

The effect of vehicles on spray drying of rifampicin inhalable microparticles: In vitro and in vivo evaluation

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ABSTRACT

Background and the purpose of the study: The aim of this study was to evaluate the effect of solvents used in the spray drying and the aerodynamic properties of the rifampicin microparticles and pulmonary absorption of the microparticles.

Methods: Different mixtures of dichloromethane and water were used as solvents for spray drying of rifampicin microparticles. The water to dichloromethane ratios were 25:75, 50:50, 75:25, 80:20, 90:10 and 100:0. The solutions were dried at inlet temperature of 70 °C. The powder properties of the samples were examined by laser diffraction, scanning electron microscopy (SEM), helium densitometer and infrared spectroscopy (IR). The aerosolization performance of these formulations was investigated using an Andersen cascade impactor. Pulmonary absorptions of formulations were examined by the in situ pulmonary absorption described by Enna and Schanker method. The plasma concentration time profiles of rifampicin were constructed 8 hours following the intravenous and the intrapulmonary administrations. The pharmacokinetics parameters, C_{max} , T_{max} , $t_{1/2}$, AUC, mean residence time (MRT), K_a and K_e were determined for each formulations.

Results and major conclusions: The T_{max} values for the samples decreased by increase in the amount of water in the initial feed. The T_{max} values for the spray dried samples from the different mixtures of dichloromethane and water were 60(min) and 30(min) respectively. The solvent mixture as the spray drying vehicle played an important role in the in vitro and in vivo lung deposition. The type of spray drying vehicle showed significant effect on the aerodynamic behavior and pharmacokinetic parameters of the particles. The pulmonary absorption of drug revealed the possibility of achieving the minimal inhibitory concentration (MIC) of the antibiotics. The spray drying vehicle only affected absorption patterns of the formulations and it did not have any effect on the elimination rate of particle.

Key words: Rifampicin, dry powder inhaler, aerodynamic behavior, pulmonary absorption.

INTRODUCTION

Respiratory drug delivery is more sophisticated today. The advantages of this route such as the very rapid onset of action, very small dose and reduced cost (1-2) have essentially remained the same. It also minimizes the risk of unwanted systemic effects, which is particularly important in the therapy with steroids, antibiotics and other potentially harmful drugs. Anti-infectious agents such as pentamidine, colistine, aminoglycosides and amphotericin B have been administered by aerosol. Local therapy with inhaled antibiotics has demonstrated improvements in pulmonary functions (3-4). While maintenance treatment with inhaled, and nebulized antibiotics is common practice in patients (5), dry powder inhalation might be a suitable and highly efficient alternative

for nebulization of antibiotic drugs (6).

Dry powder inhalers (DPIs) for Pulmonary delivery of drug have been developed as an alternative to the pressurized metered dose inhalers (pMDIs) because they eliminate the requirement of hand to mouth coordination (7). The powder formulation is composed of a micronized drug powder, either alone or in combination with carrier particles. Spray drying is a routine method in most of the industrial set ups and it converts a solution into powder in one step process and produces fine and dustless or agglomerated powders, generally hollow, and approximately spherical in shape with a narrow size range (8,9). Spray drying vehicle can significantly affect the aerodynamic behavior, particle size distribution, shape and density of the

particles which in turn influence the efficacy of inhaled aerosols (10-13). Another factor which plays an important role in the efficacy of inhaled aerosols is the carrier of DPIs (14).

In this study the lung deposition profiles of rifampicin microparticles produced by spray drying method with different ratio of water and dichloromethane were investigated.

MATERIALS AND METHOD

Materials

Rifampicin powder was supplied by Sigma (Texas, San Antonio). Lactose monohydrate was purchased from DMV (Amsterdam, the Netherlands). All solvents which were used supplied by Merck (Frankfurt, Germany) and were at least analytical grade.

Methods

Spray Drying

Solutions (6 g/100ml) of rifampicin in different water: dichloromethane ratios (Table1) were spray dried using a lab scale spray drier (Buchi 191, Buchi, Switzerland).

Preparation of blends

Powder formulations containing rifampicin and lactose with ratio 1:1 were prepared. In each mixing process 1 gram of spray dried rifampicin sample was blended with 1 gram of lactose in a turbula mixer (Dorsa Iran) at 46 Rev/min for 30 min.

Analytical method

The concentration of rifampicin in plasma samples was measured by microbiological method (15). Briefly a paper disk (8 mm in diameter) was immersed in the plasma and then the disk was placed on a heart infusion agar plate prepared by overlaying of 5ml of heart infusion agar containing ca. 5×10^4 spores of *Bacillus subtilis* (as an indicator bacterium) per ml onto 10 ml of a basal heart infusion agar layer. Following overnight culture at 37 °C, the concentration of rifampicin in the plasma samples was determined from the diameters of the zone of growth inhibition using standard curves. Rifampicin standards were prepared in blank plasma.

Particle size analysis

The particle size of samples were determined by laser light scattering (Malvern mastersizer x, Malvern, UK). Approximately 20 mg of sample was suspended in water and sonicated at 25 °C for 4 min. A few drop of each sample was poured into the small volume cell of the instrument to obtain

an obscuration of sample between 18 and 20 %. The analysis was carried out in triplicate for each sample.

Scanning electron microscopy

Morphology of each sample was examined by scanning electron microscopy (SEM) (Philips XL 30 scanning microscope, Philips, The Netherlands) at 25 Kev. Samples were gold coated prior to analysis (SCD005 Sputter coater, Bal-Tec, Germany).

Drug content determination

Quantification of rifampicin-Lactose blend content uniformity and in vitro lung deposition was carried out by UV-VIS spectrophotometer at 475 nm.

Particle density

The bulk density of the samples was determined by measurement of the volume of a known mass of the material that had been poured in to a 25 ml graduated cylinder. The true density was also determined using a helium pycnometer (Multipycnometer, Quantachrome, USA). Each sample was analysed in triplicates.

In Vitro deposition

One capsule, containing 10 mg of rifampicin was introduced to an Andersen cascade impactor via a Spinhaler (dahlia, India). After aerosolization of the powders for 4 sec at a flow rate of 60 l/min, the inhaler, capsule shell, throat, preseparator, the seven stages and plates and filter were washed with dichloromethane as the solvent. The aerodynamic characteristics of rifampicin in each sample were determined as follows:

Fine Particle Dose (FPD) was the amount of drug deposited on stage 1 to the filter. The effective cut-off diameter of stage 1 of Anderson cascade impactor at 60 l/min has been reported < 6.18 μ m (16).

Fine Particle Fraction (FPF) was calculated as the percentage of the ratio of the FPD to the total amount of the drug recovered per capsule.

The Emitted Dose (ED) was defined as the total drug recovered from throat, preseparator, seven stages and plates and filter. The percentage emitted was calculated as the ratio of ED to the total drug which was recovered per capsule and expressed as percentage.

Dispersibility was defined as the ratio of FPF per ED percentage.

In vivo studies

Male Wister rats (The Pasteur institute, Iran), weighing 250-300 g, were anaesthetized with an intraperitoneal injection of ketamin (50mg/kg)

and xylene (10mg/kg). All animals were fasted for 16 h before the experiments; they were allowed free access to water.

Drug administration

Four mg of drug as the powder was introduced into the lung through the obtuse syringe which was connected through the tracheal cannula to a depth of 2.5 cm below the tracheal incision. The tip of the syringe was located 1-2 mm above the bifurcation of the trachea. The powder was introduced over a period of 1-2 sec, to the rat which was maintained at an angle of 80°. Then, the tubing was withdrawn completely and 45sec after administration of the powder the animal was positioned to an angle of 10°.

Rifampicin solution in PBS (4mg/0.2ml) was intravenously administered into the caudal vein by bolus injection.

Absorption studies

Absorption of rifampicin from rat lung was investigated by the reported method (17). All animals were fasted for 16 h before the experiments but had free access to water. After the animal was secured on its back on animal board, the trachea was exposed through a longitudinal incision along the ventral aspect of the neck. The trachea was then cut transversely, halfway through, between the fourth and fifth tracheal rings caudal to the thyroid cartilage. For determination of the drug concentrations in plasma, 250 µL blood samples were taken 10, 30, 60, 120, 240, 360 and 480 min after dosing from jugular vein centrifuged at 1800 × g for 10 min, and the plasma was separated and stored at -30 °C until analysis.

Pharmacokinetic parameters

The pharmacokinetic parameters, C_{max} (maximum plasma concentration) and T_{max} (the peak plasma concentration time) were obtained from the plasma concentration-time curve (18). AUC_{0-t} and $AUC_{t-\infty}$ were calculated by numerical integration using a linear trapezoidal formula. The mean residence time (MRT) was determined using $AUMC/AUC$. The K_a (absorption rate constant) was determined using the residual method, K_e was calculated from terminal section of the plasma concentration-time curve and $T_{1/2}$ was calculated from $0.693/k_e$.

RESULTS AND DISCUSSION

Physical characterization

Table 2 shows the data of the particle size distribution for all samples. The commercial rifampicin was shown to have a volume median

diameter ($d_{50\%}$) of 80.24 µm with a mode at 78.22 µm. This sample was not suitable for inhalation.

Table 1. Spray drying vehicle with different solvent ratios

Code	Solvents	
	Water (%)	Dichloromethane (%)
W/DCM 100:0	100	0
W/DCM 90:10	90	10
W/DCM 80:20	80	20
W/DCM 75:25	75	25
W/DCM 50:50	50	50
W/DCM 25:75	25	75

Table 2. Particle size distribution and densities of the samples (Mean, n=3)

Sample	Particle size (µm)			Density g/ml	
	$d_{50\%}$	Mode(s)	Bulk	True	
Rifampicin commercial	80.24	78.22	1.008	1.122	
W/DCM 25:75	6.20	6.33	0.178	1.382	
W/DCM 50:50	6.25	6.53	0.132	1.318	
W/DCM 75:25	6.35	6.65	0.091	1.285	
W/DCM 80:20	5.82	5.61	0.050	1.253	
W/DCM 90:10	6.30	6.68	0.091	1.272	
W/DCM 100:0	6.33	6.71	0.150	1.266	

The spray drying process produced microparticles with different densities depending on the nature of the vehicle. No definite crystal structure was shown by these particles (Fig.1). SEM pictures in Fig. 1 show the morphologies of the microparticles fabricated under different conditions. It is clear that solvent mixture affected the shape of the microparticles. W/DCM 25:75 showed better spherical shape particles with smoothness on surface, and without any porous structure. SEM pictures of W/DCM 50:50, W/DCM 75:25, W/DCM 80:20 and W/DCM 90:10 showed non spherical shape with roughness on surface. W/DCM 100:0 showed moderate spherical shape with the minimum roughness on surface. Both true density and bulk density values of the commercial powder were different from those spray dried samples. Increasing the amount of dichloromethane more than to 25% in the initial feed resulted in more dense samples (Table 2). Increasing the percentage of water from 25% to 80% decreased the bulk and true density value. W/DCM 80:20 showed the minimum density value. It is possible that increase in the percentage of water to 80% resulted in increase in the amount of drug molecules which entered from organic phase to watery phase. Due to hydrophobicity of the drug molecules, they did not have any affinity for the inorganic phase. For this reason, during the

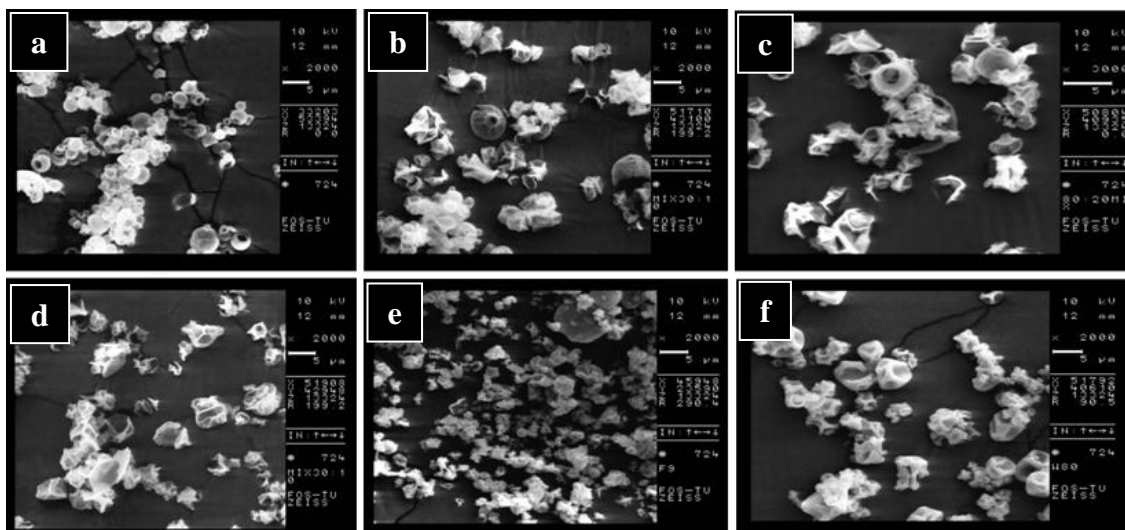


Figure 1. SEM photograph of the samples: a: W/DCM 25:75 b: W/DCM 50:50 c: W/DCM 75:25 d: W/DCM 80:20 e: W/DCM 90:10 f: W/DCM 100: 0

Table 3. Fine particle fraction and dispersibility of the samples

Percentage of water in the spray drying vehicle	FPF%	ED%	Dispersibility	Percentage remained in capsule%
25	37	79	45	21
50	45	77	58	23
75	49	70	70	30
80	58	80	73	20
90	44	67	64	33
100	39	60	65	40

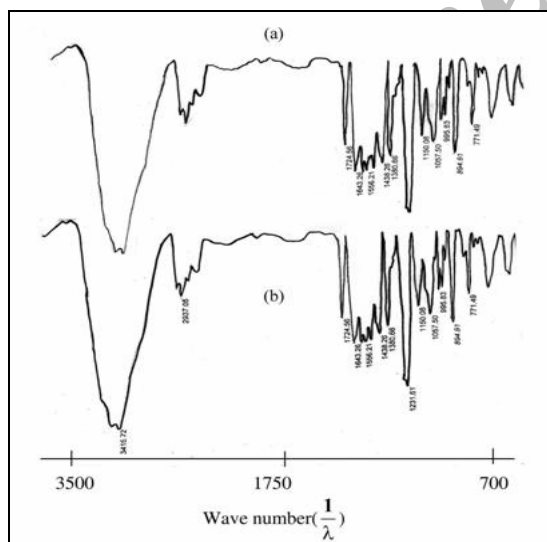


Figure 2. Infrared spectra of the samples: a) commercial b) after spray drying from water at 70 °C (the spectra of the other spray dried samples were similar)

spray drying process, the vehicle excludes from the particles easier and faster. Rapid exclusion of vehicle resulted in low denser microparticles. When 20% DCM was used as the solvent mixture, particles had small size and low density and

interaction between two solvents was maximum at the ratio of 80:20. It is possible that 20% DCM at 70°C accelerated exclusion of the spray drying vehicle from the microparticles during the spray drying process and led to the production of reduced size and low density microparticles. The mixture of the solvent had significant effects on the shape of the particles (Fig 1). According to the Fig. 1 it is clear that in comparison to other solvent mixture, particles in W/DCM 80:20 more porous. Increase in the percentage of water to more than 80% resulted in increase of the density and the size of the microparticles. Decreasing the percentage of organic phase to 10% resulted in disappearance of the effect of this phase. Under these conditions the number of hydrophobic molecules decreased and the solvent did not separate easily from the particles and as the result, values of the density and size of the microparticles increased again. Increasing the water to more than 80% resulted in the decrease of the effect of DCM and increase in the size and the density of particles. The infrared spectra of the samples were not different (Fig. 2), before and after spray drying process indicating the stability of drug with different ratios of the solvent mixture.

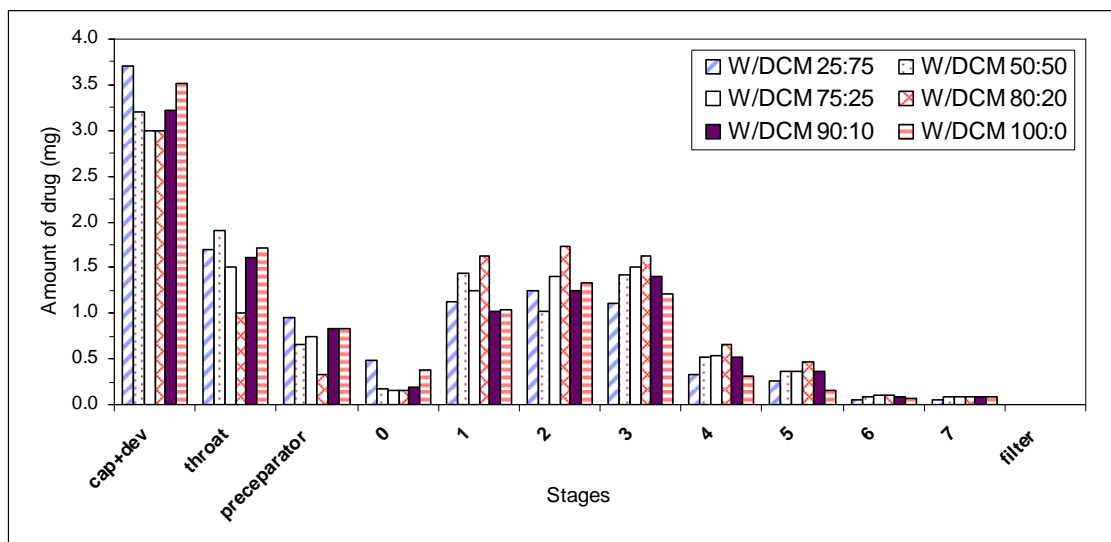


Figure 3. In vitro deposition profile of the samples: W/DCM 25:75, W/DCM 50:50, W/DCM 75:25, W/DCM 80:20, W/DCM 90:10, W/DCM 100:0

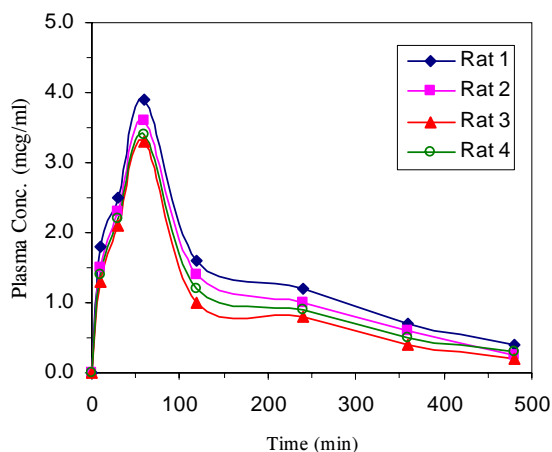


Figure 4. Plasma concentration time profiles for W/DCM in 4 rats after intratracheal administration of 4mg rifampicin micro particle

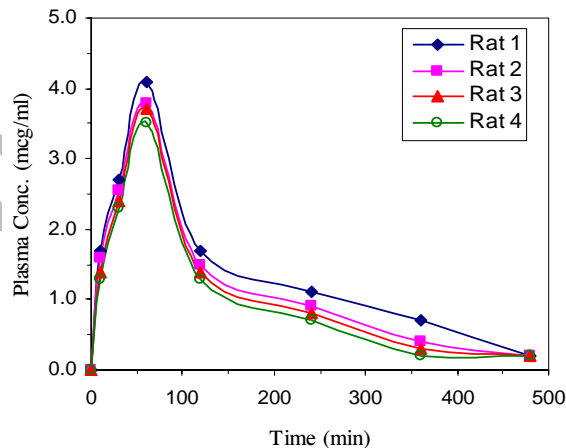


Figure 5. Plasma concentration time profiles for W/DCM 50:50 in 4 rats after intratracheal administration of 4mg rifampicin micro particle

In vitro deposition

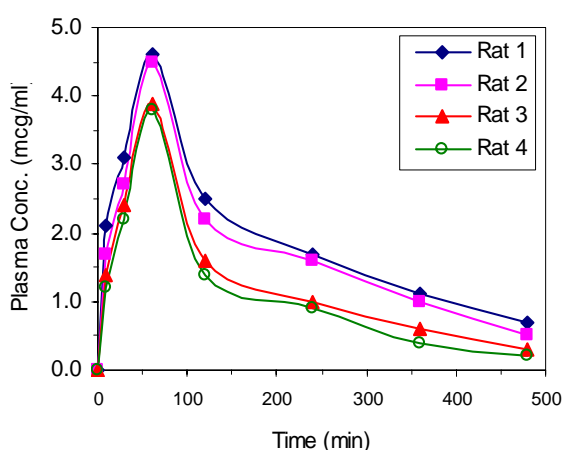
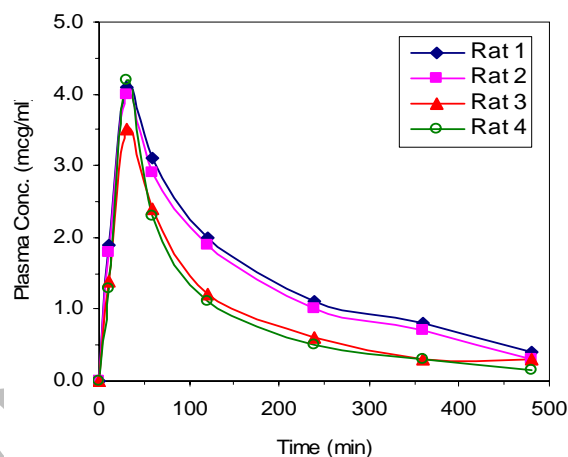
Deposition data for each micronized rifampicin powder after aerosolization of the samples at 60 L/min through a spinhaler[®], using an Andersen cascade impactor are presented in Fig 3. The amount of rifampicin deposited in various stages of the Andersen cascade impactor varied for different samples. These results suggest that the drug particles aerosolized from spray dried samples have different aerodynamic properties. A comparison of the effects of two types of solvents on the physicochemical properties of rifampicin indicated many changes in bulk densities and in vitro deposition profile. Spray dried samples processed from W/DCM 80:20 solution produced significantly ($p > 0.05$) higher percentage emission and higher FPF than the others. The amount of drug remained in the capsules for each samples is

shown in Table 3. Application of water and dichloromethane in the solvent mixture with ratio of 80:20 as the spray drying vehicle resulted microparticles with lower affinity to remain in the capsule shell. From Table 3 it is clear that increase in the percentage of water from 0 to 75% decreased the values of the ED and increased the amount of the FPF. Increasing the percentage of water to more than 80% decreased the values of the ED and FPF.

Increasing the amount of water to more than 80% resulted in production of more hydrophilic particles with high affinity to remain in the capsule shell. This phenomenon may be attributed to the material of the capsule shell and the water content of the conventional gelatin capsule. There are several reports that confirm this hypothesis (19, 20). It is possible that application of hydroxy

Table 4. Pharmacokinetic parameters for the samples

Sample W/DCM	T _{max} (h)	C _{max} (µg/ml)	K _a (1/h)	K _e (1/h)	T _{1/2} (h)	MRT (h)	AUC(0-480 min) µgh/ml	F Abs Bioavailability
25:75	1	3.6	1.07	0.347	1.99	2.88	9.391	0.66
50:50	1	3.7	1.80	0.301	2.25	3.32	9.288	0.65
75:25	1	3.9	2.25	0.344	2.01	2.90	10.21	0.72
80:20	1	4.2	2.70	0.330	2.10	3.03	11.85	0.84
90:10	0.5	4.3	3.5	0.315	2.20	3.174	8.742	0.61
100:0	0.5	4.5	5.4	0.319	2.17	3.134	8.671	0.61
IV	-	-	-	0.4464	1.83	2.240	14.2	1

**Figure 6.** Plasma concentration time profiles for W/DCM 75:25 in 4 rats after intratracheal administration of 4mg rifampicin micro particle**Figure 7.** Plasma concentration time profiles for W/DCM 80:20 in 4 rats after intratracheal administration of 4mg rifampicin micro particle

propyl methyl cellulose (HPMC) capsules which have been developed as an alternative material to gelatin (21) is a suitable way to improve the FPF of rifampicin particles which are produced by spray drying of aqueous solution of this drug, because the HPMC capsule contains much less water compared with the hard gelatin capsule and it retains the designated hardness even at moisture levels of 2% (22).

Decreasing the bulk and true densities and affinity for remaining in the capsule shell are the main reasons for increase in the FPF of the spray dried powders when the mixture of the organic and inorganic solution were used as the spray drying vehicle. The effects of these parameters were maximum when the ratio of water to dichloromethane was 80:20. These results show that selection of appropriate vehicle for spray drying plays the significant role in the aerodynamic properties of the produced microparticles.

In vivo pulmonary absorption

The pulmonary absorption of drugs using different vehicles was evaluated following administration of 4 mg of rifampicin powder as

single dose via intra tracheal and 4 mg of the solution as single dose via intravenous to rats and measurement of the plasma concentration of rifampicin at different time of 4 rats for W/DCM25:75, W/DCM50:50, W/DCM80:20, W/DCM90:10 are presented in Figs.4-7. The peak concentration via intra tracheal route reached 30 min to 60 min after dosing depending on the type of the spray drying vehicle. The mean concentration time profiles of rifampicin after intra tracheal administration of 4mg of the dry powder inhaler and 4mg of the solution via intravenous route are presented in Fig 8. The pharmacokinetic parameters of rifampicin are summarized in Table 4.

For W/DCM25:75 the K_a (1.07 1/h) was minimum. This result showed that the increase in the amount of DCM in the solvent mixture led to the decrease in the absorption velocity due to the lipophilicity of the powder which is produced by the more lipophilic vehicle. There were not significant differences in the values of MRT, $T_{1/2}$, K_e , for this sample in comparison with the others. Increasing the percentage of water from 25% to 50% caused an increase in the value of K_a from 1.07 1/h to 1.80 1/h. Increasing the percentage of

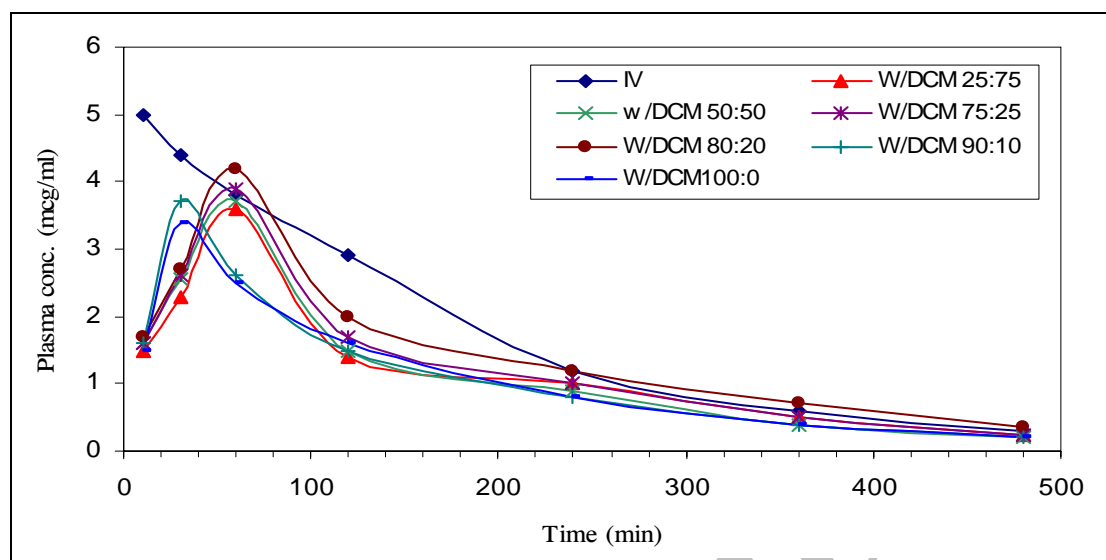


Figure 8. plasma concentration time profile of the samples of IV , W/DCM 25:75, w/DCM 50:50, W/DCM 75:25 and W/DCM 80:20

water from 25% to 75% caused an increase in the value of F (absolute bioavailability) from 0.66 to 0.72 (table 4). The T_{max} value for W/DCM 80:20 was 1h. W/DCM 80:20 showed the maximum value of the AUC and F . These results suggest that the value of AUC increased when the water and DCM were applied in the ratio of 80:20 as the spray drying vehicle. The T_{max} value for W/DCM 90:10 was 0.5h and increase in the percentage of water from 80% to 90% in the solvent mixture caused a significant decrease in the values of T_{max} and AUC and F . W/DCM 100: 0 showed the highest value of C_{max} and K_a and the T_{max} value for this sample was 0.5h. These results showed that the highest value of the absorption rate belongs to W/DCM 100: 0 and the highest value of the extent of absorption belongs to W/DCM 80:20 respectively, because W/DCM 80:20 showed the highest value of the AUC.

The value of the K_a for various samples was different. Increasing the percentage of water in the solvent mixture increased the values of K_a and C_{max} and increasing the percentage of dichloromethane in the solvent mixture increased the value of T_{max} .

These results suggest that reduction in density of the powder could improve absorption of drug from pulmonary system. Increasing the percentage of water to more than 80% decreased the value of T_{max} from 60 min to 30 min because of increase in the dissolution rate of the powder in water and increase in the hydrophilicity of the powder. From table 4 it is clear that the ratios of organic and inorganic solvents in the mixture did not have significant effects on K_e , $T_{1/2}$ and MRT

values and elimination pharmacokinetic parameters. The spray drying vehicle could only alter the absorption pharmacokinetic parameters.

CONCLUSION

A comparison of the effects of the two types of solvents on the physicochemical properties of rifampicin indicated many changes in characteristics of the samples such as in vitro deposition profile. Spray dried samples processed from W/DCM 80:20 showed significantly ($p > 0.05$) higher FPF in comparison with other samples. Therefore, selection of an appropriate solvent mixture as the spray drying vehicle could be more effective for respiratory drug delivery. The type of spray drying vehicle has significant effect on the aerodynamic behavior of the produced particles and pharmacokinetic parameters. The pulmonary absorption of drug showed the possibility of achieving the minimal inhibitory concentration (MIC) of the antibiotics in the plasma concentration time profile. It was found that the elimination pharmacokinetic parameters were not affected by the spray drying vehicle which could only alter the absorption pharmacokinetic parameters.

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