Chapter 6

FORMULATION DESIGN OF ANTITUBERCULOSIS DRY POWDER INHALERS BY SPRAYING INTO ANTISOLVENT

6.1 Introduction

Spray freeze drying was explored for pharmaceutical application in the early 1990s. It involves spraying the drug solution into a freezing medium (usually liquid nitrogen and carbon dioxide) followed by lyophilization. This process produces light and porous particles with enhanced aerosol performance as compared with spray drying and the production yield is almost 100%. The method has been applied to prepare pharmaceutical peptides and proteins particles for inhalation and also can be used for other compounds. However, this is an expensive process and would only be justifiable for expensive drugs as it requires the additional use of liquid nitrogen and the freeze drying step is more time consuming (Chan and Chew, 2003). This process is interesting for production of antitubercular drugs for inhalation. This method is may be modified for the production of inhalation particles.

6.2 Materials

Ammonium molybdate (Sigma chemical company, St. Louis, MO, USA)

Chloroform (VWR International Ltd., England)

Cholesterol from lanolin (Fluka, Switzerland)

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

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

L-α-Phosphatidylcholine from soy bean (Fluka, Switzerland)

Polyamide membrane 0.45 μm (Sartorius, Germany)

Rifampicin (Fluka, Switzerland)

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

6.3 Equipment

Analytical balance (Sartorius, Germany)

Andersen cascade impactor (Atlanta, Georgia, USA)

Cold bath (Eyela, Tokyo Rikakikai Co., Ltd., Tokyo, Japan)

Confocal laser scanning microscopy (FV300, Olympus, Japan)

Freeze dryer (Flexi-Dry, USA)

Gas chromatography (Hewlett Packard 6890 GC with Electron Capture Detector, USA)

Laser diffractometer (Mastersizer, Malvern, UK)

Pan coating (Taiyo, Japan)

Peristaltic pump (Watson-Marlow Limited,UK)

Scanning electron microscope (Jeol, Japan)

Spray nozzle (Walther Pilot, Germany)

Sputter coater (SPI supplied, USA)

Transmission electron microscope (Jeol, Japan)

Twin stage impinger (Copley instrument, Nottingham UK)

Ultrasonic bath (Tru-sweep, USA)

Vacuum pump (Gast, USA)

6.4 Methods

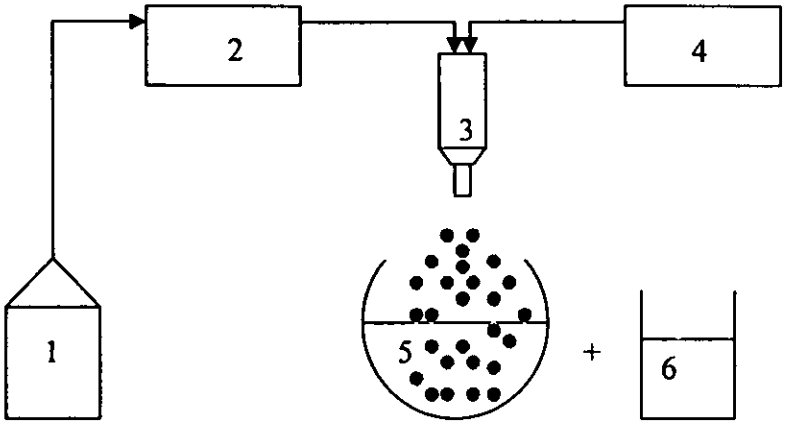
6.4.1 Production of encapsulated rifampicin

A schematic diagram of spraying into antisolvent technique that was employed in the experiments is shown in Figure 6.1.

The fundamental components of formulation are :

Rx

Cholesterol from lanolin L-α-Phosphatidylcholine from soy bean	2 g
Carrier (Trehalose or Mannose)	2 g
Rifampicin	10 mg



1. Solution formulation reservoir
2. Peristaltic pump
3. Spray nozzle
4. Air pump
5. Drug droplets spraying in antisolvent
6. Carrier solution
7. Drug powder collected after freeze drying

Figure 6.1 Schematic diagram of the spraying into antisolvent process

At the beginning of each experiment, 2 g of lipids (cholesterol and lecithin) were dissolved in 20 ml of chloroform. Then, rifampicin was added to this solution and stirred until a clear solution was obtained. The solution was then stored in a solution formulation reservoir. A volume of 400 ml water was used as an antisolvent in a pan coating having a diameter of 30 cm rotated at 50 rpm. Then, a solution of the formulation was forced through the nozzle (diameter 0.5 – 1.5 mm) with a peristaltic pump at a flow rate 0.43 ml/min. At this point, the liquid solution was delivered through

the nozzle and fine droplets of liquid were then formed from the precipitation of solute in the antisolvent. Also, the process was operated continuously until the solution formulations ran out. To ensure that the liquid formulation did not remain in the equipment, chloroform solution was fed into the tube and spray nozzle. After that, the pan coating was operated for 10 minutes in order to evaporate the chloroform. Precipitated particles suspended in antisolvent from the pan coating were collected. Homogeneous suspension was taken for 10 ml to determine a non-encapsulated rifampicin. Two grams of each carrier (trehalose or mannose) were dissolved in 10 ml of purified water until a clear solution was obtained. Then, a solution of the carrier was mixed with a formulation suspended in the antisolvent. Finally, approximately 400 ml of suspension was dried by lyophilization technique. These powder formulations were stored in a desiccator at room temperature over silica gel until further experiment will be carried out.

6.4.2 Determination of drug encapsulation

The percentage of drug encapsulation was calculated using the following equation:

$$\% \text{ encapsulation} = \frac{(\text{Total amount of drugs particles suspended in antisolvent} - \text{Amount of unencapsulate drugs})}{\text{Total amount of drugs in antisolvent}} \times 100$$

The rifampicin content was determined by HPLC. 100 mg of drug was weighed into a 50 ml volumetric flask, dissolved in HPLC mobile phase and completed

to this volume. This suspension was sonicated in an ultrasonic bath for 10 minutes. The resulting suspension was then filtered through a 0.45 μm polyamide membrane filter using a Buchner funnel. The resulting solution was then assayed for rifampicin concentration by HPLC as described in Chapter 3. The concentration was calculated based on a theoretical weight of rifampicin.

Unencapsulated rifampicin was assayed by sampling 10 ml of suspension that collected immediately before mixing with the carrier in the production process. This suspension was filtered through a 0.45 μm polyamide membrane filter using a Buchner funnel. The filtrate was assayed for unencapsulated rifampicin concentration by HPLC method. Total unencapsulated rifampicin was calculated by multiplication by a dilution factor from the total suspension.

6.4.3 Effects of cholesterol and lecithin ratios on percent encapsulation

Different dry powder formulations were produced by altering the cholesterol : lecithin ratios. The compositions of dry powder formulations are shown in Table 6.1. The total weight of lipids (cholesterol and lecithin) in these formulations was 2 g. The cholesterol : lipid ratios were varied by weight and encapsulated rifampicin by spraying into antisolvent method as described in the section 6.4.1. The percentage of encapsulation was determined by the same method according to section 6.4.2. The optimum ratio of cholesterol : lecithin with the highest encapsulation of rifampicin was chosen for further study.

Table 6.1 Compositions of dry powder formulations obtained from spraying into antisolvent technique

Formulation no.	Formulation code	Cholesterol : lecithin	carrier
21	RIF-1 (1:1)	1 : 1	Trehalose
22	RIF-1 (1:2)	1 : 2	Trehalose
23	RIF-1 (1:3)	1 : 3	Trehalose
24	RIF-1 (2:1)	2 : 1	Trehalose
25	RIF-1 (3:1)	3 : 1	Trehalose
26	RIF-2 (1:1)	1 : 1	Mannose
27	RIF-2 (1:2)	1 : 2	Mannose
28	RIF-2 (1:3)	1 : 3	Mannose
29	RIF-2 (2:1)	2 : 1	Mannose
30	RIF-2 (3:1)	3 : 1	Mannose

6.4.4 The effect of drug loading in formulation sprayed into antisolvent

After obtaining the maximum encapsulation of rifampicin from section 6.4.3, the drug loading in that formulation had to be optimized. The drug loading into the microcapsules was performed by 10 mg of rifampicin initially and the manufacturing process was performed in the same manner as that described in section 6.4.1. The amount of rifampicin in the formulations was increased to 50, 100, 500, 1000 and 2000 mg as summarized in Table 6.2. All formulations were prepared by spraying into antisolvent technique as described in section 6.4.1. The formulation with the highest rifampicin loading and suitable for dry powder inhalers was selected for dry powder inhalers evaluation.

Table 6.2 Compositions of dry powder inhaler formulations containing various amounts of rifampicin using cholesterol : lecithin in a ratio of 1 : 3 by weight

Formulation no.	Formulation code	Cholesterol (g)	Lecithin (g)	Rifampicin (mg)	Carrier
31	RIF-1 (10)	0.5	1.5	10	Trehalose
32	RIF-1 (50)	0.5	1.5	50	Trehalose
33	RIF-1 (100)	0.5	1.5	100	Trehalose
34	RIF-1 (500)	0.5	1.5	500	Trehalose
35	RIF-1 (1000)	0.5	1.5	1000	Trehalose
36	RIF-1 (2000)	0.5	1.5	2000	Trehalose

6.4.5 Content uniformity of dry powder formulations

After selecting the optimal formulation obtained in previous section, such formulation was evaluated. A total of 10 doses were collected, three doses at the top, four in the middle, and three at the bottom of the bottle containing formulation. 20 mg of each sample was weighed and placed in a 50 ml volumetric flask and made up to the volume with HPLC mobile phase. Ten aliquots were taken similarly. Each solution had a theoretical concentration of rifampicin 5 μg/ml. Then, all samples were sonicated with an ultrasonic bath for 20 minutes and were filtered through a 0.45 μm polyamide membrane filter using a buchner funnel. Finally, the resulting solutions were assayed for rifampicin by HPLC according to the determination method of rifampicin in Chapter 3. The mean actual drug content was expressed as a percentage of the theoretical drug content.

6.4.6 Particle size distribution of encapsulated particles

Particle size analysis was performed by suspending about 100 mg of dry powder formulation in water containing 1% w/v of Tween 80 which was pre-saturated with the powder under investigation. In this case, the carriers could dissolve in this medium but encapsulated drugs could not dissolve completely. Then, the experimental were performed according to section 4.4.2 as described before.

6.4.7 Morphology of encapsulated particles

The particle surface topography and texture of encapsulated rifampicin and carriers were assessed qualitatively using SEM according to section 4.4.3. All micrographs of these particles were taken at an acceleration voltage of 10 keV.

6.4.8 Lipid structure of the formulation sprayed into antisolvent by transmission electron microscopy (TEM)

Spraying into antisolvent formulation was determined the structure of lipid encapsulated rifampicin by TEM. Spraying into antisolvent formulation of rifampicin [RIF-1 (50)] was formulated by the same method that described in the section 6.4.1 except mixing with carrier solution and freezing drying process. The suspension of rifampicin microcapsules was mixed with 5% w/v of ammonium molybdate (in 1% w/v trehalose solution) at the volume ratio of 2:1. Following these, the suspension was left at room temperature for about 1 hour. After that, this mixture was loaded dropwisely onto a 200 mesh-carbon-grid on a filter paper and then drying at room temperature for about 3

hours. The resulted thin film was then determined by TEM at 100 keV (Ghanta *et al.*, 1996 ; Stahlberg *et al.*, 1999).

6.4.9 Lipid structure of the formulation sprayed into antisolvent using confocal laser scanning microscopy

For staining of lipid in formulation sprayed into antisolvent was stained with Nile blue A. The suspension of RIF-1 (50) formulation was mixed with 1% Nile blue A at the volume ratio about 1:1. Visualization of fluorescent signals and transmitted light was performed with confocal laser scanning microscope equipped with an argon laser.

6.4.10 Determination of chloroform in formulation sprayed into antisolvent

One dose of formulation sprayed into antisolvent (20 mg) was weighed and taken into a 10 ml volumetric flask, dissolved it in water and completed to 10 ml. This suspension was sonicated until a clear solution was obtained. This solution was analyzed to determine chloroform content by the following method (Golfinopoulos *et al.*, 1998 ; Kostopoulou *et al.*, 2000) :

Liquid-liquid extraction was used to extract chloroform from the sample solution. The method involved extraction of a 10 ml sample with 2 ml of freshly distilled n-hexane. One µl of the extract in hexane was then injected into a gas chromatography. The injection technique used on-column and split mode technique. The chloroform was analyzed using a Hewlett Packard Purge-and-Trap concentrator 7695 fitted with a 5 ml

sample container and 30 cm absorbent trap, a Hewlett Packard Gas Chromatograph 6890 supported by a ^{63}Ni Electron Capture Detector. The Purge-and-Trap Concentrator and GC were supported by the HP G 1909 PAT control software and HP 3365 Chemstation System software, respectively. A 30 m × 0.25 mm i.d. × 0.25 μm film thickness fused silica capillary column Rtx-5MS was use to complete separation in this study. Helium was used as the carrier and nitrogen as the make-up gas. The GC and purge-and-trap conditions used are shown in Table 6.3.

Table 6.3 Gas chromatographic conditions

Gas chromatographic conditions	
Carrier gas flow	6 ml/min
Make-up gas flow	44 ml/min
Oven temperature	40°C (10 min), to 100°C (5 min) at 3 deg/min, to 180°C at 5 deg/min
Injector temperature	40°C
Detector temperature	300°C
Purge-and-trap conditions	
Purge	40 ml/min He, 11 min, ambient temperature
Desorb	30 ml/min He, 180°C for 4 min (preheat 175°C)
Bake	220°C, for 10 min
Temperature	Line 200°C, valve 200°C, moisture control system line 200°C

Standard solutions preparation as the follows :

A methanol stock solution of chloroform was prepared by adding chloroform via a syringe to a 10 ml volumetric flask filled with methanol. Known volumes of chloroform standard stock solutions up to about 20 μl were added to 100 ml or more of organic free water in a volumetric flask to give standard water solutions.

Mixing was accomplished by inverting the flask three or four times. These aqueous standards were stand still for a few minutes or long enough to fill several 5 ml syringes fitted with valves.

6.4.11 *In vitro* deposition of rifampicin in dry powder formulations by TSI

In vitro deposition of rifampicin from dry powder inhaler formulation was carried out using a TSI. Each deposition experiment involved aerosolisation six times, each containing a dose of 20 mg powder, equivalent to 250 μg rifampicin. Approximately 20 mg of dry powder blends were weighed accurately and placed in a glass inhalers device. After that, the experimental were performrd according to section 5.4.5 as described before. Deposition of rifampicin in lower stage was considered to be the ED, FPD and FPF as described in section 4.4.5.

6.4.12 *In vitro* deposition of rifampicin in dry powder formulations by ACI

The depositions of dry powder formulations were assessed *in vitro* again using an Andersen Mark II cascade impactor. Twenty mg of dry powder formulation was weighed by analytical balance and placed in a glass inhaler device. After that, the experimental were performed according to section 4.4.7 as described before. The ED, MMAD and GSD were calculated according to the equation in section 4.4.7.

6.4.13 Stability of the formulation sprayed into antisolvent after storage

The formulation sprayed into antisolvent was stored in a desiccator at room temperature. The %content of the drugs and MMAD were determined after a storage period of 3 months according to section 4.4.8.

6.5 Results and Discussion

6.5.1 The effects of cholesterol : lecithin ratio on percent encapsulation of rifampicin

The effects of cholesterol : lecithin ratio on %encapsulation are shown in Table 6.4. The total content of rifampicin in the formulations is small (10 mg). The unencapsulated rifampicin may still dissolve in an antisolvent (H_2O) and rifampicin does not form particles in an antisolvent because the solubility of rifampicin is about 2.8 mg/ml of water at room temperature, pH 7.5 (Gallo and Radaelli, 1976). We found that cholesterol and lecithin at a ratio of 1:3 have the highest %encapsulation (about 95%) following this procedure. Similar results were obtained either using trehalose or mannose as carriers (Table 6.4). This suggests that carrier does not affect on %encapsulation of rifampicin because the carrier solution was added after the microcapsules were formed. The %yield is varied between 40 and 70. The production process is an opening system, so the solution formulation was lost between the sprayed processed into antisolvent. In addition, the distance between spray nozzle and antisolvent must be appropriate. If the distance is far, the spraying droplets are smaller from evaporation while the closer the

larger spray droplet will be obtained. Unfortunately, some small droplets were flied out the pan coating resulting in the lost and low %yield was obtained.

Type of carriers did not affect on %encapsulation as shown in Table 6.4 but influenced the physical characteristics and quality of dry powder inhalers. Figure 6.3 shows photographs of all formulations. The formulations were formulated with trehalose as carrier [Figure 6.2 (A)] have a good flow characteristics such as bulky, suitable particles size and easier in freeze drying process. In contrast, all those formulations formulated with mannose as carrier have poor flow ability and large in size. The moisture sorption may be a factor involving the aggregation (Figure 6.2 (A)). In addition, the freeze drying process of formulations with mannose carrier took longer time than the formulations using trehalose as carrier (about 4 days for formulations with mannose and 1-2 days for formulations with trehalose). As a result, the formulations with mannose as carriers are excluded as dry powder inhalers and will not be further considered in this study.

The lipid ratio of cholesterol and lecithin of 1:3 have the highest %encapsulation when total weight of lipid was kept constant. The ratios were chosen to formulate dry powder inhaler which employed the same method and will be further studied in the next section.

Table 6.4 %Encapsulation and %yield of dry powder formulations by spraying into antisolvent (mean ± SD, n = 1-6)

Formulation code	Theoretical total weight (g)	Actual total weight (g)	%yield	%encapsulation
RIF-1 (1:1)	4.0100	2.3471 ± 0.3359[b]	58.53 ± 7.61[d]	88.07 ± 0.33[d]
RIF-1 (1:2)	4.0100	1.7216 ± 0.6144[b]	42.93 ± 4.32[d]	94.20 ± 0.46[d]
RIF-1 (1:3)	4.0100	2.7075 ± 0.0830[b]	52.50 ± 11.35[d]	95.36 ± 1.43[d]
RIF-1 (2:1)	4.0100	2.4325[a]	60.66± 4.47[c]	93.77 ± 0.51[d]
RIF-1 (3:1)	4.0100	1.6194 ± 0.3841[b]	40.38 ± 8.74[d]	91.36 ± 1.02[d]
RIF-2 (1:1)	4.0100	2.7220 ± 0.1004[b]	67.88 ± 1.02[d]	86.30 ± 2.71[d]
RIF-2 (1:2)	4.0100	2.9141[a]	72.67 ± 4.49[c]	91.58 ± 0.32[c]
RIF-2 (1:3)	4.0100	2.2404[a]	55.87 ± 8.26[c]	93.53 ± 0.46[c]
RIF-2 (2:1)	4.0100	1.9571 ± 0.0514[b]	48.81 ± 0.74[d]	90.59 ± 0.11[d]
RIF-2 (3:1)	4.0100	2.1264[a]	53.03 ± 1.65[d]	90.20 ± 0.09[d]

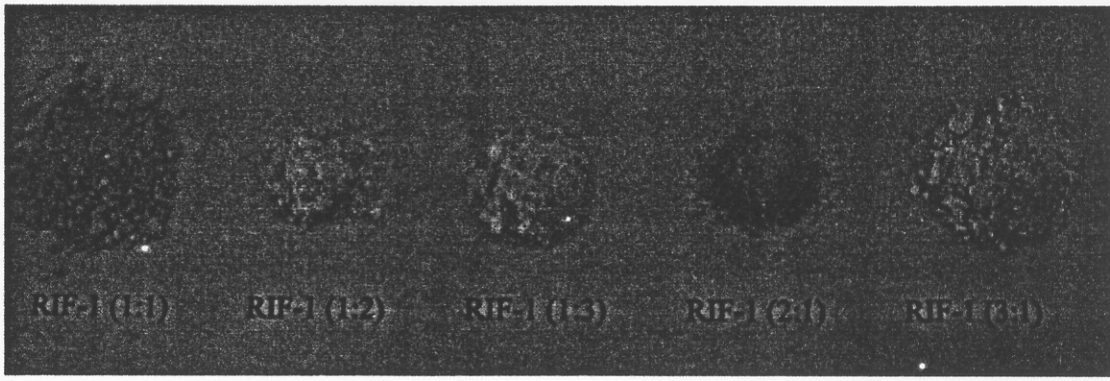
[a] n = 1

[b] n = 2

[c] n = 3

[d] n = 6

(A)



(B)

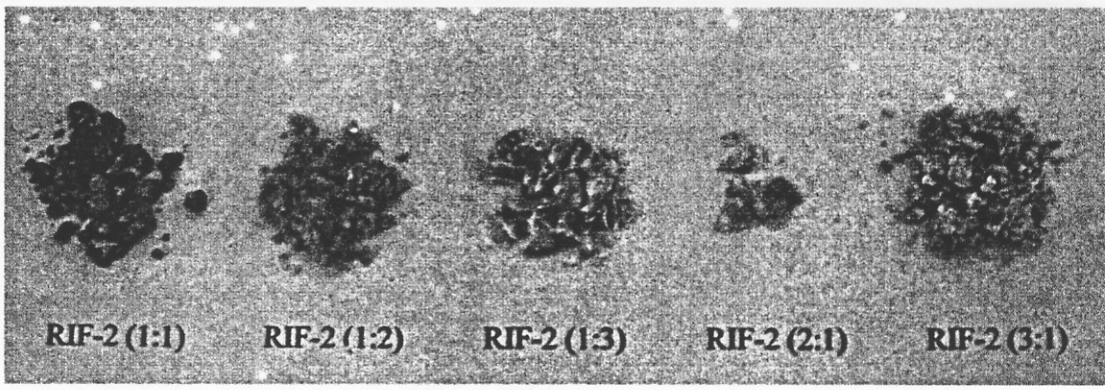


Figure 6.2 Photographs of dry powder inhaler formulations by spraying into antisolvent (A) using trehalose and (B) using mannose as carrier

6.5.2 Study of drug loading

Table 6.5 shows the amount of drug which was loaded in the formulation. The results suggest that total of rifampicin which can be loaded in these formulation is 50 mg. When the amount of rifampicin is increased, the efficiency of encapsulation cannot be increased. Higher amounts of rifampicin (at 1000 and 2000 mg) reaches the maximum solubility of rifampicin in water, Unencapsulated rifampicin became particles suspended

in the antisolvent. Prior to analysis the unencapsulated rifampicin, the suspensions were aliqout and diluted with HPLC mobile phase until a clear solution was obtained and then analysis was carried out with HPLC.

We selected RIF-1 (50) formulation (formulation no. 32) to evaluate for use as a dry powder inhaler. This formulation optimizes encapsulated rifampicin with physical properties such as bulk and small particle size.

Table 6.5 Drug loading of dry powder formulations (mean ± SD, n = 6)

Formulation code	**%encapsulation**	**drug loading**
RIF-1 (10)	94.50 ± 1.38	9.45 ± 0.13
RIF-1 (50)	82.76 ± 2.76	41.22 ± 1.59
RIF-1 (100)	35.35 ± 5.79	35.35 ± 5.79
RIF-1 (500)	8.90 ± 1.04	44.52 ± 5.20
RIF-1 (1000)	2.73 ± 0.46	27.35 ± 4.60
RIF-1 (2000)	1.87 ± 0.33	37.5 ± 6.65

6.5.3 Content uniformity of dry powder formulations and determine the chloroform content

The content of formulation RIF–1 (50) is 79.64 % ± 2.65 (mean ± SD) when compared with theoretical content. The %content was low and varied when SD value is considered due to the lost of liquid formulation during spraying process. Due to the spraying into antisolvent is an open system, the formulation solution was lost from the pan coating. After that, the suspension formulation in antisolvent was mixed with a

carrier solution therefore the suspension was diluted when compared to the theoretical weight.

To ensure that chloroform was completely evaporated from the formulation, the chloroform content was determined. The result shows that chloroform content of this formulation was not detected (the limit of detection is 0.25 ng/ml). Therefore, the chloroform may be completely disappeared evaporated, hence it can be ensured that the formulation is safe for inhalation.

6.5.4 Particle size distribution measurement

Figure 6.3 shows particle size distribution of RIF-1 (50) formulation. The volume median diameter is 4.51 ± 0.97 (mean ± SD, n = 3). This particle size is expected to be deposited in the lower airways. This production method can be used to formulate the appropriate size of microcapsule for used as DPIs.

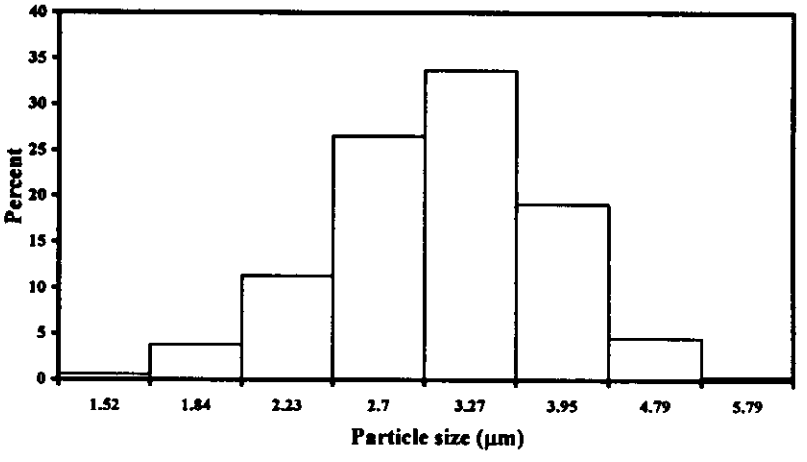
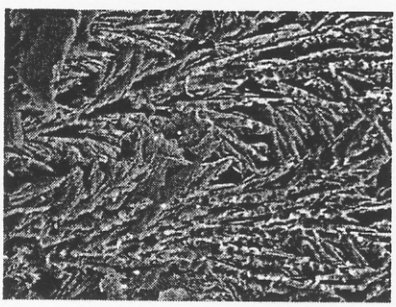


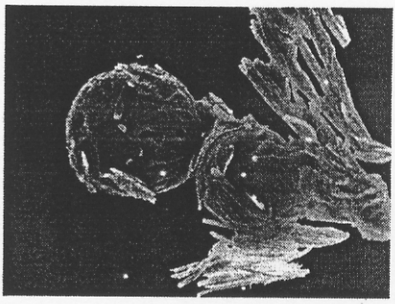
Figure 6.3 Particle size distribution based on volume of RIF-1 (50) formulation using Malvern laser light diffraction technique

6.5.5 Morphology of dry powder formulations

Figure 6.4 (A) and (B) shows the morphology of RIF-1 (50) formulation which was prepared by spraying into antisolvent. The irregular shapes particles shown in may be as a result of carriers. When the surfaces were zoomed, it was found that spherical particles (Figure 6.4 B). These particles should be encapsulated rifampicin. Figure 6.4 A shows SEM pictures of the porous texture of the trehalose cake containing encapsulated rifampicin. The result was similar to the study of Winden *et al.* (1999) who formulated liposome consisting DPPC : dipalmitoylphosphatidylglycerol (sodium salt) : cholesterol in the ratio of 10 : 1 : 4 freeze-dried in trehalose (3 g/g phospholipid). The results show the porous texture of trehalose in Figure 6.5 A. The lipid may form unilamellar or multilamellar. To confirm this hypothesis, TEM was employed.



(A) RIF-1 (50)

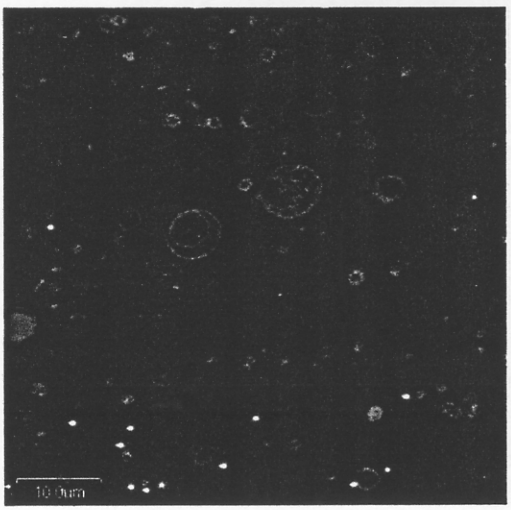


(B) RIF-1 (50)

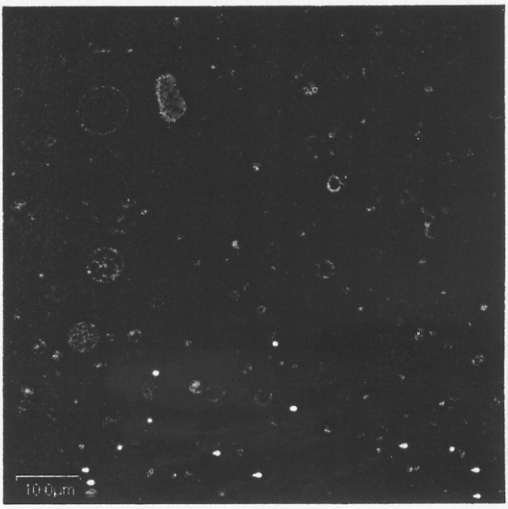
Figure 6.4 Electron micrographs of (A) RIF-1 (50) × 1500 and (B) RIF-1 (50) × 3500

6.5.6 Characterization of lipid structure of the formulation sprayed into antisolvent

Figures 6.5 (A), (B) show the lipid layers of formulation sprayed into antisolvent. These particles have a size range between 1-10 μm. The most particles is about 1-5 μm corresponding with the particle size obtained from laser light diffraction. The microencapsulated drug is unilamellar but some particle was multilamellar with a large particle. Transmission electron micrograph of these particles is shown in Figure 6.5 (C). This picture confirmed the microcapsule of this formulation.



(A)



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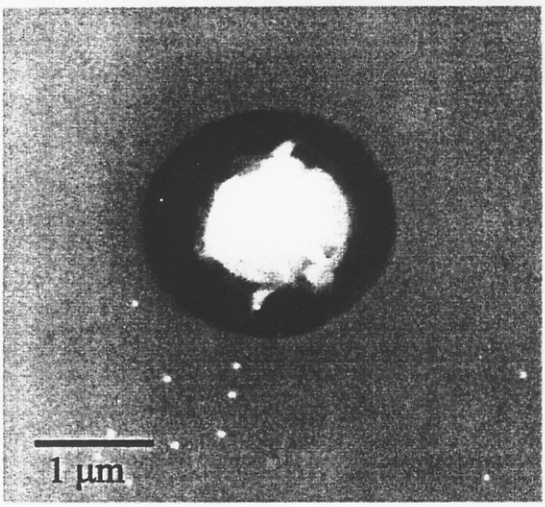

(C)

Figure 6.5 Photographs of lipid layers of the spraying into antisolvent obtained from confocal laser scanning microscope (A) and (B), obtained from transmission electron microscope (C)

6.5.7 *In vitro* deposition of rifampicin in dry powder formulation by TSI

The ED of RIF-1 (50) formulation is 249.15 ± 26.34 μg (mean ± SD, n = 6), with FPD of 170.35 ± 15.37 μg (mean ± SD, n = 6) and FPF is 68.55 ± 3.92% (mean ± SD, n = 6). These results show that the production process was produced the high FPF for used as DPIs.

6.5.8 *In vitro* deposition of rifampicin in dry powder formulation by ACI

Particle size distribution of RIF-1 (50) formulation is shown in Figure 6.6. The ED is 192.63 ± 14.85 μg, MMAD of this formulation is 2.66 ± 0.62 μm (mean ± SD, n = 6) and GSD is 1.70 ± 0.14 (mean ± SD, n = 6). These values were obtained from Figure 6.7. RIF-1 (50) formulation has suitable size for lung delivery because aerodynamic diameter of this formulation is lower than 5 μm and can be deposited to the stage 4-7 of the ACI.

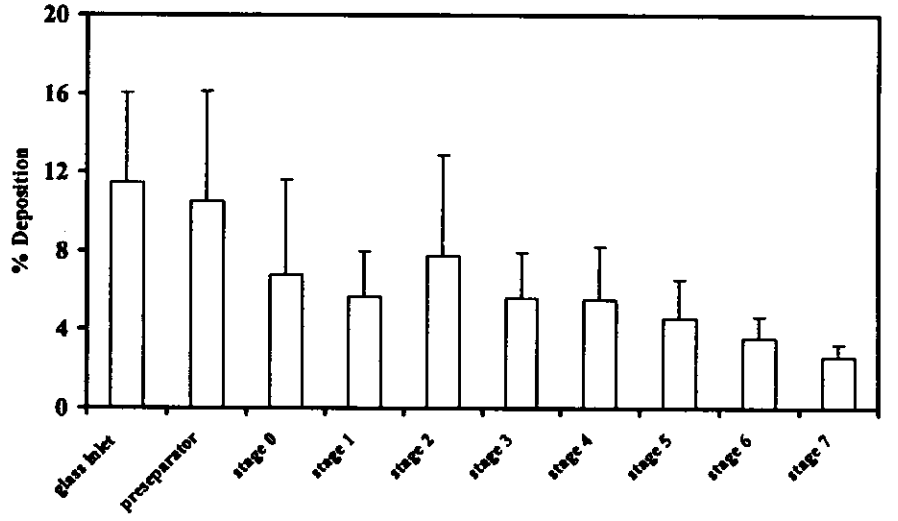

Figure 6.6 Size distribution of RIF-1 (50) formulation on each stage of ACI as aerosolized at a flow rate of 60 l/min (mean ± SD, n = 6)

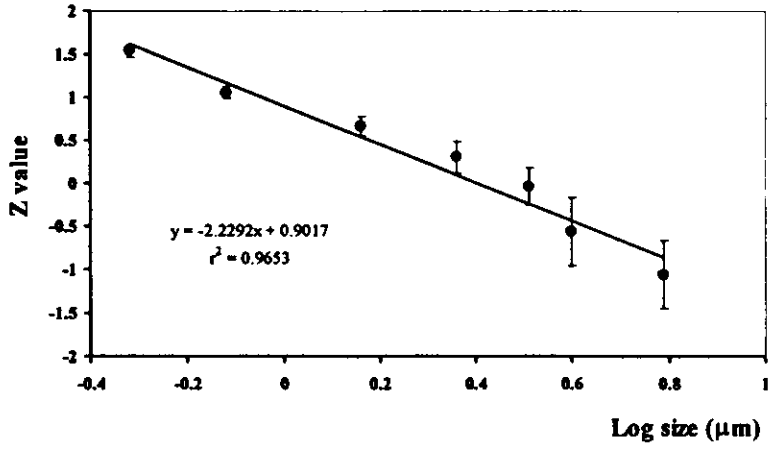

Figure 6.7 Relationship between Z value and cut off aerodynamic diameter (log scale) on each stage of the ACI of formulation RIF-1 (50) as aerosolised at a flow rate 60 l/min

6.5.9 Stability of formulation sprayed into antisolvent after storage

After storage the percentage of content and MMAD of rifampicin is shown in Table 6.6. The initial of %content and MMAD were obtained from section 6.5.3 (uniformity of dosage unit, n = 10) and section 6.5.8 (*in vitro* deposition by ACI, n = 6).

Table 6.6 Drug contents (mean ± SD, n = 6) and MMAD (mean ± SD, n = 6) of RIF-1 (50) formulation after storage 3 months at room temperature

%content		MMAD (μm)	
Initial	**After 3 months**	**Initial**	**After 3 months**
79.64 ± 2.65	67.48 ± 2.70	2.66 ± 0.62	6.23 ± 1.14[#]

[#] $P < 0.05$

The MMAD values of the initial RIF-1 (50) particles are shown in Table 6.6. When compared to RIF-1 (50) particles after 3 months storage, MMAD values were significantly higher than at initial when the carrier was trehalose. The results show that the significantly storage affected the MMAD values of these particles. It must be noted that this formulation is sensitive to moisture sorption and severe aggregation occurred. The low flowability and the aggregation behavior were also observed during the experimental handling of the RIF-1 (50) formulation. On the other hand, the initial formulation at first showed no tendency to aggregation and was deposited in the lower stages of the ACI. The shifting of the size distribution to larger ranges with increasing moisture content in the sample indicates an increase in the aggregation of the mocrospheres. The %contents of RIF-1 (50) after storage decreased significantly from 79.64 ± 2.65% to 67.48 ± 2.67%. The results show that a low content of rifampicin degraded faster than a high content rifampicin according to the study of intra- or inter-day precision of rifampicin. From Figure 3.4, the low concentration of rifampicin was degraded faster than that of high concentration.