J. Pharm. Pharmacol. 2000, 52: 771–778
Received November 1, 1999
Accepted March 20, 2000

A Human Oral-throat Cast Integrated with a Twin-stage Impinger for Evaluation of Dry Powder Inhalers

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Abstract

The aim of this study was to investigate the applicability of replacing the glass throat from a twin-stage impinger (TSI) with a human oral-throat cast. Monodisperse aerosols were used to calibrate the human oral cast-TSI at 60 L min^{-1} and cut-off in particle size was compared with that of the TSI described in the British Pharmacopoeia which employs a glass throat. The amount of salbutamol sulphate (and lactose) delivered by the Cyclohaler depositing on various elements of the in-vitro model were determined.

The calibration of the model containing a human oral-throat cast at 60 L min^{-1} gave a particle size cut-off of 5·2 μm which was less than that of the TSI (6·3 μm). The oral-throat cast trapped more drug than the glass throat model with a formulation that employed the larger carrier (63–90 μm; $P<0{\cdot}05$) while it trapped a lesser amount of drug with those filled with the lower size carrier (Lactochem, micronised lactose). The greater amount of lactose in the formulation that employed the larger-sized carrier (63–90 μm) was deposited closer to the inlet of the oral-throat cast than to the inlet of the glass throat model.

Replacement of the glass throat in the TSI by the human oral-throat cast, leads to a change in deposition efficiency, with the cast having a higher filter efficiency and hence more aerosol particles being captured before their entry into the TSI. This should be investigated further to determine whether such a model might provide a more realistic assessment of the in-vivo characteristics of an aerosol in comparison with the TSI currently being employed, which utilises the glass throat as the portal of entry.

Very few guidelines have been established for the in-vitro deposition testing of dry powder inhalers (Byron et al 1994; European Pharmacopoeia 1997; British Pharmacopoeia 1999). This is due primarily to the great diversity both in the delivery device (each having a different aerosolisation mechanism and principle of dose metering) and the type of formulation. In practice, the in-vitro determination of deposition has been carried out employing impactors and impingers described in official compendia, such as the USP (United States Pharmacopeia (1999). At present the European and British Pharmacopoeias describe the use of impingers and impactors to determine whether dry powder inhalers (DPIs) fulfil required specifications.

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The oropharynx is the main part of the upper respiratory tract. It has unique geometric features, which lead to aerosolised particles impacting extensively in this region, resulting in only a relatively small fraction of the particles entering the lower airways. As part of its structural design, a glass throat is incorporated as the portal of entry to the twin-stage impinger (TSI) described in the British Pharmacopoeia 1999, in an attempt to mimic the oropharynx, whilst the USP describes a metal induction port with a 90° sharp bend for the cascade impactor system (Figure 1). Hence the rationale for the design of the in-vitro test is an attempt to simulate patient use as far as practicable with the hope of achieving correlation of the results obtained from such tests with performance in-vivo. However, previous studies have claimed a poor correlation between in-vitro and in-vivo test results for drugs administered by a DPI (Vidgren et al 1987; Pitcairn et al 1994). In an attempt to improve the correlation between such data, previous work-

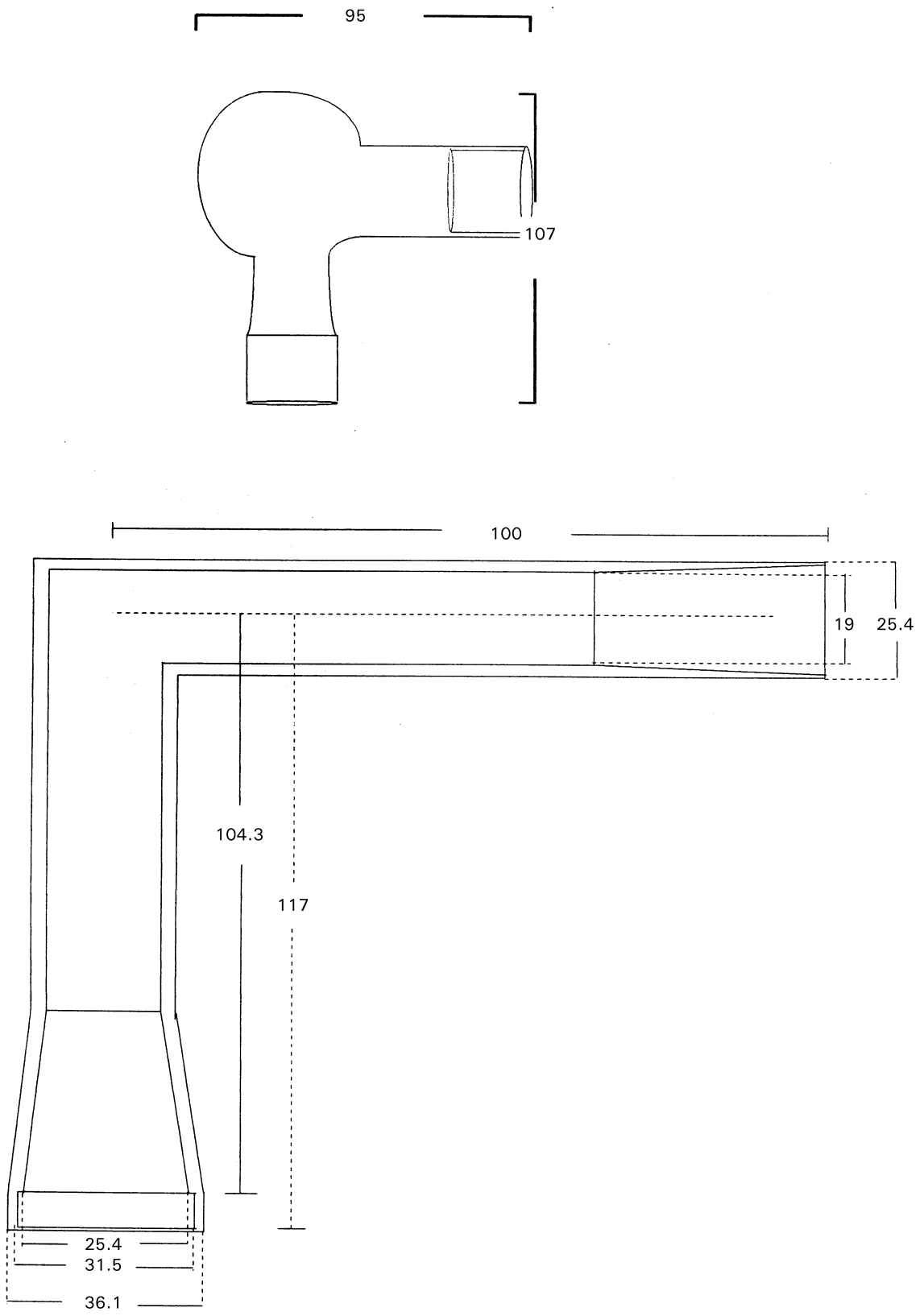

Figure 1. Top: glass throat employed in the BP twin-stage impinger. Bottom: sample induction port for a USP cascade impactor. Units in mm from USP 1995.

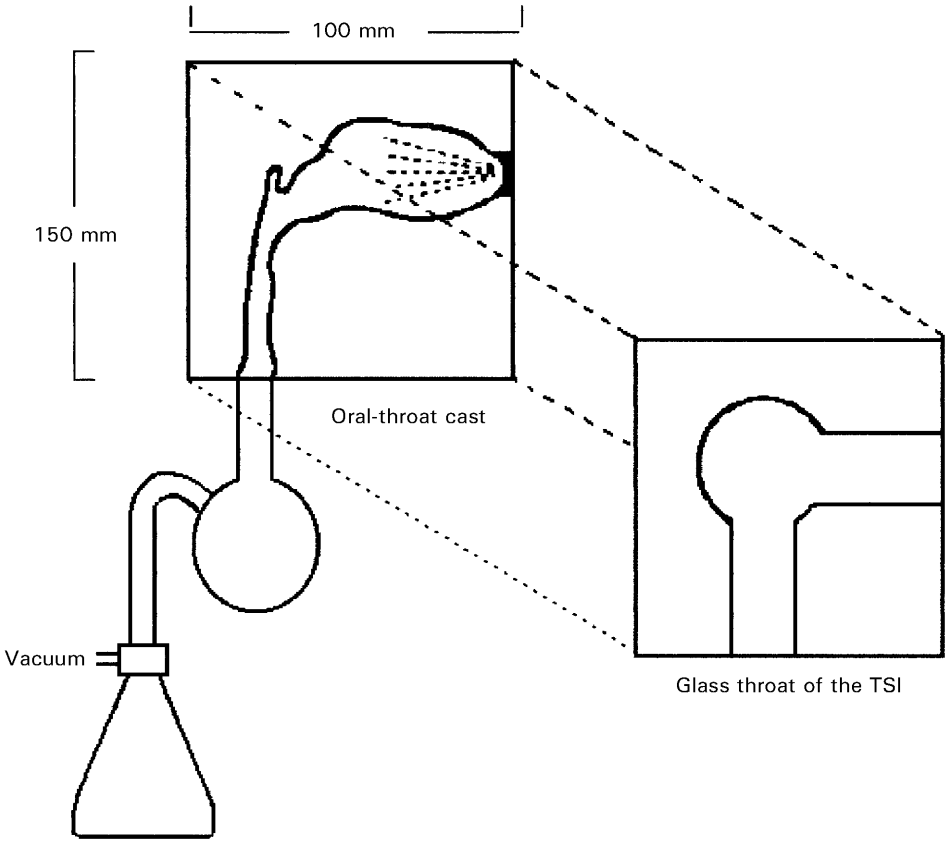

Figure 2. The oral-throat cast, obtained by magnetic resonance image from an adult male, integrated with the twin-stage impinger system. The relative position of the oral-throat cast and the glass throat is depicted by the dotted lines.

ers have combined an oral cast with a sizing unit to create a more realistic in-vitro model of the upper airways (Cheng et al 1990; Niven et al 1994; Olsson et al 1996; Schrerer et al 1994). Berg et al (1998) employed different oral-throat casts to evaluate the dose exiting from different metered-dose inhaler formulations. However, calibration of the oral-throat cast has not been performed. Moreover, the dimensions of the employed cast were not illustrated and the investigators considered only drug deposition without considering the carrier.

The aim of this study was to investigate the applicability and consequences of replacing the glass throat from a TSI with a human oral-throat cast (Figure 2) by determining the in-vitro deposition of salbutamol sulphate and lactose (employed as a carrier) from four different dry powder formulations. A secondary objective was to determine the effects of wetting the internal surface of the cast upon the deposition of the drug and excipient.

Materials and Methods

Materials

Stearic acid was obtained from Aldrich (WI). Micronised salbutamol sulphate was supplied by Glaxo-Wellcome (Ware, UK). Lactochem lactose (medium grade) was purchased from Borculo Whey Ltd (Chester, UK). Coarse lactose (63–90 μm) was obtained from Meggle (Wesserburg, Germany). Micronised lactose was prepared from Meggle lactose by a jet mill microniser (Rheinfelden, Switzerland). Glucose was obtained from Fisons (Loughborough, UK). All other chemicals were of analytical grade. All HPLC reagents were purchased from Rathburn (Walkerburn, Scotland).

Oral-throat cast

A clear polyester cast of the upper airways of an adult male (53 years) was made at Johns Hopkins University as described previously (Cheng et al 1990). As a life sized model, the cast included the oral cavity, tongue, nasopharynx, larynx and upper trachea. In brief, it was manufactured by taking a magnetic resonance image (MRI) of the upper airways, which was subsequently traced on each side of two pieces of thick clear plastic. A high-speed cutting tool was then employed to fashion the entire model. This method ultimately provided an accurate geometry of the oral-throat cast as con-

firmed by computerised tomography. The entrance and exit of the oral-throat cast could be connected to a dry powder device and an impactor system in series. The dimensions of the cast and its integration with the TSI are shown in Figure 2.

Calibration of the oral-throat cast-TSI

The cast was attached to the TSI in place of the glass throat. The monodisperse NaCl–stearic acid aerosols, after preparation as previously described by Onyechi et al (1994) and sizing by a Malvern 2600 laser diffraction (Malvern Instruments, Worcester, UK), were passed into the human oral cast-TSI. Air was drawn through the oral cast-TSI at a flow rate of 60 L min^{-1} for 20 s and the samples collected in the upper and lower stages were analysed by atomic emission spectroscopy (Instrumentation Laboratory, MA). The percentage of upper-stage deposition (y) was plotted as a function of logarithmic particle size (x) and this plot was used to construct the collection efficiency curve. The size cut-off was defined as the point of 50% deposition in the upper stage of the impinger.

In vitro deposition of dry powder formulations using an oral-throat cast-TSI

Four formulations were used: three formulations were made in-house as described previously (Srichana et al 1998) and those formulations contained 63–90-μm medium-grade lactose, Lactochem lactose (10–50 μm) or micronised lactose (70% < 10 μm) mixed with micronised salbutamol sulphate (2·77 ± 0·11 μm) in a ratio 1 : 67·5, drug : lactose. The formulation was prepared by mixing drug with lactose in a Turbula mixer for 2 h. The fourth formulation was that employed in Rotacaps 400. All formulations contain a unit dose of drug and lactose 27·4 mg filled in capsule size 2. All experiments were run at room temperature (17–22°C) and 40–55% r.h.

Table 1. Dimensions (mm, unless otherwise stated) of the glass throat employed in a twin-stage impinger and the oral-throat cast.

Part	Glass throat	Oral-throat cast
Entrance to the back of the throat	95·0	75·0
Diameter of the mouth	16·0	25·0
Distance from the top to the lower palate	45·7	44·6
Distance from left to right of the oral cavity	45·7	43·5
Length of the larynx and trachea	61·0	115·0
Total volume of the upper airways (mL)	102	86

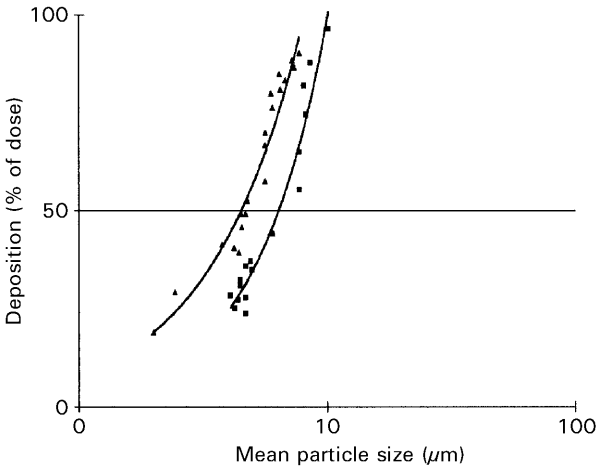

Figure 3. The percentage deposition of monodisperse aerosols captured by the upper stage of a twin-stage impinger (■) or oral-throat cast-TSI (▲) plotted as a function of particle size at a flow rate of 60 L min^{-1}.

A capsule containing one of the four formulations was aerosolised, using the Cyclohaler device (Pharbita, Zaandam, The Netherlands), by drawing air through the TSI at a flow rate of 60 L min^{-1} for 10 s. Drug and lactose deposition on each stage of the impinger was determined by HPLC.

Salbutamol was analysed by a validated HPLC (LDC Analytical CM 4000, USA) method using a UV detector (Spectromonitor 3100) set at a wavelength of 276 nm. The assay conditions were: stationary phase, Hypersil C18 (15 cm × 4·6 mm i.d.); mobile phase, methanol:acetate buffer (pH 4·5) 54 : 46 by volume; flow rate, 0·8 mL min^{-1}; injection volume, 100 μL; integrator, LDC Analytical CI-4100; internal standard, 0·2 μg mL^{-1} ethyl paraben; limit of detection, 0·1 μg mL^{-1}.

Lactose was analysed by HPLC (LDC Analytical CM 4000, USA) using a refractive index detector (Erma Inc., Japan). The conditions were: stationary phase, Phenomenex amino silane (30 cm × 4·6 mm i.d.); mobile phase, acetonitrile : deionised water 75 : 25 by volume; flow rate, 1 mL min^{-1}; injection volume, 100 μL; integrator, LDC Analytical CI-4100; internal standard, 2 μg mL^{-1} glucose monohydrate; limit of detection, 2 μg mL^{-1}.

The fine particle fraction (FPF) and fine particle lactose (FPL) were calculated as the amount of drug and lactose, respectively, depositing in the lower stage of the TSI, expressed as a percentage of the nominal dose. Each formulation was tested six times.

Dry and lubricated oral-throat cast-TSI deposition with different dry powder formulations

For each test, a capsule containing one of the four formulations was placed in the Cyclohaler device. The cast was used either dry or lubricated with an

Table 2. Percentage nominal dose of the drug deposition in various parts of the TSI and oral-throat cast-TSI systems after delivering the dry-powder formulations via the Cyclohaler.

Formulation	TSI			Dry cast-TSI			Lubricated cast-TSI			Total emission
	Throat	Upper stage	Lower stage	Throat	Upper stage	Lower stage	Throat	Upper stage	Lower stage	
63–90-μm lactose	10·80 (0·18)	33·60 (1·81)	19·90 (1·23)	30·90 (2·64)	21·10 (2·37)	10·90 (1·59)	NA	NA	7·29 (0·44)	63·10–64·30
Rotacaps 400	18·34 (0·67)	35·15 (1·67)	20·74 (1·68)	30·28 (0·48)	30·78 (1·52)	16·73 (1·60)	NA	NA	16·15 (0·71)	73·50–77·00
Lactochem lactose	14·40 (0·68)	33·90 (1·48)	30·70 (2·17)	30·80 (2·20)	22·60 (2·61)	28·90 (1·67)	NA	NA	28·90 (0·89)	79·00–81·90
Micronised lactose	20·00 (2·63)	20·71 (1·00)	40·49 (1·11)	32·77 (1·28)	11·83 (1·26)	40·06 (1·96)	NA	NA	39·38 (1·11)	81·20–84·50

NA, amount of drug was not assayed. Results are presented as mean ± s.d., n = 6.

Table 3. Percentage nominal dose of lactose deposition in various parts of the TSI and oral-throat cast-TSI systems after delivering the dry powder formulations via the Cyclohaler.

Formulation	TSI			Dry cast-TSI			Lubricated cast-TSI			Total emission
	Throat	Upper stage	Lower stage	Throat	Upper stage	Lower stage	Throat	Upper stage	Lower stage	
63–90-μm lactose	20·55 (1·11)	42·30 (3·44)	ND	21·22 (1·70)	42·67 (6·37)	ND	NA	NA	ND	62·80–64·00
Rotacaps 400	23·37 (2·40)	44·77 (1·96)	1·60 (0·16)	23·55 (2·55)	42·52 (3·44)	1·53 (0·19)	NA	NA	0·46 (0·11)	67·60–69·70
Lactochem lactose	24·33 (3·29)	51·85 (4·63)	3·18 (0·14)	26·15 (1·03)	49·18 (5·24)	2·44 (0·15)	NA	NA	0·91 (0·20)	77·77–79·30
Micronised lactose	31·33 (3·07)	45·93 (4·55)	12·98 (0·69)	37·04 (3·70)	41·11 (1·43)	11·60 (0·62)	NA	NA	10·15 (0·28)	89·75–90·24

NA, amount of lactose was not assayed. ND, lactose was not detected. Results are presented as mean ± s.d., n = 6.

excess of oleic acid (BDH, UK) by manually manoeuvring the cast so that all surfaces were wetted with oil. The oral-throat cast was orientated such that excess oil could drain from the internal surface for 30 min before undertaking deposition studies. Air was drawn through the device at 60 L min^{-1} for 10 s. Drug and lactose analyses were performed only on the fluid plus washings from the lower stage of the impinger. The FPF of drug was expressed as a percentage of the nominal dose. The lactose was expressed as the mean of percentage weight of the nominal amount.

Data analysis

All data were analysed by analysis of variance (ANOVA) using Microsoft Excel for Windows. The level of significance was set at 0·01 and 0·05 as appropriate.

Results and Discussion

Calibration of the oral cast-TSI

The calibration of the model comprising the human oral-throat cast fitted in series with a TSI operated at 60 L min^{-1} gave a size cut-off for the upper stage of 5·2 μm (Figure 3). This value which was less than the experimentally derived value for the TSI with the glass throat (6·3 μm) operated at 60 L min^{-1}. The cut-off of the TSI and glass throat was sharper than that of the oral-throat cast-TSI. The difference in cut-off value and the higher number of particles being deposited was presumably due to the geometry of the oral-throat cast as compared with that of the BP glass throat (Table 1). The cut-off diameter of the TSI was found, therefore, to be reduced when the glass throat was replaced with the oral-throat cast. Also it might have been expected that when the oral-throat cast was lubricated, the cut-off point would further decrease compared with that obtained when dry. However, this study was not performed due to the interference of oleic acid in the analytical methods employed to assay drug and lactose in the calibration procedures and deposition studies. Table 1 shows that the glass throat had a higher volume (102 mL) than the oral-throat cast (86 mL) but the latter had a narrower laryngeal and tracheal region than the corresponding outlet cone of the glass throat

(Figures 1 and 2). The larynx and trachea in the cast were also much longer than in the glass throat. All these differences in geometry would be expected to affect the air-flow pattern in the oral cavity and, hence, the aerosolisation and deposition in the glass throat/oral-throat cast.

Drug and lactose deposition in the glass throat and oral-throat cast

The oral cast trapped three times more drug from the formulation containing 63–90-μm lactose than the glass throat while it trapped only up to twice that from the Rotacaps and formulations which incorporated either the Lactochem lactose or the micronised lactose (Table 2). The dry oral-throat cast trapped around 30% of the nominal dose of the drug for all four formulations. The amount of drug depositing in the upper stage of the TSI was influenced by the amount impacting in the glass throat or oral-throat cast. The percentage of drug removed by the oral-throat cast and upper stage of the TSI was approximately 50% of the nominal dose, except when micronised lactose was employed in the formulation. The micronised carrier and incorporated drug penetrated deeper into the impinger resulting in a higher deposition in the lower stage (Tables 2 & 3).

The percentage of lactose retained in the oral-throat cast from all formulations, with the exception of the one containing micronised lactose, was no different ($P > 0{\cdot}05$) to the amounts deposited in the glass throat from the same formulations (Table 3). Although the cast and glass throat clearly have different geometries, similar deposition of lactose was obtained. However, a significantly higher percentage of lactose was found to be retained in the oral-throat cast when the micronised lactose formulation was aerosolised compared with the percentage of lactose that was deposited in the glass throat ($P < 0{\cdot}05$). If micronised lactose was emitted as aggregates it might be expected that larger amounts of lactose would be retained in the upper stage. The oral-throat cast removed a higher percentage of fine lactose carrier than the glass throat when either Lactochem lactose or micronised lactose was employed as the carrier (Table 3). More than 85% of the emitted lactose was removed from the air stream in the throat part (whether glass or cast) and upper stage of the impinger. However, only 77% of micronised lactose deposited in the oral-throat cast, the rest penetrated to the lower stage of the impinger.

TSI. Lower stage deposition

The change in cut-off value for the upper stage of the TSI from that conferred by the glass throat, to that when the oral-throat cast was employed, and the possible further decrease in cut-off when oleic acid was utilised to wet the cast, does add an extra level of complexity to interpreting the data. Nevertheless, it is still possible to compare the changes in drug and lactose deposition profiles in the different models between formulations. Drug deposition on the lower stage of the TSI using the 63–90-μm lactose carrier decreased from 19·9% to 10·9% when the glass throat was replaced by the human oral-throat cast. The lower-stage deposition of salbutamol sulphate from the Lactochem lactose and micronised lactose formulations was not significantly different ($P > 0{\cdot}05$) when the glass throat or oral-throat cast were employed (Table 2). There was a distinct reduction in the lower-stage deposition of the drug using an oral-throat cast in place of a glass throat when the Rotacaps formulation was aerosolised ($P < 0{\cdot}01$). It can thus be deduced that, when the smaller-sized carrier was employed in the formulation, the oral-throat cast and glass throat were filtering drug to the same extent, whereas the glass throat removed less drug than the human oral-throat cast from the dry powder inhaler formulation containing carrier within the size range 40–90 μm.

The lactose carrier was not detected in the lower stage of the impinger when the 63–90-μm lactose formulation was delivered whereas with the Rotacaps, 430 μg of lactose was detected in the lower stage of the TSI and 410 μg was found for the oral-throat cast-TSI ($P > 0{\cdot}05$). It clearly showed that lactose deposition in the lower stage of the oral-throat cast-TSI was significantly lower than that obtained with the formulations containing either Lactochem or micronised lactose ($P < 0{\cdot}01$) (Table 3).

Dry and lubricated oral-throat cast deposition after aerosolisation of dry powder inhaler

A series of liquids (water, glycerol, polyethylene glycol, mineral oil and oleic acid) with a range of surface tensions (30–73 mN m^{-1}) were selected to investigate their effect as a possible wetting fluid for the oral-throat polyester cast in an effort to simulate more closely the conditions in-vivo. Niven et al (1994) used PEG 600 : PEG 1000 in a ratio of 1 : 1 to lubricate a silicone cast. However, with the polyester cast in this study the PEG mixture formed visible beads on the surface and the mixture was therefore not considered suitable. Another study by Miller & Purrington (1996) used mineral oil to lubricate a rubber cast. In our study, when the mineral oil was used to lubricate the oral-throat cast, air bubbles in the oil proved difficult to eliminate while retaining a uniform coating. In a comparative study using oleic acid or glycerol it

was found that oleic acid lubricated the cast better than glycerol. Water did not spread over the surface of the cast because of its high surface tension and it also dried quickly under the air flow conditions employed. When air was drawn through the impinger system the lubricating liquid needed to be retained on the surface throughout the experiment. The effect of both surface tension and spreading coefficient on the tested surface had to be considered in tandem when selecting a suitable wetting fluid. When oleic acid was placed on the surface of polyester, it was found to spread as a uniform film, and it proved successful in sustaining a coating on the oral cast throughout the experiment.

The internal surfaces of the oropharyngeal cavities are sufficiently wet, such that the airborne particles cannot be re-entrained into the flowing airstream. The inertial deposition at the back of the throat is normally expected to predominate for large particles at high flow rates. Drug deposition in the lower stage of the lubricated oral-throat cast-TSI was not found to be different from that when a dry oral-throat cast was employed for the smaller-sized carrier formulations, despite the former model having a possible lower cut-off for the upper stage than the latter (Table 2). However, the difference in drug deposition in the cast was pronounced when 63–90-μm lactose was employed as a carrier. It can be concluded, therefore, that a greater proportion of larger-sized carrier with any adhering micronised drug impacted upon, and was trapped by, the wet surface of the oral cast while the smaller carrier excipient particles with any adherant drug were trapped in much lower amounts (Table 2).

When the four formulations were used to determine the lower-stage deposition of lactose in the lubricated or non-lubricated cast-TSI combination, it was found that with the coarse carrier, lactose could not be detected. However, when the formulation containing the Lactochem or micronised lactose were aerosolised, lactose was detected in higher quantities when the dry cast-TSI rather than the lubricated-cast-TSI was employed. The reason for this finding might be due in part to the different physical properties of materials since it is known that powders adhere more easily to a wet surface than to a dry surface.

The lubricated oral-throat cast reduced the FPF of salbutamol sulphate from the 63–90-μm lactose formulation even further than the dry cast in comparison with the glass throat. Hence, the in-vitro test of the TSI may markedly overestimate the FPF due to airway geometry and lack of lubrication. While there were no differences in terms of drug deposition for any of the three models (TSI, dry and lubricated oral-throat cast-TSI) in the case of the formulation containing Lactochem lactose or micronised lactose, there was a difference in the amount of lactose deposited when the oral-throat cast was lubricated.

From these studies, it can be concluded that the oral cast influenced the amount of powder, both drug and excipient, reaching the lower stage of the impinger in the case of some formulations. This suggests that an oral-throat cast in conjunction with an impactor may prove to be a better predictive tool and provide more realistic in-vitro data for the evaluation of dry powder inhalers. However, the oral-throat cast used in this study was taken from one individual and concerns as to whether it truly represents a typical oral cavity have not been considered. The influence of the size of oral cavity (e.g. male/female, age), diseases of the oral cavity and of surgery and birth defects are just some of the issues that require further investigation. The glass throat is preferred because of its simplicity of design. However, for certain formulations there are significant differences in lower-stage deposition (including that from Rotacaps) with the possible consequence of incorrectly estimating potential respirable fraction.

Acknowledgements

The authors wish to thank the late Prof. David Swift, Johns Hopkins Institute and Colin Dickens, AEA, for the loan of the oral-throat cast employed in these studies.

References

Berg, E., Rossborg, C., Lindqvist, E., Bisgaard, H. (1998) Dose exiting models of the human throat – a comparison of five different models. Proceeding of Drug Delivery to the Lungs IX, The Aerosol Society, Bristol, pp 29–32

British Pharmacopoeia (1999)Aerodynamic assessment of fine particles. Appendix XII F, A203–A212

Byron, P. R., Kelly, E. L., Kontny, M. J., Lovering, E. G., Poochikian, G. K., Sethi, S., Thiel, C .G., Vads, E. B. (1994) Recommendations of the USP advisory panel on aerosols on the USP general chapters on aerosols and uniformity of dosage units. Pharm. Forum 20: 698–705

Cheng, Y. S., Yamada, Y., Yeh, H. C. (1990) Deposition of ultrafine aerosols in a human oral cast. Aerosol Sci. Technol. 12: 1075–1081

European Pharmacopoeia (1997) Preparations for inhalations: aerodynamic assessment of fine particles. pp 143–150

Miller, N.C., Purrington, A. M. (1996) A cascade impactor entry port for MDI sprays with collection characteristics imitating a physical model of the human throat. Pharm. Res. 13: 391–397

Niven, R. W., Lott, F. D., Ip, A. Y., Somaratne, K. D., Kearney, M. (1994) Development and use of an in vitro system to evaluate inhaler devices. Int. J. Pharm. 101: 81–87

Olsson, B., Borgstrom, L., Bondesson, E. (1996) Effect of inlet throat on the correlation between measured fine particle dose and lung deposition. Proceeding of Respiratory Drug Delivery V. Illinois, pp 273–281

Onyechi, J. O., Martin, G. P., Marriott, C., Murphy, L. (1994) Deposition of dry powder aerosols in the cascade impactor at different flow rates. J. Aerosol Med. 7: 181–184

Pitcairn, G., Lunghetti, G., Ventura, P., Newman, S. (1994) A comparison of the lung deposition of salbutamol inhaled from a new dry powder inhaler at two inhaled flow rates. Int. J. Pharm. 102: 11–18

Schrerier, H., Mobley, W. C., Concessio, N., Hickey, A. J., Niven, R. W. (1994) Formulation and in vitro performance of liposome powder aerosols. STP Pharm. Sci. 4: 38–44

Srichana, T., Martin, G. P., Marriott, C. (1998) On the relationship between drug and lactose carrier. Int. J. Pharm. 167: 1–13

United States Pharmacopeia 23-National Formulary 18 (1999) Physical tests and determinations of aerosols. (Suppl. 10) pp 4933–4949

Vidgren, M., Karkikainen, A., Paronen, T. P., Karjalainen, P. (1987) Respiratory tract deposition of ^{99m}Tc labelled drug particles administered via a dry powder inhaler. Int. J. Pharm. 39: 101–105