FORMULATION AND EVALUATION OF RIFAMPICIN-LOADED POLYMERIC PARTICLES FOR PULMONARY DELIVERY

By

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This thesis is dedicated to …

My late father, my mother, my late brother, my wife and my sons

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LIST OF ABBREVIATION

Tuberculosis (TB)

World Health Organization (WHO)

Directly observed therapy, short-course (DOTS)

Isoniazid (H)

Rifampicin (R)

Pyrazinamide (Z)

Streptomycin (S)

Ethambutol (E)

Antitubercular drugs (ATD)

Metered dose inhalers (MDIs)

Dry powder inhalers (DPIs)

Chlorofluorocarbon (CFC)

Hydrofluoroalkanes (HFAs)

Poly (Lactic acid) (PLA)

Poly (glycolic acid) (PGA)

Poly (lactic-co-glycolic acid) (PLGA)

methoxypolyethyleneglycol distearoyl-phosphatidylethanolamine (mPEG-DSPE)

Food and drug administration (FDA)

Oil in water (O/W)

Water in oil (W/O)

Water in oil in water (W/O/W)

Oil in oil (O/O)

Polyvinyl alcohol (PVA)

Drug Loading (DL)

Entrapment efficiency (EE)

Scanning electron microscopy (SEM)

Transmission electron microscopy (TEM)

Photon correlation spectroscopy (PCS)

Volume mean diameter D[4, 3]

Mass median diameter D(v, 0.5)

The size of particle for which 10% of the sample is below this size D(v, 0.1) (the

Size of particle for which 90% of the sample is below this size) D(v, 0.9)

Differential scanning calorimetry (DSC)

Fourier transformed infrared (FTIR)

Glass transition temperatures (Tg)

Exothermic crystallization (Tc)

Mass median aerodynamic diameter (MMAD)

Geometric standard deviation (GSD)

Emitted dose (ED)

Fine particle fraction (FPF)

Effective cut-off diameter (ECD)

Oleic acid-albumin-dextrose-catalase (OADC)

Dimethyl sulphoxide (DMSO).

Pure culture of the sensitive strain (H37Rv)

Pure culture of the resistant strain (JB74)

Minimum inhibiting concentration (MIC)

LIST OF PUBLICATIONS

1 Abdullah. J.M.A., Darwis. Y., and Tan, Y.T.F., (2003). Formulation and characterization of rifampicin-loaded Poly (ethylene oxide)-Block distearoyl phosphoidylethanolamine (mPEG-DSPE) polymeric nanoparticles. *14 international symposium of microencapsulation*, 4-6 September 2003, Singapore, Malaysia.

2 J.M.A. Abdulla., Y.T. F. Tan, and Y. Darwis., (2003). Entrapment of rifampicin in polylactic-co-glycolide: preparation and characterization. Malaysian pharmaceutical society Pharmacy scientific conference. 10-12. Octobar 2003, Selangor, Malaysia.

3 J.M.A. Abdulla., Y.T. F. Tan, and Y. Darwis., (2004). An *in vitro* study of the release of rifampicin from poly (d.l-lactide-co-glycolide) microspheres. *4th Malaysian pharmaceutical society Pharmacy scientific conference*. 6-8, August 2004, Kuala Lumpur, Malaysia.

4 J.M.A. Abdulla., H.H. Haris., P. Ibrahim., Y.T. F. Tan and Y. Darwis., (2004). Susceptibility of *mycobacterium tuberculosis* to rifampicin loaded Methoxy poly- (ethylene oxide)- block-distearoylphosphatidyl ethanolamine. *National TB symposium.* 5-6 Octobar 2004, Penang, Malaysia.

FORMULASI DAN PENILAIAN PARTIKEL POLIMERIK BERMUATAN RIFAMPISIN UNTUK PENGHANTARAN PULMONARI

ABSTRAK

Partikel polimerik dibangunkan menggunakan polimer bioterdegradasikan PLGA dan mPEG-DSPE. Pengaruh berbagai parameter formulasi ke atas ciri-ciri fisikal partikel polimerik dinilai. Parameter formulasi yang dinilai untuk PLGA ialah jenis polimer (RG 502, RG 503H dan RG 504), kepekatan PVA (2.5 dan 5 % w/v) dan perkadaran drug dengan polimer (0.2:1, 0.5:1 and 1:1). Parameter formulasi yang dinilai untuk mPEG-DSPE ialah jenis polimer ($mPEG_{2000}$-DSPE dan $mPEG_{5000}$-DSPE), perkadaran drug dengan polimer (1:5, 1:10 and 1.5:10) dan keliangan turas (0.22 dan 0.45 μm). Formulasi disediakan menggunakan kaedah pemeruapan pelarut dan amaun rifampicin terperangkap di dalam partikel polimer ditentukan menggunakan UV spektrofotometer. Purata saiz partikel mPEG-DSPE (241.5 nm) lebih kecil berbanding saiz partikel PLGA (3.7 μm). Hasil mikropartikel PLGA (90.71 %) tidak dijejas oleh semua factor. Di antara PLGA yang diselidiki, PLGA 503H mempunyai kecekapan pemerangkapan tertinggi iaitu 79.59 % pada kepekatan 5 % dan perkadaran drug dengan polimer 0.2:1. Kecekapan pemerangkapan tertinggi mPEG-DSPE ialah 100% pada perkadaran drug dengan polimer 1:5 dan keliangan turas 0.45 μm. Jenis polimer dan keliangan turas tidak ada kesan ke atas kecekapan pemerangkapan, hasil dan muatan drug. Walaubagaimanapun, perkadaran drug dengan polimer berkadar negatif dengan kecekapan pemerangkapan nanopartikel. Analisis termal menggunakan DSC memperlihatkan Tg nanopartikal tersesar ke nilai rendah. Walaubagaimanapun, spectra FTIR tidak memperlihatkan cirri-ciri puncak drug dan polimer tersesar dan ini bermakna tiada interaksi kimia antara drug dan polimer dalam polimerik partikel.

Pelepasan drug dari PLGA mikropartikel sangat perlahan berbanding mPEG-DSPE nanopartikel. Pelepasan berkadar negative dengan jenis PLGA dan berkadar positif dengan perkadaran drug dengan polimer. Kesan cetusan pelepasan diperlihatkan semasa pekadaran drug dengan polimer mencapai 1:1. Di antara PLGA-PLGA, pelepasan drug dari PLGA 503H mikropartikel berlaku paling cepat (14.11 % dalam masa 12 jam). Pelepasan dari PLGA sesuai dengan kinetik tertib sifar manakala PLGA 502 dan 503H masing-masing mengikut kinetik bieksponential. Sebaliknya, pelepasan dari mPEG-DSPE nanopartikles mengikut kenetik tertib pertama dan pelepasan drug paling cepat (58%) berlaku dalam masa 12 jam. Jenis mPEG-DSPE yang digunakan tiada kesan ke atas profile pelepasan drug dari nanopartikel. Walaubagaimanapun, peningkatan perkadaran drug dengan polimer dan penignkatan keliangan turas akan memanjangkan masa pembebasan drug dari nanopartikel.

MMAD mPEG-DSPE yang dihasilkan oleh nebulizer (2.6 μm) dan Rotahaler® (5.8 μm) yang dicirikan menggunakan NGI adalah lebih kecil dari pada aerosol MMAD PLGA 503H yang dihasilkan oleh nebulizer (6.9 μm) dan Rotahaler® (10.6 μm). Sebagai tambahan, FPF mPEG-DSPE (≈ 40 %) lebih tinggi dari pada FPF PLGA 503H (≈15 %). Seterusnya, kaedah perkadaran agar 1% digunakan untuk menguji keterentanan rifampisin terhadap mikobaterium. MIC mPEG-DSPE untuk strain sensitif drug (H37Rv) (10 μg/ml) dan strain rintang drug (JB74) (25 μg/ml) adalah rendah dari pada rifampisin mentah (masing-masing 35 dan 200 μg/ml). Oleh itu, boleh diambil kesimpulan bahwa mPEG-DSPE nanopartikel adalah pembawa yang sesuai untuk penghantaran rifampisin ke pulmonary.

FORMULATION AND EVALUATION OF RIFAMPICIN-LOADED POLYMERIC PARTICLES FOR PULMONARY DELIVERY

ABSTRACT

Polymeric particles were developed using PLGA and mPEG-DSPE biodegradable polymers. The influence of various formulation parameters on physical characteristics of polymeric particles was investigated. The formulation parameters investigated for PLGA were polymer type (RG 502, RG 503H and RG 504), PVA concentration (2.5 and 5 % w/v) and drug to polymer ratio (0.2:1, 0.5:1 and 1:1). The formulation parameters investigated for mPEG-DSPE were polymer type ($mPEG_{2000}$-DSPE and $mPEG_{5000}$-DSPE), drug to polymer ratio (1:5, 1:10 and 1.5:10) and filter porosity (0.22 and 0.45 μm). The formulations were prepared using a solvent evaporation method and the amount of rifampicin encapsulated in polymeric particles was quantified using a UV spectrophotometry. The mean particle size of mPEG-DSPE (241.5 nm) was smaller than PLGA (3.7 μm). The PLGA microparticles yield (90.71 %) was not affected by all factors. Among the PLGA studied, PLGA 503H had the highest entrapment efficiency with 79.59 % at a PVA concentration of 5 %w/v and drug polymer ratio of 0.2:1. The highest entrapment efficiency of mPEG-DSPE nanoparticles was 100 % at a drug to polymer ratio of 1:5 and filter porosity 0.45 μm. Polymer type and filter porosity had no effect on entrapment efficiency, yield and drug loading. However, drug to polymer ratio was negatively correlated with the entrapment efficiency of nanoparticles. Thermal analysis using DSC showed the Tg of nanoparticles shifted to a lower value. However, the FTIR spectra showed no shift in the characteristic peaks of drug and polymer which indicated no chemical interaction between drug and polymer in polymeric particles.

Drug release from PLGA microparticles was much slower than mPEG-DSPE nanoparticles. The release was negatively correlated with PLGA type and positively correlated with drug to polymer ratio. The burst effect was seen when drug to polymer ratio reached 1:1. Drug release from PLGA 503H microparticles was the fastest (14.11 % in 12 hours) among PLGAs. The release from PLGA 504 fitted zero order kinetics whereas PLGA 502 and 503H followed biexponential first order kinetics. Conversely, the release from mPEG-DSPE followed the first order release kinetics and the fastest drug released form nanoparticles (58%) occurred in 12 hours. The mPEG-DSPE type used had no effect on the drug release profile from nanoparticles. However, increasing drug to polymer ratio and filter porosity would prolong the release of drug from nanoparticles.

The MMAD of mPEG-DSPE generated by nebulizer (2.6 μm) and Rotahaler® (5.8 μm) characterized by NGI was smaller than the MMAD of PLGA 503H aerosols produced by nebulizer (6.9 μm) and Rotahaler® (10.6 μm). In addition, the FPF of mPEG-DSPE (≈ 40 %) was higher than the FPF of PLGA 503H (≈15 %). Furthermore, 1% agar proportional method was used to test the susceptibility of rifampicin against mycobacteriums. The MIC values of mPEG-DSPE for drug sensitive strain (H37Rv) (10 μg/ml) and drug resistant strain (JB74) (25 μg/ml) were lower than raw rifampicin (35 and 200 μg/ml respectively). Therefore, it can be concluded that the mPEG-DSPE polymer is a suitable carrier for pulmonary delivery of rifampicin.

APPENDICES

Appendix 2.1.1: Statistical analysis of drug yield values of rifampicin loaded PLGA microparticles

Homogeneous Subsets

Scheffe [a]

CODE	N	Subset
		1
F6	3	87.8500
F15	3	88.1833
F14	3	88.4467
F5	3	88.6667
F9	3	89.0167
F18	3	89.1500
F17	3	89.5800
F12	3	90.3000
F8	3	90.6233
F3	3	90.9333
F2	3	91.0233
F11	3	91.5100
F4	3	92.4133
F7	3	92.8067
F13	3	92.9467
F1	3	93.0000
F16	3	93.0833
F10	3	93.2233
Sig.		.504

Means for groups in homogeneous subsets are displayed

a Uses Harmonic Mean Sample Size = 3.000

Appendix 2.1.2: Statistical analysis of drug loading values of rifampicin-loaded PLGA microparticles

Homogeneous Subsets
Scheffe [a]

CODE	N	Subset												
		1	2	3	4	5	6	7	8	9	10	11	12	13
F4	4	12.23												
F1	4	12.46	12.46											
F13	4		13.27	13.27										
F10	4		13.40	13.40	13.40									
F7	4			13.97	13.97									
F16	4				14.25									
F5	4					23.57								
F2	4						24.72							
F14	4							26.45						
F8	4							26.64						
F11	4							26.65						
F17	4								28.53					
F6	4									32.58				
F3	4										36.03			
F15	4											39.00		
F12	4											39.23		
F9	4												40.20	
F18	4													42.435
Sig.		1.00	0.05	0.46	0.13	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00

Means for groups in homogeneous subsets are displayed
a Uses Harmonic Mean Sample Size = 4.000

Appendix 2.1.3: Statistical analysis of drug entrapment values of rifampicin-loaded PLGA microparticles

Homogeneous Subsets
Scheffe [a]

CODE	N	Subset												
		1	2	3	4	5	6	7	8	9	10	11	12	13
F6	4	57.23												
F5	4		62.70											
F3	4		65.50	65.50										
F2	4			67.49	67.49									
F4	4			67.82	67.82									
F15	4				68.75	68.75								
F1	4				69.53	69.53	69.53							
F14	4				70.13	70.13	70.13	70.13						
F12	4					70.85	70.85	70.85						
F9	4					71.55	71.55	71.55	71.55					
F8	4						72.41	72.41	72.41	72.41				
F11	4							73.08	73.08	73.08	73.08			
F13	4								73.95	73.95	73.95	73.95		
F10	4									74.88	74.88	74.88	74.88	
F18	4										75.66	75.66	75.66	
F17	4											76.63	76.63	
F7	4												77.75	77.75
F16	4													79.59
Sig.		1.00	0.08	0.36	0.14	0.08	0.06	0.05	0.29	0.24	0.17	0.13	0.07	0.78

Means for groups in homogeneous subsets are displayed
a Uses Harmonic Mean Sample Size = 4.000

Appendix 2.2.1: Physical characterizations of rifampicin loaded PLGA microparticles at 2.5 % PVA

MICROPARTICLE YIELDING									
Code	Formulation	Polymer Molecular weight	Drug: Polymer (w/w)	1	2	3	4	Mean	SD
F1	PLGA 502	17,000	(0.2:1)	92.92	94.58	91.50		93.00	1.54
F2	//	//	(0.5:1)	92.27	90.13	90.67		91.02	1.11
F3	//	//	(1:1)	91.55	90.95	90.30		90.93	0.63
F4	PLGA 504	48,000	(0.2:1)	92.08	92.58	92.58		92.42	0.29
F5	//	//	(0.5:1)	88.40	91.20	86.40		88.67	2.41
F6	//	//	(1:1)	87.80	87.45	88.30		87.85	0.43
F7	PLGA 503H	36,000	(0.2:1)	92.25	94.25	91.92		92.81	1.26
F8	//	//	(0.5:1)	88.73	90.87	92.27		90.62	1.78
F9	//	//	(1:1)	88.05	90.10	88.90		89.02	1.03
DRUG LOADING									
F1	PLGA 502	17,000	(0.2:1)	12.46	12.62	12.36	12.40	12.46	0.11
F2	//	//	(0.5:1)	24.27	25.06	24.68	24.88	24.72	0.34
F3	//	//	(1:1)	35.98	36.18	35.94	36.02	36.03	0.11
F4	PLGA 504	48,000	(0.2:1)	12.20	12.24	12.30	12.18	12.23	0.05
F5	//	//	(0.5:1)	23.92	23.38	23.60	23.38	23.57	0.26
F6	//	//	(1:1)	32.46	32.38	32.62	32.84	32.58	0.20
F7	PLGA 503H	36,000	(0.2:1)	14.02	13.82	13.78	14.26	13.97	0.22
F8	//	//	(0.5:1)	26.58	26.14	27.02	26.82	26.64	0.38
F9	//	//	(1:1)	40.30	39.96	40.18	40.34	40.20	0.17
DRUG ENTRAPMENT									
F1	PLGA 502	17,000	(0.2:1)	69.53	70.42	68.97	69.19	69.53	0.64
F2	//	//	(0.5:1)	66.25	68.41	67.38	67.92	67.49	0.93
F3	//	//	(1:1)	65.41	65.78	65.34	65.48	65.50	0.19
F4	PLGA 504	48,000	(0.2:1)	67.65	67.87	68.20	67.54	67.82	0.29
F5	//	//	(0.5:1)	63.63	62.19	62.78	62.19	62.70	0.68
F6	//	//	(1:1)	57.03	56.89	57.31	57.70	57.23	0.36
F7	PLGA 503H	36,000	(0.2:1)	78.02	76.91	76.69	79.36	77.74	1.22
F8	//	//	(0.5:1)	72.24	71.05	73.44	72.90	72.41	1.03
F9	//	//	(1:1)	71.73	71.13	71.52	71.81	71.55	0.30

Appendix 2.2.2: Physical characterizations of rifampicin loaded PLGA microparticles at 5 % PVA

MICROPARTICLE YIELDING									
Code	Formulation	Polymer Molecular weight	Drug: Polymer (w/w)	1	2	3	4	Mean	SD
F10	PLGA 502	17,000	(0.2:1)	91.67	94.17	93.83		93.22	1.36
F11	//	//	(0.5:1)	92.87	91.33	90.33		91.51	1.28
F12	//	//	(1:1)	90.10	90.45	90.35		90.30	0.18
F13	PLGA 504	48,000	(0.2:1)	92.67	92.67	93.50		92.94	0.48
F14	//	//	(0.5:1)	93.07	85.07	87.20		88.44	4.14
F15	//	//	(1:1)	85.15	89.90	89.50		88.18	2.63
F16	PLGA 503H	36,000	(0.2:1)	93.17	93.50	92.58		93.08	0.46
F17	//	//	(0.5:1)	88.67	90.07	90.00		89.58	0.79
F18	//	//	(1:1)	87.35	90.20	89.90		89.15	1.57
DRUG LOADING									
F10	PLGA 502	17,000	(0.2:1)	13.36	13.38	13.40	13.44	13.40	0.03
F11	//	//	(0.5:1)	26.22	27.02	26.58	26.78	26.65	0.34
F12	//	//	(1:1)	39.06	39.04	39.66	39.16	39.23	0.29
F13	PLGA 504	48,000	(0.2:1)	13.24	13.52	13.22	13.08	13.27	0.18
F14	//	//	(0.5:1)	26.26	26.72	26.44	26.36	26.45	0.20
F15	//	//	(1:1)	38.78	39.60	38.60	39.00	39.00	0.44
F16	PLGA 503H	36,000	(0.2:1)	14.26	14.60	13.96	14.18	14.25	0.27
F17	//	//	(0.5:1)	28.42	28.60	28.40	28.70	28.53	0.14
F18	//	//	(1:1)	42.36	42.58	42.34	42.46	42.44	0.11
DRUG ENTRAPMENT									
F10	PLGA 502	17,000	(0.2:1)	74.68	74.79	74.91	75.13	74.88	0.19
F11	//	//	(0.5:1)	71.90	74.09	72.88	73.43	73.07	0.93
F12	//	//	(1:1)	70.54	70.51	71.63	70.72	70.85	0.53
F13	PLGA 504	48,000	(0.2:1)	73.81	75.37	73.70	72.92	73.95	1.03
F14	//	//	(0.5:1)	69.64	70.86	70.12	69.91	70.13	0.52
F15	//	//	(1:1)	68.37	69.81	68.05	68.76	68.75	0.77
F16	PLGA 503H	36,000	(0.2:1)	79.64	81.54	77.97	79.20	79.59	1.48
F17	//	//	(0.5:1)	76.34	76.82	76.28	77.09	76.63	0.39
F18	//	//	(1:1)	75.53	75.92	75.49	75.71	75.66	0.20

Appendix 2.3: Statistical analysis of particle size values of rifampicin-loaded PLGA microparticles

Homogeneous Subsets
Scheffe [a]

CODE	N	Subset												
		1	2	3	4	5	6	7	8	9	10	11	12	13
F15	5	2.36												
F18	5		2.914											
F14	5		2.93											
F13	5			3.17										
F17	5				3.70									
F16	5					4.16								
F12	5					4.21								
F6	5						4.60							
F11	5							4.79						
F5	5								4.98					
F9	5								4.98					
F4	5								5.05	5.05				
F10	5									5.15				
F8	5										5.47			
F7	5											5.80		
F3	5											5.83		
F2	5												6.25	
F1	5													6.49
Sig.		1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.97	0.57	1.00	1.00	1.00	1.00

Means for groups in homogeneous subsets are displayed
a Uses Harmonic Mean Sample Size = 5.000

Appendix 2.4.1: Particle Size distribution of rifampicin loaded PLGA microparticles at 2.5 % PVA

No	Code	Copolymer Type	Drug: Polymer (w/w)	D(0.1) µm	D(0.5) µm	D(0.9) µm	D(4,3) µm	Span
1	F1	PLGA502	(0.2:1)	4.08	5.70	9.75	6.50	2.08
2	F1	//	//	4.11	5.70	9.56	6.44	1.98
3	F1	//	//	4.12	5.75	9.73	6.51	2.04
4	F1	//	//	4.04	5.66	9.79	6.49	2.11
5	F1	//	//	4.05	5.69	9.88	6.52	2.11
Mean				4.08	5.70	9.74	6.49	2.06
±SD				0.04	0.03	0.12	0.03	
1	F2	PLGA502	(0.5:1)	3.86	5.35	9.46	6.21	2.30
2	F2	//	//	3.87	5.39	9.57	6.26	2.30
3	F2	//	//	3.88	5.41	9.62	6.29	2.32
4	F2	//	//	3.90	5.41	9.43	6.23	2.27
5	F2	//	//	3.92	5.43	9.40	6.24	2.27
Mean				3.89	5.40	9.50	6.25	2.29
±SD				0.02	0.03	0.09	0.03	
1	F3	PLGA502	(1:1)	3.80	5.10	8.53	5.80	2.28
2	F3	//	//	3.82	5.15	8.67	5.84	2.21
3	F3	//	//	3.82	5.17	8.72	5.85	2.26
4	F3	//	//	3.86	5.17	8.45	5.81	2.22
5	F3	//	//	3.85	5.17	8.51	5.83	2.18
Mean				3.83	5.15	8.58	5.83	2.23
±SD				0.02	0.03	0.11	0.02	
1	F4	PLGA504	(0.2:1)	4.57	5.00	5.39	5.02	0.43
2	F4	//	//	4.66	5.10	5.65	5.14	0.47
3	F4	//	//	4.51	5.00	5.67	5.05	0.49
4	F4	//	//	4.39	4.95	5.74	5.02	0.53
5	F4	//	//	4.83	4.89	5.36	5.02	0.45
Mean				4.59	4.99	5.56	5.05	0.47
±SD				0.17	0.08	0.17	0.05	
1	F5	PLGA504	(0.5:1)	4.42	4.95	5.68	5.01	0.64
2	F5	//	//	4.41	4.95	5.70	5.01	0.69
3	F5	//	//	4.37	4.90	5.62	4.96	0.63
4	F5	//	//	4.38	4.90	5.60	4.96	0.70
5	F5	//	//	4.38	4.89	5.57	4.95	0.63
Mean				4.39	4.92	5.63	4.98	0.66
±SD				0.02	0.03	0.05	0.03	
1	F6	PLGA504	(1:1)	4.10	4.50	5.06	4.55	0.58
2	F6	//	//	4.11	4.51	5.08	4.56	0.59
3	F6	//	//	4.12	4.51	5.07	4.56	0.62
4	F6	//	//	4.17	4.60	5.17	4.65	0.60
5	F6	//	//	4.18	4.62	5.20	4.67	0.63
Mean				4.14	4.55	5.12	4.60	0.60
±SD				0.04	0.06	0.06	0.06	
1	F7	PLGA503H	(0.2:1)	4.98	5.70	6.70	5.78	0.63
2	F7	//	//	5.14	5.70	6.40	5.75	0.56
3	F7	//	//	5.20	5.80	6.53	5.85	0.45
4	F7	//	//	5.47	5.79	6.22	5.82	0.39
5	F7	//	//	4.37	5.50	7.57	5.79	0.51
Mean				5.03	5.70	6.68	5.80	0.51
±SD				0.41	0.12	0.53	0.04	
1	F8	PLGA503H	(0.5:1)	4.51	5.30	6.51	5.43	0.93
2	F8	//	//	4.43	5.30	6.70	5.47	0.95
3	F8	//	//	4.45	5.33	6.75	5.50	0.96
4	F8	//	//	4.49	5.33	6.65	5.48	0.96
5	F8	//	//	4.49	5.30	6.65	5.48	0.96
Mean				4.47	5.31	6.65	5.47	0.95
±SD				0.03	0.02	0.09	0.03	
1	F9	PLGA503H	(1:1)	3.62	4.50	6.65	4.91	2.15
2	F9	//	//	3.58	4.50	6.88	4.96	2.13
3	F9	//	//	3.60	4.55	7.01	5.02	2.05
4	F9	//	//	3.62	4.55	6.89	5.01	2.08
5	F9	//	//	3.61	4.54	6.86	4.99	2.13
Mean				3.61	4.53	6.86	4.98	2.11
±SD				0.02	0.03	0.13	0.04	

Appendix 2.4.2: Particle Size distribution of rifampicin loaded PLGA microparticles at 5 % PVA

	Code	Copolymer Type	Drug: Polymer (w/w)	D(0.1) µm	D(0.5) µm	D(0.9) µm	D(4,3) µm	Span
1	F10	PLGA502	(0.2:1)	1.6	3.98	9.88	5.15	0.99
2	F10	//	//	1.63	4.11	9.75	5.16	0.96
3	F10	//	//	1.65	4.01	9.85	5.17	0.98
4	F10	//	//	1.59	3.93	9.89	5.13	1.02
5	F10	//	//	1.58	3.95	9.93	5.15	1.02
Mean				1.61	4.00	9.86	5.15	0.99
±SD				0.03	0.07	0.07	0.01	
1	F11	PLGA502	(0.5:1)	1.3	3.59	9.55	4.81	1.05
2	F11	//	//	1.26	3.59	9.52	4.79	1.06
3	F11	//	//	1.29	3.54	9.51	4.78	1.06
4	F11	//	//	1.31	3.58	9.42	4.77	1.02
5	F11	//	//	1.33	3.61	9.51	4.81	1.01
Mean				1.30	3.58	9.50	4.79	1.04
±SD				0.03	0.03	0.05	0.02	
1	F12	PLGA502	(1:1)	1.19	3.1	8.25	4.18	0.93
2	F12	//	//	1.21	3.18	8.24	4.21	0.94
3	F12	//	//	1.19	3.17	8.36	4.24	0.95
4	F12	//	//	1.23	3.18	8.28	4.23	0.89
5	F12	//	//	1.24	3.2	8.21	4.21	0.90
Mean				1.21	3.17	8.27	4.21	0.92
±SD				0.02	0.04	0.06	0.02	
1	F13	PLGA504	(0.2:1)	2.55	3.01	3.85	3.13	0.16
2	F13	//	//	2.49	3.1	3.94	3.17	0.19
3	F13	//	//	2.48	3.08	3.98	3.18	0.23
4	F13	//	//	2.46	3.08	4.1	3.21	0.27
5	F13	//	//	2.52	3.06	3.89	3.15	0.11
Mean				2.50	3.07	3.95	3.17	0.19
±SD				0.04	0.03	0.10	0.03	
1	F14	PLGA504	(0.5:1)	2.11	2.89	3.97	2.99	0.25
2	F14	//	//	2.07	2.8	3.99	2.95	0.26
3	F14	//	//	2.08	2.79	3.85	2.9	0.26
4	F14	//	//	2.03	2.83	4.01	2.95	0.25
5	F14	//	//	2.02	2.77	3.77	2.85	0.24
Mean				2.06	2.82	3.92	2.93	0.25
±SD				0.04	0.05	0.10	0.05	
1	F15	PLGA504	(1:1)	1.72	2.23	3.01	2.32	0.21
2	F15	//	//	1.76	2.22	3.08	2.35	0.22
3	F15	//	//	1.7	2.21	3.06	2.32	0.21
4	F15	//	//	1.71	2.32	3.11	2.38	0.22
5	F15	//	//	1.73	2.38	3.23	2.44	0.22
Mean				1.72	2.27	3.10	2.36	0.22
±SD				0.02	0.07	0.08	0.05	
1	F16	PLGA503H	(0.2:1)	2.95	3.89	5.41	4.08	0.30
2	F16	//	//	3.12	3.91	5.32	4.11	0.22
3	F16	//	//	3.29	4.2	5.19	4.22	0.23
4	F16	//	//	3.61	4.11	5.2	4.3	0.13
5	F16	//	//	3.2	3.91	5.19	4.1	0.58
Mean				3.23	4.00	5.26	4.16	0.29
±SD				0.24	0.14	0.10	0.09	
1	F17	PLGA503H	(0.5:1)	2.28	3.45	5.48	3.73	0.38
2	F17	//	//	2.23	3.39	5.44	3.68	0.43
3	F17	//	//	2.2	3.41	5.47	3.68	0.43
4	F17	//	//	2.19	3.42	5.48	3.69	0.41
5	F17	//	//	2.23	3.4	5.49	3.7	0.41
Mean				2.23	3.41	5.47	3.70	0.41
±SD				0.04	0.02	0.02	0.02	
1	F18	PLGA503H	(1:1)	0.88	2.21	5.64	2.91	0.67
2	F18	//	//	0.9	2.2	5.59	2.89	0.73
3	F18	//	//	0.89	2.28	5.57	2.91	0.75
4	F18	//	//	0.91	2.25	5.6	2.92	0.72
5	F18	//	//	0.91	2.24	5.67	2.94	0.72
Mean				0.90	2.24	5.61	2.91	0.72
±SD				0.01	0.03	0.04	0.02	

Appendix 2.5: Thermal analysis of raw rifampicin, freeze-dried rifampicin, raw polymer, physical mixture, blank polymer

No	Raw Rifampicin		Freeze-dried Rifampicin	
	Tċ	Decomposition	Tċ	Decomposition
1		231.53	172.21	239.99
2		228.62	173.99	235.34
3		229.82	171.93	231.01
Mean		229.99	172.71	235.44
SD		1.46	1.11	4.45

No	PLGA502	PLGA504	PLGA503H
	Tg	Tg	Tg
Sample of Raw Polymer			
1	48.39	52.21	55.12
2	49.01	53.8	54.31
3	47.29	53.9	54.88
Mean	48.23	53.30	54.77
SD	0.87	0.95	0.42
Sample of Physical Mixture			
1	50.03	53.73	54.35
2	48.88	55.13	54.17
3	48.93	51.91	55.28
Mean	49.28	53.59	54.60
SD	0.65	1.61	0.60
Sample of Blank Polymer			
1	51.32	58.13	58.05
2	53.1	54.45	58.27
3	49.19	57.6	57.07
Mean	51.20	56.73	57.80
SD	1.96	1.99	0.64

Appendix 2.6: Thermal analysis of rifampicin-loaded PLGA microparticles

No	R/PLGA502		R/PLGA504		R/PLGA503H	
	Tg	Tċ	Tg	Tċ	Tg	Tċ
Sample of R/PLGA Microparticles at (0.2:1) Weight Ratio						
1	50.01	164.6	54.33	169.75	55.33	158.57
2	49.15	167.12	53.25	170.29	55.35	162.63
3	47.37	165.03	53.81	169.9	56.77	153.94
Mean	48.84	165.58	53.80	169.98	55.82	158.38
SD	1.35	1.35	0.54	0.28	0.83	4.35
Sample of R/PLGA Microparticles at (0.5:1) Weight Ratio						
1	51.31	166.95	55.53	173.72	57.56	167.63
2	52.11	169.02	53.2	170.11	54.22	162.31
3	51.96	167.98	53.96	172.81	57.64	164.61
Mean	51.79	167.98	54.23	172.21	56.47	164.85
SD	0.43	1.04	1.19	1.88	1.95	2.67
Sample of R/PLGA Microparticles at (1:1) Weight Ratio						
1	53.01	178.34	56.31	178.05	58.36	169.14
2	52.09	175.99	55.83	175.17	57.21	166.38
3	52.79	177.3	54.78	174.66	58.3	164.12
Mean	52.63	177.21	55.64	175.96	57.96	166.55
SD	0.48	1.18	0.78	1.83	0.65	2.51

Appendix 2.7.1: Statistical analysis of drug yield values of rifampicin-loaded mPEG-DSPE nanoparticles

Homogeneous Subsets
Scheffe [a]

CODE	N	Subset for alpha = .05			
		1	2	3	4
F24	4	69.8075			
F25	4	71.0025			
F30	4	73.1700	73.1700		
F31	4	73.3925	73.3925		
F22	4	75.0350	75.0350		
F21	4	77.4200	77.4200	77.4200	
F28	4	78.6100	78.6100	78.6100	
F27	4	82.2600	82.2600	82.2600	82.2600
F23	4		86.2300	86.2300	86.2300
F29	4		86.9375	86.9375	86.9375
F20	4			89.4175	89.4175
F26	4				93.1850
Sig.		.117	.051	.153	.267

Means for groups in homogeneous subsets are displayed
a Uses Harmonic Mean Sample Size = 4.000

Appendix 2.7.2: Statistical analysis of drug loading values of rifampicin loaded mPEG-DSPE nanoparticles

Homogeneous Subsets
Scheffe [a]

CODE	N	Subset for alpha = .05			
		1	2	3	4
F27	4	10.7175			
F21	4	11.4000	11.4000		
F30	4	12.1825	12.1825		
F24	4		12.5650		
F28	4			14.3275	
F22	4			14.5225	
F31	4			15.1775	
F25	4			15.9475	
F26	4				18.6525
F20	4				19.3025
F23	4				19.5800
F29	4				19.5825
Sig.		.150	.471	.069	.789

Means for groups in homogeneous subsets are displayed
a Uses Harmonic Mean Sample Size = 4.000

Appendix 2.7.3: Statistical analysis of drug entrapment values of rifampicin loaded mPEG-DSPE nanoparticles

Homogeneous Subsets

Scheffe [a]

CODE	N	Subset for alpha = .05		
		1	2	3
F22	4	83.5450		
F31	4	85.0925		
F28	4	86.3350		
F25	4	86.8450	86.8450	
F24	4		96.3150	96.3150
F21	4			97.0550
F27	4			97.3800
F30	4			97.8500
F29	4			100.2600
F23	4			101.8900
F20	4			103.5550
F26	4			103.9700
Sig.		.992	.058	.261

Means for groups in homogeneous subsets are displayed

a Uses Harmonic Mean Sample Size = 4.000

Appendix 2.8: Physical characterizations of rifampicin loaded mPEG-DSPE nanoparticles

Code	Filter Porosity (µm)	Rifampicin Conc (mg/ml)	Copolymer Conc (mg/ml)	Drug: Polymer (w/w)	1	2	3	4	Mean	SD
NANOPARTICLE YIELDING										
F20	0.45	0.2	1	(1:5)	91.83	90.92	83.17	91.75	89.42	4.19
F21	//	0.2	2	(1:10)	76.82	75.86	81.14	75.86	77.42	2.52
F22	//	0.3	2	(1.5:10)	75.48	76.48	75.70	72.48	75.03	1.76
F23	0.22	0.2	1	(1:5)	84.00	92.67	74.92	93.33	86.23	8.66
F24	//	0.2	2	(1:10)	68.59	68.23	71.77	70.64	69.81	1.69
F25	//	0.3	2	(1.5:10)	71.57	71.09	69.61	71.74	71.00	0.97
F26	0.45	0.2	1	(1:5)	91.00	94.33	94.33	93.08	93.19	1.57
F27	//	0.2	2	(1:10)	82.36	82.68	82.23	81.77	82.26	0.38
F28	//	0.3	2	(1.5:10)	79.22	78.09	74.74	82.39	78.61	3.16
F29	0.22	0.2	1	(1:5)	94.00	93.58	74.92	85.25	86.94	8.97
F30	//	0.2	2	(1:10)	72.23	72.77	75.00	72.68	73.17	1.24
F31	//	0.3	2	(1.5:10)	73.96	72.83	72.43	74.35	73.39	0.91
DRUG LOADING										
F20	0.45	0.2	1	(1:5)	19.26	19.30	19.30	19.35	19.30	0.04
F21	//	0.2	2	(1:10)	11.67	11.22	11.22	11.49	11.40	0.22
F22	//	0.3	2	(1.5:10)	15.01	14.32	13.94	14.82	14.52	0.48
F23	0.22	0.2	1	(1:5)	19.19	19.41	19.49	20.23	19.58	0.45
F24	//	0.2	2	(1:10)	12.27	12.86	12.09	13.04	12.56	0.46
F25	//	0.3	2	(1.5:10)	16.19	16.32	15.96	15.32	15.95	0.44
F26	0.45	0.2	1	(1:5)	18.66	18.62	18.68	18.65	18.65	0.03
F27	//	0.2	2	(1:10)	10.76	10.71	10.71	10.69	10.72	0.03
F28	//	0.3	2	(1.5:10)	14.46	14.33	13.88	14.64	14.33	0.32
F29	0.22	0.2	1	(1:5)	20.21	19.96	18.11	20.05	19.58	0.99
F30	//	0.2	2	(1:10)	12.39	11.97	11.98	12.39	12.18	0.24
F31	//	0.3	2	(1.5:10)	15.36	13.75	15.81	15.79	15.18	0.98
DRUG ENTRAPMENT										
F20	0.45	0.2	1	(1:5)	103.3	103.5	103.5	103.8	103.5	0.19
F21	//	0.2	2	(1:10)	99.34	95.56	95.50	97.82	97.06	1.87
F22	//	0.3	2	(1.5:10)	86.35	82.40	80.19	85.24	83.54	2.79
F23	0.22	0.2	1	(1:5)	99.88	101.0	101.4	105.2	101.8	2.34
F24	//	0.2	2	(1:10)	94.04	98.56	92.70	99.96	96.32	3.49
F25	//	0.3	2	(1.5:10)	88.16	88.87	86.92	83.43	86.84	2.42
F26	0.45	0.2	1	(1:5)	104.0	103.8	104.1	103.9	103.9	0.14
F27	//	0.2	2	(1:10)	97.76	97.30	97.30	97.16	97.38	0.26
F28	//	0.3	2	(1.5:10)	87.15	86.35	83.64	88.20	86.33	1.95
F29	0.22	0.2	1	(1:5)	103.4	102.2	92.70	102.6	100.2	5.07
F30	//	0.2	2	(1:10)	99.48	96.16	96.22	99.54	97.85	1.92
F31	//	0.3	2	(1.5:10)	86.12	77.08	88.65	88.52	85.09	5.47

Appendix 2.9: Statistical analysis of particle size and polydispersity values of rifampicin loaded mPEG-DSPE nanoparticles

Particle Size

Homogeneous Subsets)
Scheffe [a]

CODE	N	Subset for alpha = .05		
		1	2	3
F29	3	162.9667		
F30	3	186.2333	186.2333	
F31	3	194.0333	194.0333	
F26	3	210.6667	210.6667	
F23	3	225.7000	225.7000	
F27	3	231.6333	231.6333	
F28	3	232.5667	232.5667	
F20	3	235.7333	235.7333	
F24	3	247.5333	247.5333	
F21	3	258.0667	258.0667	258.0667
F25	3		318.0667	318.0667
F22	3			395.6333
Sig.		.525	.111	.082

Means for groups in homogeneous subsets are displayed
a Uses Harmonic Mean Sample Size = 3.000

Polydispersity

Homogeneous Subsets
Scheffe [a]

CODE	N	Subset for alpha = .05
		1
F20	3	.4333
F26	3	.4833
F24	3	.5367
F23	3	.5467
F25	3	.5467
F22	3	.5533
F21	3	.5633
F27	3	.5733
F29	3	.6033
F28	3	.6067
F31	3	.6233
F30	3	.6267
Sig.		.350

Means for groups in homogeneous subsets are displayed
a Uses Harmonic Mean Sample Size = 3.000

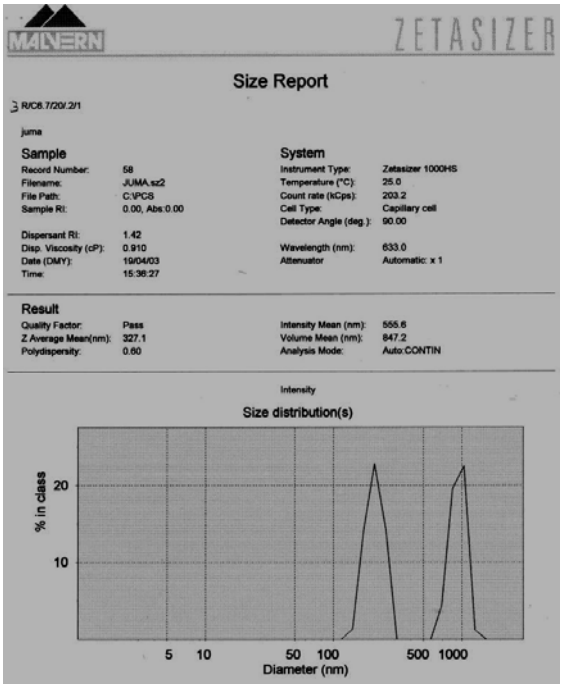
Appendix 2.10: Particle size and polydispersity values of rifampicin-loaded $mPEG_{2000}$-DSPE and $mPEG_{5000}$-DSPE nanoparticles

Code	Filter Porosity (µm)	Rifampicin Conc (mg/ml)	Copolymer Conc (mg/ml)	Drug: Polymer (w/w)	1	2	3	Mean	SD
PARTICLE SIZE									
F20	0.45	0.2	1	(1:5)	137.80	288.50	280.90	235.7	84.9
F21	//	0.2	2	(1:10)	233.90	198.30	342.00	258.0	74.8
F22	//	0.3	2	(1.5:10)	409.50	396.60	380.80	395.6	14.3
F23	0.22	0.2	1	(1:5)	227.50	228.30	221.30	225.7	3.83
F24	//	0.2	2	(1:10)	269.40	251.60	221.60	247.5	24.1
F25	//	0.3	2	(1.5:10)	327.10	321.60	305.50	318.0	11.2
F26	0.45	0.2	1	(1:5)	225.30	208.60	198.10	210.6	13.7
F27	//	0.2	2	(1:10)	231.10	236.20	227.60	231.6	4.32
F28	//	0.3	2	(1.5:10)	240.00	232.00	225.70	232.5	7.17
F29	0.22	0.2	1	(1:5)	125.10	165.80	198.00	162.9	36.5
F30	//	0.2	2	(1:10)	203.70	189.50	165.50	186.2	19.3
F31	//	0.3	2	(1.5:10)	191.00	206.10	185.00	194.0	10.8
POLYDESPERSITY									
F20	0.45	0.2	1	(1:5)	0.40	0.41	0.49	0.43	0.05
F21	//	0.2	2	(1:10)	0.66	0.41	0.62	0.56	0.13
F22	//	0.3	2	(1.5:10)	0.53	0.61	0.52	0.55	0.05
F23	0.22	0.2	1	(1:5)	0.60	0.48	0.56	0.55	0.06
F24	//	0.2	2	(1:10)	0.58	0.55	0.48	0.54	0.05
F25	//	0.3	2	(1.5:10)	0.60	0.56	0.48	0.55	0.06
F26	0.45	0.2	1	(1:5)	0.53	0.48	0.44	0.48	0.05
F27	//	0.2	2	(1:10)	0.58	0.67	0.47	0.57	0.10
F28	//	0.3	2	(1.5:10)	0.62	0.60	0.60	0.61	0.01
F29	0.22	0.2	1	(1:5)	0.56	0.59	0.66	0.60	0.05
F30	//	0.2	2	(1:10)	0.68	0.64	0.56	0.63	0.06
F31	//	0.3	2	(1.5:10)	0.64	0.62	0.61	0.62	0.02

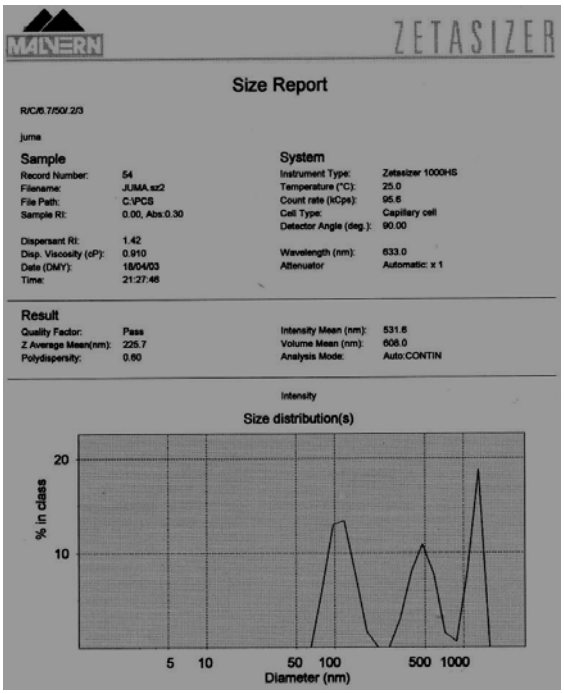
Appendix 2.11: Thermal analysis of rifampicin-loaded $mPEG_{5000}$-DSPE nanoparticles

No	$mPEG_{2000}$-DSPE	$mPEG_{5000}$-DSPE
	Tg	Tg
Sample of Raw Polymer		
1	52.84	55.12
2	51.81	56.58
3	52.69	54.5
Mean	52.45	55.40
SD	0.56	1.07
Sample of Physical Mixture		
1	52.30	55.8
2	52.60	55.9
3	52.91	55.18
Mean	52.60	55.63
SD	0.31	0.39
Sample of R/$mPEG_{5000}$-DSPE Nanoparticles		
1	49.85	50.41
2	48.19	51.87
3	48.52	50.22
Mean	48.85	50.83
SD	0.88	0.90

Appendix 2.12: Bimodal particle size distribution of rifampicin loaded $mPEG_{5000}$-DSPE nanoparticles prepared at drug to polymer weight ratio of 1.5:10



Appendix 2.13: Trimodal particle size distribution of rifampicin loaded Mpeg 5000-DSPE nanoparticles prepared at drug to polymer weight ratio of 1.5:10



Appendix 3.1: Percentages of drug released from rifampicin-loaded PLGA microparticles with different weight ratios

R/PLGA 502 Microparticles

Time (days)	PLGA 502 (0.2:1)					PLGA 502 (0.5:1)					PLGA 502 (1:1)				
	F10a	F10b	F10c	average	SD	F11a	F11b	F11c	average	SD	F12a	F12b	F12c	average	SD
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
0.125	0.64	0.17	0.57	0.46	0.26	5.43	5.11	5.33	5.29	0.16	10.23	10.03	10.16	10.14	0.10
0.25	0.67	0.62	7.25	2.85	3.81	15.14	15.36	15.04	15.18	0.16	18.67	17.87	19.08	18.54	0.62
0.5	7.24	7.88	7.39	7.50	0.34	17.32	17.14	17.62	17.36	0.24	27.81	28.11	24.96	26.96	1.74
1	13.83	14.55	12.17	13.52	1.22	21.43	21.83	21.64	21.63	0.20	33.24	33.14	33.19	33.19	0.05
2	22.59	23.17	22.40	22.72	0.40	33.93	32.85	34.40	33.73	0.79	43.09	42.37	43.19	42.88	0.44
3	30.77	28.96	31.20	30.31	1.19	39.68	39.61	39.50	39.60	0.09	51.58	51.02	52.02	51.54	0.50
4	34.89	33.26	34.10	34.09	0.81	45.11	46.46	44.68	45.42	0.93	58.16	57.40	58.06	57.87	0.42
5	39.38	40.55	39.17	39.70	0.74	50.79	51.75	50.68	51.07	0.59	63.04	61.45	64.03	62.84	1.30
6	45.25	44.83	44.89	44.99	0.22	56.75	57.14	56.46	56.79	0.34	65.89	66.56	65.79	66.08	0.42
7	50.75	52.52	50.61	51.29	1.07	60.75	61.11	60.86	60.90	0.18	69.18	69.64	69.52	69.45	0.24
8	58.78	58.57	58.00	58.45	0.40	64.71	64.04	64.25	64.33	0.35	71.73	72.73	71.96	72.14	0.52
9	63.06	64.44	64.50	64.00	0.82	69.32	69.25	69.43	69.33	0.09	73.67	74.23	73.44	73.78	0.41
10	68.05	67.25	67.48	67.59	0.41	72.86	71.50	73.18	72.51	0.89	75.71	76.02	75.79	75.84	0.16
11	70.15	68.85	70.37	69.79	0.82	74.46	73.25	74.61	74.11	0.75	77.27	77.86	76.94	77.36	0.47
12	73.26	72.25	73.55	73.02	0.68	75.21	74.93	75.07	75.07	0.14	79.29	78.88	79.06	79.07	0.20
13	74.28	74.86	74.64	74.59	0.29	75.82	75.46	76.11	75.80	0.32	80.10	79.97	80.46	80.18	0.25
14	76.74	75.43	77.03	76.40	0.85	76.00	75.54	76.43	75.99	0.45	80.36	80.87	80.43	80.55	0.28
15	78.11	77.53	78.84	78.16	0.65	76.07	76.79	77.43	76.76	0.68	81.51	81.25	81.40	81.39	0.13
17	78.26	79.71	78.84	78.93	0.73	77.18	77.75	77.86	77.60	0.36	82.96	84.31	82.86	83.38	0.81
19	80.36	80.43	80.28	80.36	0.07	78.68	78.64	78.71	78.68	0.04	86.76	86.79	86.73	86.76	0.03
21	82.35	82.24	82.46	82.35	0.11	80.07	80.18	79.96	80.07	0.11	88.97	89.01	88.93	88.97	0.04
23	83.07	82.89	83.25	83.07	0.18	82.09	82.14	82.04	82.09	0.05	90.92	91.05	90.79	90.92	0.13
25	85.17	85.35	84.99	85.17	0.18	84.77	84.61	84.93	84.77	0.16	91.93	91.86	91.99	91.93	0.06
27	86.87	86.73	87.02	86.87	0.14	86.07	86.11	86.04	86.07	0.04	92.23	92.14	92.32	92.23	0.09
29	87.09	86.87	87.31	87.09	0.22	87.88	87.79	87.96	87.88	0.09	92.60	92.63	92.58	92.60	0.03
31	88.07	87.89	88.25	88.07	0.18	89.48	89.50	89.46	89.48	0.02	92.95	92.91	92.98	92.95	0.04

Continue

R/PLGA 504 Microparticles

Time (days)	PLGA 504 (0.2:1)					PLGA 504 (0.5:1)					PLGA 504 (1:1)				
	F13a	F13b	F13c	average	SD	F14a	F14b	F14c	average	SD	F15a	F15b	F15c	average	SD
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
0.125	0.38	0.00	0.69	0.36	0.34	3.63	2.82	3.99	3.48	0.60	13.00	15.32	14.62	14.31	1.19
0.25	0.69	0.60	0.77	0.69	0.08	3.95	4.35	4.03	4.11	0.21	15.09	16.44	15.26	15.59	0.74
0.5	2.98	2.27	2.82	2.69	0.37	4.35	4.31	4.47	4.38	0.08	17.66	18.80	18.24	18.23	0.57
1	6.50	5.28	7.51	6.43	1.11	9.09	7.07	4.68	6.95	2.21	19.34	20.55	18.54	19.48	1.01
2	8.56	7.81	9.07	8.48	0.63	13.33	12.65	13.78	13.25	0.57	22.53	21.89	22.89	22.43	0.51
3	13.38	12.39	14.26	13.34	0.93	16.38	15.95	16.86	16.39	0.46	23.15	23.41	22.95	23.17	0.23
4	17.20	18.00	16.92	17.38	0.56	20.54	19.54	20.95	20.34	0.73	24.43	24.70	24.26	24.46	0.22
5	17.89	19.82	18.44	18.72	0.99	21.81	21.52	22.29	21.87	0.39	28.22	25.74	28.91	27.62	1.67
6	22.78	22.90	22.69	22.79	0.10	23.84	23.29	23.78	23.64	0.30	30.51	27.03	30.77	29.44	2.09
7	23.32	23.79	23.65	23.59	0.24	25.11	25.12	25.35	25.19	0.14	32.42	30.77	32.23	31.81	0.90
8	25.38	26.07	24.63	25.36	0.72	26.63	26.28	27.54	26.82	0.65	34.45	34.01	35.07	34.51	0.53
9	27.52	28.38	27.39	27.76	0.54	29.38	30.19	28.60	29.39	0.80	35.79	36.38	38.26	36.81	1.29
10	29.97	30.75	29.77	30.16	0.52	32.17	32.58	31.87	32.21	0.36	37.64	38.49	38.85	38.33	0.62
11	31.27	31.47	31.36	31.37	0.10	33.37	33.70	33.16	33.41	0.27	40.86	40.81	40.63	40.77	0.12
12	32.80	32.96	33.80	33.19	0.54	35.54	35.55	35.09	35.39	0.26	42.48	41.07	42.95	42.16	0.98
13	35.09	35.93	34.80	35.28	0.59	38.04	37.83	38.51	38.13	0.35	45.13	43.33	45.88	44.78	1.31
14	38.07	38.30	37.33	37.90	0.51	41.99	41.70	41.88	41.86	0.15	48.43	47.35	48.79	48.19	0.75
15	42.43	41.25	40.74	41.47	0.87	48.01	46.88	47.43	47.44	0.56	50.15	49.72	50.62	50.16	0.45
17	46.02	45.84	46.25	46.04	0.21	55.18	54.67	54.38	54.75	0.40	58.24	57.18	58.55	57.99	0.72
19	50.51	50.62	50.41	50.51	0.11	58.71	58.62	58.80	58.71	0.09	63.95	64.08	63.83	63.95	0.13
21	52.74	52.26	53.21	52.74	0.48	62.17	61.99	62.36	62.17	0.18	68.87	68.72	69.03	68.87	0.15
23	59.30	59.09	59.51	59.30	0.21	68.16	68.25	68.08	68.16	0.09	77.06	77.01	77.11	77.06	0.05
25	65.12	65.32	64.92	65.12	0.20	73.30	73.59	73.01	73.30	0.29	81.42	81.49	81.36	81.42	0.06
27	70.23	70.12	70.34	70.23	0.11	78.98	79.12	78.84	78.98	0.14	86.20	85.92	86.48	86.20	0.28
29	74.50	74.59	74.42	74.50	0.09	83.03	83.13	82.93	83.03	0.10	87.86	87.77	87.95	87.86	0.09
31	78.01	78.08	77.94	78.01	0.07	84.30	84.50	84.09	84.30	0.20	89.01	88.88	89.13	89.01	0.13

Continue

R/PLGA 503H Microparticles

Time (days)	PLGA 503H (0.2:1)					PLGA 503H (0.5:1)					PLGA 503H (1:1)				
	F16a	F16b	F16c	average	SD	F17a	F17b	F17c	average	SD	F18a	F18b	F18c	average	SD
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
0.125	0.61	0.85	0.64	0.70	0.13	14.59	14.73	14.49	14.60	0.12	11.91	11.21	11.82	11.64	0.38
0.25	0.66	1.62	1.30	1.19	0.49	17.50	17.53	17.40	17.48	0.07	19.13	18.84	19.08	19.02	0.15
0.5	14.53	13.95	13.85	14.11	0.36	17.85	21.04	18.27	19.05	1.73	28.56	28.30	28.29	28.38	0.15
1	24.58	23.88	24.80	24.42	0.48	39.31	39.31	38.99	39.20	0.18	49.20	49.60	49.44	49.41	0.20
2	49.58	47.84	49.25	48.89	0.93	55.08	55.15	55.01	55.08	0.07	64.25	63.89	64.48	64.21	0.30
3	57.68	58.19	56.96	57.61	0.62	62.03	61.68	62.20	61.97	0.27	75.47	76.20	76.06	75.91	0.39
4	60.75	62.86	62.11	61.91	1.07	66.27	66.73	66.23	66.41	0.27	78.56	77.62	78.33	78.17	0.49
5	62.92	63.63	62.80	63.12	0.45	70.09	69.60	69.71	69.80	0.26	80.14	80.19	80.09	80.14	0.05
6	66.34	66.35	65.98	66.22	0.21	71.84	71.49	71.77	71.70	0.19	80.21	80.69	80.40	80.43	0.24
7	68.37	68.83	68.31	68.50	0.28	73.14	73.46	72.97	73.19	0.25	81.18	81.34	81.23	81.25	0.09
8	69.76	70.18	69.68	69.87	0.27	74.96	74.51	75.25	74.91	0.37	81.60	82.08	82.03	81.90	0.26
9	70.88	70.53	71.09	70.83	0.28	76.54	76.26	76.75	76.52	0.25	82.55	82.81	82.62	82.66	0.13
10	73.32	72.28	73.18	72.93	0.57	77.49	77.14	77.66	77.43	0.27	83.23	83.75	83.07	83.35	0.36
11	75.84	73.74	75.28	74.95	1.08	78.89	78.65	79.10	78.88	0.23	84.03	84.32	83.80	84.05	0.26
12	77.23	76.68	77.03	76.98	0.28	79.70	79.70	79.98	79.79	0.16	84.93	84.95	85.09	84.99	0.09
13	80.31	77.86	78.35	78.84	1.29	81.38	81.31	81.56	81.42	0.13	85.78	85.87	85.68	85.78	0.09
14	81.77	79.96	82.82	81.52	1.45	84.01	83.42	83.77	83.73	0.30	86.91	87.03	86.84	86.93	0.10
15	83.87	82.19	84.29	83.45	1.11	84.89	85.27	84.78	84.98	0.26	87.48	87.74	87.67	87.63	0.13
17	85.43	84.01	85.34	84.92	0.79	87.76	86.78	87.13	87.23	0.50	90.38	89.36	89.58	89.77	0.53
19	85.89	85.27	86.52	85.89	0.63	88.32	88.39	88.25	88.32	0.07	90.72	90.61	90.83	90.72	0.11
21	88.48	88.20	88.76	88.48	0.28	89.39	89.34	89.45	89.39	0.05	92.71	93.23	92.19	92.71	0.52
23	90.01	90.08	89.94	90.01	0.07	90.11	90.15	90.08	90.11	0.04	93.16	93.21	93.11	93.16	0.05
25	89.87	89.80	89.94	89.87	0.07	90.04	90.08	90.01	90.04	0.04	93.21	93.23	93.18	93.21	0.02
27	89.87	89.94	89.80	89.87	0.07	90.08	90.04	90.11	90.08	0.04	93.21	93.16	93.25	93.21	0.05
29	89.98	90.08	89.87	89.98	0.10	89.73	89.41	90.04	89.73	0.32	93.21	93.11	93.30	93.21	0.09
31	89.87	89.94	89.80	89.87	0.07	89.94	89.87	90.01	89.94	0.07	93.22	93.21	93.23	93.22	0.01

Continue
Rifampicin (reference)

Time (days)	Rifampicin (reference)				
	F00a	F00b	F00c	average	SD
0	0	0	0	0	0
0.125	19.03	18.82	18.33	18.73	0.36
0.25	37.00	34.58	36.74	36.10	1.33
0.5	57.59	58.04	56.79	57.47	0.63
1	86.55	84.45	87.09	86.03	1.40
2	90.96	89.51	90.51	90.33	0.74
3	91.37	93.02	93.40	92.60	1.08
4	93.12	93.23	93.82	93.39	0.38
5	93.17	93.47	94.46	93.70	0.67
6	94.87	94.77	95.03	94.89	0.13
7	94.79	95.24	95.20	95.08	0.25
8	95.05	95.07	95.00	95.04	0.04
9	94.88	94.68	94.64	94.74	0.13
10	94.75	94.59	94.70	94.68	0.09
11	94.72	94.53	94.64	94.63	0.09
12	94.55	94.68	94.59	94.61	0.07
13	94.66	94.72	94.70	94.69	0.03
14	94.87	94.74	94.83	94.81	0.07
15	94.83	94.40	94.79	94.67	0.24
17	94.83	94.48	94.66	94.66	0.18
19	94.64	94.66	94.62	94.64	0.02
21	94.63	94.59	94.66	94.63	0.04
23	94.56	94.52	94.61	94.56	0.04
25	94.50	94.48	94.53	94.50	0.03
27	94.51	94.55	94.48	94.51	0.04
29	94.52	94.49	94.55	94.52	0.03
31	94.58	94.59	94.57	94.58	0.01

Appendix 3.2: Statistical analysis of the percentage release of rifampicin loaded PLGA microparticles

12 hour

Homogeneous Subsets as follow

Scheffe [a]

CODE	N	Subset for alpha = .05					
		1	2	3	4	5	6
F13	3	2.6900					
F14	3	4.3767	4.3767				
F10	3		7.5033				
F16	3			14.1100			
F11	3				17.3600		
F15	3				18.2333		
F17	3				19.0533		
F12	3					26.9600	
F18	3					28.3833	
F00	3						57.4733
Sig.		.738	.062	1.000	.733	.880	1.000

Means for groups in homogeneous subsets are displayed

a Uses Harmonic Mean Sample Size = 3.000

15th day

Homogeneous Subsets as follow

Scheffe [a]

CODE	N	Subset for alpha = .05							
		1	2	3	4	5	6	7	8
F13	3	41.473							
F14	3		47.440						
F15	3			50.163					
F11	3				76.763				
F10	3				78.160				
F12	3					81.386			
F16	3					83.450	83.450		
F17	3						84.980		
F18	3							87.630	
F00	3								94.673
Sig.		1.000	1.000	1.000	.533	.095	.408	1.000	1.00

Means for groups in homogeneous subsets are displayed

a Uses Harmonic Mean Sample Size = 3.000

Appendix 3.3.1: Statistical analysis of the $T_{50\%}$ of rifampicin loaded PLGA microparticles

Homogeneous Subsets
Scheffe [a]

CODE	N	Subset for alpha = 0.05								
		1	2	3	4	5	6	7	8	9
F00	3	9.9200								
F18	3	24.960	24.960							
F17	3		40.320	40.320						
F16	3			50.880						
F12	3				67.680					
F11	3					115.36				
F10	3						163.36			
F15	3							357.20		
F14	3								376.80	
F13	3									457.20
Sig.		.059	.051	.389	1.000	1.000	1.000	1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed
a Uses Harmonic Mean Sample Size = 3.000

Appendix 3.3.2: Sstatistical analysis of the release rate constant $K_2\alpha$ and $K_2\beta$ of rifampicin loaded PLGA 502 and 503H microparticles

Homogeneous Subsets
Scheffe [a]

$K_2\alpha$	No	Subset for alpha = .05				$K_2\beta$	No	Subset for alpha = .05	
CODE		1	2	3	4	CODE		1	2
F10	3	.1960				F11	3	.0427	
F11	3	.3109	.3109			F10	3	.0442	
F12	3	.5335	.5335	.5335		F18	3	.0584	.0584
F16	3		.8889	.8889		F16	3	.0590	.0590
F17	3			.9564		F17	3	.0649	.0649
F18	3			.9656		F12	3	.0694	.0694
F00	3				2.8035	F00	3		.0890
Sig.		.499	.056	.237	1.000	Sig.		.110	.051

Means for groups in homogeneous subsets are displayed
a Uses Harmonic Mean Sample Size = 3.000

Appendix 3.3.3: Statistical analysis of the release rate constant (K_o) of rifampicin loaded PLGA 504microparticles

Homogeneous Subsets
Scheffe [a]

CODE	N	Subset for alpha = .05		
		1	2	3
F13	3	2.4406		
F15	3		2.6002	
F14	3			2.6949
Sig.		1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed
a Uses Harmonic Mean Sample Size = 3.000

Appendix 3.3.4: Statistical analysis of the Lag time of rifampicin loaded PLGA nanoparticles

Homogeneous Subsets
Scheffe [a]

CODE	N	Subset for alpha = .05					
		1	2	3	4	5	6
F15	3	123.4433					
F14	3		-47.6767				
F13	3			-39.9900			
F12	3				-6.6700		
F11	3				-2.8167	-2.8167	
F18	3					-1.0733	-1.0733
F17	3					-.4067	-.4067
F10	3					1.5200	1.5200
F00	3					1.8667	1.8667
F16	3						3.8567
Sig.		1.000	1.000	1.000	.353	.140	.102

Means for groups in homogeneous subsets are displayed
a Uses Harmonic Mean Sample Size = 3.000

Appendix 3.4.1: The percentage of drug released from rifampicin loaded $mPEG_{2000}$-DSPE nanoparticles

Time (days)	$mPEG_{2000}$-DSPE (1:5)/ 0.45µm					$mPEG_{2000}$-DSPE (1:10)/ 0.45µm					$mPEG_{2000}$-DSPE (1.5:10)/ 0.45µm				
	F20a	F20b	F20c	average	SD	F21a	F21b	F21c	average	SD	F22a	F22b	F22c	average	SD
0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
0.083	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
0.166	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
0.333	14.75	16.10	18.25	16.37	1.76	14.34	16.80	19.18	16.78	2.42	18.13	16.80	19.57	18.17	1.39
0.5	36.27	32.78	37.18	35.41	2.32	29.93	39.77	37.23	35.64	5.11	33.27	34.59	31.70	33.19	1.44
1	70.29	68.93	70.44	69.89	0.83	70.15	75.81	74.06	73.34	2.90	55.55	57.12	55.02	55.90	1.09
2	86.47	84.58	88.23	86.43	1.82	84.99	93.20	92.34	90.18	4.51	76.75	78.83	79.33	78.30	1.37
3	100.0	100.0	100.0	100.00	0.00	100.0	100.0	100.0	100.00	0.00	94.61	100.0	100.0	98.20	3.11
4	94.08	92.12	94.86	93.69	1.41	85.74	96.28	96.35	92.79	6.11	92.46	94.00	96.54	94.33	2.06
5	95.29	94.10	96.08	95.15	0.99	74.55	98.58	97.15	90.09	13.48	99.82	97.43	98.83	98.69	1.20
6	94.69	93.87	95.15	94.57	0.65	77.92	99.59	97.15	91.55	11.87	100.0	98.22	99.61	99.28	0.94

Time (days)	$mPEG_{2000}$-DSPE (1:5)/ 0.22µm						$mPEG_{2000}$-DSPE (1:10)/ 0.22µm						$mPEG_{2000}$-DSPE (1.5:10)/ 0.22µm					
	F23a	F23b	F23c	F23ar	average	SD	F24a	F24b	F24c	F24ar	average	SD	F25a	F25b	F25c	F25br	average	SD
0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
0.083	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
0.166	14.37	16.87	16.84	14.68	15.69	1.35	13.45	13.09	16.82	15.51	14.72	1.76	0.72	1.94	1.91	1.48	1.51	0.57
0.333	18.08	19.08	19.02	18.47	18.66	0.48	23.32	32.82	24.38	29.15	27.42	4.40	11.22	15.28	12.59	12.49	12.89	1.71
0.5	55.98	47.25	58.19	59.14	55.14	5.42	58.35	60.13	52.79	60.84	58.03	3.65	34.85	52.62	53.67	47.36	47.13	8.64
1	91.47	87.95	90.69	93.44	90.89	2.28	93.49	89.03	87.33	90.56	90.10	2.61	91.96	84.75	96.05	87.57	90.08	4.96
2	85.78	97.95	97.87	98.07	94.92	6.09	92.19	96.18	87.87	94.79	92.76	3.65	90.55	100.0	100.0	88.88	94.86	5.98
3	94.17	100.0	98.34	98.83	97.84	2.54	100.0	100.0	100.0	100.0	100.00	0.00	100.0	98.53	94.14	100.0	98.17	2.77
4	100.0	94.07	99.99	100.01	98.52	2.97	94.22	99.02	89.03	98.24	95.13	4.58	97.79	90.58	97.00	97.27	95.66	3.40
5	97.79	99.55	98.11	99.89	98.84	1.04	92.81	93.23	89.03	92.85	91.98	1.97	97.48	90.58	93.18	92.80	93.51	2.88
6	96.48	97.38	98.58	98.56	97.75	1.01	88.26	93.23	91.16	93.26	91.48	2.36	97.00	90.58	97.48	91.45	94.13	3.62

Appendix 3.4.2: The percentage of drug released from rifampicin loaded $mPEG_{5000}$-DSPE nanoparticles

Time (days)	mPEG5000-DSPE (1:5)/ 0.45μm					mPEG5000-DSPE (1:10)/ 0.45μm					mPEG5000-DSPE (1.5:10)/ 0.45μm				
	F26a	F26b	F26c	average	SD	F27a	F27b	F27c	average	SD	F28a	F28b	F28c	average	SD
0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
0.083	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
0.166	0.00	0.00	0.00	0.00	0.00	0.62	0.00	0.00	0.21	0.36	0.00	0.00	0.00	0.00	0.00
0.333	7.21	6.32	5.67	6.40	0.77	13.46	11.09	12.78	12.44	1.22	0.00	1.94	4.24	2.06	2.12
0.5	27.34	28.43	29.08	28.28	0.88	32.51	29.77	29.41	30.56	1.69	14.82	15.48	22.46	17.59	4.23
1	62.15	64.87	55.79	60.94	4.66	66.25	66.53	65.72	66.17	0.41	57.22	59.50	52.66	56.46	3.49
2	87.25	84.31	82.98	84.85	2.19	85.92	90.96	93.92	90.27	4.04	71.81	73.78	75.12	73.57	1.66
3	100.0	99.13	94.80	97.98	2.79	100.0	100.0	100.0	100.00	0.00	93.11	89.82	93.69	92.21	2.09
4	98.74	100.0	100.0	99.58	0.73	98.97	93.22	93.92	95.37	3.14	95.80	94.38	96.86	95.68	1.25
5	97.69	96.45	93.38	95.84	2.22	83.27	91.26	92.19	88.91	4.90	99.36	98.54	99.17	99.02	0.43
6	94.46	94.27	91.96	93.56	1.39	87.52	91.50	94.61	91.21	3.56	100.0	100.0	100.0	100.00	0.00

Time (days)	mPEG5000-DSPE (1:5)/ 0.22μm						mPEG5000-DSPE (1:10)/ 0.22μm						mPEG5000-DSPE (1.5:10)/ 0.22μm				
	F29a	F29b	F29c	F29ar	average	SD	F30a	F30b	F30c	F30ar	average	SD	F31a	F31b	F31c	average	SD
0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
0.083	0.00	0.00	0.00	0.12	0.03	0.06	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
0.166	0.00	10.51	7.26	5.53	5.82	4.40	1.30	2.14	0.00	0.55	1.00	0.93	5.99	4.47	6.59	5.68	1.09
0.333	15.24	17.41	21.31	20.14	18.53	2.73	23.78	21.74	26.00	23.36	23.72	1.76	16.45	13.82	9.05	13.11	3.75
0.5	51.34	42.82	51.74	43.49	47.35	4.85	47.60	60.54	51.20	53.83	53.29	5.46	44.01	45.88	43.76	44.55	1.16
1	75.95	83.17	84.62	82.57	81.58	3.85	85.26	88.00	91.00	89.54	88.45	2.45	92.43	80.94	81.41	84.93	6.50
2	86.19	98.27	94.25	90.01	92.18	5.22	89.32	94.23	93.71	93.61	92.72	2.28	97.97	93.54	87.35	92.95	5.33
3	90.09	97.77	93.66	95.30	94.21	3.22	99.99	100.0	100.0	99.98	99.99	0.01	99.12	98.36	93.06	96.84	3.30
4	91.45	99.50	98.16	100.0	97.28	3.96	99.94	94.17	96.60	100.0	97.68	2.83	97.39	94.22	91.47	94.36	2.96
5	89.42	100.0	99.13	98.04	96.65	4.88	99.22	94.62	97.00	98.81	97.41	2.10	100.0	100.0	100.0	100.00	0.00
6	100.0	99.24	100.0	101.6	100.22	1.02	94.57	96.25	96.73	95.19	95.69	0.98	99.64	99.93	99.53	99.70	0.21

Appendix 3.4.3: The percentage of drug released from rifampicin (reference) microparticles

Time (day)	Rifampicin (reference)					
	F19a	F19b	F19c	F19c	average	SD
0	0.00	0.00	0.00	0.00	0.00	0.00
0.083	2.23	0.00	3.37	1.84	1.86	1.40
0.166	11.09	15.18	18.10	10.12	13.62	3.71
0.333	27.75	29.33	26.10	32.55	28.93	2.75
0.5	54.91	66.02	57.68	52.94	57.89	5.76
1	99.80	100.00	100.00	100.00	99.95	0.10
2	100.00	96.63	96.84	98.27	97.93	1.56
3	99.01	95.90	94.31	96.84	96.52	1.96
4	98.99	98.80	96.21	96.24	97.56	1.54
5	99.25	97.83	97.26	95.37	97.43	1.61
6	99.44	98.07	96.84	97.05	97.85	1.19

Appendix 3.5: Statistical analysis of the percentage release of rifampicin loaded mPEG-DSPE nanoparticles

12 hour

Homogeneous Subsets as follow

Scheffe [a]

CODE	N	Subset for alpha = .05				
		1	2	3	4	5
F28	3	17.5867				
F26	3	28.2833	28.2833			
F27	3	30.5633	30.5633	30.5633		
F22	3	33.1867	33.1867	33.1867		
F20	3	35.4100	35.4100	35.4100	35.4100	
F21	3	35.6433	35.6433	35.6433	35.6433	
F31	3		44.5500	44.5500	44.5500	44.5500
F25	4			47.1250	47.1250	47.1250
F29	4			47.3475	47.3475	47.3475
F30	4				53.2925	53.2925
F23	4					55.1400
F19	4					57.8875
F24	4					58.0275
Sig.		.054	.126	.100	.059	.369

Means for groups in homogeneous subsets are displayed

a Uses Harmonic Mean Sample Size = 3.391

b The group sizes are unequal. The harmonic mean of the group sizes is used

Type I error levels are not guaranteed

2nd day

Homogeneous Subsets as follow

Scheffe [a]

CODE	N	Subset for alpha = .05		
		1	2	3
F28	3	73.5700		
F22	3	78.3033	78.3033	
F26	3	84.8467	84.8467	84.8467
F20	3	86.4267	86.4267	86.4267
F21	3		90.1767	90.1767
F27	3		90.2667	90.2667
F29	4		92.1800	92.1800
F30	4		92.7175	92.7175
F24	4		92.7575	92.7575
F31	3		92.9533	92.9533
F25	4			94.8575
F23	4			94.9175
F19	4			97.9350
Sig.		.199	.078	.178

Means for groups in homogeneous subsets are displayed

a Uses Harmonic Mean Sample Size = 3.391

b The group sizes are unequal. The harmonic mean of the group sizes is used

Type I error levels are not guaranteed

Appendix 3.6.1: Statistical analysis of the $T_{50\%}$ of rifampicin loaded mPEG-DSPE nanoparticles

Homogeneous Subsets
Scheffe [a]

CODE	N	Subset for alpha = .05					
		1	2	3	4	5	6
F24	3	10.7267					
F19	3	10.8500					
F30	3	11.4067					
F23	3	11.6900					
F25	3	12.0500					
F29	3	12.5400					
F31	3	13.6267	13.6267				
F21	3		16.5200	16.5200			
F20	3			17.0600	17.0600		
F27	3			18.5400	18.5400	18.5400	
F26	3				20.1033	20.1033	20.1033
F22	3					20.8800	20.8800
F28	3						22.1033
Sig.		.133	.135	.638	.094	.409	.652

Means for groups in homogeneous subsets are displayed
a Uses Harmonic Mean Sample Size = 3.000

Appendix 3.6.2: Statistical analysis of the release rate constant (k_1) of rifampicin loaded mPEG-DSPE nanoparticles

Homogeneous Subsets
Scheffe [a]

CODE	N	Subset for alpha = .05		
		1	2	3
F28	3	.7567		
F22	3	.8167		
F26	3	1.0367		
F20	3	1.1000		
F27	3	1.2933	1.2933	
F21	3	1.3100	1.3100	
F29	3	1.4367	1.4367	
F24	3	1.5567	1.5567	1.5567
F30	3	1.5900	1.5900	1.5900
F31	3	1.6300	1.6300	1.6300
F23	3		2.1400	2.1400
F25	3			2.4000
Sig.		.068	.086	.089

Means for groups in homogeneous subsets are displayed
a Uses Harmonic Mean Sample Size = 3.000

Appendix 3.6.3: Statistical analysis of the Lag time of rifampicin loaded mPEG-DSPE nanoparticles

Homogeneous Subsets
Scheffe [a]

CODE	N	Subset for alpha =0.05	
		1	2
F24	3	.6400	
F30	3	.8800	
F29	3	1.3533	1.3533
F19	3	1.7233	1.7233
F22	3	1.8567	1.8567
F31	3	2.2600	2.2600
F23	3	2.3367	2.3367
F20	3	2.4633	2.4633
F21	3	3.0367	3.0367
F28	3	3.7500	3.7500
F26	3	3.8467	3.8467
F27	3	3.9433	3.9433
F25	3		4.7267
Sig.		.094	.080

Means for groups in homogeneous subsets are displayed
a Uses Harmonic Mean Sample Size = 3.000

Appendix 3.7.1: Correlation of particle size and size distribution with release kinetic parameter of rifampicin loaded PLGA 504 microparticles

		P. Size	Span	ko	T50%	Lag-time
P. Size	Pearson Correlation	1	-.444	-.386	.811**	.982**
	Sig. (2-tailed)	.	.097	.305	.008	.000
	N	15	15	9	9	9
Span	Pearson Correlation	-.444	1	.959**	-.843**	-.296
	Sig. (2-tailed)	.097	.	.000	.004	.439
	N	15	15	9	9	9
ko	Pearson Correlation	-.386	.959**	1	-.833**	-.225
	Sig. (2-tailed)	.305	.000	.	.005	.561
	N	9	9	9	9	9
T50%	Pearson Correlation	.811**	-.843**	-.833**	1	.709*
	Sig. (2-tailed)	.008	.004	.005	.	.032
	N	9	9	9	9	9
Lag-time	Pearson Correlation	.982**	-.296	-.225	.709*	1
	Sig. (2-tailed)	.000	.439	.561	.032	.
	N	9	9	9	9	9

** Correlation is significant at the 0.01 level (2-tailed)
* Correlation is significant at the 0.05 level (2-tailed

Appendix 3.7.2: Correlation of particle size and size distribution with release kinetic parameter of rifampicin loaded PLGA 502, and 503H microparticles

		P. Size	Span	$k_2\alpha$	$k_2\beta$	T50%	Lag-time
P. Size	Pearson Correlation	1	.195	-.886**	-.592**	.920**	.052
	Sig. (2-tailed)	.	.302	.000	.010	.000	.837
	N	30	30	18	18	18	18
Span	Pearson Correlation	.195	1	-.609**	-.316	.421	-.662**
	Sig. (2-tailed)	.302	.	.007	.201	.082	.003
	N	30	30	18	18	18	18
$k_2\alpha$	Pearson Correlation	-.886**	-.609**	1	.841**	-.725**	.341
	Sig. (2-tailed)	.000	.007	.	.000	.000	.130
	N	18	18	21	21	21	21
$k_2\beta$	Pearson Correlation	-.592**	-.316	.841**	1	-.707**	.007
	Sig. (2-tailed)	.010	.201	.000	.	.000	.978
	N	18	18	21	21	21	21
T50%	Pearson Correlation	.920**	.421	-.725**	-.707**	1	-.084
	Sig. (2-tailed)	.000	.082	.000	.000	.	.717
	N	18	18	21	21	21	21
Lag-time	Pearson Correlation	.052	-.662**	.341	.007	-.084	1
	Sig. (2-tailed)	.837	.003	.130	.978	.717	.
	N	18	18	21	21	21	21

** Correlation is significant at the 0.01 level (2-tailed)

Appendix 3.7.3: Correlation of particle size and size distribution and release kinetic parameter of rifampicin loaded mPEG-DS nanoparticles

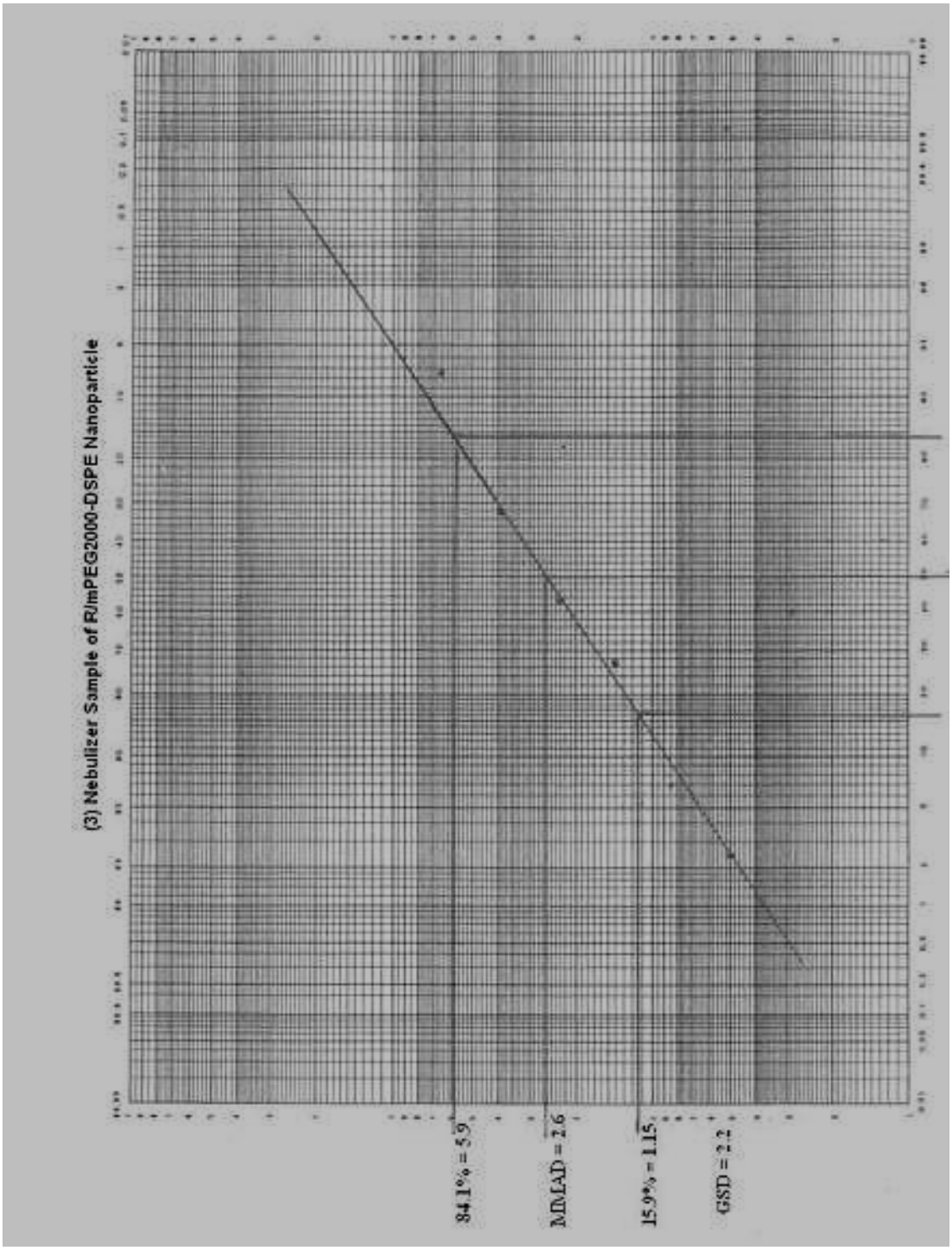
		P. Size	Polydispersity	k_1	T50%	Lag-time
P. Size	Pearson Correlation	1	.000	-.089	.312	.082
	Sig. (2-tailed)	.	.998	.607	.064	.633
	N	36	36	36	36	36
Polydispersity	Pearson Correlation	.000	1	.045	-.134	-.148
	Sig. (2-tailed)	.998	.	.794	.434	.390
	N	36	36	36	36	36
k_1	Pearson Correlation	-.089	.045	1	-.655**	.280
	Sig. (2-tailed)	.607	.794	.	.000	.076
	N	36	36	41	41	41
T50%	Pearson Correlation	.312	-.134	-.655**	1	.423**
	Sig. (2-tailed)	.064	.434	.000	.	.006
	N	36	36	41	41	41
Lag-time	Pearson Correlation	.082	-.148	.280	.423**	1
	Sig. (2-tailed)	.633	.390	.076	.006	.
	N	36	36	41	41	41

** Correlation is significant at the 0.01 level (2-tailed)

Appendix 4.1: Cumulative percentage values generated from nebulizer and dry powder inhaler through ACI applied on log probability paper to obtained MMAD and GSD

Cumulative %									
R/mPEG$_{2000}$-DSPE Nanoparticle Formulation									
Nebulizer Samples					Dry Powder Sample				
1	2	3	4	ECD	1	2	3	4	ECD
100.0	100.0	100.0	100.0	N.D	100.00	100.00	100.00	100.0	N.D
96.32	93.93	95.07	94.78	11.0	63.83	63.35	63.20	63.05	7.80
90.65	88.74	87.98	89.82	6.60	41.12	40.47	40.70	41.19	4.60
69.43	65.48	65.08	67.19	3.90	22.39	21.92	21.32	22.42	2.70
45.14	47.61	41.10	49.99	2.30	15.46	15.18	14.64	15.57	1.60
24.72	27.16	27.02	28.56	1.40	8.22	7.88	7.01	8.02	0.96
8.06	10.93	8.02	9.56	0.84	3.01	2.90	2.72	3.08	0.57
4.56	6.44	3.77	5.96	0.51	1.19	0.97	0.92	1.13	0.33
2.47	2.15	1.23	2.69	N.D	0.45	0.33	0.33	0.37	N.D
R/mPEG$_{5000}$-DSPE Nanoparticle Formulation									
Nebulizer Samples					Dry Powder Sample				
1	2	3	4	ECD	1	2	3	4	ECD
100.0	100.0	100.0	100.0	N.D	100.00	100.00	100.00	100.0	N.D
92.01	94.43	92.86	95.00	11.0	63.32	62.83	63.43	63.01	7.80
83.86	83.13	86.91	89.61	6.60	41.18	40.59	41.04	40.88	4.60
70.96	71.24	59.43	65.48	3.90	22.03	21.58	21.63	22.38	2.70
52.52	46.47	34.88	45.06	2.30	15.25	15.24	14.76	15.10	1.60
37.37	27.68	17.66	27.54	1.40	7.97	7.68	7.24	8.06	0.96
9.96	6.81	6.46	9.06	0.84	2.90	3.01	2.80	3.19	0.57
2.88	3.24	3.45	4.47	0.51	0.84	0.91	0.84	1.01	0.33
2.31	1.18	2.60	3.13	N.D	0.20	0.26	0.16	0.18	N.D
R/PLGA 503H Microparticle Formulation									
Nebulizer Samples					Dry Powder Sample				
1	2	3	4	ECD	1	2	3	4	ECD
100.0	100.0	100.0	100.0	N.D	100.00	100.00	100.00	100.0	N.D
82.64	73.38	77.30	82.12	11.0	30.82	34.74	37.01	36.77	7.80
40.57	43.10	50.76	52.51	6.60	15.67	18.22	19.18	19.78	4.60
9.58	25.33	28.38	21.38	3.90	2.73	4.18	4.90	5.02	2.70
1.62	6.63	4.60	4.30	2.30	1.01	1.68	1.92	1.73	1.60
0.42	0.46	0.23	0.94	1.40	0.04	0.04	0.02	0.03	0.96
0.35	0.16	0.11	0.18	0.84	0.00	0.01	0.01	0.00	0.57
0.04	0.16	0.07	0.07	0.51	0.00	0.00	0.00	0.00	0.33
0.04	0.16	0.00	0.07	N.D	0.00	0.00	0.00	0.00	N.D

Appendix 4.2: Log probability paper graph displayed the calculated MMAD and GSD



Appendix 4.3: Data from log probability paper to obtain the average of MMAD and GSD for (**a**) nebulizer and (**b**) dry powder samples

(a)

Formula	NO	MMAD	average	SD	84.1%	15.9%	GSD	average	SD
R/mPEG2000-DSPE									
	1	2.5	2.56	0.11	5.60	1.10	2.26	2.40	0.11
	2	2.6			6.50	1.05	2.49		
	3	2.7			6.40	1.15	2.36		
	4	2.45			5.90	0.95	2.49		
R/mPEG5000-DSPE									
	1	2.1	2.56	0.41	6.60	0.65	3.19	2.58	0.41
	2	2.55			5.60	1.09	2.27		
	3	3.1			7.80	1.35	2.40		
	4	2.5			6.00	1.00	2.45		
R/PLGA 503H									
	1	7.2	6.88	0.32	11.20	4.40	1.60	1.75	0.11
	2	7			11.40	3.40	1.83		
	3	6.9			11.30	3.60	1.77		
	4	6.45			11.20	3.45	1.80		

(b)

Formula	NO	MMAD	average	SD	84.1%	15.9%	GSD	average	SD
R/mPEG2000-DSPE									
	1	6.10	5.775	0.43	12.20	1.70	2.68		1
	2	5.40			11.80	1.70	2.63		2
	3	5.40			11.70	1.75	2.59		3
	4	6.20			12.25	1.75	2.65		4
R/mPEG5000-DSPE									
	1	6.00	5.975	0.40	12.00	1.90	2.51	2.56	0.06
	2	5.40			11.70	1.70	2.62		
	3	6.30			12.00	1.90	2.51		
	4	6.20			12.10	1.80	2.59		
R/PLGA 503H									
	1	11.20	10.550	0.64	13.10	5.20	1.59	1.67	0.05
	2	11.00			12.45	4.45	1.67		
	3	10.00			12.50	4.40	1.69		
	4	10.00			12.50	4.25	1.71		

Appendix 4.4: Values of (%) deposition generated from nebulizer sample

Formulations	Stages	1	2	3	4	Mean	SD
R/mPEG$_{2000}$-DSPE	nebulizer	39.82	36.56	40.32	38.89	38.90	1.67
	thr + pre-sep	2.22	3.85	2.94	3.19	3.05	0.68
	Stage 1	3.41	3.29	4.23	3.03	3.49	0.52
	Stage 2	12.77	14.76	13.67	13.83	13.75	0.81
	Stage 3	14.62	11.34	14.31	10.52	12.70	2.07
	Stage 4	12.29	12.97	8.40	13.09	11.69	2.22
	Stage 5	10.03	10.29	11.34	11.61	10.82	0.77
	Stage 6	2.10	2.85	2.54	2.20	2.42	0.34
	Stage 7	1.26	2.72	1.52	2.00	1.87	0.64
	Filter	1.49	1.36	0.74	1.64	1.31	0.40
R/mPEG$_{5000}$-DSPE	nebulizer	38.30	32.90	35.32	41.56	37.02	3.75
	thr + pre-sep	4.93	3.74	4.62	2.92	4.05	0.91
	Stage 1	5.03	7.58	3.85	3.15	4.90	1.95
	Stage 2	7.96	7.98	17.77	14.10	11.95	4.84
	Stage 3	11.38	16.62	15.88	11.94	13.95	2.68
	Stage 4	9.34	12.61	11.14	10.24	10.83	1.39
	Stage 5	16.91	14.00	7.24	10.80	12.24	4.16
	Stage 6	4.37	2.40	1.95	2.68	2.85	1.06
	Stage 7	0.35	1.38	0.55	0.78	0.76	0.45
	Filter	1.43	0.79	1.68	1.83	1.43	0.46
R/PLGA 503H	nebulizer	41.36	32.87	40.23	35.97	37.61	3.92
	thr+ pre-sep	10.18	17.87	13.57	11.49	13.27	3.36
	Stage 1	24.67	20.33	15.87	18.94	19.95	3.66
	Stage 2	18.17	11.93	13.38	19.92	15.85	3.81
	Stage 3	4.67	12.55	14.21	10.93	10.59	4.17
	Stage 4	0.70	4.14	2.61	2.15	2.40	1.42
	Stage 5	0.05	0.20	0.07	0.49	0.20	0.20
	Stage 6	0.18	0.00	0.02	0.07	0.07	0.08
	Stage 7	0.00	0.00	0.04	0.00	0.01	0.02
	Filter	0.02	0.11	0.00	0.04	0.04	0.05

Appendix 4.5: Values of (%) deposition generated from dry powder sample

Formulations	Stages	1	2	3	4	Mean	SD
R/mPEG$_{2000}$-DSPE	Inhelator	6.29	6.33	6.50	6.49	6.40	0.11
	thr + pre-sep	33.90	34.33	34.41	34.55	34.30	0.28
	Stage 1	21.28	21.43	21.04	20.44	21.05	0.43
	Stage 2	17.55	17.38	18.12	17.54	17.65	0.32
	Stage 3	6.49	6.31	6.24	6.41	6.36	0.11
	Stage 4	6.78	6.84	7.14	7.06	6.96	0.17
	Stage 5	4.89	4.67	4.01	4.62	4.54	0.38
	Stage 6	1.71	1.81	1.69	1.83	1.76	0.07
	Stage 7	0.69	0.59	0.55	0.71	0.64	0.08
	Filter	0.42	0.31	0.31	0.35	0.35	0.05
R/mPEG$_{5000}$DSPE	Inhelator	6.69	6.50	6.90	7.32	6.85	0.35
	thr+ pre-sep	34.22	34.76	34.05	34.28	34.33	0.30
	Stage 1	20.66	20.79	20.85	20.51	20.70	0.15
	Stage 2	17.87	17.77	18.07	17.14	17.71	0.40
	Stage 3	6.32	5.94	6.40	6.74	6.35	0.33
	Stage 4	6.79	7.06	7.00	6.53	6.85	0.24
	Stage 5	4.73	4.37	4.14	4.52	4.44	0.25
	Stage 6	1.92	1.96	1.82	2.02	1.93	0.09
	Stage 7	0.59	0.61	0.63	0.77	0.65	0.08
	Filter	0.19	0.24	0.15	0.17	0.19	0.04
R/PLGA 503H	Inhelator	5.83	4.62	5.79	5.81	5.52	0.60
	thr+ pre-sep	65.14	62.24	59.34	59.56	61.57	2.72
	Stage 1	14.26	15.76	16.80	16.00	15.71	1.06
	Stage 2	12.19	13.39	13.45	13.90	13.23	0.73
	Stage 3	1.62	2.38	2.81	3.10	2.48	0.64
	Stage 4	0.91	1.56	1.79	1.61	1.47	0.38
	Stage 5	0.03	0.03	0.02	0.02	0.02	0.01
	Stage 6	0.00	0.00	0.01	0.00	0.00	0.00
	Stage 7	0.00	0.00	0.00	0.00	0.00	0.00
	Filter	0.00	0.00	0.00	0.00	0.00	0.00

Appendix 4.6: Statistical analysis applied for MMAD, GSD, ED and FPF from nebulizer and dry powder inhaler formulations

MMAD

Homogeneous Subsets

Scheffe [a]

CODE	N	Subset for alpha = .05			
		1	2	3	4
R/Mpeg$_{2000}$DSPE Nebulizer	4	2.5625			
R/mPEG$_{5000}$DSPE Nebulizer	4	2.5625			
R/mPEG$_{2000}$DSPE Powder	4		5.7750		
R/mPEG$_{5000}$DSPE Powder	4		5.9750	5.9750	
R/PLGA 503H Nebulizer	4			6.8875	
R/PLGA 503H Powder	4				10.5500
Sig.		1.000	.992	.142	1.000

Means for groups in homogeneous subsets are displayed

a Uses Harmonic Mean Sample Size = 4.000

GSD

Homogeneous Subsets

Scheffe [a]

CODE	N	Subset for alpha =.05	
		1	2
R/PLGA 503H Powder	4	1.6650	
R/PLGA 503H Nebulizer	4	1.7500	
R/mPEG$_{2000}$DSPE Nebulizer	4		2.4000
R/mPEG$_{5000}$DSPE Powder	4		2.5575
R/mPEG$_{5000}$DSPE Nebulizer	4		2.5775
R/mPEG$_{2000}$DSPE Powder	4		2.6375
Sig.		.994	.653

Means for groups in homogeneous subsets are displayed

a Uses Harmonic Mean Sample Size = 4.000

EMITTED DOSE

Homogeneous Subsets

Scheffe [a]

CODE	N	Subset for alpha = .05	
		1	2
R/mPEG$_{2000}$DSPE Nebulizer	4	61.1032	
R/mPEG$_{5000}$DSPE Nebulizer	4	62.3931	
R/PLGA 503H Nebulizer	4	62.9800	
R/mPEG$_{2000}$DSPE Powder	4		93.1475
R/mPEG$_{5000}$DSPE Powder	4		93.5975
R/PLGA 503H Powder	4		94.4875
Sig.		.930	.983

Means for groups in homogeneous subsets are displayed

a Uses Harmonic Mean Sample Size = 4.000

Continue

The fractions emitted dose of particle size deposited in cascade impactor.

The fine particle fraction (FPF)

Homogeneous Subsets

Scheffe [a]

CODE	N	Subset for alpha = .05		
		1	2	3
R/PLGA 503H Powder	4	17.1650		
R/PLGA 503H Nebulizer	4		13.3150	
R/mPEG$_{5000}$DSPE Powder	4		38.1025	
R/mPEG$_{2000}$DSPE Powder	4		38.3150	
R/mPEG$_{2000}$DSPE Nebulizer	4			40.8075
R/mPEG$_{5000}$DSPE Nebulizer	4			42.0725
Sig.		1.000	.077	.996

Means for groups in homogeneous subsets are displayed

a Uses Harmonic Mean Sample Size = 4.000

The fractions emitted dose in deposited stage 1-3 0r 2-3

Homogeneous Subsets

Scheffe [a]

CODE	N	Subset for alpha = .05		
		1	2	3
R/mPEG$_{2000}$DSPE Nebulizer	4	29.9450		
R/PLGA 503H Powder	4	28.9o75		
R/mPEG$_{5000}$DSPE Nebulizer	4	34.9475	34.9475	
R/mPEG$_{5000}$DSPE Powder	4		38.4300	38.4300
R/mPEG$_{2000}$DSPE Powder	4			38.6600
R/PLGA 503H Nebulizer	4			46.3950
Sig.		.642	.059	.996

Means for groups in homogeneous subsets are displayed

a Uses Harmonic Mean Sample Size = 4.000

The fractions emitted dose deposited in through and pre-separator

Homogeneous Subsets

Scheffe [a]

CODE	N	Subset for alpha = .05		
		1	2	3
R/mPEG$_{2000}$DSPE Nebulizer	4	3.0500		
R/mPEG$_{5000}$DSPE Nebulizer	4	4.0525		
R/PLGA 503H Nebulizer	4		13.2775	
R/mPEG$_{2000}$DSPE Powder	4			34.2975
R/mPEG$_{5000}$DSPE Powder	4			34.3275
R/PLGA 503H Powder	4			61.5961
Sig.		.986	1.000	1.000

Means for groups in homogeneous subsets are displayed

a Uses Harmonic Mean Sample Size = 4.000

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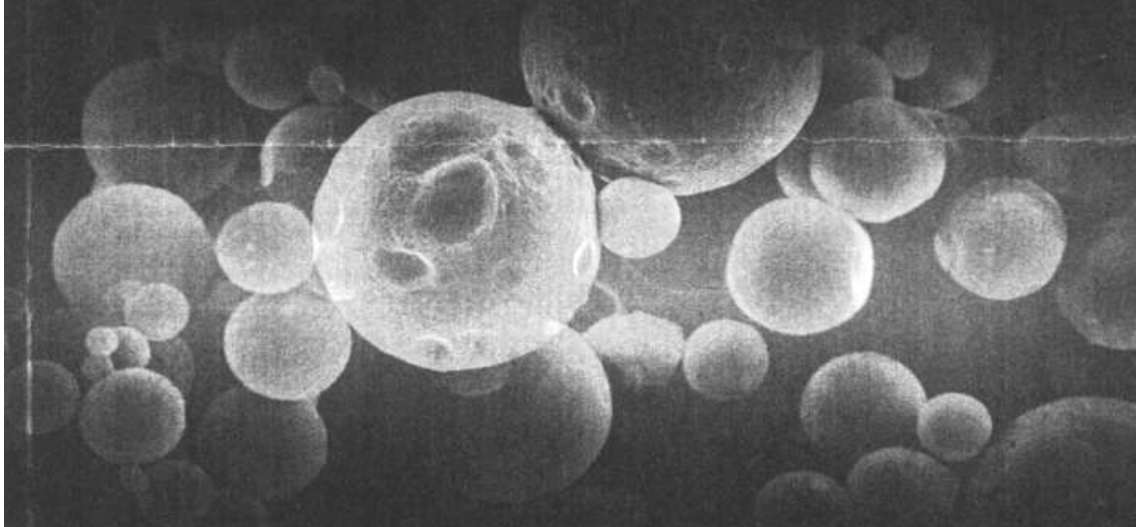

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FORMULATION AND CHARACTERIZATION OF RIFAMPICIN-LOADED POLY(ETHYLENE OXIDE)-BLOCK-DISTEAROYLPHOSPHATIDYLETHANOLAMINE (MPEG-DSPE) COPOLYMER NANOPARTICLES

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Rifampicin is an antibiotic that is mainly used for the treatment of tuberculosis (TB), leprosy, prophylaxis of meningococcal meningitis and haemophilus influenzae infection. Rifampicin is slightly soluble in water, thus its dissolution in biological liquids is particularly difficult. Targeting rifampicin to the alveolar macrophages in microparticle sizes in aerosol dosage form is deemed to be a recent approach to TB therapy.

The aim of this study is to formulate rifampicin-loaded poly-(ethylene oxide)-block-distearoyl phosphatidyl-ethanolamine (mPEG-DSPE) copolymer nanoparticles of controlled size range. The effects of copolymer type, drug-copolymer ratio and membrane filter porosity on particle size and physicochemical characterization are also examined. Rifampicin-loaded copolymer nano-particles were prepared by solvent evaporation method using different rifampicin to copolymer ratios. The formulations could be freeze-dried and stored in the lyophilized form. The physico-chemical characteristics of the drug- loaded nanoparticles were examined using FTIR and DSC.

Rifampicin showed the characteristic band at 1732 cm^{-1} of carbonyl ketone and 1649 cm^{-1} of carbonyl amide. These bands, especially the carbonyl amide band, were also evident in the rifampicin-loaded nanoparticles without major shift. DSC results showed that samples of mPEG-DSPE 2000 or physical mixture of mPEG-DSPE 2000 and rifampicin have the same peak at 56°C, but the peak for nanoparticles was shifted down to 53°C. Similarly, samples of mPEG-DSPE 5000 or physical mixture of mPEG-DSPE 5000 and rifampicin have the same peak at 60°C, but the peak for nanoparticles was shifted down to 53°C. Since rifampicin melts at about 185°C with decomposition, there was no peak in the DSC thermogram. However, when rifampicin was encapsulated in the micellar form, it resulted in the downward shift of the copolymer peak.

The drug recovery of rifampicin in mPEG-DSPE nanoparticles depended on both the drug:copolymer molar ratio and the amount of rifampicin used. This relationship can be expressed by a mathematical model and illustrated by response surface and contour plot. When analyzed using photon correlation spectroscopy, the particle sizes of drug-loaded mPEG-DSPE 2000 nanoparticle were in the range of 220-450nm, while those with mPEG-DSPE 5000 were in the range of 170-270nm, indicating the nanoparticle size reduced as the PEG chain of the copolymer increased.

ACKNOWLEDGEMENT

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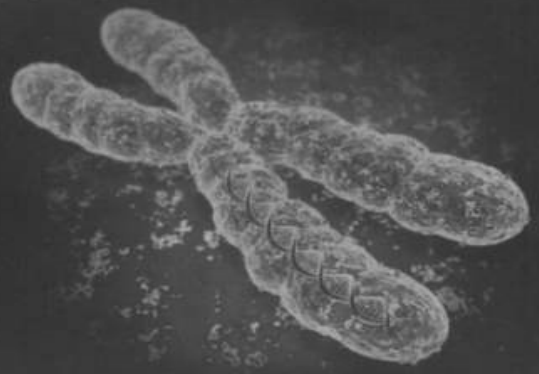

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Oral Presentation PT5

Entrapment Of Rifampicin In Polylactic-Co-Glycolide: Preparation And Characterization

JMA Abdulla, YTF Tan, Y Darwis
School of Pharmaceutical Sciences, Universiti Sains Malaysia, 11800 Penang, Malaysia

Polylactide-co-glycolide (PLGA) polymers have been extensively investigated for site-specific delivery of drugs. Targeting rifampicin to the lung in the rifampicin-PLGA microsphere forms may improve the efficacy and reduce the toxic side-effects of the drug. The objectives of this study were to prepare rifampicin-PLGA microspheres using PLGA RG502, RG503H and RG504 and to evaluate the physicochemical properties of these microspheres by DSC and FTIR. Microspheres were prepared by solvent evaporation technique and the amount of rifampicin entrapped was determined using UV spectrophotometry. The results showed that the percent yields of rifampicin-PLGA microspheres were in the range of 89 – 93%. The entrapment efficiency was directly correlated with the drug to polymer ratios used. The highest efficiency (46.8 %) was found at 1:5 ratio. DSC results showed that there was no difference in the endothermic transition temperatures of blank PLGA microspheres, physical mixture of rifampicin and blank PLGA microspheres, and rifampicin-PLGA microspheres. No peak was observed in the rifampicin DSC thermogram because the drug melts at about 185°C with decomposition. However, the exothermic transition peaks were observed at 159, 172 and 173°C in the rifampicin-PLGA RG502, RG503H and RG504 microspheres, respectively. The FTIR spectrum of rifampicin showed the characteristic bands at 3490 for OH, 1732 for carbonyl ketone, 1649 for carbonyl amide and 1573 for aromatic ring. These bands were also observed in the rifampicin-PLGA microspheres without a major shift. The sizes of all rifampicin-PLGA microspheres were smaller than 3μm. In conclusion, DSC and FTIR results showed the presence of rifampicin in PLGA microspheres and that the rifampicin-PLGA microspheres were small enough for lung delivery.

Oral Presentation PT6

The Significance And Correlation Of *In Vivo* Investigations And *In Vitro* Characterization Of Lipid-Based Matrices Incorporated With A Model Drug

YW Choy, AKK Nurzalina, KH Yuen
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Paracetamol was incorporated into the lipid base (G50/13) through the melt-fusion technique and filled into hard gelatin capsules. The freshly prepared and incrementally aged samples (week 1, 2, 3, 8, 10, 14 and 22) at 3 different temperatures (4°C, room temperature and 37°C) were subjected to characterization studies including thermal analysis by Differential Scanning Calorimetry (DSC) and *in vitro* release studies by dissolution test. *In vivo* studies were then carried out on healthy human volunteers on the samples that showed significant structural modifications and *in vitro* drug release alterations. Pharmacokinetic parameters, namely, maximum plasma concentrations (C_{max}), time to reach maximum plasma concentration (T_{max}) and total area under the plasma concentration-time curve ($AUC_{0-\infty}$), were estimated from the plasma concentration-time data. The thermal profiles showed that ageing the samples at all temperatures increased their crystallinity and polymorphic stability and is more apparent for the samples aged at 37°C. The storage at 37°C also increased the drug release profile and quickened the rate of change within the matrices that leads to such increases. Electron microscopy studies showed that structural modification at the macromolecular level for samples stored at 37°C contributed significantly to the increase in the drug release rate. In addition, after a certain period of storage, the release behaviour seemed to reach an equilibrium and further ageing did not alter the profile. Although significant structural modifications and drug release alterations were observed *in*

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AN *IN VITRO* STUDY OF THE RELEASE OF RIFAMPICIN FROM POLY (D,L-LACTIDE-CO-GLYCOLIDE) MICROSPHERES

J. M. A. Abdulla, Y. T. F. Tan & Y. Darwis

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Poly (D,L-lactide-co-glycolide) (PLGA), a biocompatible polymer, is deemed a potential carrier for delivering drugs to the lungs. In this study the release of rifampicin from PLGA 502 (MW 17,000), PLGA 503H (MW 36,000) and PLGA 504 (MW 48,000) microspheres were examined. In addition, the effects of the pH of dissolution media (3 and 7) and the ratio of drug to polymer (1:1, 1:2 and 1:5) were also investigated. Rifampicin release from microspheres was evaluated using a shaking water bath at 37 °C and the drug was quantified by UV spectrophotometer. The results showed that a decrease in MW of PLGA increased drug release, while an increase in the polymer to drug ratio, decreased drug release. In general, at both pH 3 and 7 there was an initial faster drug release in the first 12 hours, followed by a slower release in the subsequent 2 weeks. At pH 7, the drug release from PLGA 502, 503H and 504 were 26.9 %, 28.3 % and 18%, respectively. However, at higher ratios of 1:2 and 1:5, rifampicin release from PLGA 502, 503H and 504 decreased to (17.3 and 7.5 %), (19 and 14 %), and (4.3 and 2.6 %), respectively in the first 12 hours. Maximum release of 40 to 87 % from different ratios and molecular weights of PLGA microspheres were achieved in a period of 2 weeks. In the acidic pH, drug release from PLGA 504 microspheres for all ratios showed an initial release of 1 - 14 %, followed by a later release of 18 - 35 %.

Keywords: Rifampicin, poly (D,L-lactide-co-glycolide), PLGA

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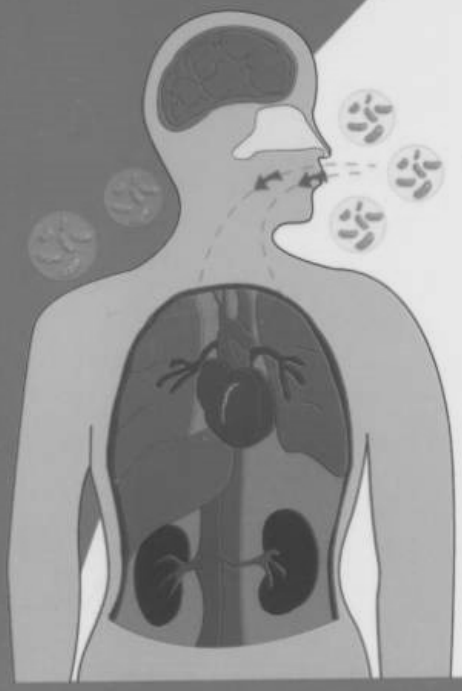

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SUSCEPTIBILITY OF *MYCOBACTERIUM TUBERCULOSIS* TO RIFAMPICIN LOADED POLY- (ETHYLENE OXIDE)- BLOCK-METHOXYDISTEAROYLPHOSPHATIDYLETHANOLAMINE

J. M. A. ABDULLA, H. H. HARIS, P. IBRAHIM, Y. T. F. TAN AND Y. DARWIS
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BACKGROUND

Rifampicin is the first line drug recommended by World Health Organisation (W.H.O) for the treatment of tuberculosis. However, relatively high doses of orally administered drug are often required to maintain therapeutic concentrations for longer periods, which leads to several side effects. Therefore, to overcome this problem, delivering rifampicin directly to the lungs using biodegradable polymer as drug carrier may improve therapeutic efficacy and reduce side effects of the drug.

OBJECTIVE

The main purpose of this study is to compare the minimum inhibitory concentration (MIC) of rifampicin and rifampicin loaded poly-(ethylene glycol)-block-methoxydistearoylphosphatidylethanolamine 5000 (mPEG-DSPE 5000) nanoparticles on a drug-resistant strain of *Mycobacterium tuberculosis* (clinical isolate, JB74).

MATERIALS & METHODS

Rifampicin loaded-mPEG-DSPE 5000 nanoparticles were prepared by solvent evaporation method. The nanoparticles were then freeze-dried and stored in the lyophilized formed until used. Susceptibility of *Mycobacterium tuberculosis* to rifampicin, mPEG-DSPE 5000 copolymer and rifampicin-loaded-mPEG-DSPE 5000 nanoparticles was examined using the 1% proportional method. The MIC of all samples was obtained after 2 weeks incubation of the cultured plates.

RESULTS

It was found that the MIC of rifampicin (200μg/ml) was eight times higher than rifampicin-loaded-mPEG-DSPE 5000 nanoparticles (25μg/ml). No inhibition of growth was observed for the isolate using mPEG-DSPE 5000 copolymer alone.

CONCLUSIONS

The rifampicin loaded-mPEG-DSPE 5000 nanoparticles have shown better antimycobacterial activity against drug-resistant *Mycobacterium tuberculosis* compared to rifampicin alone. This may indicate that the nanopaticles could be used to improve the efficacy of rifampicin in the treatment of tuberculosis.

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This is to certify that

Juma Masoud Abdulla Abdulla

has delivered a presentation on

"Formulation and Evaluation of Rifampicin Loaded – Polymeric Particles for Pulmonary Delivery"

at the
School of Pharmaceutical Sciences,
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CHAPTER 1
GENERAL INTRODUCTION

1.1 Tuberculosis

Tuberculosis (TB) is a chronic communicable disease caused by the bacterium (*Mycobacterium tuberculosis*) and usually occurs in the lungs (the initial site of infection), but it also can occur in other organs. The complex nature of this pathogen and its ability to evade the immune system has prevented the development of an effective vaccine. TB is a highly contagious, persistent disease characterized by the formation of hard greyish nodules, or tubercles (Pandey *et al.,* 2003).

The World Health Organization (WHO) on 23 April 1993 declared tuberculosis as global public health emergency (Brennan, 1997; Makino *et al*, 2004). The disease infects over 1.8 billion people worldwide and it is responsible for 1.5 million deaths annually (Pandey *et al*., 2003). Frieden *et al*. (2003) also affirmed *Mycobacterium tuberculosis* as being a leading cause of infectious mortality after HIV AIDS worldwide. Frieden *et al*. (2003) noted that there were an estimated of 8–9 million new cases of tuberculosis in 2000, 3–4 million cases were sputum-smear positive. Most cases (5–6 million) were in people aged 15–49 years. Duncan and Barry (2004) said that according to a recent report compiled by the World Health Organization (WHO), the total number of new cases of tuberculosis (TB) worldwide in 2002 had risen to approximately 9 million. This is despite the success of widespread of the 'DOTS' (directly observed therapy, short-course) strategy, now covering 180 countries and accessible by over 70% of the world's population.

Despite the availability of effective therapeutic regimens for the treatment of TB, treatment failure and emergence of drug resistant are still problematic. This treatment failure is related in part to patient non-compliance (due to frequent administration of anti-TB drugs). Patient-compliance can be improved by the use of sustained release antitubercular drugs formulations, which reduce the dosing frequency of the drugs. Such system can be designed to target specific regions of the lung, and therefore allow controlled drug delivery to lung, or to the systemic circulation via the lung (Fu *et al*., 2002; Prabakaran *et al*., 2004).

1.2 Drug Therapy in Pulmonary Tuberculosis

The goals of drug therapy are to ensure cure without relapse, to prevent death, to stop transmission and to prevent the emergence of multi-drug resistance tuberculosis (Frieden *et al*, 2003). Directly Observed Treatment, Short-course (DOTS) therapy, which lasts for 6 or 8 months, given under direct observation, is one of the most important components of WHO strategy against tuberculosis.

Tuberculosis is treated in two phases. The initial phase for 2 months involves concurrent use of at least 3 drugs to reduce the bacterial population rapidly and prevent drug resistant bacteria emerging. The second continuation phase for 4-6 months involves fewer drugs and is used to eliminate any remaining bacteria and prevent recurrence. Direct observation of therapy is considered essential to ensure compliance during treatment of tuberculosis. Five drugs are considered essential first line for treatment of tuberculosis (Academy of Medicine of Malaysia 2nd edition. 2002). These are isoniazid (H), rifampicin (R), pyrazinamide (Z), streptomycin (S) (which are bactericidal) and ethambutol (E)

(which is bacteriostatic) are used in various combinations as part of WHO recommended treatment regimens. Isoniazid, rifampicin and pyrazinamide are components of all antituberculosis drug regimens currently recommended by WHO. In supervised regimens change of drug regimen should be considered only if the patients fail to respond after 5 months of DOTS.

Patients who cannot comply reliably with the treatment regimen drug administration needs to be fully supervised (directly observed therapy, DOTS) The patients are given daily doses of SHRZ or EHRZ or HRZ under supervision i.e directly observed by health personnel or trained person for the first 2 months followed by HR or SHR or HR, 2 –3 times a week for a further of 4 months (Academy of Medicine of Malaysia 2nd edition. 2002). Frieden et al., (2003) reported that the DOTS method could ensure high rates of treatment completion, reduce development of acquired drug resistance, and prevent relapse.

Second line drugs in TB therapy are reserved for use only if the bacteria are resistant to the first line agents or if the patient experiences toxic side effects to them. The 2nd line drugs are much less active and have a much higher toxicity. Examples of second line drugs are ofloxacin/Ciprofloxacin, ethionamide, aminosalicylate, cycloserine, amikacin/Kanamycin and capreomycin (Pandey *et al*., 2003).

1.3 Respiratory System and Lung Anatomy

The respiratory system consists of the conducting airway and respiratory regions (Figure 1.1). The conducting airway essentially consists of nasal cavity, nasopharynx, bronchi and bronchioles. Airways distal to the bronchioles constitute the respiratory region, which include the respiratory bronchioles, the alveolar ducts and the alveolar sacs. The latter structures (the alveoli), which are the important parts in this study, are composed almost exclusively of a nonciliated epithelial membrane. The alveolar walls contain a dense network of capillaries and connective tissue fibers (Suarez and Hickey, 2000).

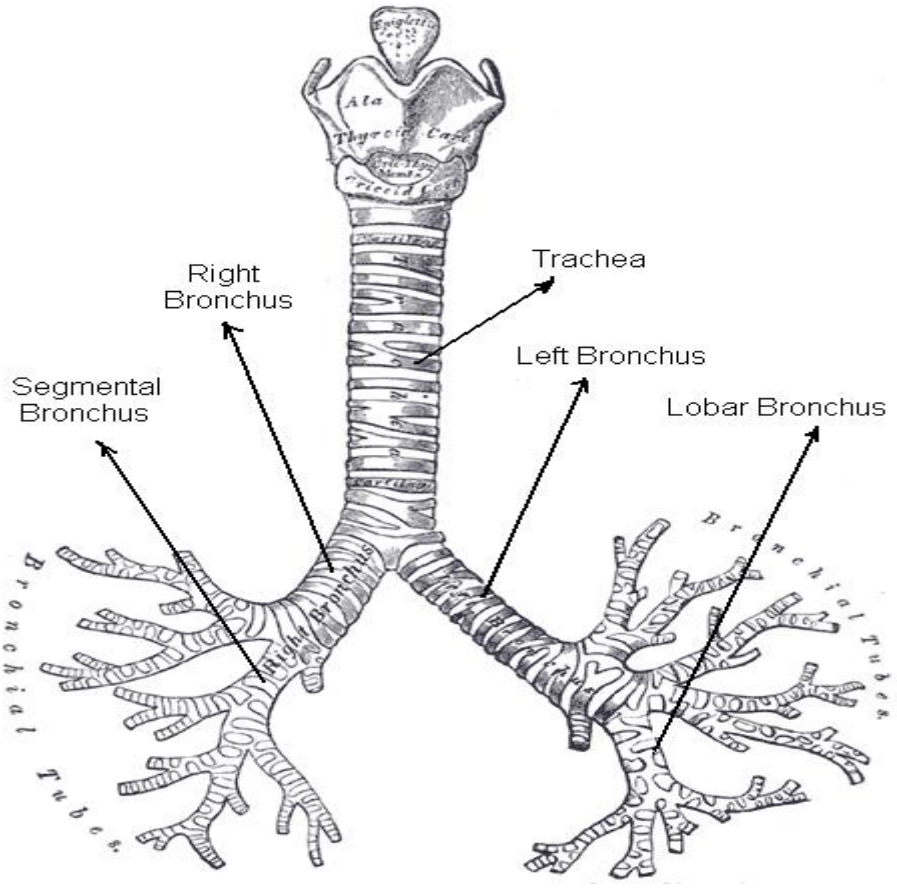

Figure1.1: Front view of cartilages of larynx, trachea, and bronchial tree (Gray, 2001)

The lungs have in fact been demonstrated an efficient port of entry to the bloodstream due to: (i) the tremendous surface area of the alveoli (100 m^2), immediately accessible to drug; (ii) a relatively low metabolic activity locally, as well as a lack of first-pass hepatic metabolism; and (iii) the elevated blood flow (5 l/min) which rapidly distributes molecules throughout the body (Fehrenbach, 2001).

The lungs have two separate circulations. The bronchial circulation, which involves small systemic arteries from the aorta supplies oxygen for the relatively high metabolic needs for lungs. The pulmonary circulation, which serves respiratory function, begins in the pulmonary artery; bring venous blood from the right atrium. The pulmonary arteries subdivide extensively and finally terminate in a dense capillary network around the alveoli. Venous blood returns to the left atrium via veins, which coalesce and eventually form the pulmonary venous system. The venous blood from the bronchial circulation returns to the system circulation via the azygous and pulmonary veins (Gray, 2001).

1.4 Pulmonary Drug Delivery Systems

Growing attention has been given to the potential of a pulmonary route as an non-invasive administration for systemic delivery of therapeutic agents due to the fact that the lungs could provide a large absorptive surface area (up to 100 m^2) with extremely thin (0.1 μm – 0.2 μm) absorptive mucosal membrane and good blood supply. Controlled release polymeric systems are approaches that help for improving the duration and effectiveness of inhaled drugs (Fu *et al*., 2002).

Targeting delivery of drugs to the diseased lesions is one of the most important aspects of drug delivery systems. The systems should have novel properties such as increase efficiency of drug delivery, improve release profiles and drug targeting to the diseased site. Among the different dosage forms reported, nanoparticles and microparticles sized polymeric systems occupy unique position in drug delivery technology (Majeti and Kumar, 2000).

The advantages of sustained drug delivery to the respiratory tract are numerous, and include extended duration of action, reduction in drug use, improved management of therapy, improved compliance, reduction in side effects and together with potential cost savings that exist for sustained release therapy (Cook *et al*., 2005).

Malo *et al*. (1989) showed that four times daily treatment of asthma with a corticosteroid resulted in less nocturnal cough attacks and relapses when compared to a twice daily schedule, with no change in the side effect profile. However, excessive dosing frequency is a well-documented cause of non-compliance in patients. In another study Mann *et al*. (1992) reported that, inhaler under-usage was greater with four times daily versus twice daily treatment (57.1% versus 20.2%). Even in twice daily dosing, just 40% of patients complied with the given protocol, despite extensive education at the study onset. An inhaled sustained release formulation, administered once daily, would therefore provide benefit to non-compliant patient groups owing to the convenience of reduced dosing frequency (Cook *et al*., 2005).

Deol and Khuller. (1997) encapsulated antitubercular drugs (ATD) in liposomes. Sustained release of such drugs in the lung would be particularly beneficial since they could be delivered to and retained at the targeted receptors for a prolonged period of time and thus minimize the biodistribution throughout the systemic circulation (Zeng *et al.*, 1995). This strategy helps to improve patient compliance in terms of reducing the dosage frequency, and can contribute in minimizing the risk of emergence of drug-resistance and potential toxicity (Makino *et al*., 2004).

1.5 Advantage of Pulmonary Delivery

The pulmonary delivery route has attracted much attention, as well as nasal, rectal, injections and oral routes, to improve the quality of life of patients, because no dose repeated are required. Further, this route is desirable for delivering drugs because of the following advantages over other routes.

(1) The surface area of a lung is extremely large (approximately 100 m^2) and the mucosal permeation of drug substances is comparatively easy, because the vascular system is well developed and the wall of the alveolus is extremely thin (Yamamoto *et al.*, 2005).

(2) The activity of drug-metabolizing enzymes with intracellular or extracellular is relatively low, it avoids hepatic first-pass metabolism (Suarez and Hickey, 2000).

(3) A very rapid onset of action with very small dose. An oral dose of bronchodilator may take 2–3 h to be fully effective while an inhaled dose usually takes a minimum of 15–30 min (Zeng *et al*., 1995).

(4) Reduces exposure of drug to the systemic circulation and potentially minimizes adverse effects and lower dosage regimens may provide considerable cost saving especially with expensive therapeutic agents (Joshi and Misra, 2001).

1.6 Pulmonary Delivery Devices

Local delivery of medication to the lung is highly desirable, especially in patients with specific pulmonary diseases like cystic fibrosis, asthma, chronic pulmonary infections, or lung cancer. Aerosols are an effective method to deliver therapeutic agents to the respiratory tract. Metered dose inhalers (MDIs), dry powder inhalers (DPIs) or nebulizers are commonly used for this purpose (Finlay, 2001).

There are numerous commercially available devices, and their design is an important factor governing aerosol size and fluid output. Although pressurised metered dose inhalers are the most commonly used inhalation drug delivery system, other delivery systems, such as dry powder inhalers and nebulizers, are widely used as propellant-free alternatives to MDIs (McCallion *et al*., 1996a). Gupta and Hickey, (1991) reported that nebulizer inhalers compared to MDIs or DPIs, generate smaller particles, which are better penetration to the distal region of the lungs and, thus, are more suitable for systemic delivery.

1.6.1 Metered Dose Inhalers (MDIs)

The metered dose inhalers (MDIs) were the first apparatus, which is both reliable and practical (Timsina *et al*., 1994). The fundamental components of

MDIs are an actuator, a metering valve, and a pressurized container that holds the micronized drug suspension or solution, propellant, and surfactant. The high vapor pressure propellant supplies the energy for dispersion in these delivery systems (Suarez and Hickey, 2000). Chlorofluorocarbon (CFC) as a propellant for MDIs has been widely used for pulmonary drug delivery devices (Yamamoto *et al*., 1999).

Chlorofluorocarbons based metered-dose therapeutic aerosols are in the process of being reformulated with more environmentally friendly propellants, such as hydrofluoroalkanes (HFAs). CFCs were reported to destroy ozone layer in the stratosphere and allow excessive ultraviolet radiation to reach the earth's atmosphere (Tashkin, 1999). HFAs were investigated as possible substitutes for CFCs because they shared similar desirable characteristics but non-ozone depleting. Despite the similarities with the CFCs, many additional difficulties were observed. HFAs were demonstrated to have toxic effects, modified the solubilities of drug and incompatibility with MDI components such as valves and container walls (Crowder *et al*., 2001).

The main disadvantages of MDI, especially in young children and elderly who have difficulty to administer the drug alone since it require patient’s hand and breathe coordination. Another disadvantage is release the aerosol at high velocity. This ballistic effect causes deposition of approximately 65% of the medication in the upper respiratory tract (mouth, oropharynx and larynx). It became also known that only a small fraction (10-20%) of the emitted dose reaches the lower airways. The remainder deposits in the extrathoracic and

upper airways, are swallowed and subsequently absorbed in the gastrointestinal tract. The low temperature of the CFCs or HFAs discharged from a pMDI frequently also causes children to abruptly stop inhaling. All the disadvantages lead to a suboptimal delivery of drugs to the airways and thereby reduced therapeutic efficacy (Biddiscombe *et al*., 1993).

1.6.2 Dry Powder Inhalers

Dry powder inhalers (DPIs) can be divided into two classes: passive and active.

1. Passive devices depend on the inhalation ability of patient's to provide the energy needed for dispersion.
2. Active powder-dispersion devices, similar to propellant-driven metered-dose inhalers, which use an external energy source to help the patient to accomplish some part of the aerosol dispersion (Crowder, 2004).

DPIs are the most recent developed devices in respiratory therapy. The majority of these devices are breath-activated inhalers that rely on the patient's inspiratory flow to deaggregate and deliver the drug for inhalation, thereby eliminating the requirement of inhalation coordination inherent in pMDI use. However, with DPIs there is the need to generate at least moderate inspiratory flow in order to accomplish effective drug delivery. The drug in a DPI is in the form of a finely milled powder in large aggregates, either alone or in combination with some carrier substance (Byron *et al*., 1990).

Most of the particles are initially too large to be carried into the lower airways, but the turbulent air stream created in the inhaler during inhalation causes the

aggregates to break up into primary particles sufficiently small to be carried into the lower airways. Therefore, the deposition pattern of the particles depends on the inspiratory flow generated by the patient. A very low inspiratory flow is likely to move the dose from the inhaler into the patient's mouth, with very low deposition in the pulmonary air-ways. Shear, turbulence, and mechanical intervention may be used to aid in the dispersion of aerosols from dry powders (Suarez and Hickey, 2000).

Dry powder generation is often hindered by aggregation of the small particles (Brown, 1987), which is in turn exacerbated by the hygroscopic nature of the drug and its electrostatic charge. The reduction of powder hygroscopic and electrostatic charge may enhance the future prospects of aerosol powder formulation (Ferron, 1977).

1.6.3 Nebulizers

Nebulizers use ultrasound or compressed gas to produce aerosol droplets in the respirable size range from liquids, usually aqueous solutions of drugs. They are widely used therapeutically to deliver corticosteroids, antiallergics, anticholinergics, antibiotics, mucolytics and other agents to the respiratory tract (British National Formulary, 1994). Further, the nebulizers are adaptable to very fine suspensions as well as aqueous solution (Yamamoto *et al*., 1999).

Nebulizers have the advantage over MDIs and DPIs that the drug may be inhaled during normal breathing through a mouth-piece or facemask. Thus, they can be employed to deliver aerosolized drug to patients, such as children, the

elderly and patients with arthritis, who experience difficulties using other devices. Nebulizers can also deliver relatively large volumes of drug solutions and suspensions. They are frequently used for drugs that can not be conveniently formulated into an MDI or DPI or where the therapeutic dose is too large for delivery with the alternative systems (McCallion *et al*., 1996a).

1.7 Preparation Techniques for Pulmonary Drug Delivery System

Different drug carriers/delivery systems have been used for controlled drug delivery. In the last two decades, synthetic biodegradable polymers have been increasingly used as carrier to deliver drugs, because they are free from most of the problems associated with the natural polymers. Poly (amides), poly (amino acids), poly (alkyl-α-cyano acrylates), poly (esters), poly (orthoesters), poly (urethanes), poly (acrylamides) and ligands of carbonyl-methoxypolyethyleneglycol (mPEG) and distearoylphosphatidylethanolamine (DSPE) have been used to prepare various drug-loaded devices to improve therapy. Amongst them, the thermoplastic aliphatic poly (esters) such as PLA, PGA, especially PLGA and niosomes (Non-ionic surfactant vesicles), as well as mPEG and DSPE based polymeric micelles have generated so much interest due to their excellent biocompatibility and biodegradability. However, recent approaches to improve patient compliance have involved instituting intermittent drug delivery regimens with the use of polymers by cleaving conventional antitubercular drugs to various types of carrier systems (Dutt and Khuller, 2001; Zhang *et al*., 2003).

1.7.1 Microspheres

Microspheres are defined as homogenous monolithic spherical colloidal particles made of single or multiple type of polymers, typically with a particle size in the range of 1-200 μm, ideally <125 μm (Jain, 2000). Microspheres in strict sense are monolithic. However, the terms microcapsules and microspheres are often used synonymously. In addition, some related terms are used as well, for example, “micro beads” and “beads”. The term sphere and spherical particles are also used for a large size and rigid morphology (Majeti and Kumar, 2000). Microspheres have been used widely as drug carriers for controlled drug release (Hincal and Calis, 1999). Polymers, which have been extensively investigated for drug carriers, are (lactic acid) (PLA), poly (glycolic acid) (PGA) poly (lactic-co-glycolic acid) (PLGA). These polymers have excellent biocompatibility, mechanical strength, ease of fabrication, prolonged in vivo degradation kinetics, and changeable biodegradability properties (Pandey *et al*., 2003 and Zheng *et al*., 2004). The polymers have been fabricated into a variety of devices, such as microspheres, micelles, liposomes, nanospheres, film, implants, and pellets. Furthermore, their application in humans has been approved by food and drug administration (FDA). However, the disadvantages of this types of polymeric system particularly that of PLGA are low entrapment efficiency, burst release, instability of entrapped hydrophilic protein, and its incomplete release (Zheng *et al*., 2004).

1.7.2 Microparticle Preparation

The preparations of lung based drug delivery system have involved several processes. Hincal and Calis’s. (1999) reported that a wide range of

microencapsulation techniques. The selection of the technique depends on the nature of the polymer, the drug, the intended use and the duration of therapy (O'Donnell and McGinity, 1997). In preparing controlled release microspheres for efficient entrapment of the active substance, the choice of the method is importance. The microencapsulation methods for hydrophobic biodegradable polymers such as poly (lactide-co-glycolide) and poly (lactic acid) as matrix materials are:

a) Emulsion-Solvent Evaporation and Solvent Extraction.
b) Phase Separation (Coacervation).
c) Interfacial Polymerisation
d) Spray Drying.

1.7.2 (a) Solvent Evaporation and Extraction Process

The solvent evaporation method is widely used to produce microspheres. There are two systems from which to choose, oil in water (O/W) or water in oil (W/O) and (W/O/W). The choice of a particular method is usually determined by the solubility characteristics of the drug.

i. Single Emulsion Process

The method is ideal for water-insoluble drugs in which polymer are first dissolved in volatile organic solvent. The drug is then added to the polymer solution to produce a solution or dispersion of the drug particles. This polymer–solvent–drug solution/dispersion is then emulsified (with appropriate stirring and temperature conditions) in a larger volume of water in presence of an emulsifier to yield an o/w emulsion. The emulsion is then subjected to solvent removal by either evaporation or extraction process to harden the oil droplets. The solid microspheres obtained are then washed and collected by filtration, sieving, or

centrifugation. The microspheres are then dried under appropriate conditions or lyophilised to give the final free flowing microsphere product (Bodmeier and McGinity, 1988; Torres *et al*., 1996; Jain, 2000).

It should be noted that the solvent evaporation process in a way is similar to the extraction method, in the sense that the solvent must first diffuse out into the external aqueous dispersion medium before it could be removed from the system by evaporation (Arshady, 1991; Wu, 1995).

In order to increase the encapsulation of the water-soluble drugs, an oil-in-oil (O/O) emulsification method was developed (Arshady, 1991 and Ramírez *et al*., 1999). A water-miscible organic solvent is employed to solubilise the drug in which polymers are also soluble. This solution is then dispersed into oil such as light mineral oil in presence of an oil soluble surfactant like Span to yield the (O/O) emulsion. Microspheres are finally obtained by evaporation or extraction of the organic solvent from the dispersed oil droplets and the oil is washed off by solvents like n-hexane. This process is also sometimes referred as water-in-oil (W/O) emulsification method (Jalil and Nixon, 1990a).

ii. Double / Multiple Emulsion Process

The process is best suited to encapsulate water-soluble drugs like peptides, proteins, and vaccines, unlike the o/w method which is ideal for water-insoluble drugs. The method is that a buffered or plain aqueous solution of the drug (sometimes containing a viscosity building and/or stabilizing protein like gelatin) is added to an organic phase consisting of polymer solution in organic solvent

with vigorous stirring to form the first w/o emulsion. This emulsion is added gently with stirring into large volume water containing an emulsifier like PVA to form the w/o/w emulsion. The emulsion is then subjected to solvent removal by either evaporation or extraction process. The solid microspheres obtained are then washed and collected by filtration, sieving, or centrifugation. The microspheres are then dried under appropriate conditions or lyophilized to give the final free flowing microsphere product (Jain, 2000).

1.7.2 (b) Phase Separation (Coacervation)

Coacervation is a process in which a homogeneous solution of macromolecules undergoes liquid-liquid phase separation, giving rise to a polymer rich dense phase. Coacervation has been classified into simple and complex processes depending on the number of participating macromolecules. In simple polyelectrolyte coacervation, addition of salt or alcohol normally promotes coacervation. In complex coacervation, two oppositely charged macromolecules (or a polyelectrolyte and an oppositely charged colloid) could undergo coacervation through associative interactions (Mohanty *et al*., 2004).

The process consists of decreasing the solubility of the encapsulating polymer by addition of a third component to the polymer solution in an organic solution (Jalil and Nixon, 1990a). At a particular point, the process yields two liquid phases (phase separation): the polymer containing coacervate phase and the supernatant phase depleted in polymer. The drug which is dispersed/dissolved in the polymer solution is coated by the coacervate. Thus, the coacervation process includes the following three steps: (i) phase separation of the coating

polymer solution, (ii) adsorption of the coacervate around the drug particles, and (iii) solidification of the microspheres (Jain, 2000).

The main disadvantages of this method are tendency to produce agglomerated particles, problem in mass production, requires large quantities of organic solvent, and difficult to remove residual solvents from the final microsphere product (Takada *et al*., 1995).

1.7.2 (c) Interfacial Polymerization

The method involves the condensation of two monomers at the interface of the organic and aqueous phases. Polyamide capsules are a good example of this system (Conti *et al.,* 1992). The surface polymerization of the monomer surfactants is the advanced method of this technique for preparation of nanocapsules (Shapiro and Pykhteeva, 1998).

1.7.2 (d) Spray Drying

The spray drying technique appears to be attractive for the preparation of microparticles (Baras *et al*., 2000). It can be used for the microencapsulation of antigens. The technique consists of spraying an emulsion of polymer and drug through the nozzle of a spray dryer apparatus; the solvent evaporates very quickly, leaving solid microparticles (Pavanetto *et al*., 1992). The spray drying process involves the following four sequential stages: atomization of the product into a spray nozzle, spray air contact, drying of the sprayed droplets and collection of the solid product obtained. Due to the rapid evaporation of the solvent, the temperature of the droplets can be kept below the drying air

temperature, and for this reason spray-drying can be applied to heat-sensitive materials (Broadhead *et al*., 1992).

The main advantages of the spray drying technique are applicable to both heat resistant and heat sensitive drugs, as well as water-soluble and water-insoluble drugs (Jain, 2000 and Mu *et al*., 2005). However, the method is associated with some drawback that included a significant loss of the product during spray-drying, due to adhesion of the microparticles to the inside wall of the spray-drier apparatus, and agglomeration of the microparticles (Takada *et al*., 1995). Another limitation of spray drying is its unsuitability for substances sensitive to mechanical shear of atomization (Maa and Prestrelski, 2000) and amorphous materials which are hygroscopic, more cohesive and difficult to flow and disperse (Hak and Nora, 2003)**.**

1.7.3 Poly (Lactic-Co-Glycolic Acid) (PLGA)

Poly(lactide-co-glycolide) PLGA is a highly biocompatible and biodegradable synthetic polymer, which is hydrolytically degraded into non-toxic oligomer and finally to lactic acid and glycolic acid (Ito and Makino, 2004). In general, poly lactic-co-glycolic acid (PLGA), poly lactic acid (PLA) and poly glycolic acid (PGA) are block copolymers of lactic and/or glycolic acid (Figure 1.2), with the monomers linked by ester bands. The final hydrolytic products are monomers glycolic and lactic acid. Both monomers enter the tricarboxylic acid cycle and can be eliminated from the body as carbon dioxide and water (Jain, 2000).

Chemically, lactic acid, which is a composite of PLGA, contains one more side methyl group and is more hydrophobic than glycolic acid. Therefore, the higher content of lactide, the more hydrophobic is the polymer, the lower water uptake and the slower the degradation rate. In addition, lactic acid in the polymer can either be in its optically active form (L) or as a racemate (D, L), which affects the crystallinity of the polymer. Besides hydrophobicity and crystallinity, MW and poyldispersity are also important molecular properties affecting polymer performance. Several other important bulk properties, like glass transition temperature, melting point, and solubility in organic solvents, water uptake rate and biodegradation rate are closely related to the molecular properties of PLGA polymers (Jain, 2000).

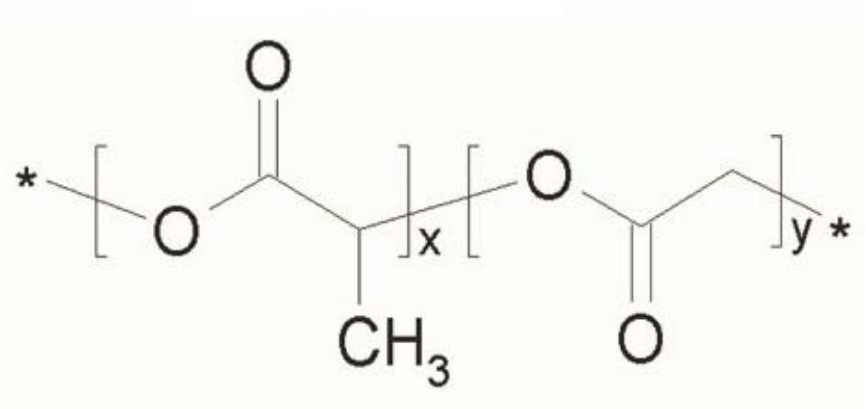

Figure 1.2: Chemical structure of poly lactic-co-glycolic acid (PLGA)

1.7.4 PLGA Microparticles for Lung Delivery

Most previous studies of polymeric pulmonary drug delivery have utilized PLGA since it is readily available and has a long history of safety in humans (Fu *et al*., 2002).

Masinde and Hickey. (1993) prepare poly (lactic acid) (PLA) microspheres with particle sizes between 1 and 11 μm by a solvent evaporation technique. The microspheres were suspended in a non-surfactant solution and subsequently atomized using a jet nebulizer. The particles generated were suitable for drug delivery to the lower airways, having a median diameter of 2 μm and geometric standard deviation of 2.4 μm. Zeng *et al*. (1995) studied tetrandrine antisilicotic alkaloid entrapped in albumin microspheres for delivery to the alveolar region. They observed tetrandrine metabolized in alveolar and incorporate into alveolar macrophages.

Lai *et al*. (1993) reported prolonged protection against bronchoconstriction challenge in rats at least 12 h post-administration with PLGA/isoproterenol microspheres. Edwards *et al*. (1997) studied sustained release of insulin in rats with large porous particles fabricated from PLGA, and showed reduced macrophage uptake and immune response to the larger particles relative to non-porous controls. El-Baseir and Kellaway. (1998) studied the *in vitro* sustained release of beclomethasone diproprionate and nedocromil sodium entrapped in PLA microparticles for 8 and 6 days respectively. However, pulmonary administration of PLA microspheres to rabbits was associated with inflammation at sites adjacent to microparticle deposition, raised neutrophil count and incidence of haemorrhage (Armstrong *et al*., 1996).

PLGA has many limitations as a carrier for drugs in the lungs. First, small amount of PLGA microspheres degrade over the period of weeks to months, but typically deliver drugs are released for a shorter period of time. Such a pattern

would lead to an unwanted build-up of polymer in the lungs upon repeat administration (Cook *et al*., 2005). Second, bulk degradation of PLGA microspheres creates an acidic core, which can damage pH sensitive drugs such as peptides and proteins. Surface eroding polymers, such as polyanhydrides, lessen the effect of acidic build-up by increased diffusion rates of soluble fragments away from the particle. Third, PLGA microspheres have hydrophobic surfaces, which result in sub-optimal particle flight into the deep lung (due to particle agglomeration by van der Waals forces) (Fu *et al*., 2002). Additionally, hydrophobic surfaces lead to rapid opsonization (protein adsorption), resulting in a rapid clearance by alveolar phagocytic cells (Cook *et al*., 2005).

1.7.5 Polymeric Nanoparticles

Nanoparticles are colloidal particles ranging in size from 10 to 1000 nm, and they are extensively employed for targeted drug delivery systems. Nanoparticles have several advantages over conventional drug carriers; small particle size, ease of administration, drug targeting to the specific body site, solubilization of hydrophobic drug, avoid the reticuloendothelical system (RES), and reduced side effects of anticancer drugs (Lee *et al*., 2003).

Various drug delivery and drug targeting systems are currently developed or under development. Among drug carriers are soluble polymers, insoluble or biodegradable natural and synthetic polymers, microcapsules, nanocapsules, cells, cell ghosts, lipoproteins, liposomes, and micelles. Each of those carrier types offers its own advantages and has its own shortcomings, so the choice of

a certain carrier for each given case can be made only taking into account the whole bunch of relevant considerations (Torchilin, 2001).

Among the various drug delivery systems considered for pulmonary application, biodegradable polymeric nanoparticles demonstrate several potential advantages. In comparison to liposomal formulations, polymeric nanoparticles may exhibit a greater stability in the face of extreme forces generated during the nebulization process, thus eliminating the possibility of drug leakage. A further advantage of nanoparticle formulations is the fact that particles with a diameter of <1 μm are more easily incorporated in the `respirable percentage' of aerosolized droplets (droplets exhibiting a mass median aerodynamic diameter (MMAD) of 1–5μ μm) (Lea *et al*., 2003).

Drug targeting systems like liposomes (Codde *et al*., 1993) or prodrugs (O'Hare *et al*., 1989) have been limited with some disadvantages such as instability of carriers in the body fluid, rapid elimination by undesirable organs, difficulties in modifying macromolecular carriers, possibility of drug inactivation during chemical attachment, liberation rate of drug from the macromolecular-drug conjugates and biodegradation. Drug carrier systems of core-shell type nanoparticles reported by Peracchia *et al*. (1997), or polymeric micelles reported by Yokoyama *et al*. (1990), were attempted to solve the problems mentioned above. Nanoparticles based on core-shell structure or polymeric micelles have many advantages such as long circulation in the body, better drug solubility, drug stability and high drug encapsulation. However, polymeric micelles or core-shell type nanoparticles are found to have limited application

for specific drug targeting due to the drug may be freely diffused throughout the body (Jeong *et al*., 2005).

Recently, block copolymers or polymeric conjugates were synthesized to make core-shell type nanoparticles and polymeric micelle. Polymeric micelles represent a separate class of micelles and are formed from polymers consisting of both hydrophilic and hydrophobic monomer units and they are more stable compared to micelles (Torchilin, 2001: Torchilin, 2002). Polymeric micelles have a hydrophobic core and a hydrophilic outer shell, in which hydrophobic segments form the inner-core of the structure, acts as a drug incorporation site, especially for hydrophobic drugs (Jeong *et al*., 1998). At present, polymeric micelles seem to be one of the most advantageous carriers for the delivery of water-insoluble drugs (Deol and Khuller, 1997; Jones and Leroux, 1999).

Use of lipid moieties as hydrophobic blocks capping PEG chains can provide additional advantages for particle stability when compared with conventional amphiphilic polymeric micelles due to the existence of two fatty acid acyls which might contribute considerably to an increase in the hydrophobic interactions between the polymeric chains in the micelle's core (Torchilin, 2002).

Diacyllipid–PEG conjugates micelles have been introduced into the area of controlled drug delivery as polymeric surface modifiers for liposomes (Klibanov *et al*., 1990). Interestingly, diacyllipid–PEG molecule itself represents a characteristic amphiphilic polymer with a bulky hydrophilic (PEG) portion and short but extremely hydrophobic diacyllipid part. The diacyllipid–PEG

conjugates were found to form micelles of different sizes in an aqueous environment (Lasic *et al*., 1991). PEG–PE micelles can efficiently incorporate sparingly soluble drugs (Weissig *et al*., 1998a). It seems that the use of PEG-diacyllipid conjugates, which represent micelle-forming amphiphilic polymers with larger hydrophilic blocks and more lipophilic hydrophobic blocks, might result in colloidal particles, which are more stable under physiologic conditions (Torchilin, 1999).

1.7.6 PEG-PE Nanoparticles Preparation

There are two principal methods for the preparation of polymeric micelles, the direct dissolution method and the dialysis method. In each particular case, the choice of the method is usually determined by the extent of the solubility of a micelle-forming in an aqueous medium. If the polymer is marginally soluble in water, the direct dissolution method is employed, whereas if the polymer is poorly soluble in water, the dialysis method is usually employed (Allen *et al*., 1999).

In direct dissolution method, a polymer is dissolved in an aqueous medium at normal or elevated temperature and at a concentration well above its CMC value. Usually, in direct dissolution method the copolymer produce micelles spontaneously in aqueous solution, but in some cases the copolymer and water are mixed at elevated temperatures to ensure micellization (Allen *et al*., 1999; Torchilin, 2001). This method is frequently applied for micelle preparation from block co-polymers possessing a certain degree of solubility in water (Torchilin, 2001).