Chapter 4

FORMULATION DESIGN OF ANTITUBERCULOSIS DRY POWDER INHALERS BY PHYSICAL MIXING

4.1 Introduction

Dry powder formulations for inhalation are often composed of fine drug particles and inert coarse carrier particles. DPIs have some advantages over MDIs such as the former do not require breath coordination with actuation. However, this is also one of their main limitations since they often require high inspiratory flow rates to achieve good dispersion of the powder discharge (Bosquillon *et al.*, 2001).

DPIs are generally formulated by mixing a cohesive preparation of micronised drug particles with large carrier particles. The aerodynamic diameters of a drug particle need to be between 1 and 5 μm for deep lung deposition and absorption in the lungs. The carrier particles should be physiologically inert, not impair the drug bioavailability, chemically compatible with the drug and able to aid the flow and dispersion of highly cohesive drug particles (Larhrib *et al.*, 2003). Adhesion forces between the drug and coarse carrier are required to produce dose uniformity. The fine drug particles are expected to adhere to the carrier surface to form ordered mixtures. To obtain a homogeneous, stable ordered blend depends on drug percentage, drug/carrier size ratio, on the shape and surface of the particles. The carrier particles are used to improve the

flow of the drug particles (Flament *et al.*, 2004). However, such adhesion forces should not be too high otherwise the forces imparted by inspiration cannot deaggregate the drug-carrier complex so that the drug can proceed to the lower airways whilst the carrier is deposited only in the upper airways. If the force of adhesion is high, a low efficiency of drug delivery to the lung will be obtained. Adhesion forces are primarily a summation of van der Waals, electrostatic and capillary forces. These forces are affected by the materials, shape and size of the particle; roughness and contamination of the surface; relative humidity; temperature; duration of contact and initial contact velocity (Srichana, 1998).

4.2 Materials

Acetonitrile (J.T.Baker, NJ, USA)

Chloroform (VWR International Ltd., England)

D-(+)-Lactose monohydrate (Fluka, Switzerland)

D-(+)-Mannose (Sigma chemical company, St. Louis, MO, USA)

D-(+)-Trehalose dihydrate (Sigma chemical company, St. Louis, MO, USA)

Hexane 95% (J.T. Baker, NJ, USA)

Isonicotinic acid hydrazide (Sigma chemical company, St. Louis, MO, USA)

Rifampicin (Fluka, Switzerland)

Sodium dihydrogen phosphate (Riedel-de Haën, Germany)

Span 80 (Srichand united dispensary, Co. Ltd., Bangkok, Thailand)

Tween 80 (Srichand united dispensary, Co. Ltd., Bangkok, Thailand)

4.3 Equipment

Andersen cascade impactor (Atlanta, Georgia, USA)

Grinding mill (Fritsch, Germany)

Laser diffractometer (Mastersizer, Malvern, UK)

Scanning electron microscope (Jeol, Japan)

Sputter coater (SPI supplied, USA)

Twin stage impinger (Copley instrument, Nottingham, UK)

Ultrasonic bath (Tru-sweep, USA)

Vacuum oven (Precision Scientific, Inc., Chicago, USA)

Vacuum pump (Gast, USA)

V-shape mixer (Superline, Japan)

4.4 Methods

4.4.1 Preparation of the micronised and fine particles

Rifampicin, isoniazid and sugar carriers (trehalose, mannose and lactose) were dried at 37°C for 12 hours in a vacuum oven. Each drug and carrier was reduced in size by a grinding mill for 3 hours to obtain micronised particles (particle size ranges of 1-5 μm). Fine carrier particles (particle size ranges of about 10-20 μm) were prepared by

a similar method to the micronised particles except the grinding time was reduced to 2 hours. All the powders were stored in glass tight containers, which were placed in a desiccator at room temperature over silica gel until required for further use.

4.4.2 Particle size distribution measurement

The particle sizes based on volume distribution of micronised drugs and carriers were measured by laser diffraction using an independent particle size model fitted with a 100 mm lens and an obscuration was kept between 10 and 30%.

Dispersion of rifampicin (100 mg/50 ml) was prepared in aqueous 1% Tween 80, which dispersion of isoniazid was prepared in 1% Span 80 in hexane. Either micronised or fine carriers (trehalose, mannose and lactose) were dispersed in 50 ml of chloroform containing 1% w/v of Span 80 separately. All suspensions were sonicated in an ultrasonic bath for 10 minutes. Before performing sample measurement, a Malvern sample bath was filled with about 900 ml of the same medium that dispersed the drug or carriers. Background measurement was taken and then the sample was added and mixed homogeneously with the medium. Each size measurement was taken in triplicate.

4.4.3 Charactcrization of particle morphology

Visualization of particle size and morphology of micronised rifampicin, micronised isoniazid and all carriers were achieved by scanning electron microscopy (SEM). A small amount of each sample was scattered on an aluminium stub, the latter surface covered with clear double-sided adhesive tape. In order to obtain uniformly

scattered samples the aluminium stub was tapped gently on its edge with a spatula. The particles were then coated with a 15 to 20 nm layer of gold using a sputter coater on an argon atmosphere (50 Pa) at 50 mA for 50 seconds. All micrographs were taken at an acceleration voltage of 10 or 15 keV.

4.4.4 Preparation of formulations

The formulations in Table 4.1 were prepared by mixing 0.6 g of rifampicin or isoniazid with 1.0 g of each carrier (trehalose, mannose and lactose) in a screw cap tube and mixed for 5 minutes at room temperature. The tubes containing the formulation were fixed on V-shape mixer with tape and operated at 50 rpm for 2 hours. The drug : carrier ratio was 1 : 1.67 (w/w) in all formulations. These powder formulations are equal to 100 doses (16 mg per dose). All formulations were stored in a desiccator at room temperature over silica gel.

4.4.5 Effects of carrier size on deposition *in vitro*

The details of formulations are shown in Table 4.1. Formulation code -1, -2 or -3 are using trehalose, mannose or lactose as carrier, respectively. Formulation code (A), (B) or (C) are using fine fine carrier, fine+micronised carrier (1:1) and micronised carrier, respectively.

Powder blends of 16 mg of each formulation were weighed and placed in a glass inhaler device. The glass inhaler device is shown in Figure 4.1. It was designed and made in house at Prince of Songkla University to introduce a powder blend into a

twin stage impinger (TSI) at a flow rate of 60 l/min. The sample port allowed powder to be introduced into the device prior to aerosolisation. A plastic grid, size 74 μm, was fitted as a round disc to deaggregate the large particle size. The bleed holes served to reduce the resistance of the device and generate turbulence inside the device. The glass device was fitted with a moulded rubber mouthpiece adaptor that fitted the inlet part of the TSI. The diagrammatic representation of the TSI is show in Figure 4.2.

Table 4.1 Compositions of the dry powder formulations obtained from physical mixing

Formulation no.	Formulation code	Compositions		
		Micronised drug	Fine particles carrier	Micronised particles carier
1	RIF-1 (A)	Rifampicin 0.6 g	Trehalose 1.0 g	-
2	RIF-1 (B)	Rifampicin 0.6 g	Trehalose 0.5 g	Trehalose 0.5 g
3	RIF-1 (C)	Rifampicin 0.6 g	-	Trehalose 1.0 g
4	RIF-2 (A)	Rifampicin 0.6 g	Mannose 1.0 g	-
5	RIF-2 (B)	Rifampicin 0.6 g	Mannose 0.5 g	Mannose 0.5 g
6	RIF-2 (C)	Rifampicin 0.6 g	-	Mannose 1.0 g
7	RIF-3 (A)	Rifampicin 0.6 g	Lactose 1.0 g	-
8	RIF-3 (B)	Rifampicin 0.6 g	Lactose 0.5 g	Lactose 0.5 g
9	RIF-3 (C)	Rifampicin 0.6 g	-	Lactose 1.0 g
10	INH-1 (A)	Isoniazid 0.6 g	Trehalose 1.0 g	-
11	INH-1 (B)	Isoniazid 0.6 g	Trehalose 0.5 g	Trehalose 0.5 g
12	INH-1 (C)	Isoniazid 0.6 g	-	Trehalose 1.0 g
13	INH-2 (A)	Isoniazid 0.6 g	Mannose 1.0 g	-
14	INH-2 (B)	Isoniazid 0.6 g	Mannose 0.5 g	Mannose 0.5 g
15	INH-2 (C)	Isoniazid 0.6 g	-	Mannose 1.0 g
16	INH-3 (A)	Isoniazid 0.6 g	Lactose 1.0 g	-
17	INH-3 (B)	Isoniazid 0.6 g	Lactose 0.5 g	Lactose 0.5 g
18	INH-3 (C)	Isoniazid 0.6 g	-	Lactose 1.0 g

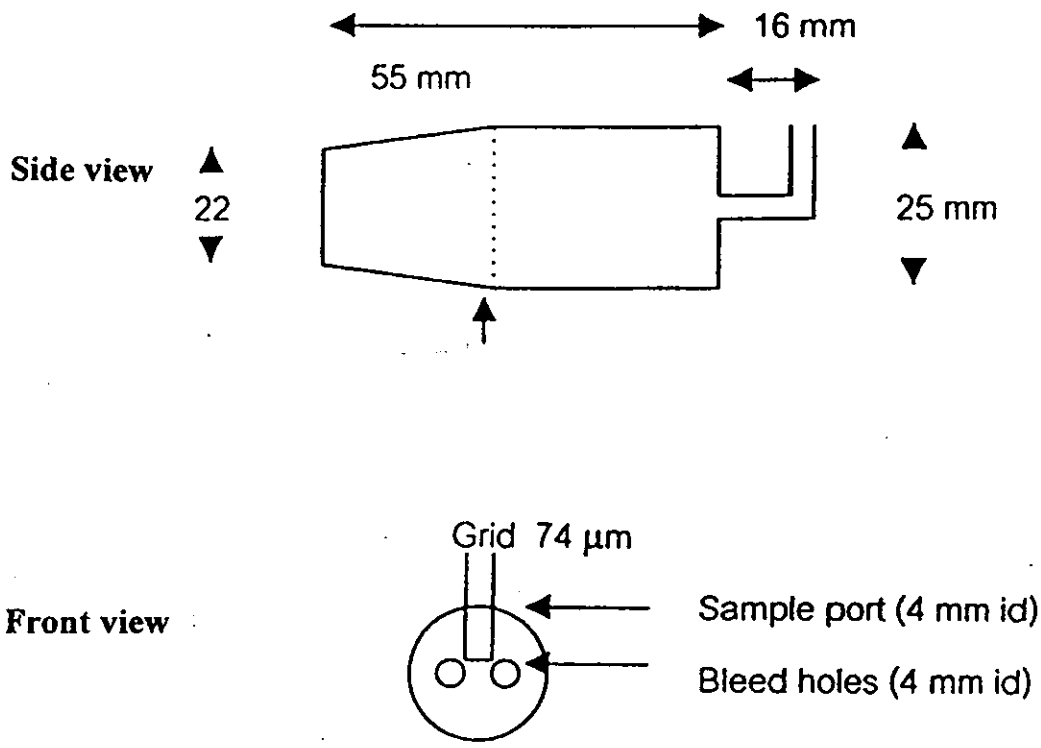


Figure 4.1 Diagrammatic representation of glass inhaler device showing dimensions which fitted the glass throat of TSI or Andersen cascade impactor (ACI) (Srichana *et al.*, 2003)

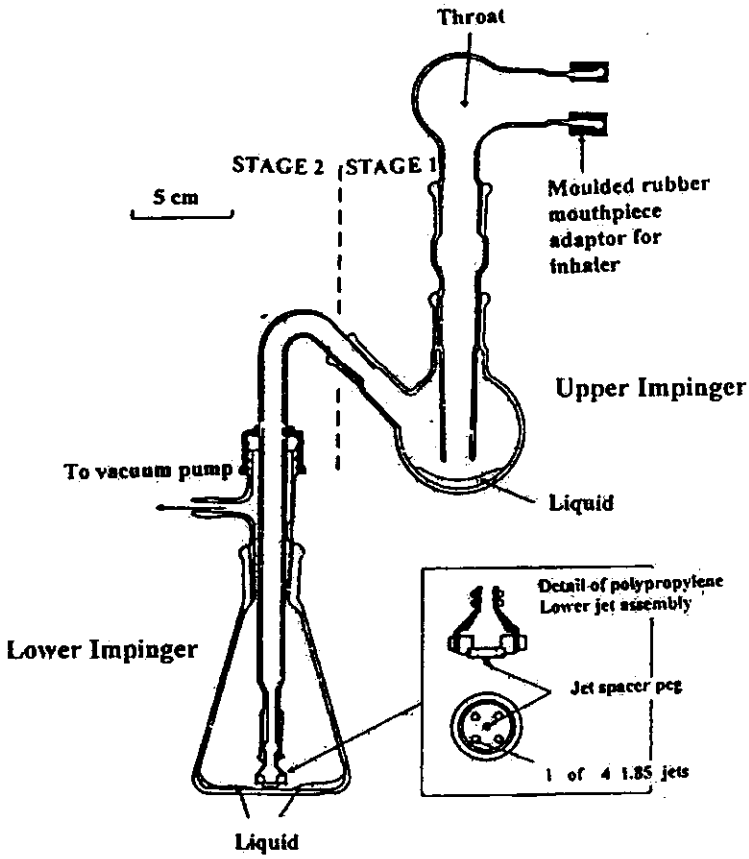


Figure 4.2 Diagrammatic representation of the TSI (Adapted from Hallworth and Westmoreland, 1987)

For each determination, 7 ml of HPLC mobile phase was placed in the stage 1 and 30 ml in stage 2 of the TSI. The airflow was drawn through the device at a flow rate of 60 l/min for 10 seconds. This was delivered into the TSI and the experiment was carried out 6 times. For each delivery, the apparatus was rinsed out with a HPLC mobile phase. Stage 1 washings included those from the rubber adaptor and stage 2 included those from the inside and outside of the stage 2 inlet tube assembly. Then the eluent was adjusted to the appropiate volume with the same solvent. The quantities of drug recovered separately from stage 1 and stage 2, were determined by HPLC. The results were calculated as the emitted dose (ED), which is the sum of the drug collected from the mouthpiece of the inhaler device and those deposited in the upper and lower stages. The fine particle dose (FPD) defined as the amount of drug recovered from the lower stages of the impinger, of which the aerodynamic diameter was less than 6.4 μm at an air flow rate of 60 l/min. Fine particle fraction (FPF) was the ratio of FPD to ED.

4.4.6 Content uniformity of the powder blends

Homogeneity of powder blends is required for DPIs that contain inactive excipient or active added substances. In this case the powder of each formulation was examined by sampling the dry powder formulation following the USP acceptance. A total of 10 doses were collected, three doses at the top, four in the middle, and three at the bottom of the powder blends.

In formulations of rifampicin, 16 mg of powder blend (1 dose) was weighed accurately and put into a 100 ml volumetric flask. HPLC mobile phase was used

to dissolve rifampicin and made up completed to 100 ml. The 100 ml of this solution was further diluted by pipetting 1 ml aliquat and transferring to 10 ml volumetric flask and completed to 10 ml with the same solvent.

In a formulation of isoniazid the sample was collected in a similar manner to that of rifampicin except that the drug was dissolved and diluted with water. All solutions were assayed according to the HPLC method described in Chapter 3. The mean actual drug content was expressed as a percentage of the theoretical drug content and the %RSD of these values was used to assess the homogeneity of the blends. The content uniformity should lie within the range of 75% to 125% of the theoretical content according to USP 24. This is a general criteria for DPI otherwise directed in the individual monograph.

4.4.7 *In vitro* deposition of drugs in dry powder formulations by ACI

The deposition of each dry powder drug was assessed *in vitro* using a Andersen Mark II cascade impactor. Cascade impactors are instruments designed to measure the size distribution of aerosolised particles. The ACI consists of an inlet, preseperator and eight stages of impactor together with the plate. Some selected formulations after being assessed by the TSI were determined again by ACI based on the formulations having a high lower deposition in the TSI (>60%). In the case of rifampicin formulations, each powder formulation was weighed by an analytical balance for 16 mg and placed in a glass inhaler device. The air flow was drawn through the device at a standard flow rate of 60 l/min at a flow time 10 seconds. This was delivered into ACI and

the experiment was carried out 6 times. For each delivery, the powder deposited on the different stages was recovered by washing each plate and the above stage with a HPLC mobile phase. The powder deposited in the glass device, inlet and preseparator was also collected in a separated container. All eluent was adjusted to the appropriate volume with the same solvent.

In the case of isoniazid formulations a similar method was applied but using water to dissolve and dilute instead of HPLC mobile phase of rifampicin. The drug concentrations in these samples were assayed by HPLC according to HPLC method as described in Chapter 3.

The ED was determined as the percent of total powder mass exiting the inhaler device. The mass of powder deposited on each stage of the Andersen impactor was calculated as a percentage and plotted against the log (effective cut-off diameter). The y axis was obtained by transformation of the percent cumulative oversize on various stage of the ACI to a probability scale (z value) and x axis was on logarithm of size. The mass median aerodynamic diameter (MMAD) corresponds to z value of 0 and the geometric standard deviation (GSD) was obtained by the square root of the size of z value of 1 divided by the size obtained at z value of -1 that can be illustrated by the following equation;

$$GSD = \sqrt{\frac{SizeX}{SizeY}}$$

Where size X is the particle size for which the line crosses the 84% mark (z value = 1) and size Y the 16% mark (z value = -1).

4.4.8 Stability of the power blends after storage

The selected formulations evaluated by ACI were stored in a desiccator at room temperature. After storage for 3 months, the drug contents and MMAD were determined as described previously. Then a pair *t*-test was performed to compare the MMAD of the formulations before and after storage at the significance level of $P < 0.05$ using the program of SPSS version 10.0.

4.5 Results and Discussion

4.5.1 Particle size distribution measurement

The volume median diameters of rifampicin, isoniazid and all carriers obtained from laser light diffraction are summarised in Table 4.2. The examples of volume particle size distribution of all drugs and carriers are shown in Figure 4.3-4.10.

Grinding mills have been widely applied in different fields such as the mining, chemical and pharmaceutical industries. A grinding mill consists of a rotating cylindrical drum with mortar. Particle size reduction is dependent on operational conditions such as rotational speed, mill size and sample charge (Mori *et al.*, 2004). The atmospheric moisture affects the particle size aggregation. Therefore, prior to milling process, particle was dried in a vacuum oven.

Pharmaceutical powders smaller than 5 μm are predicted to be able to enter the lower airways (Hickey, 1992). Table 4.2 shows the mean of the volume median diameters. Micronised drugs have a particle size in a range of 1-5 μm while the carriers have two ranges of particle size. Both carriers can be used to prepare the formulations with a micronised drug. Reducing the carrier particle size has also been exploited in an attempt to improve *in vitro* drug deposition. A more practical approach, which might be employed industrially to improve drug delivery efficiency from DPI, might involve manipulation of the powder formulations. Such an approach could include the addition of fine particles of a third component or micronised carrier to the powder formulations (Tee *et al.*, 2000).

The grinding mill process produced micronised drugs and micronised sugar carriers with a volume median diameter ranging between 3.35 and 6.04 μm. The fine carriers have a volume median diameter ranging between 15.21 and 17.88 μm when using a grinding time shorter than micronised drugs or micronised sugar carriers. The micronised rifampicin and isoniazid exhibited particle size less than 5 μm over 99% (Figure 4.3 and 4.4), suggesting that these materials were suitable for use as an inhalation aerosol. The particles of fine trehalose and mannose had a particle size over 10 μm for 100% (Figure 4.5 and 4.7) whereas over 85% of the lactose present in the size over 10 μm (Figure 4.9). Such a difference in the particle size of carriers is important for the effects of the deposition in lower airway.

Table 4.2 The volume median diameter of drugs and carriers (mean ± SD, n = 3)

Materials	Mean of volume median diameter (μm)
Micronised rifampicin	3.35 ± 2.23
Micronised isoniazid	4.07 ± 2.03
Fine trehalose	15.21 ± 1.42
Micronised trehalose	4.84 ± 1.81
Fine mannose	16.91 ± 1.44
Micronised mannose	3.75 ± 1.73
Fine lactose	17.88 ± 2.25
Micronised lactose	6.04 ± 0.48

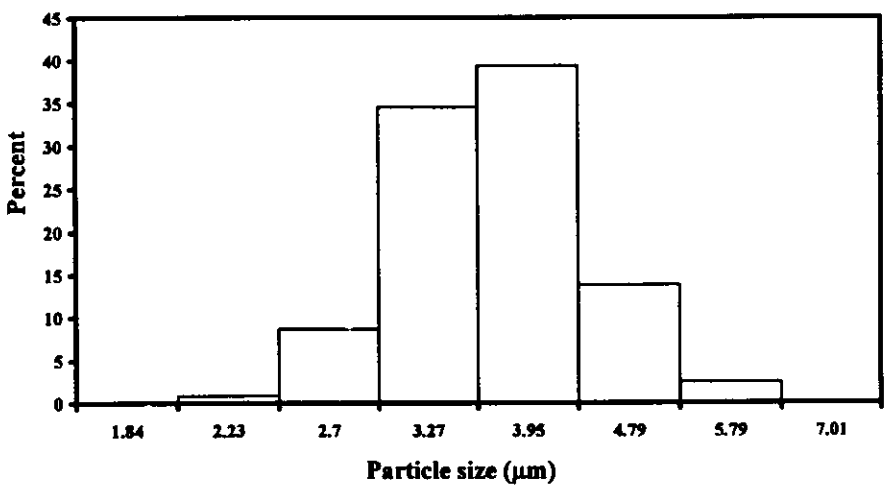


Figure 4.3 Particle size distribution based on volume of micronised rifampicin using Malvern laser light diffraction technique

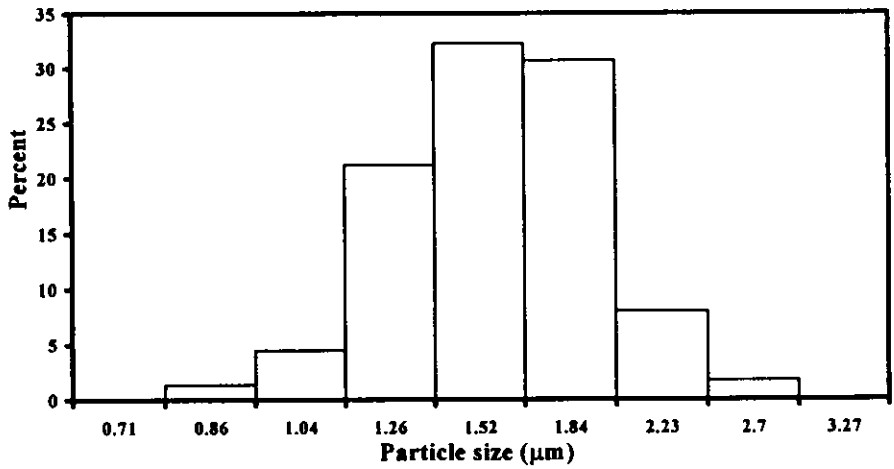


Figure 4.4 Particle size distribution based on volume of micronised isoniazid using Malvern laser light diffraction technique

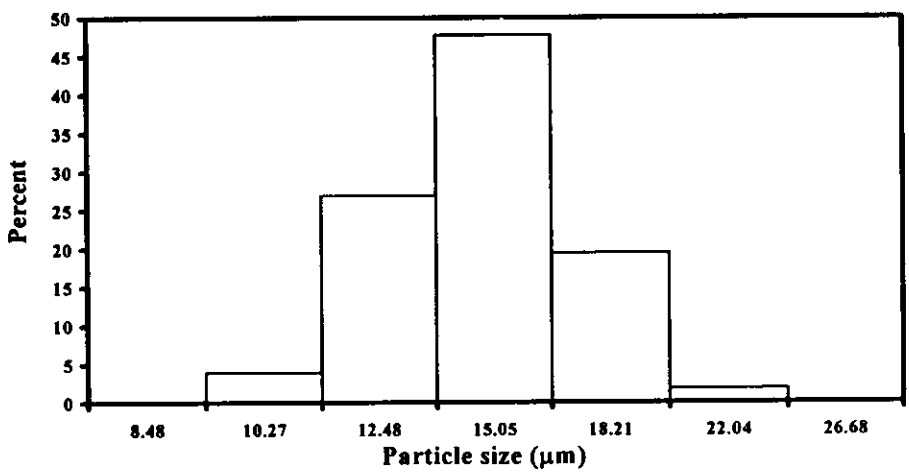


Figure 4.5 Particle size distribution based on volume of fine trehalose using Malvern laser light diffraction technique

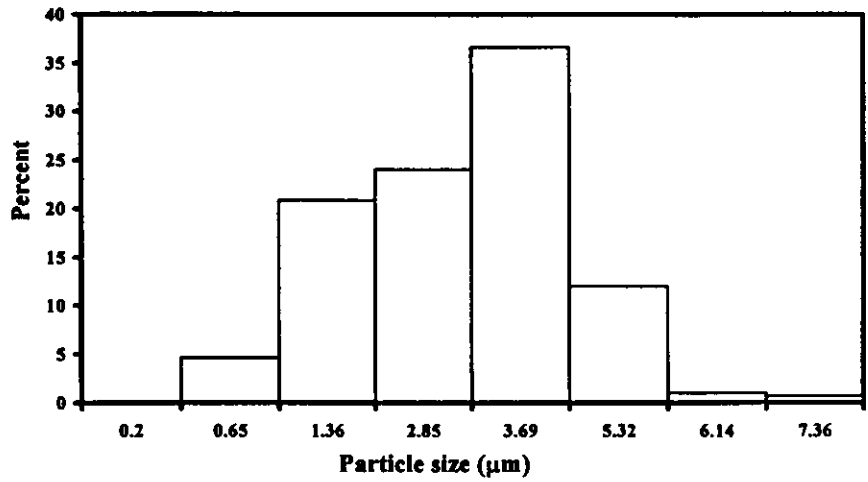


Figure 4.6 Particle size distribution based on volume of micronised trehalose using Malvern laser light diffraction technique

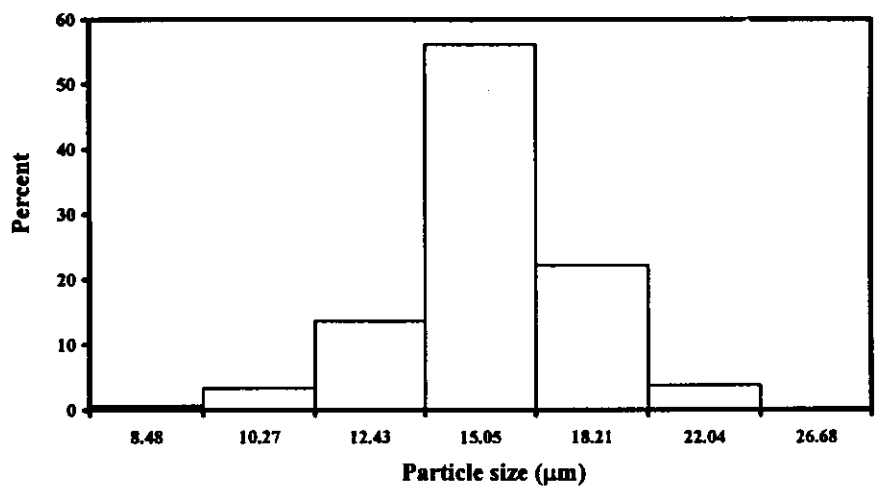


Figure 4.7 Particle size distribution based on volume of fine mannose using Malvern laser light diffraction technique

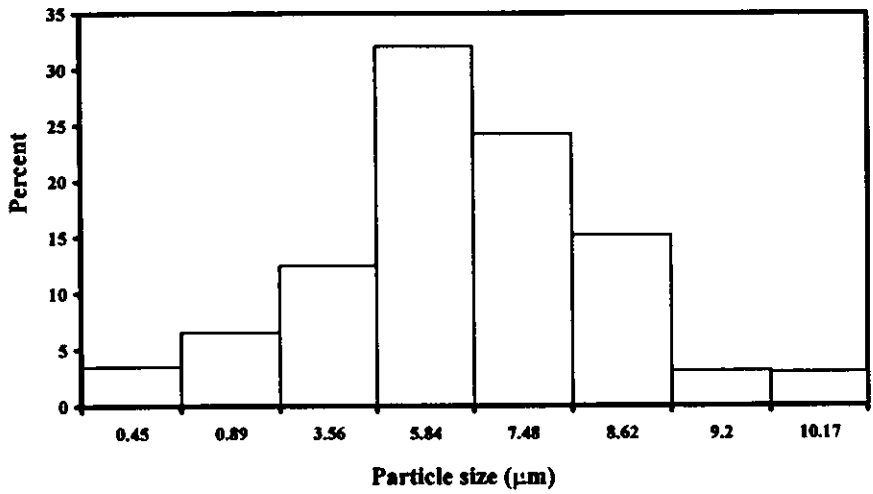


Figure 4.8 Particle size distribution based on volume of micronised mannose using Malvern laser light diffraction technique

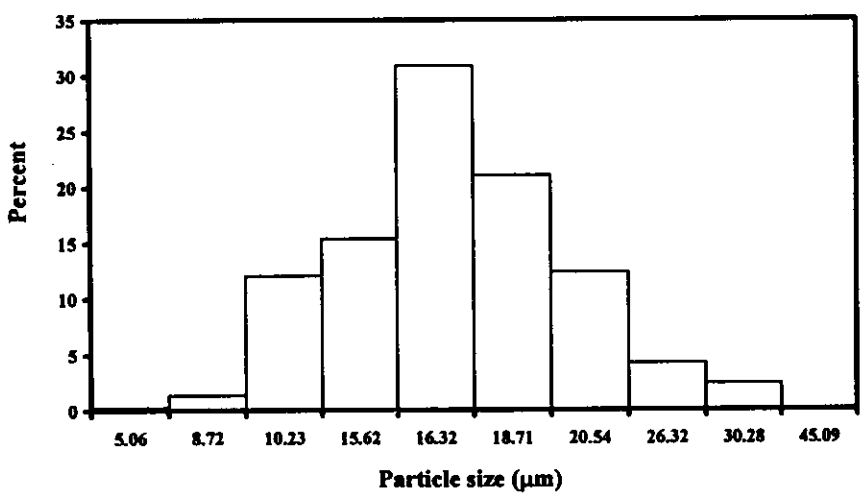


Figure 4.9 Particle size distribution based on volume of fine lactose using Malvern laser light diffraction technique

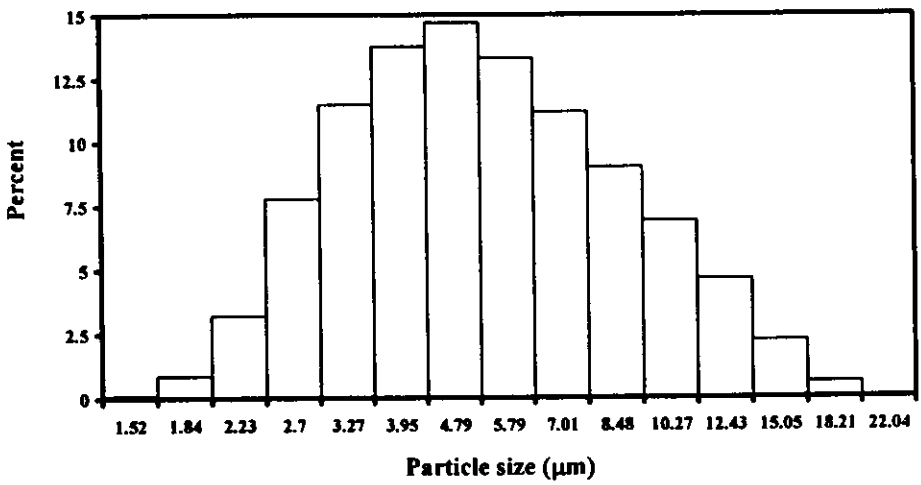
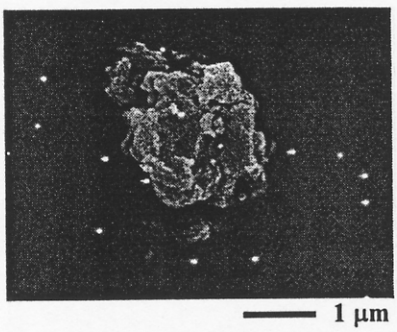


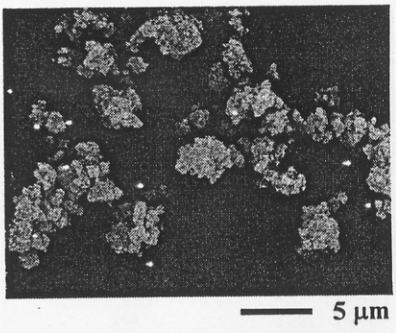
Figure 4.10 Particle size distribution based on volume of micronised lactose using Malvern laser light diffraction technique

4.5.2 Morphology of particles

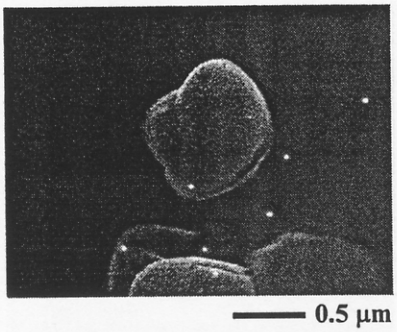
Scanning electron micrographs show that the drugs and carriers varied from irregular shapes to spherical shapes (Figures 4.11 – 4.12). All drugs and carriers particles were aggregated from individual particles. All particles varied between 1-20 μm according to particle size distribution.



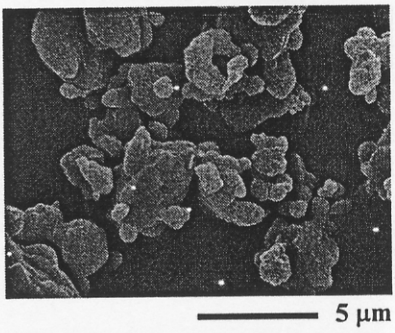
(A) Micronised rifampicin



(B) Micronised rifampicin



(C) Micronised isoniazid



(D) Micronised isoniazid

Figure 4.11 Electron micrographs of (A) micronised rifampicin × 1500, (B) micronised rifampicin × 3000, (C) micronised isoniazid × 30000 and (D) micronised isoniazid × 4000

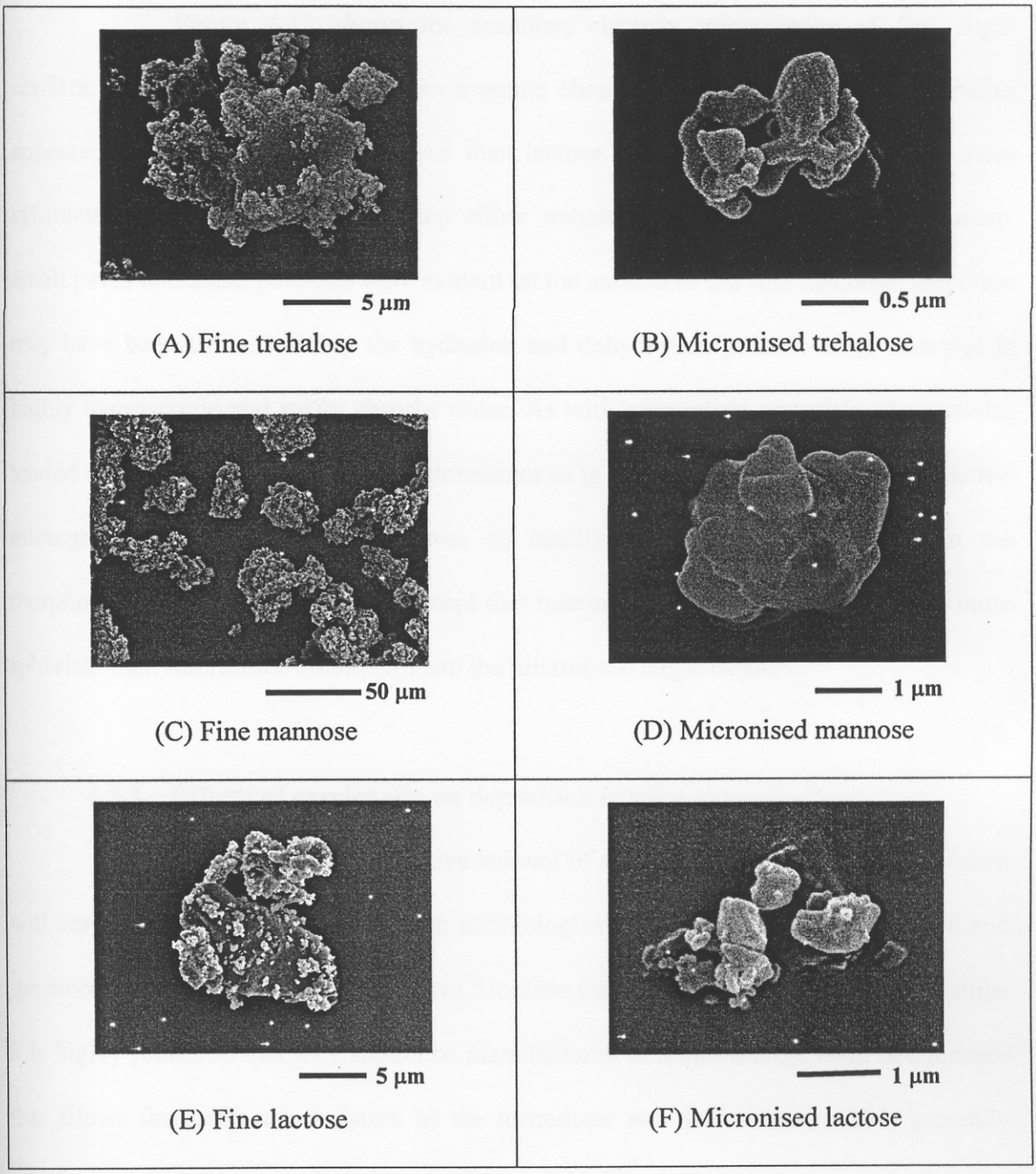


Figure 4.12 Electron micrographs of (A) fine trehalose × 4000, (B) micronised trehalose × 3000, (C) fine mannose × 500, (D) micronised mannose × 15000, (E) fine lactose × 3000 and (F) micronised lactose × 18000

Figure 4.12 shows the scanning electron micrographs of fine sugar carriers. The fine lactose exhibited an irregular shape. Trehalose and mannose particles appeared to be slightly more spherical than lactose. The mannose particles were more symmetrical and clearly rounder than either trehalose or lactose. Interestingly, many small pores and small particles were evident on the surface of the fine mannose, and these may have been formed during the hydration and dehydration process since mannose is highly hygroscopic and easily absorbs water. As with micronised materials, the particles tended to be cohesive and formed agglomerates as is apparent from the scanning electron micrographs (Figure 4.11). There was no readily distinguishable difference in the morphology of these fine particles except that micronised isoniazid appeared to be more spherical than micronised rifampicin and the micronised sugar carriers.

4.5.3 Effects of carrier size on deposition *in vitro*

A therapeutically effective amount of an active pharmaceutical preparation will vary in composition depending on the biological activity of the drug employed and the amount needed in a unit dosage form. Because the subject compounds are dispersible, it is highly preferred that they should be manufactured in a unit dosage form in a manner that allows for ready manipulation by the formulator and the consumer. This generally means that a unit dosage will be between about 0.5 mg and 15 mg of total materials in the drug powder composition, preferable between about 2 mg and 10 mg. Generally, the amount of drug in the composition will vary from about 0.05% w/w to about 99.0% w/w.

Preferably the composition will be about 0.2% w/w to about 97.0% w/w drug (Platz *et al.*, 2003).

The amount of a pharmaceutically acceptable carrier is the amount needed to provide the necessary stability, dispersibility, consistency and bulking characteristics to ensure a uniform pulmonary delivery of the composition subject in need thereof. Numerically the amount may be from about 0.05% w/w to about 99.95% w/w, depending on the activity of the drug being employed. Preferably about 5% w/w to about 95% w/w will be used (Platz *et al.*, 2003).

One dose of rifampicin is 600 mg and isoniazid is 300 mg. Generally, the dose for inhalation may be reduced to less than 40-80 times compared to conventional oral formulation. Hence, the dose of both rifampicin and isoniazid for DPIs is fixed at 6 mg. This result will be verified *in vitro* using microbial assay. Total unit dosages of these formulations are 16 mg.

The aerodynamic behavior of all formulations was estimated with TSI making it possible to study the *in vitro* deposition profile. The effects of the carriers, the ratios of micronised and fine carriers on the ED, FPD and FPF are shown in Table 4.3.

The results show that the ED with all types of carrier is high for blends obtained with different ratios of carriers; representing 5.12 to 6.49 mg (85.33-107.5%) of the theoretical dose (6 mg). The ED with all carriers was high and displayed few variations. The low variations in the ED were related to the variation in the mean content of the blend and ED obtained by ACI.

Table 4.3 Drug depositions in the TSI after aerosolization of the different blends (mean ± SD, n = 6)

Formulation code	ED (mg)	FPD (mg)	FPF (%)
RIF-1 (A)	5.71 ± 0.16	4.25 ± 0.14	75.42 ± 3.17
RIF-1 (B)	5.12 ± 0.37	2.54 ± 1.19	57.73 ± 4.67
RIF-1 (C)	5.54 ± 0.34	0.65 ± 0.26	11.75 ± 4.68
RIF-2 (A)	5.68 ± 0.29	0.77 ± 0.29	13.61 ± 5.17
RIF-2 (B)	5.95 ± 0.43	2.09 ± 0.20	37.73 ± 3.31
RIF-2 (C)	5.85 ± 0.24	4.26 ± 0.25	72.91 ± 4.89
RIF-3 (A)	5.96 ± 0.18	4.71 ± 0.24	79.04 ± 1.77
RIF-3 (B)	5.22 ± 0.29	2.72 ± 0.16	52.11 ± 0.81
RIF-3 (C)	5.75 ± 0.34	0.99 ± 0.14	17.19 ± 1.54
INH-1 (A)	5.87 ± 0.22	3.80 ± 0.18	64.74 ± 2.26
INH-1 (B)	6.05 ± 0.23	1.97 ± 0.91	38.04 ± 5.21
INH-1 (C)	5.88 ± 0.43	0.71 ± 0.18	11.99 ± 2.87
INH-2 (A)	6.49 ± 0.41	1.17 ± 0.53	21.08 ± 1.85
INH-2 (B)	5.95 ± 0.64	1.66 ± 0.85	33.20 ± 10.57
INH-2 (C)	5.89 ± 0.72	4.34 ± 0.66	73.86 ± 8.62
INH-3 (A)	5.55 ± 0.16	4.52 ± 0.32	81.50 ± 4.66
INH-3 (B)	5.80 ± 0.22	3.56 ± 0.38	61.41 ± 6.52
INH-3 (C)	5.75 ± 0.38	0.97 ± 0.55	16.85 ± 9.16

The ED was substantially lower than the theoretical dose. This may be the results of drug deposition in glass inhaler device and degradation of rifampicin. FPDs and FPFs were varied from 11.75% to 81.50% depending on the types and ratios of micronised and fine carriers. The results show that the carrier size strongly affected the *in vitro* deposition of drugs. The formulations containing fine particles of trehalose and lactose (particle size about 10-20 μm) showed the highest FPFs of drugs. In contrast, the

formulations containing micronized particles of mannose (particle size about 1-5 μm) shows the highest FPFs of the drugs (both rifampicin and isoniazid). This result can be partly explained by the fact that interaction between the drug and different types of carriers strongly affected the adhesion force of drug and carriers. According to the study of Bosquillon *et al.* (2001), the types of sugars/polyol greatly influenced the *in vitro* deposition of the powders (lactose, trehalose and mannitol presenting the best to poorest behavior in the Andersen impactor). But the physical characteristics of those powders, particle size, density, and overall morphology, as visualized by electron microscopy, were relatively similar, suggesting that the differences in respirable fractions rather resulted from differences in powder cohesiveness with the type of excipient. The study of Louey *et al.* (2003) was found that closer examination showed correlation between FPF and the presence of fine carrier particles (*e.g.* particle less than 5 μm), demonstrating the importance of fine adhered carrier particles in dispersion process. The improved dispersion, when lactose fines were associated with the carrier, was consistant with a proposed hypothesis relating to greater surface detachment and dispersion of mixed agglomerates of fine lactose and drug. The existence of larger carrier particles was found to be essential to optimize the dispersion process, with maximum drug deposition achieved with carrier systems containing around 10% fine particle and 90% coarse particle concentrations. The study of Louey *et al.* (2003) go along with our report that lactose or trehalose with particle size 10-20 μm can improved FPF of drug more than particle size 1-5 μm of carrier. Differences in hygroscopicity of the materials and thereby alteration of capillary forces and/or differences in surface properies may explain those

results (Bosquillon *et al.*, 2001). From Table 4.3 the ED reached 96.31 ± 5.08 % in all of formulations. The powder exhibited excellent aerosolization properties in the TSI. We selected the formulations that have high FPF (> 60%) in each carrier for study further. The selected powder blends were RIF-1 (A), RIF-2 (C), RIF-3 (A), INH-1 (A), INH-2 (C) and INH-3 (A).

4.5.4 Content uniformity of the powder blends

After blending the carriers with rifampicin and isoniazid, the average content and the uniformity of the drugs were measure. Figure 4.13 shows the content uniformity of all rifampicin formulations. The average rifampicin contents combined with trehalose ; RIF-1 (A), (B) and (C) and lactose ; RIF-3 (A), (B) and (C) were 103.16 ± 3.55% and 98.67 ± 3.85%, respectively. Whereas the formulations RIF-2 (A), (B) and (C) with mannose presented greater variations in drug content (85.24 ± 15%). Probably, the formulation with mannose may absorb more moisture into powder blends. Thus, content uniformity in formulation RIF-2 included in the interval of content ± 15% is different from the formulation using trehalose and lactose carriers. Figure 4.14 shows content uniformity of ail isoniazid formulations. In this case, all formulations had high content uniformity. The average isoniazid contents were 102.49 ± 2.07%, 102.17 ± 2.26% and 101.62 ± 1.88% of the INH-1, INH-2 and INH-3 formulations, respectively. The results suggest that the overall process of mixing, sampling and analysis in this study was accurate and reproducible, and uniform mixing was achieved by employing this mixing procedure. In addition, the drug distribution in blends with all carriers was more

homogeneous, probably because of its higher level of drug adhesion on the carrier and high drug contents in dosage unit. In all cases of rifampicin and isoniazid formulations, blend uniformity so called "uniformity of dosage units" was obtained because it was within the range of acceptance criteria of dosage unit in DPIs that described in USP 24. However, these rifampicin formulations could be further developed to protect the formulations against degradation for obtained high drug content (within range ± 5%).

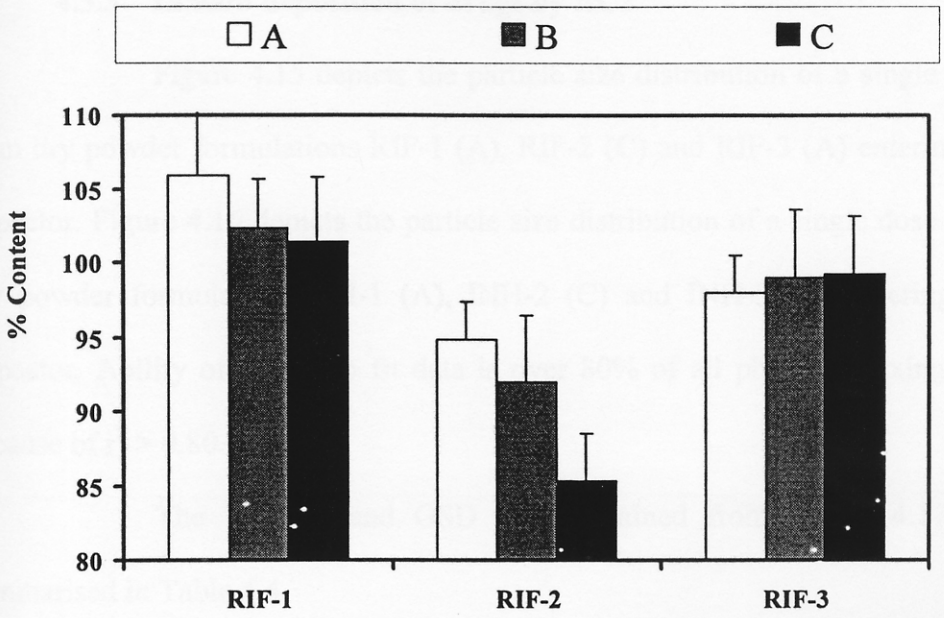


Figure 4.13 Content uniformity of formulations RIF-1, RIF-2 and RIF-3 (mean ± SD, n = 10)

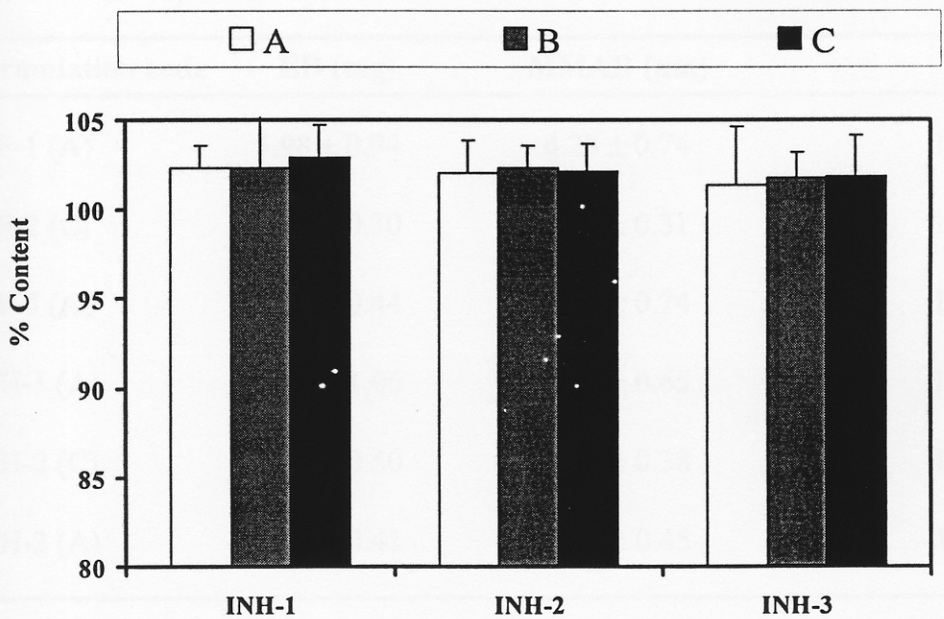


Figure 4.14 Content uniformity of formulations INH-1, INH-2 and INH-3 (mean ± SD, n = 10)

4.5.5 *In vitro* deposition of drugs by ACI

Figure 4.15 depicts the particle size distribution of a single dose delivery from dry powder formulations RIF-1 (A), RIF-2 (C) and RIF-3 (A) entering the cascade impactor. Figure 4.16 depicts the particle size distribution of a single dose delivery from dry powder formulation INH-1 (A), INH-2 (C) and INH-3 (A) entering the cascade impactor. Ability of model to fit data is over 80% of all physical mixing formulations because of $r^2 > 0.80$.

The MMAD and GSD were obtained from Figure 4.17 – 4.18 and summarised in Table 4.4.

Table 4.4 The summarised of MMAD and GSD as obtained from ACI (mean ± SD, n = 6)

Formulation code	ED (mg)	MMAD (μm)	GSD
RIF-1 (A)	5.98 ± 0.94	4.28 ± 0.74	1.68 ± 0.13
RIF-2 (C)	5.82 ± 0.30	4.41 ± 0.31	1.54 ± 0.19
RIF-3 (A)	5.80 ± 0.44	3.67 ± 0.74	1.98 ± 0.18
INH-1 (A)	4.58 ± 1.06	4.40 ± 0.65	1.62 ± 0.36
INH-2 (C)	5.02 ± 0.50	3.14 ± 0.38	1.74 ± 0.42
INH-3 (A)	5.73 ± 0.41	4.01 ± 0.45	1.97 ± 0.11

The ED varied from 4.58 to 5.98 mg when calculated from ACI. The ED was very high and varied within a narrow range as similar to the ED obtained from TSI. Figures 4.15 and 4.16 show the weight fractions according to the size distribution of the aerosolized particles of rifampicin and isoniazid with the selected formulations of difference types and ratios of micronised and fine carriers. Each bar represents the powders of certain sizes collected on a defined stage of the ACI. In the case of rifampicin, the size distribution of RIF-1 (A), RIF-2-(C) and RIF-3 (A) are in similar manner. The results suggest that the physical interaction between rifampicin and fine trehalose, micronised mannose or fine lactose was suitable for the delivery of rifampicin to the lower airway. In the case of isoniazid, the size distribution of INH-1(A), INH-2 (C) and INH-3 (A) was varied. The result may be explained by the different interaction between isoniazid and different types of carriers. Therefore, the delivery of the formulations based on aerodynamic diameters was different. However, limitations of ACI are lacks precision and fine particle collection by adhesion and electrostatic charge may occur (Boer *et al.*, 2002)

The average MMAD of each powder formulation represents in Table 4.4. The MMAD varied from 3.14 to 4.28 μm according to aerosol powders ranging from 1 to 5 μm are considered as the optimum size for deposition beyond the increasingly narrow airway into the alveoli. GSD was presented the spread of the particle (Mitchell *et al.*, 2003). All formulations had GSD values above 1.25 therefore the formulations were polydisperses (Suarez and Hickey, 2000).

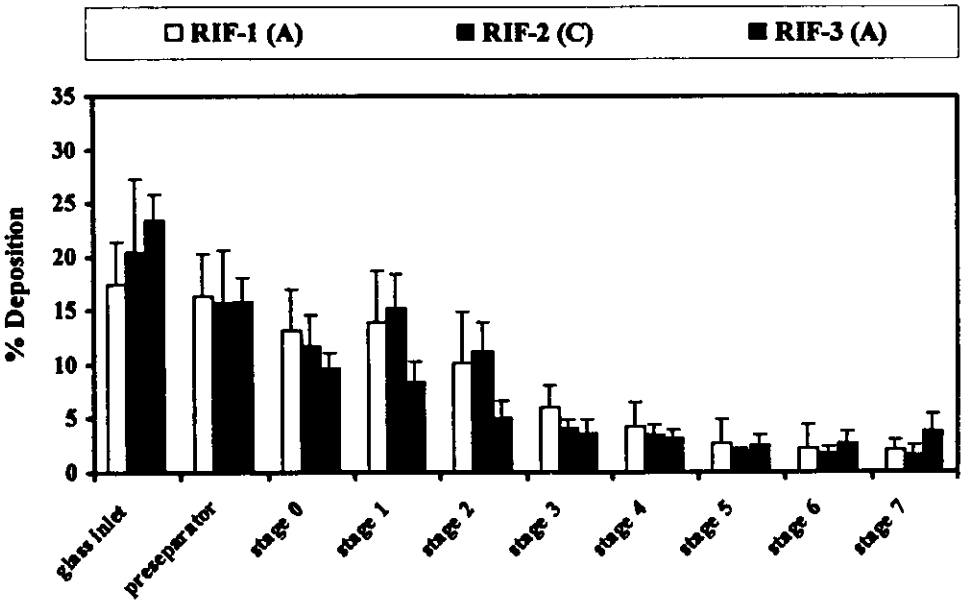


Figure 4.15 Size distribution of rifampicin formulations on each stage of the ACI as aerosolized at a flow rate of 60 l/min (mean ± SD, n = 6)

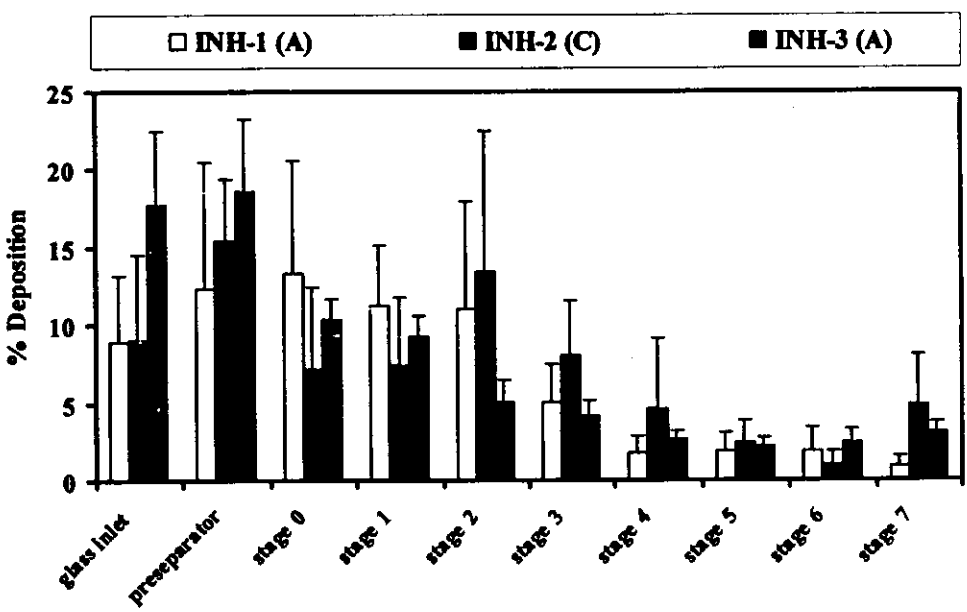
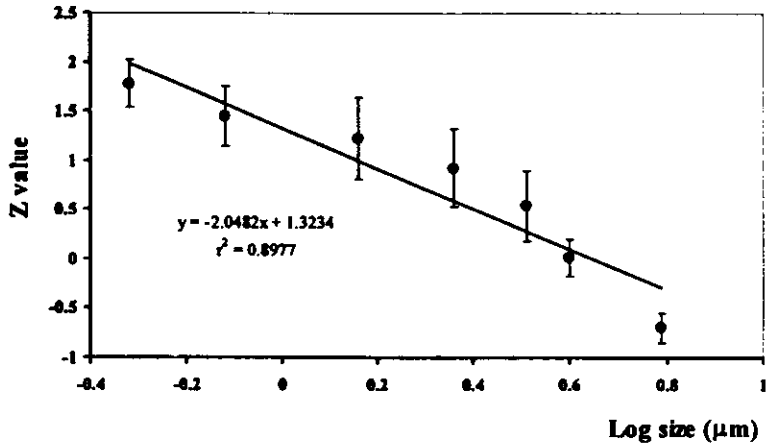
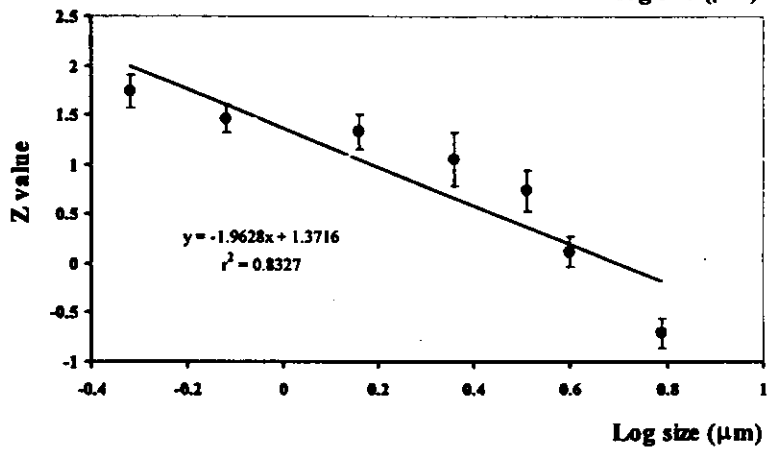


Figure 4.16 Size distribution of isoniazid formulations on each stage of the ACI as aerosolized at a flow rate of 60 l/min (mean ± SD, n = 6)

(A)



(B)



(C)

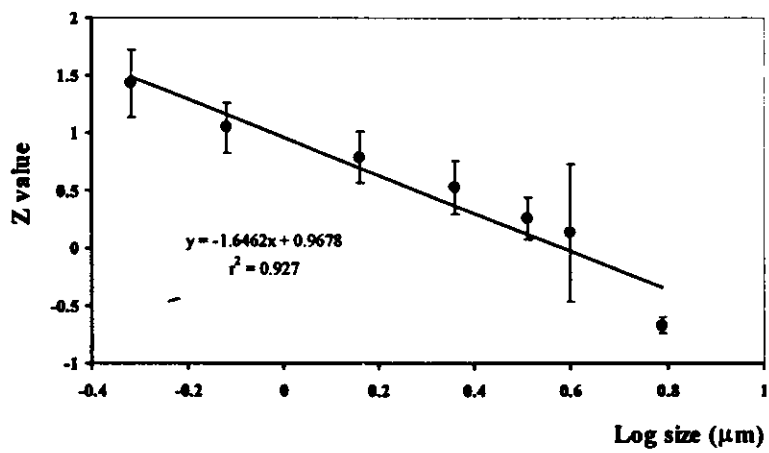
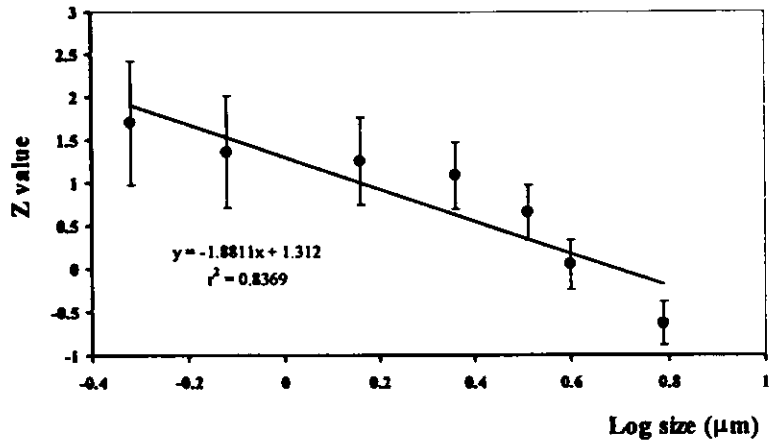
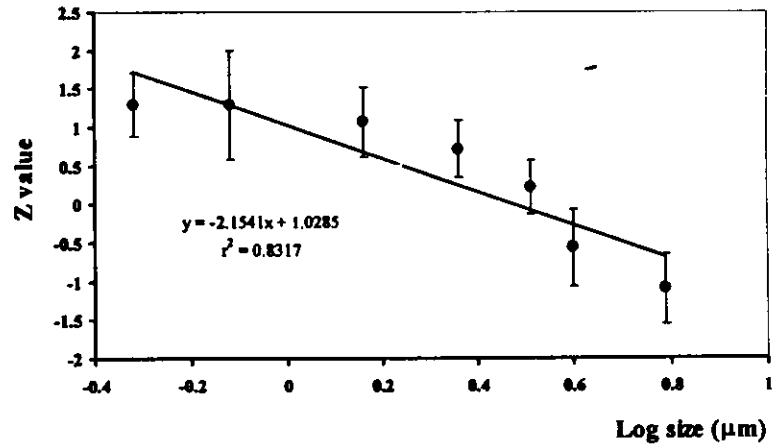


Figure 4.17 Relationship between Z value and cut off aerodynamic diameter (log scale) on each stage of the ACI of formulations ; (A) RIF-1 (A), (B) RIF-2 (C) and (C) RIF-3 (A), as aerosolized at a flow rate 60 l/min

(A)



(B)



(C)

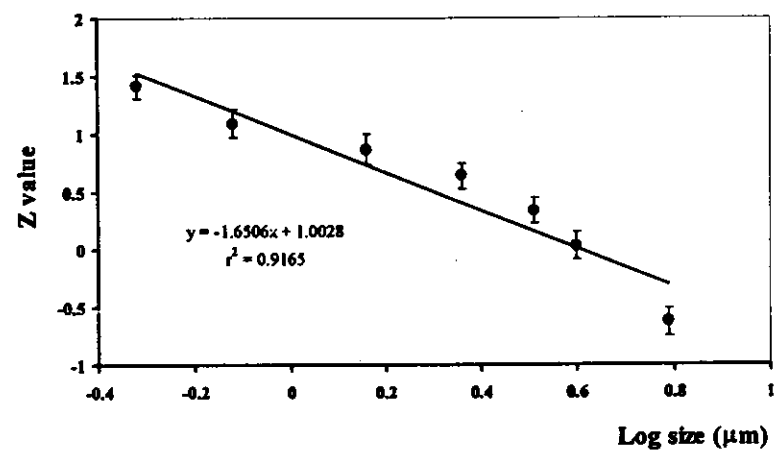


Figure 4.18 Relationship between Z value and cut off aerodynamic diameter (log scale) on each stage of the ACI of formulations ; (A) INH-1 (A), (B) INH-2 (C) and (C) INH-3 (A), as aerosolized at a flow rate 60 l/min

4.5.6 Stability of the powder blends after storage

The drug contents and MMAD after storage for 3 months at room temperature are shown in Table 4.5.

Table 4.5 Drug contents (mean ± SD, n = 3) and MMAD (mean ± SD, n = 6) of dry powder formulations after storage 3 months at room temperature

Formulation	%content		MMAD (μm)	
	Initial	After 3 months	Initial	After 3 months
RIF-1 (A)	105.81 ± 5.45	84.19 ± 8.30	4.28 ± 0.74	4.94 ± 0.47
RIF-2 (C)	85.24 ± 4.70	66.49 ± 4.87	4.41 ± 0.31	5.14 ± 2.65
RIF-3 (A)	97.88 ± 4.37	88.52 ± 10.59	3.67 ± 0.74	4.94 ± 0.47#
INH-1 (A)	102.33 ± 1.19	102.69 ± 2.02	4.40 ± 0.65	5.33 ± 1.04#
INH-2 (C)	102.16 ± 1.49	98.09 ± 3.14	3.14 ± 0.38	5.49 ± 0.55#
INH-3 (A)	101.32 ± 1.83	100.62 ± 2.35	4.01 ± 0.45	4.89 ± 0.26

#$P < 0.05$

From Table 4.5, all the rifampicin formulations showed rapid decrease in drug content. This may be the results that rifampicin was sensitive to moisture and light causing degradation (Gallo and Radaelli, 1976). Fortunately, formulated in a solid formulation, rifampicin was slower to degrade than in a solution formulation. In contrast the drug content isoniazid formulations, before and after storage are not different. The results suggest that the chemical stability of isoniazid formulations is higher than that of rifampicin formulations. MMAD of the initial formulations was compared with that storage after 3 months. The formulations containing fine trehalose [RIF-1 (A), INH-1 (A)], micronised mannose [RIF-2 (C), INH-2 (C)] or fine lactose [RIF-3 (A), INH-3 (A)] showed small MMAD values before storage (varying from 3.14 to 4.41 μm) when the formulations RIF-3 (A), INH-1 (A) and INH-2 (C) after storage are significantly larger than the initial formulations (P value < 0.05 of these formulations). The significant

increase of MMAD after storage may probably due to the formation of particle aggregates. These aggregates were presumably responsible for the large fractions of these microparticles depositing in the preseparator and the higher stages of the cascade impactor. Particles aggregation may occur by moisture sorption and electrostatic force. Therefore, these formulations had to be improved in the aggregation of particles. Aggregation of particles in the DPIs or upon their administration can influence the respirable fraction of the delivered dose. The molecular, electric, coulombic and capillary forces responsible for powder aggregation are influenced by particle size, surface morphology, surface charge and moisture composition (Philip *et al.*, 1997). Most of aerosols consist of charged particles, especially when freshly generated. When the particles are bipolar, the collisions between the particles occur more rapidly than with the particles that are uncharged. When the particles are unipolar, the collision rate is even amaller than that for uncharged particles. Thus a particle sample that is highly uipolar will be less aggregated than a microparticles sample that relatively less unipolar (i.e. more bipolar) (Philip *et al.*, 1997). In contrast, the formulations RIF-1 (A), RIF-2 (C) and INH-3 (A) showed unchanged MMAD values after storage.