dose emerging from the DPI is dependent upon the flow rate (ANOVA $p < 0.01$: 1.0 ± 0.2 mg (30 l/min, $n = 25$), 5.1 ± 0.5 mg (60 l/min, $n = 30$) and 6.2 ± 0.6 mg (120 l/min, $n = 28$)) (Table 1 and Fig. 3). At best, only 31% of the available dose is delivered to the mouth. Given

TABLE 1

Effect of flow rate on dose dispensed by Rotahaler

Flow rate (l/min)	30	60	120
Dose in capsule (mg)	19.8±0.3	20.3±0.6	20.4±0.4
Dose dispensed (mg)	1.0±0.2	5.1±0.5	6.2±0.6
Delivery (%)	4.9±1.1	25.4±2.7	30.4±2.7
n	25	30	28

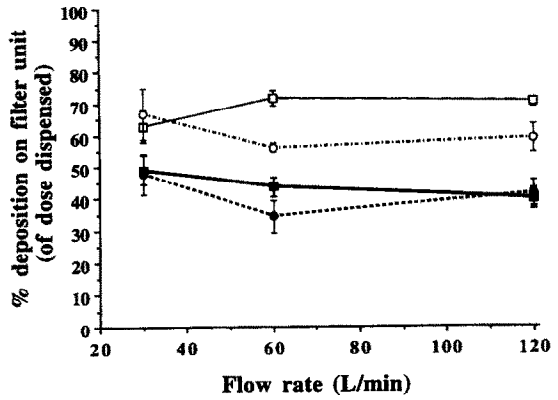

Fig. 3. The air flow rate vs the percentage of the dispensed dose recovered from the filter unit. Deposition is not significantly dependent upon the inhaled volume flow rate but is dependent upon the throat configuration used: glass-dry (□), glass-wet (■), silicone-dry (○), silicone-wet (●). Error bars are the standard error of the mean. $n \geq 6$.

that this fraction includes agglomerates that deposit in the throat, it is not surprising that a low percentage of the dose reaches the lung (Bell et al., 1971). This is indicated by the percentage of the total dose recovered from the filter unit (Table 2) which is also dependent upon the flow rate (ANOVA, $p < 0.01$ glass wet or dry, $p < 0.05$ silicone wet and $p < 0.001$ silicone dry), irrespective of the throat type or condition. However, because of the nature of the throat, the amount of powder recovered from the filter unit can be ranked: glass-dry > silicone-dry > glass-wet > silicone-wet.

The percentage of the dispensed dose recovered from the filter unit is independent of flow rate (ANOVA, p = NS) for all throat configurations (Fig. 3). This result implies that if powder can be effectively dispensed from the Rotahaler, a good percentage (40–70%, Fig. 3) can be expected to enter the respiratory tract irrespective of the inhalation volume flow rate. No information is obtained on the particle size distribution of powder reaching the filter unit but if the majority of deaggregation occurs in the device and mouth, then there is good reason to expect efficient deposition in the respiratory tract of respirable particles. It should be recognized, however, that the above results apply only to a dry powder inhaler where dispensing of the powder has been achieved by an inspiration. If, as with the propellant MDIs, the dose is propelled forwards into the mouth at velocities substantially greater than achieved through inspiration, then an entirely different set of deposition profiles is likely.

TABLE 2

% of total dose recovered from the filter unit [a]

Flow rate (l/min)		Glass throat		Silicone throat	
		Mean	SD	Mean	SD
30	wet	1.4	1.2	0.9	1.0
	dry	5.1	4.9	4.1	3.0
60	wet	8.9	3.6	7.3	4.6
	dry	21.0	14.6	14.9	4.6
120	wet	14.3	8.1	9.6	8.4
	dry	24.8	9.9	18.6	4.5

[a] The dose to the 'filter unit' includes the material deposited on the component of the artificial throat that represented the upper trachea.

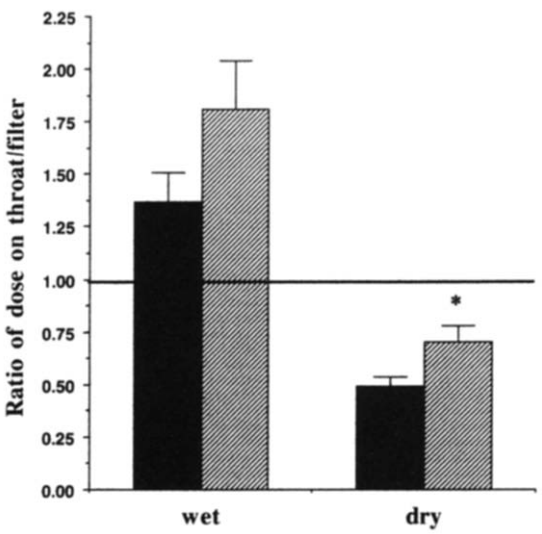

Fig. 4. The relationship between the throat/filter deposition ratio vs the type of throat used. The line denotes where the ratio of dose recovered from the filter and the throat are equal. The ratio in the silicone throat (diagonally hatched bars) is always higher than in the glass throat (filled bars), but is only significant when the surfaces are dry. Error bars are the standard error of the mean. $n \geq 18$.

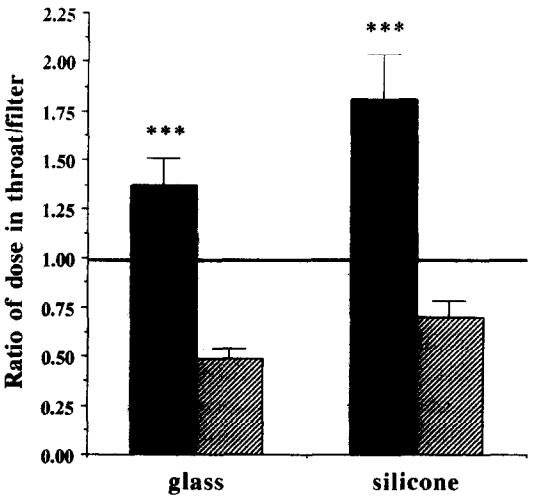

Fig. 5. The relationship between the throat/filter deposition ratio vs the condition of the throat. The line denotes where the ratio of dose recovered from the filter and the throat are equal. The ratios are significantly higher when wet (filled bars) than when dry, (diagonally hatched bars). Error bars are the standard error of the mean. $n \geqq 18$.

The ratio of the dispensed dose (throat/filter) is statistically different for the glass and silicone throat when dry (t-test, $p < 0.05$) but not when wet (Fig. 4). A smaller fraction of powder reaches the collection filter when the dry silicone throat is used vs the dry glass throat. This implies that the surface geometry of the dry throat can influence deposition but this phenomenon may be masked by the overwhelming influence of the wet condition of the throat. This point is substantiated when the glass or silicone throats are tested in the wet vs dry state. Here the results are highly significant in both cases (t-test, $p < 0.001$, silicone and glass) (Fig. 5).

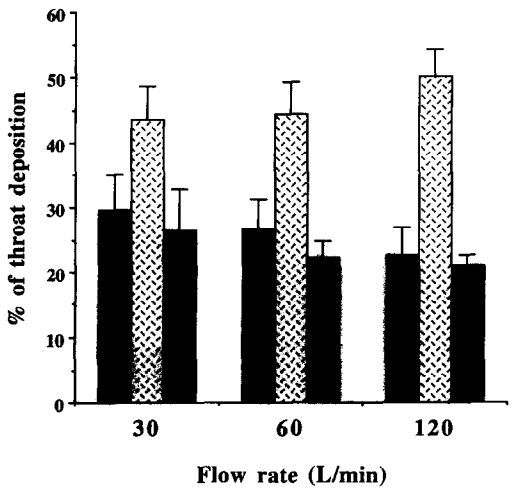

Fig. 6. The distribution of dose in the silicone throat segments. Dose in the 'upper palate' (cross-hatched bars) is greater than that of the 'lower palate' (filled bars) and 'back of oro-pharynx' (diagonally hatched bars), at all flow rates tested. There is also a tendency for more material to deposit in the 'upper palate' as the flow rate increases. Error bars are the standard error of the mean. $n \geqq 12$.

Distribution of dose in the silicone throat

The distribution is affected by the orientation of the inhaler device in the mouth. But given that most users will hold the device horizontally the following patterns are found. Visibly, the majority of powder impacts on the posterior hard palate and on the soft palate just superior to the uvula. Large agglomerates occasionally appear on the surface of the tongue that have probably been forced out of the main air stream due to turbulent eddies in the mouth or at the outer lip of the device. Some powder also impacts on the back wall of the pharynx and deposition is also noted in the piriform fossae which are two evolutionary redundant legs that lie on either side of the laryngopharynx. These observations are partially borne out by chemical assay of deposited powder as illustrated in Fig. 6 with reference to Fig. 1A. Interestingly, minimal visible deposition is noted around the vestibular and vocal folds of the larynx. Presumably, the majority of large agglomerates are 'filtered' out in the mouth and the 'smaller' agglomerates and fully deaggregated particles remain entrained in the accelerating air stream as it traverses the sinus of the larynx.

Conclusions

An artificial throat that duplicates the shape of the oral cavity and which can be broken down into several components has been created to obtain more information on the deposition of inhaled powders in the mouth than a standard glass throat. The condition, wet or dry, of either type of throat markedly affects the fraction of dose

depositing in the throat. When studying dry powder inhalers, the use of any representation of a throat in the dry state is likely to result in an overestimation of dose that might be expected to reach the lung. The use of an artificial throat that duplicates the shape, size and surface condition of a human throat may provide information that can be directly related to the use of inhaler devices in vivo.

Acknowledgement

We should like to acknowledge C.G. Pitt for his valuable comments on the content of the manuscript.

References

Bell, J.H., Hartley, P.S. and Cox, J.S.G., Dry powder aerosols I: a new powder inhalation device. *J. Pharm. Sci.*, 60 (1971) 1559–1564.

Boundy, M.G., Leith, D. and Knowles, M.R., Dispersion and size distribution of amiloride by metered dose and dry powder inhalers. *J. Aerosol Med.*, 3 (1990) 233–241.

British Pharmacopeia, Appendix XVII C, 1988.

Martin, G.P., Bell A.E. and Marriott, C., An in vitro method for assessing particle deposition from metered pressurized aerosols and dry powder inhalers. *Int. J. Pharm.*, 44 (1988) 57–63.

Sciarra, J.J. and Cutie, A., Simulated respiratory system for in vitro evaluation of two inhalation delivery systems using selected steroids. *J. Pharm. Sci.*, 67 (1978) 1428–1431.

Vidgren, M., Vidgren, P., Uotila, J. and Paronen, P., In vitro inhalation of disodium cromoglycate powders using two dosage forms. *Acta Pharm. Fenn.*, 97 (1988) 187195.