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APPENDICES

APPENDIX A

Preparation and characterization of Al(OH)₃ and chitosan conjugated PLGA microparticles as nasal vaccine carriers

Particle size and particle size distribution of PLGA particles(Figure 3.2-3.3)

Table 6.1 Particle size and particle size distribution of PLGA particles

Formulation code	Input force ¹	w/o ratio ²	(w/o)/w ratio ³	%PVA ⁴ (w/v)	Median size (µm), uniformity
P1	Probe sonication	1:10	1:2	4	0.92, 0.27
P2				2	1.55, 0.48
P3				1	2.31, 1.02
P4			1:4	4	0.68, 0.51
P5				2	1.25, 0.30
P6				1	1.96, 0.36
P7		1:5	1:2	4	3.00, 0.70
P8				2	3.54, 1.03
P9				1	4.53, 1.29
P10		1:4	4	2.32, 0.67	
P11			2	3.37, 0.99	
P12			1	4.15, 0.94	
P13		1:2.5	1:2	4	5.82, 1.69
P14				2	7.07, 1.61
P15				1	7.88, 1.47
P16		1:4	4	5.26, 1.57	
P17			2	6.39, 5.46	
P18			1	7.44, 5.14	
P19	Bath sonication	1:10	1:2	4	10.20, 2.21
P20				2	14.60, 3.41
P21				1	20.11, 1.21

¹ Input force of secondary emulsion

² Volume ratio of primary w/o emulsion

³ Volume ratio of secondary (w/o)/w emulsion

⁴ %PVA of secondary emulsion

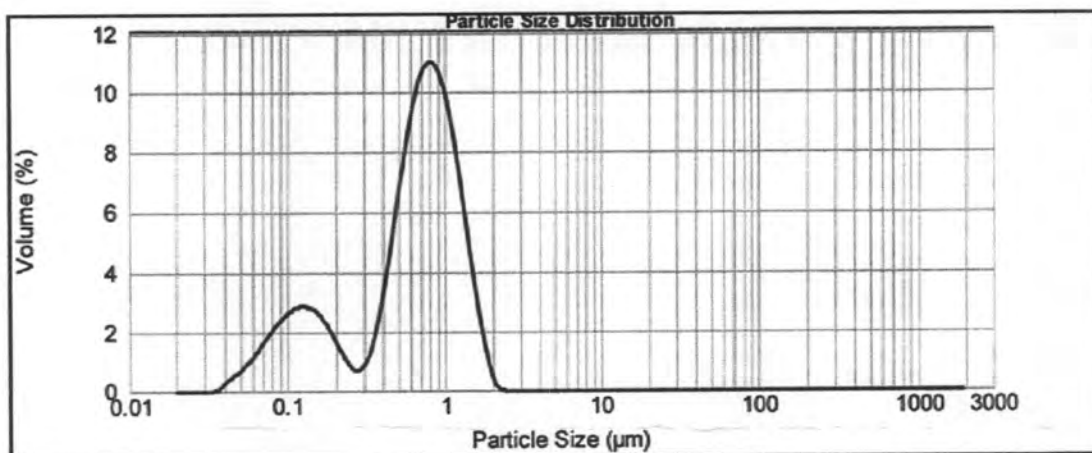


Figure 6.1 Particle size and particle size distribution of PLGA $0.68\mu\text{m}(0.51)$

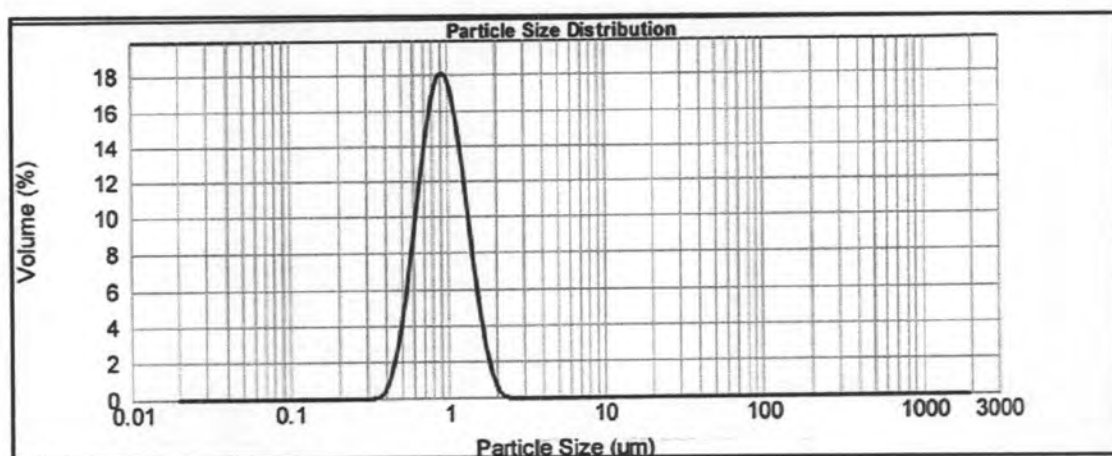


Figure 6.2 Particle size and particle size distribution of PLGA $0.92\mu\text{m}(0.27)$

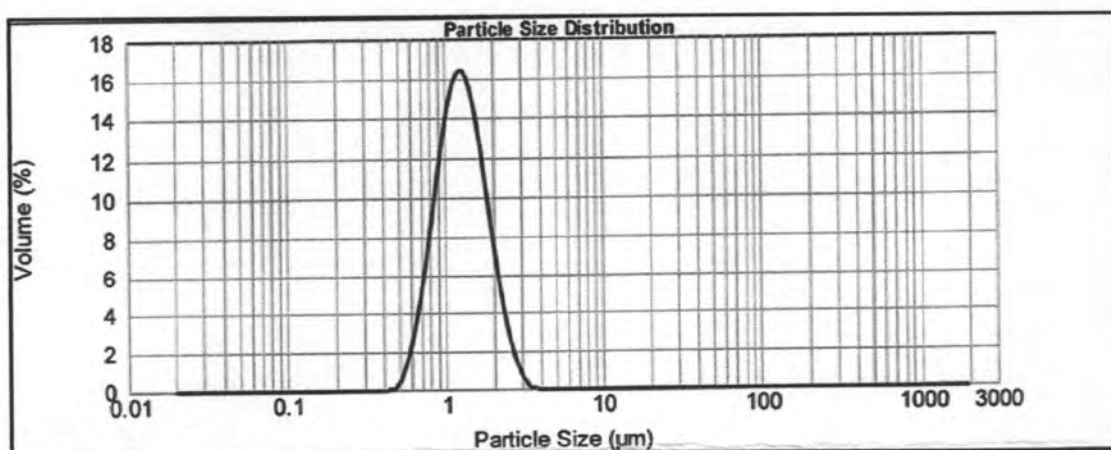


Figure 6.3 Particle size and particle size distribution of PLGA $1.25\mu\text{m}(0.30)$

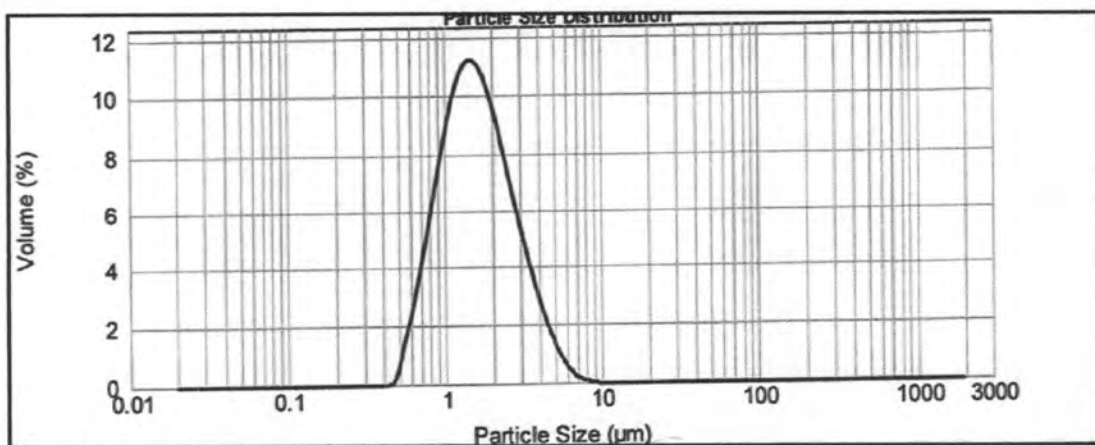


Figure 6.4 Particle size and particle size distribution of PLGA $1.55\mu\text{m}(0.48)$

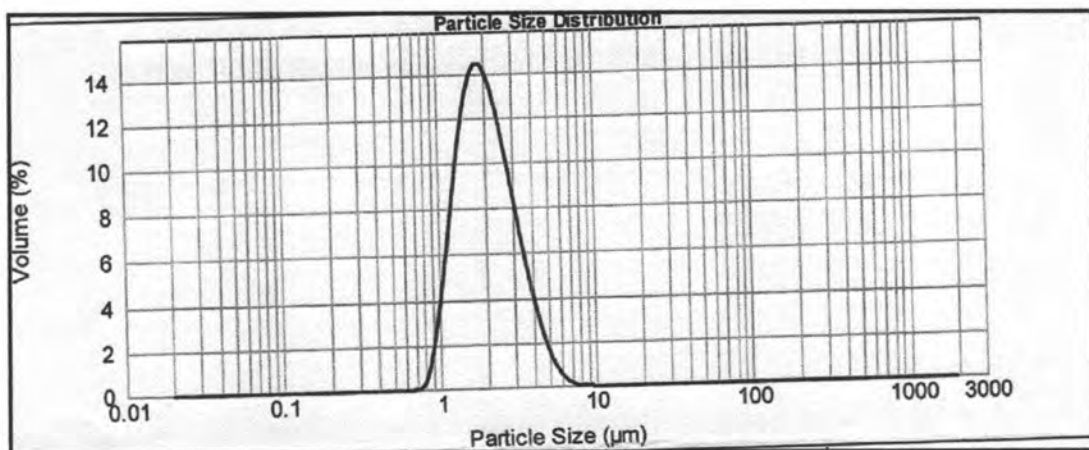


Figure 6.5 Particle size and particle size distribution of PLGA $1.96\mu\text{m}(0.36)$

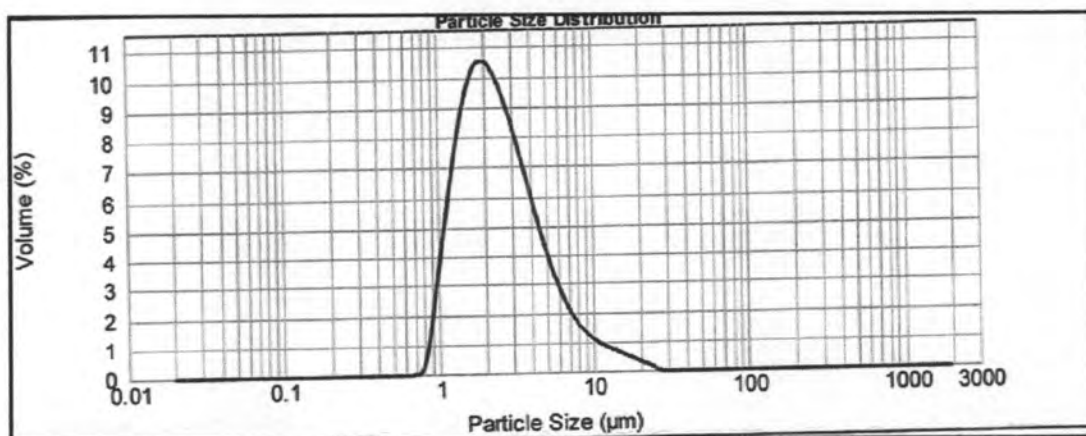


Figure 6.6 Particle size and particle size distribution of PLGA $2.32\mu\text{m}(0.67)$

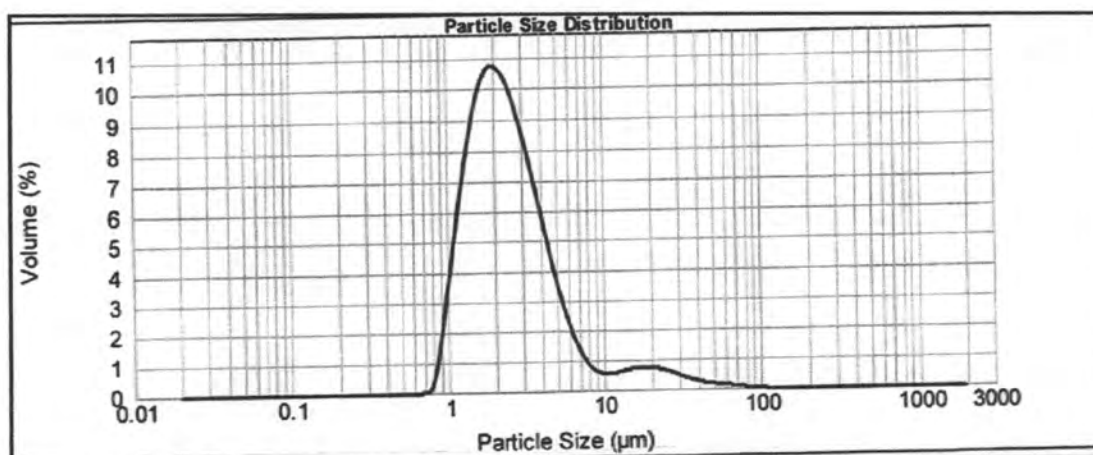


Figure 6.7 Particle size and particle size distribution of PLGA 2.31 μm (1.02)

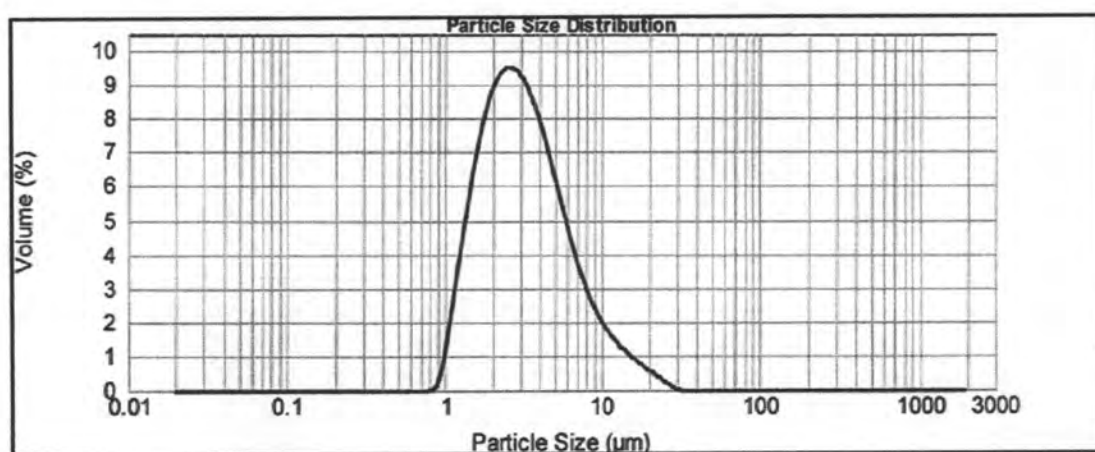


Figure 6.8 Particle size and particle size distribution of PLGA 3.00 μm (0.70)

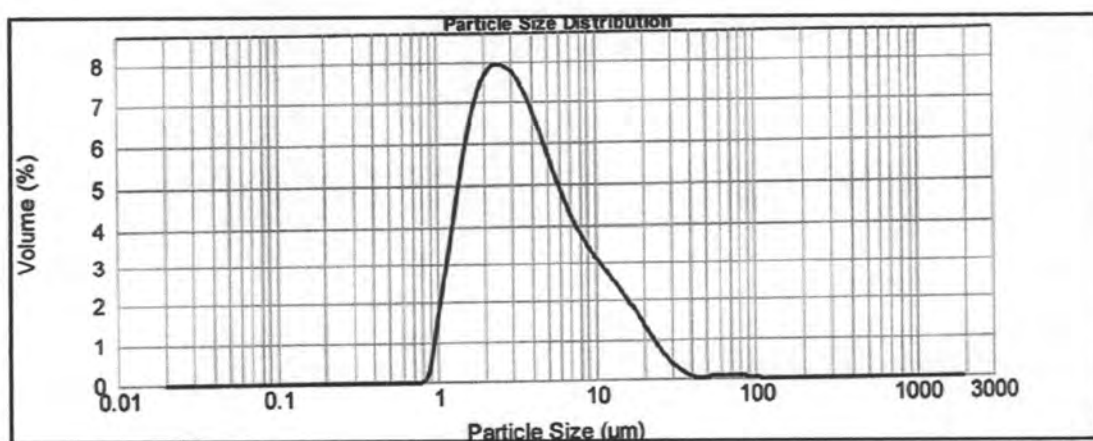


Figure 6.9 Particle size and particle size distribution of PLGA 3.37 μm (0.99)

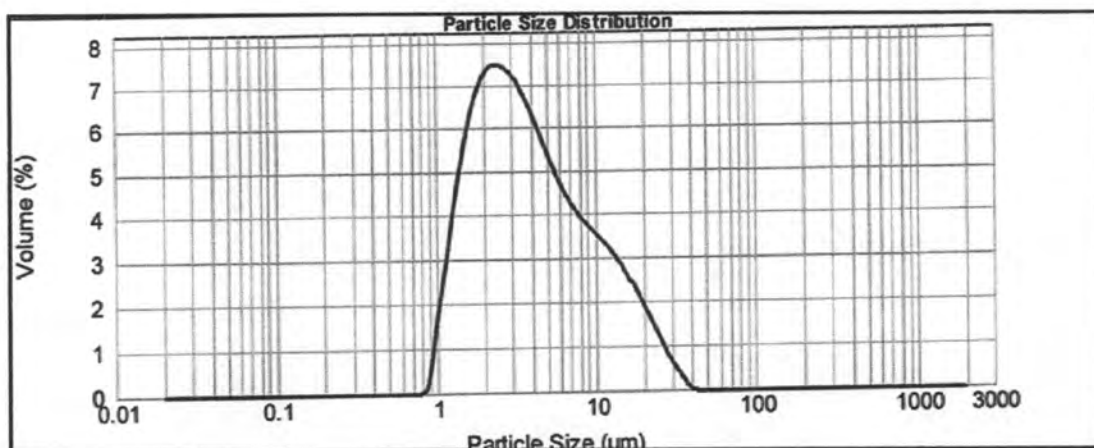


Figure 6.10 Particle size and particle size distribution of PLGA 3.54µm(1.03)

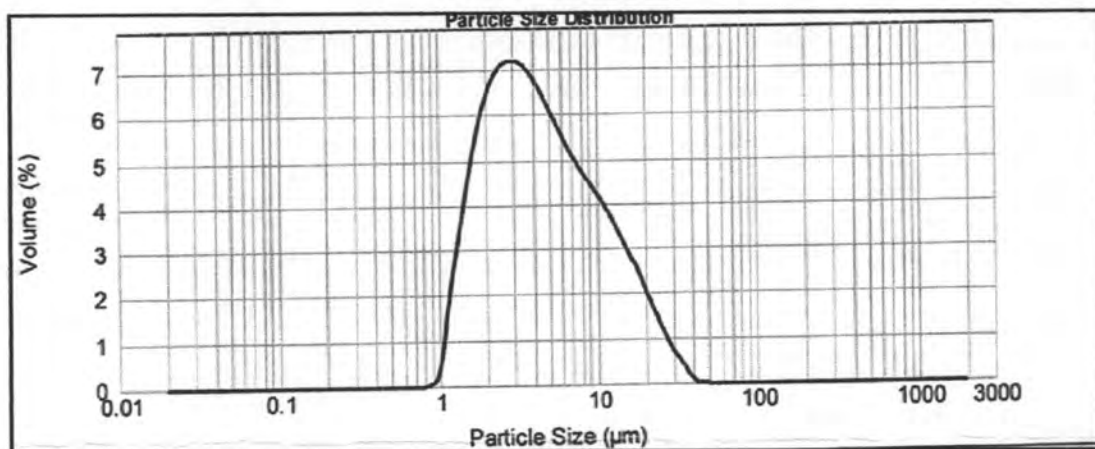


Figure 6.11 Particle size and particle size distribution of PLGA 4.15µm(0.94)

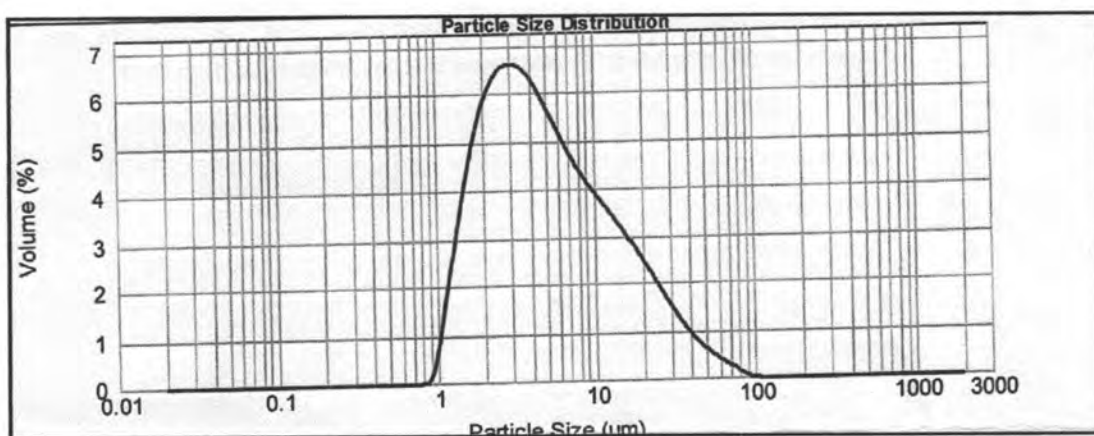


Figure 6.12 Particle size and particle size distribution of PLGA 4.53µm(1.29)

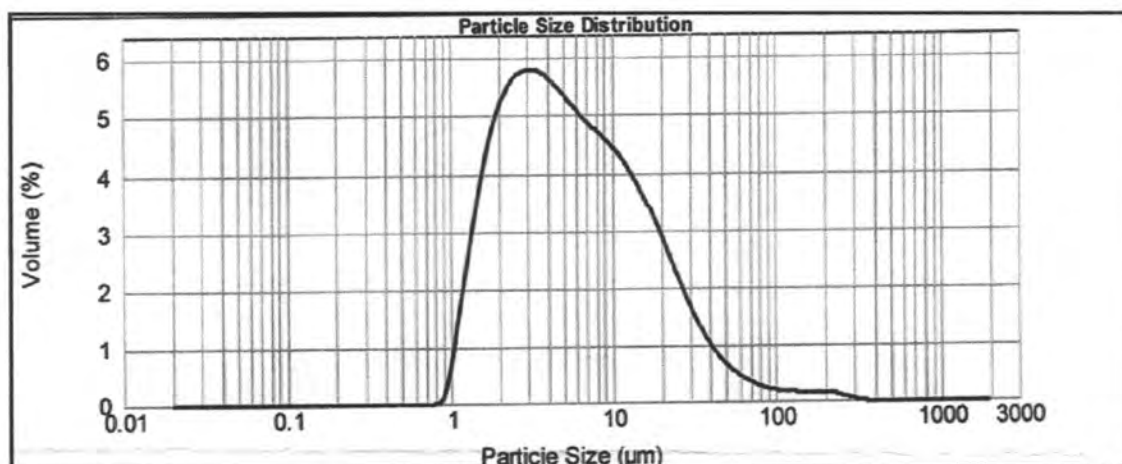


Figure 6.13 Particle size and particle size distribution of PLGA 5.26µm(1.57)

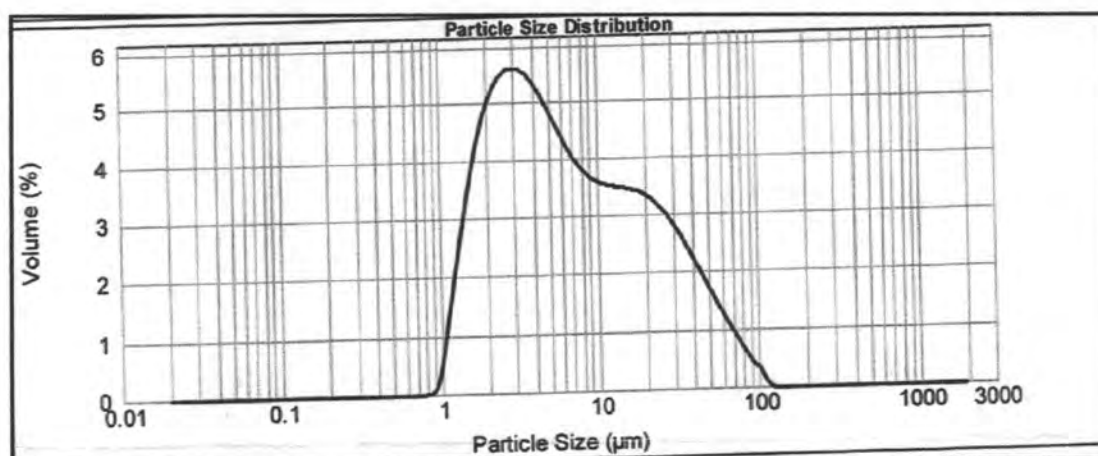


Figure 6.14 Particle size and particle size distribution of PLGA 5.82µm(1.69)

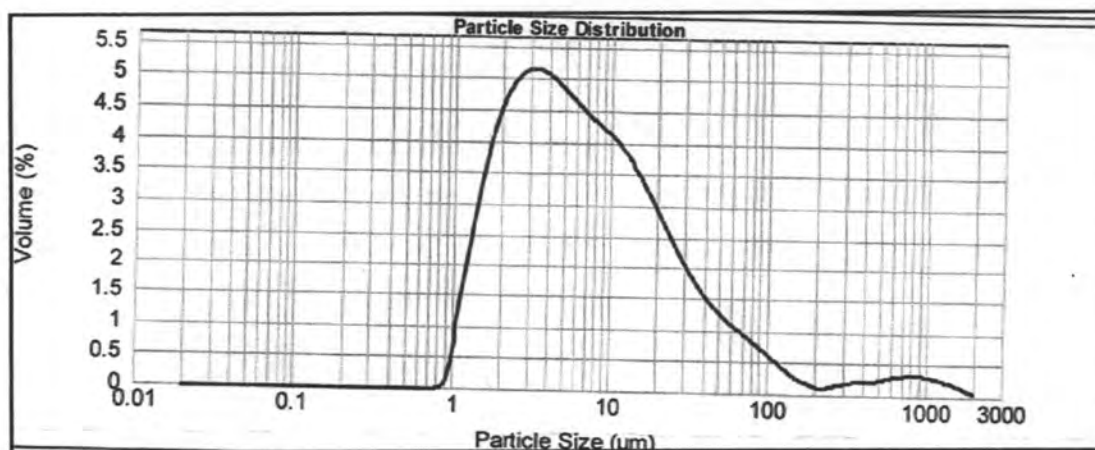


Figure 6.15 Particle size and particle size distribution of PLGA 6.39µm(5.46)

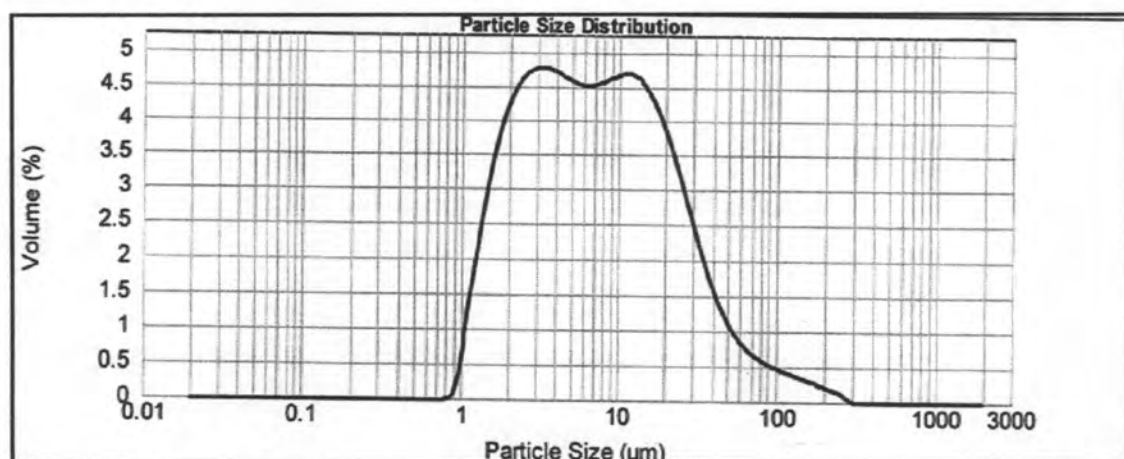


Figure 6.16 Particle size and particle size distribution of PLGA 7.07 μm (1.61)

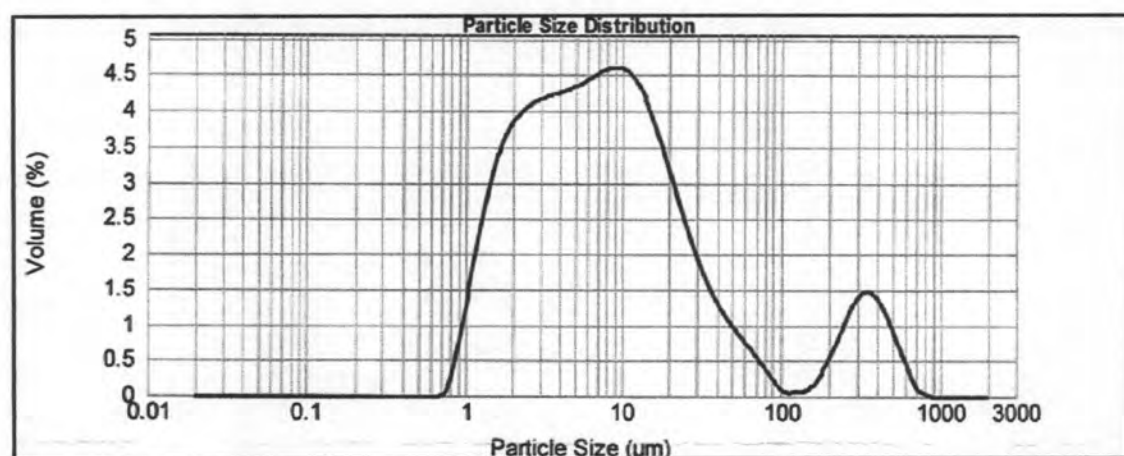


Figure 6.17 Particle size and particle size distribution of PLGA 7.44 μm (5.14)

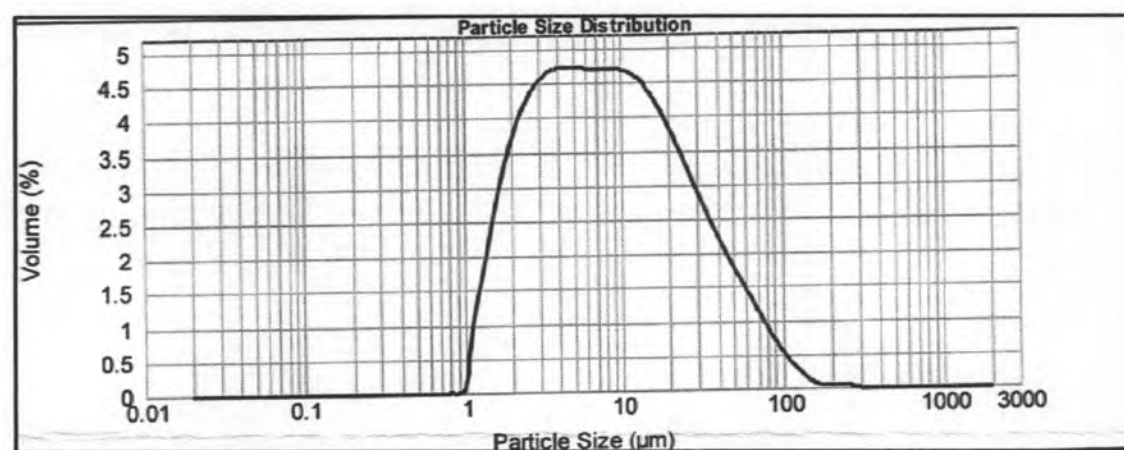


Figure 6.18 Particle size and particle size distribution of PLGA 7.88 μm (1.47)

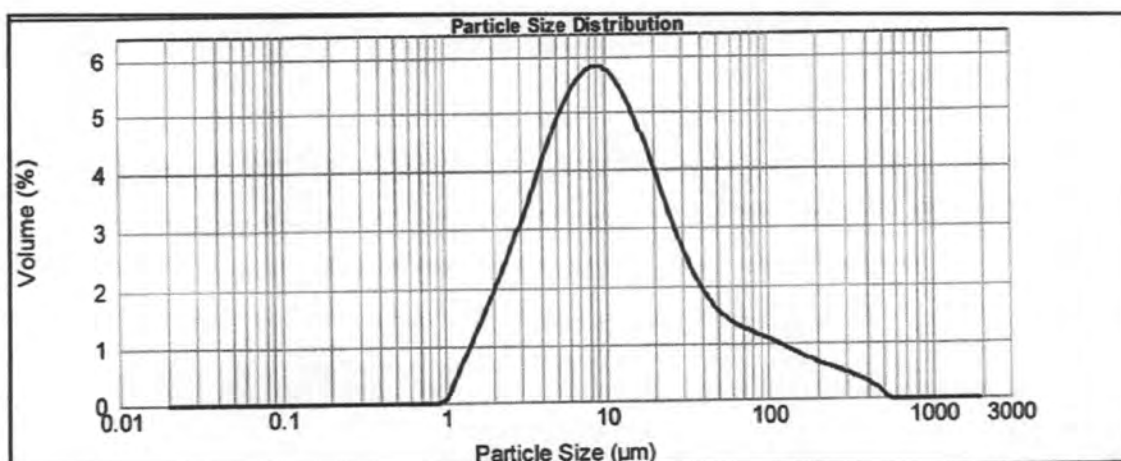


Figure 6.19 Particle size and particle size distribution of PLGA $10.20\mu\text{m}(2.21)$

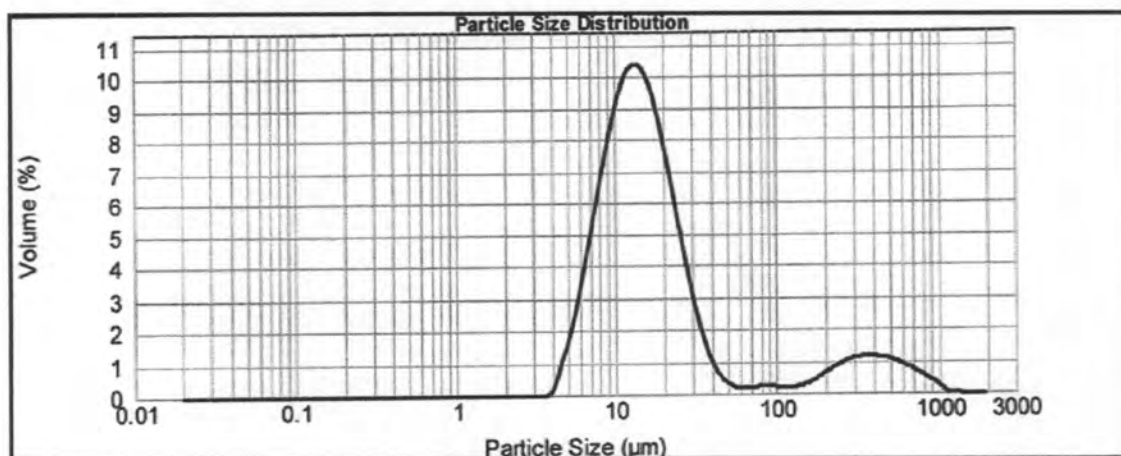


Figure 6.20 Particle size and particle size distribution of PLGA $14.60\mu\text{m}(3.41)$

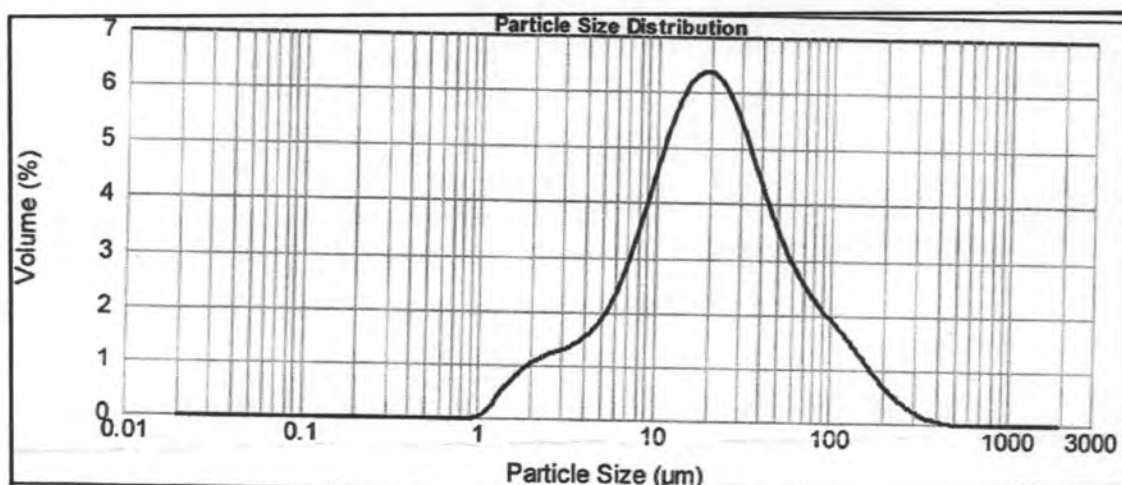


Figure 6.21 Particle size and particle size distribution of PLGA $20.11\mu\text{m}(1.21)$

Particle size and particle size distribution of conjugated particles (Table 3.1)

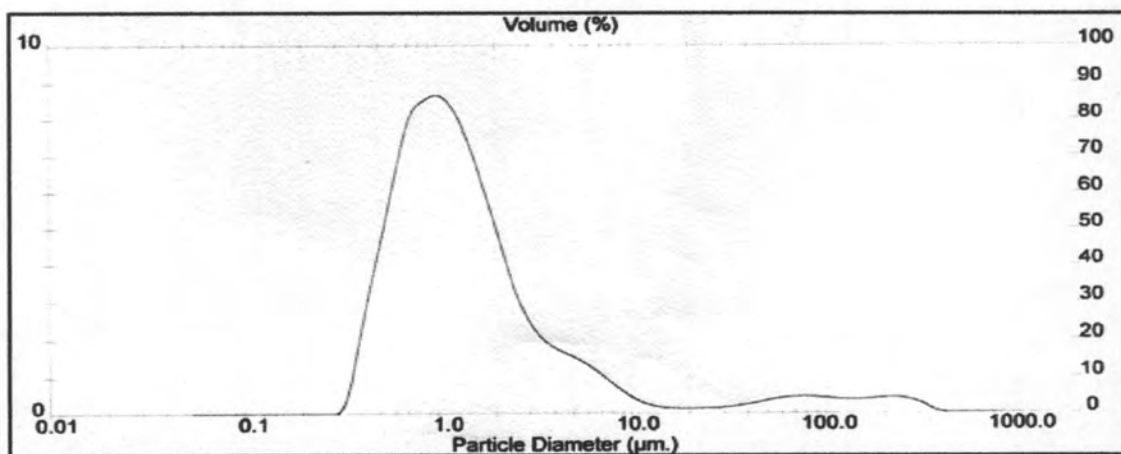


Figure 6.22 Particle size and particle size distribution of PLGA-CS of 1 μm particles 1.22 μm (7.72)

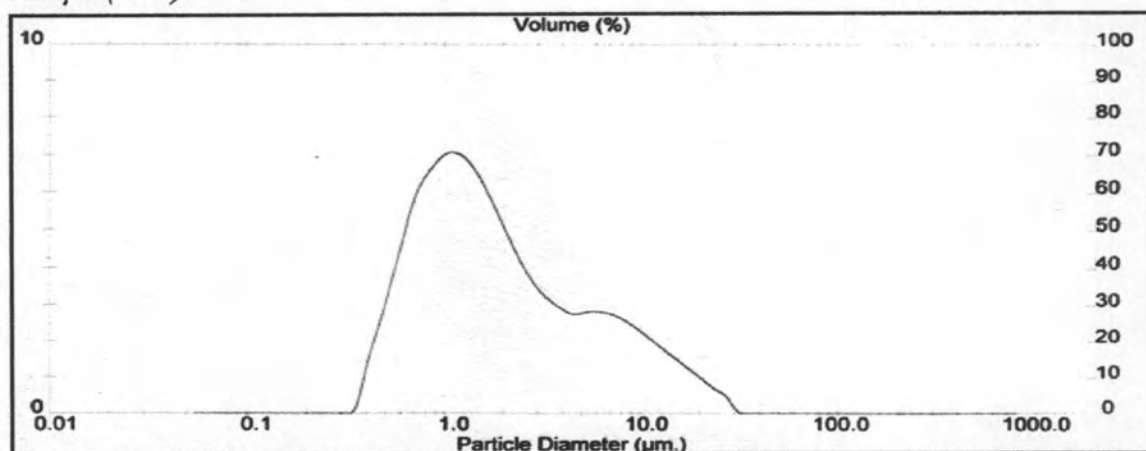


Figure 6.23 Particle size and particle size distribution of PLGA-0.75% Al(OH)₃ of 1 μm particles 1.62 μm (1.59)

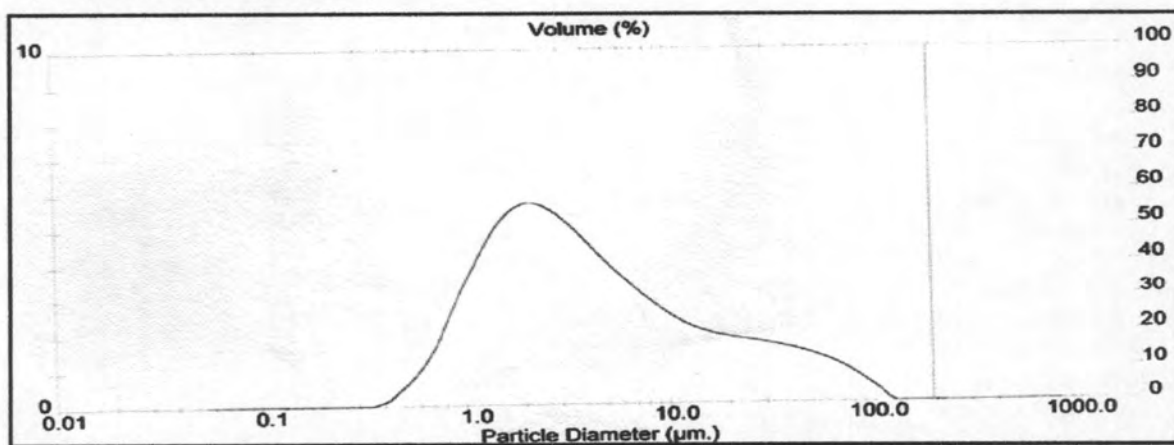


Figure 6.24 Particle size and particle size distribution of PLGA-1.5% Al(OH)₃ 1 μm particles 3.44 μm (2.22)

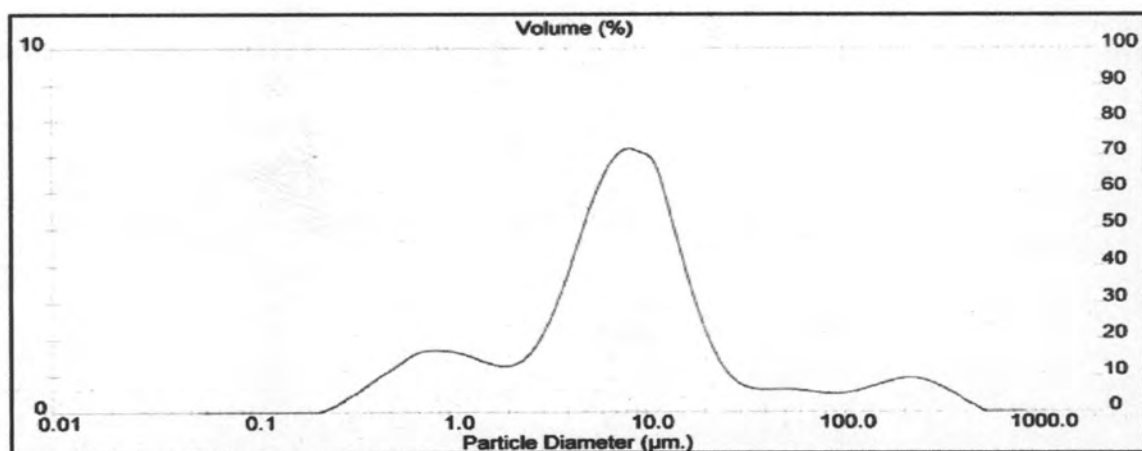


Figure 6.25 Particle size and particle size distribution of PLGA-CS of 5µm particles
7.59µm(2.52)

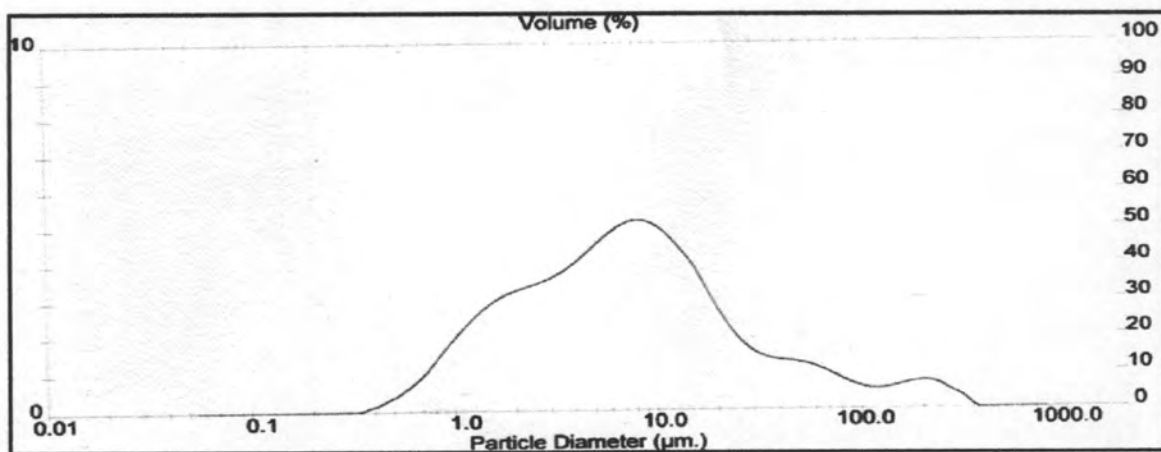


Figure 6.26 Particle size and particle size distribution of PLGA- 0.75% Al(OH)₃ of
5µm particles 6.87µm(2.34)

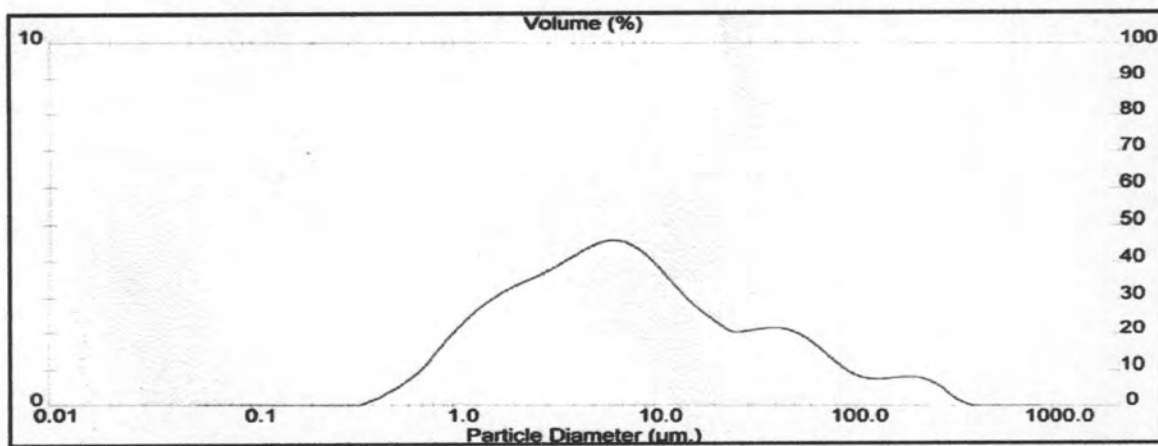


Figure 6.27 Particle size and particle size distribution of PLGA- 1.5% Al(OH)₃ of
5µm particles 6.89µm(2.96)

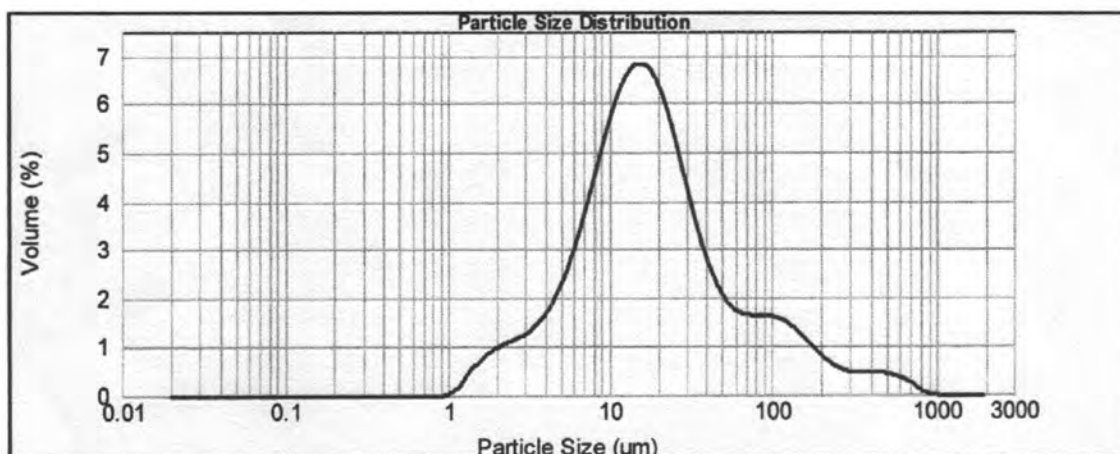


Figure 6.28 Particle size and particle size distribution of PLGA- CS of 15µm particles $17.18\mu\text{m}(2.77)$

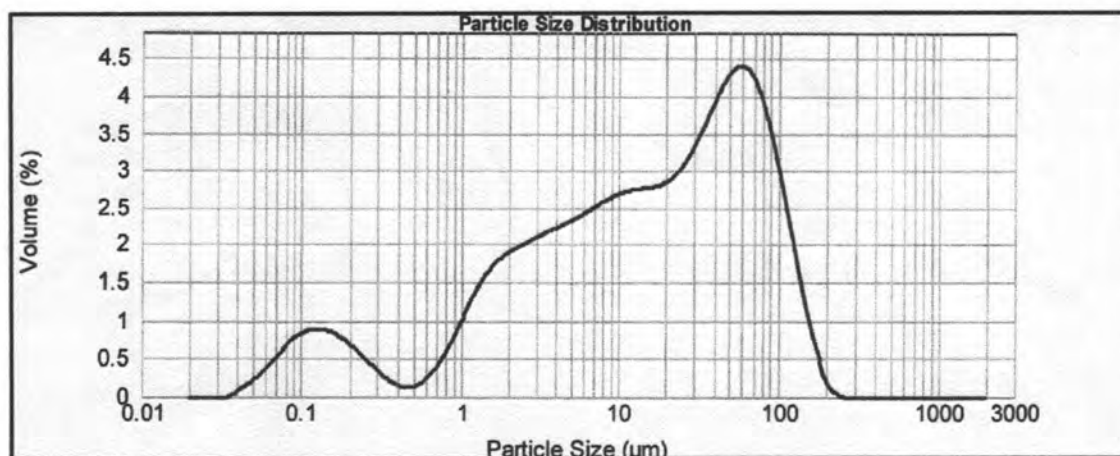


Figure 6.29 Particle size and particle size distribution of PLGA- 0.75% Al(OH)₃ of 15µm particles $16.85\mu\text{m}(1.98)$

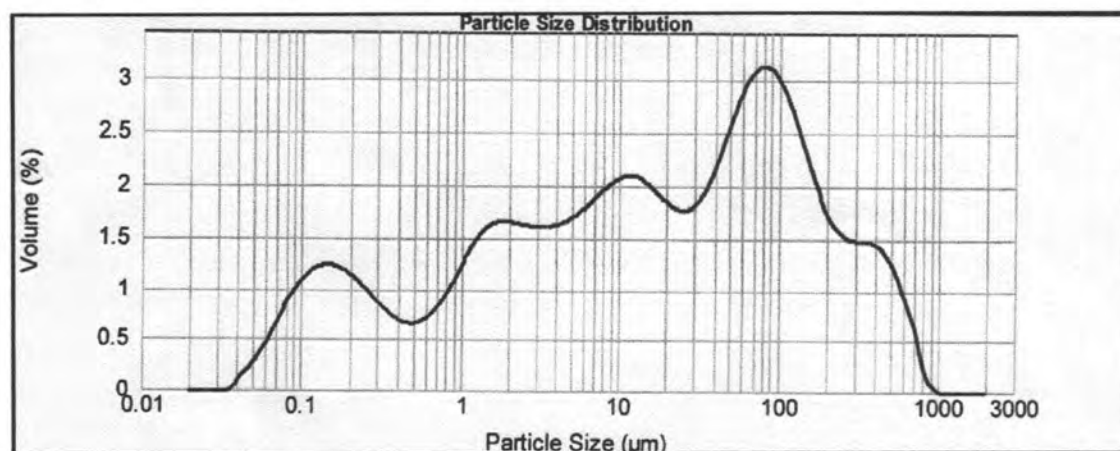
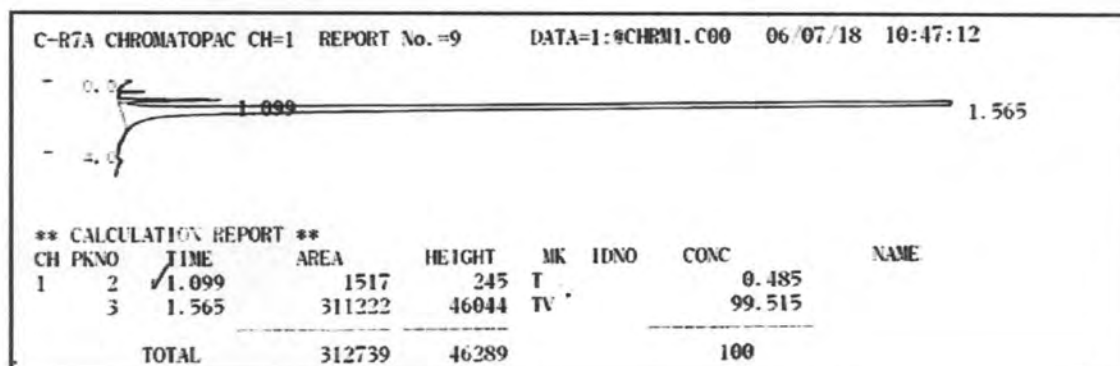
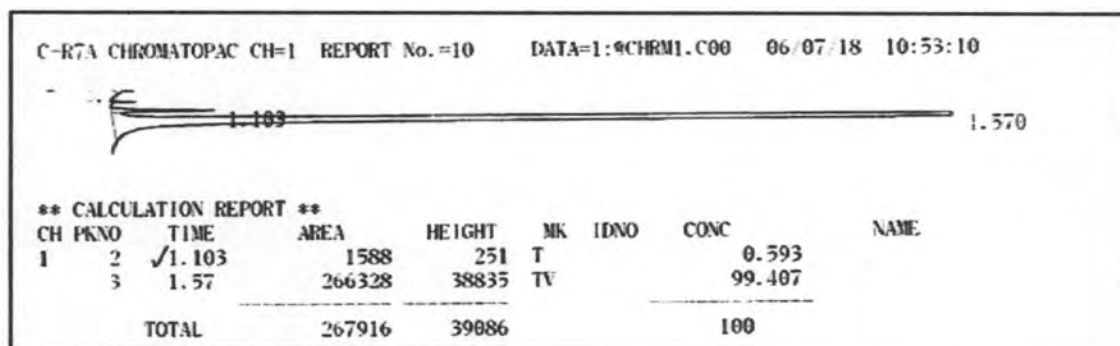
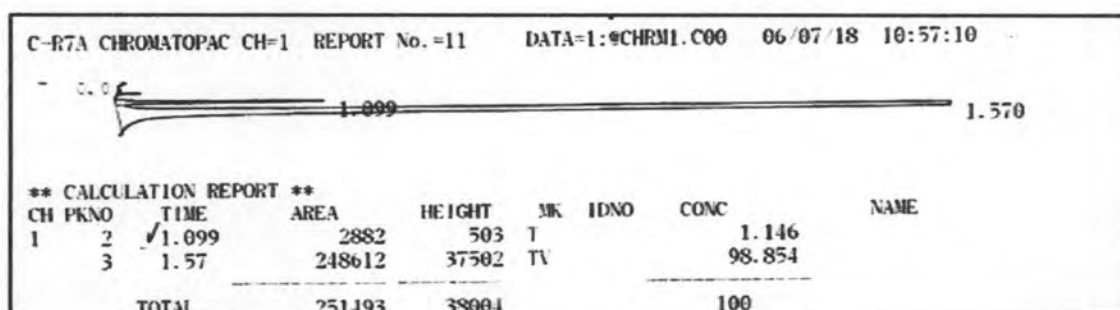


Figure 6.30 Particle size and particle size distribution of PLGA- 1.5% Al(OH)₃ of 15µm particles $16.95\mu\text{m}(1.83)$

Organic solvent residue of 1, 5 and 15 μ m PLGA particlesFigure 6.31 Solvent residue by GC of DCM in 1 μ m PLGA particlesFigure 6.32 Solvent residue by GC of DCM in 5 μ m PLGA particlesFigure 6.33 Solvent residue by GC of DCM in 15 μ m PLGA particles

Standard curve of BSA for entrapment and release study (Figure 6.9)

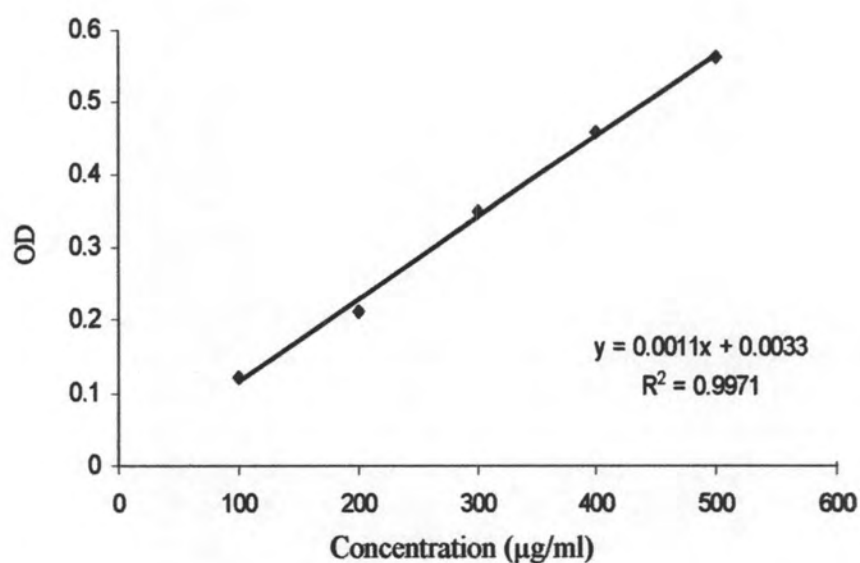


Figure 6.34 Standard curve of BSA

In vitro release study

Table 6.2 Percentage of Cumulative release

Day	%Cumulative release				
	1µm	5µm	15µm	1A*	1C*
1	6.46	10.97	7.9	3.24	3.68
4	16.05	16.34	17.82	8.16	8.99
7	19.11	18.39	19.05	11.08	13.99
14	22.59	20.06	19.87	15.34	19.52
21	24.23	21.38	21.42	17.21	22.32
28	26.39	22.93	22.97	18.22	25.22
60	32.08	27.86	24.65	27.02	30.29

1A* : 1µm PLGA conjugated 0.75% Al(OH)₃ particle

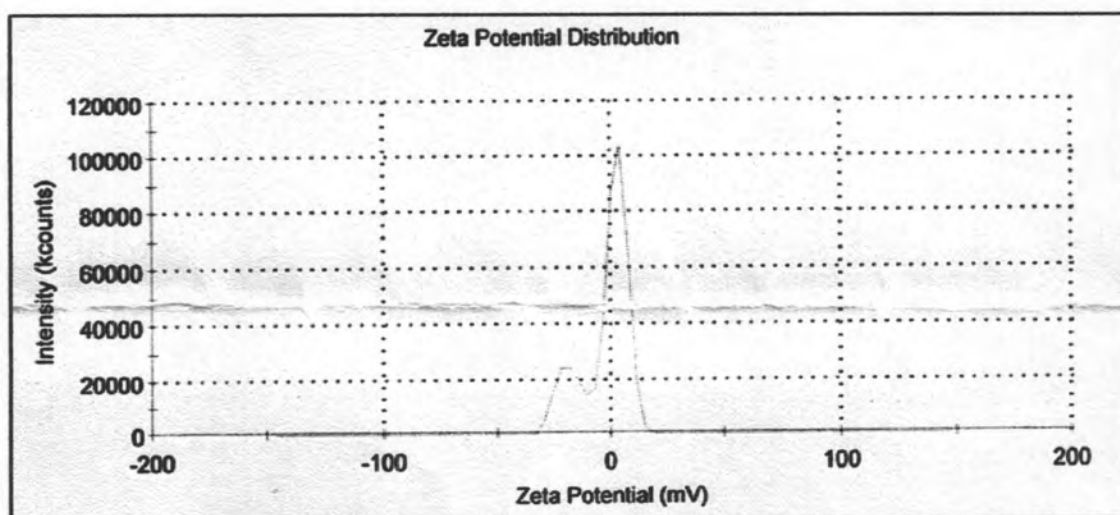
1C* : 1µm PLGA conjugated CS particle

Table 6.3 Standard deviation of cumulative release

Day	SD of cumulative release				
	1 μ m	5 μ m	15 μ m	1A*	1C*
1	1E-0.4	0.03	0.000115	0.0001	0.6
4	1E-0.4	0.0001	0.000379	5.7735E-05	5.7735E-05
7	5.7735E-05	0.000208	0.000416	0	0.65
14	5.7735E-05	5.7735E-05	0.000252	0.00011547	5.7735E-05
21	0.42	0.000265	0	5.7735E-05	5.7735E-05
28	0.000231	0.000252	0.000351	0.4	0.00015275
60	0.115	0.7	0.0003	0.1	0.15

1A* : 1 μ m PLGA conjugated 0.75% Al(OH)₃ particle

1C* : 1 μ m PLGA conjugated CS particle

Diagram of zeta potential of selected 1, 1A and 1C PLGA particles**Figure 6.35** Zeta potential diagram of 1 μ m PLGA particles

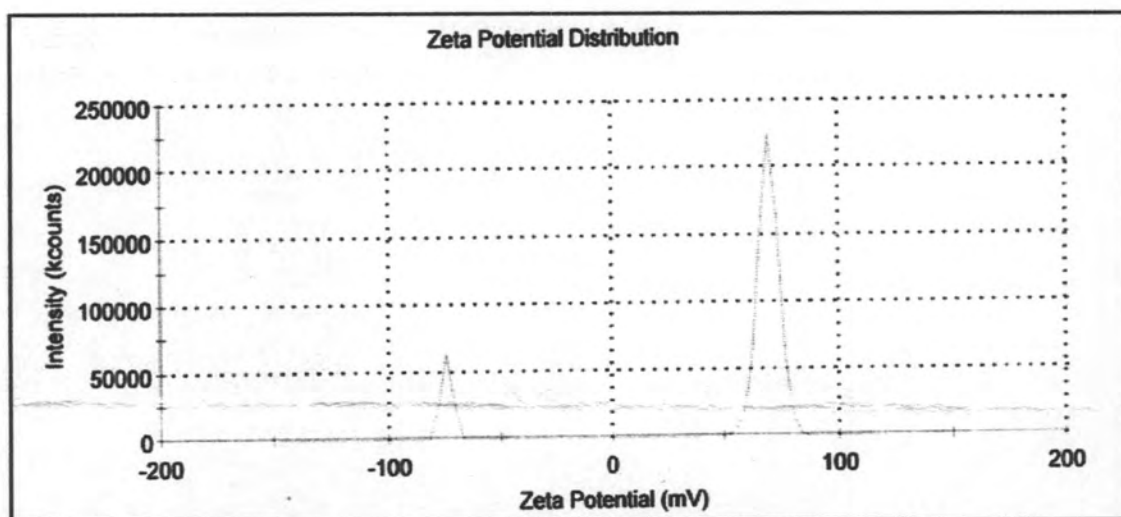


Figure 6.36 Zeta potential diagram of 1A μ m PLGA particles

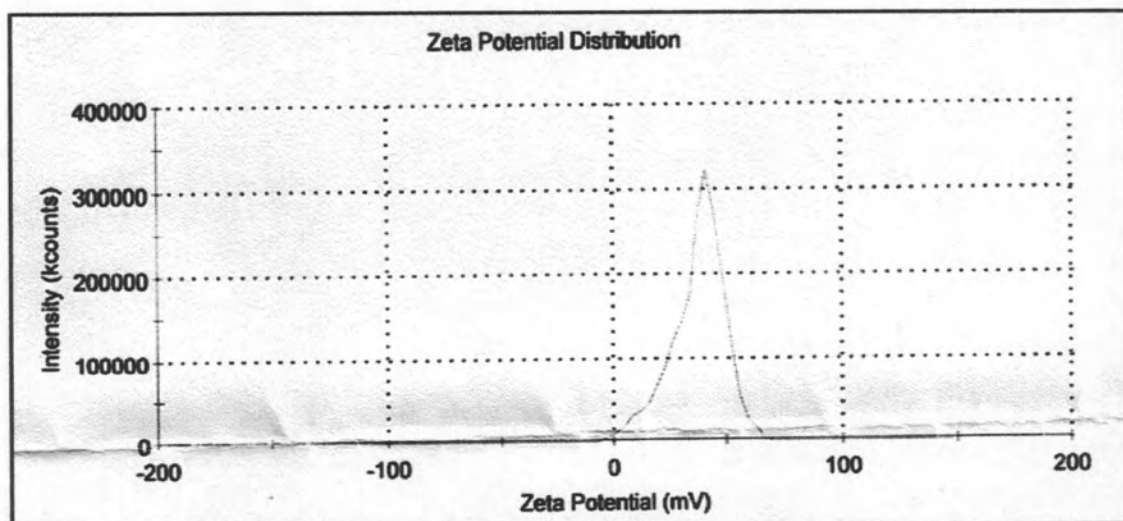


Figure 6.37 Zeta potential diagram of 1C μ m PLGA particles

APPENDIX B

Ex vivo evaluation of Al(OH)₃ and chitosan conjugated PLGA microparticles as nasal vaccine carriers in porcine nasal mucosa

Uptake study (Table 4.2)

Table 6.4 Fluorescent intensity of 1µm particles incubated porcine nasal tissue for 20 minutes

Measure	ROI MaxSize	Statistics	CHS1	CHS1	CHS1
CenterX	317.331	Integration	4215502	6697011	7310728
CenterY	317.331	Average	16.081	25.547	27.888
Area	404373.897	Max	4095	4095	4095
Perimeter	2543.616	Min	0	0	0
LengthL	635.904	Range	4095	4095	4095
LengthS	635.904	StdDev	193.406	296.779	311.224
		3StdDev	580.218	890.337	933.673

Table 6.5 Fluorescent intensity of 1µm particles incubated porcine nasal tissue for 90 minutes

Measure	ROI MaxSize	Statistics	CHS1	CHS1	CHS1
CenterX	317.331	Integration	1217313	2448192	3802702
CenterY	317.331	Average	4.644	9.339	14.506
Area	404373.897	Max	4095	4095	4095
Perimeter	2543.616	Min	0	0	0
LengthL	635.904	Range	4095	4095	4095
LengthS	635.904	StdDev	121.518	178.631	216.958
		3StdDev	364.555	535.893	650.873

Table 6.6 Fluorescent intensity of 1 μ m particles

Measure	ROI MaxSize	Statistics	CHS1	CHS1	CHS1
CenterX	317.331	Integration	12325026	13073644	12578896
CenterY	317.331	Average	47.016	49.872	47.985
Area	404373.897	Max	4095	4095	4095
Perimeter	2543.616	Min	0	0	0
LengthL	635.904	Range	4095	4095	4095
LengthS	635.904	StdDev	145.896	153.688	180.076
		3StdDev	437.687	461.065	540.229

Table 6.7 Fluorescent intensity of 5 μ m particles incubated porcine nasal tissue for 20 minutes

Measure	ROI MaxSize	Statistics	CHS1	CHS1	CHS1
CenterX	317.331	Integration	430638	318262	214580
CenterY	317.331	Average	1.643	2.089	0.819
Area	404373.897	Max	4095	4050	4095
Perimeter	2543.616	Min	0	0	0
LengthL	635.904	Range	4095	4050	4095
LengthS	635.904	StdDev	59.103	51.871	54.182
		3StdDev	177.31	155.614	162.545

Table 6.8 Fluorescent intensity of 5 μ m particles incubated porcine nasal tissue for 90 minutes

Measure	ROI Max size	Statistics	CHS1	CHS1	CHS1
CenterX	333.477	Integration	172473	370989	418961
CenterY	381.294	Average	1.039	2.355	1.598
Area	256087.22	Max	4095	4095	4095
Perimeter	2024.46	Min	0	0	0
LengthL	514.188	Range	4095	4095	4095
LengthS	498.042	StdDev	48.408	69.95	49.804
		3StdDev	145.223	209.85	149.412

Table 6.9 Fluorescent intensity of 5 μ m particles

Measure	ROI MaxSize	Statistics	CHS1	CHS1	CHS1
CenterX	317.331	Integration	7122901	2550439	14082730
CenterY	317.331	Average	27.172	9.729	53.721
Area	404373.897	Max	4095	4095	4095
Perimeter	2543.616	Min	0	0	0
LengthL	635.904	Range	4095	4095	4095
LengthS	635.904	StdDev	269.678	169.084	398.99
		3StdDev	809.033	507.251	1196.97

Table 6.10 Fluorescent intensity of 15 μ m particles incubated porcine nasal tissue for 20 minutes

Measure	ROI MaxSize	Statistics	CHS1	CHS1	CHS1
CenterX	317.331	Integration	253759	991363	55
CenterY	317.331	Average	0.968	3.782	0
Area	404373.897	Max	4095	4095	33
Perimeter	2543.616	Min	0	0	0
LengthL	635.904	Range	4095	4095	33
LengthS	635.904	StdDev	44.287	62.183	0.077
		3StdDev	132.862	186.55	0.232

Table 6.11 Fluorescent intensity of 15 μ m particles incubated porcine nasal tissue for 90 minutes

Measure	ROI MaxSize	Statistics	CHS1	CHS1	CHS1
CenterX	317.331	Integration	1889002	ND	ND
CenterY	317.331	Average	3.346	ND	ND
Area	404373.897	Max	4095	ND	ND
Perimeter	2543.616	Min	0	ND	ND
LengthL	635.904	Range	4095	ND	ND
LengthS	635.904	StdDev	90.176	ND	ND
		3StdDev	270.529	ND	ND

Table 6.12 Fluorescent intensity of 15 μ m particles

Measure	ROI MaxSize	Statistics	CHS1	CHS1	CHS1
CenterX	317.331	Integration	2800945	2489779	3274530
CenterY	317.331	Average	10.685	12.586	12.491
Area	404373.897	Max	4095	4095	4095
Perimeter	2543.616	Min	0	0	0
LengthL	635.904	Range	4095	4095	4095
LengthS	635.904	StdDev	76.438	189.01	209.814
		3StdDev	229.315	567.031	629.443

Table 6.13 Fluorescent intensity of 1C PLGA particles incubated porcine nasal tissue for 20 minutes

Measure	ROI MaxSize	Statistics	CHS1	CHS1	CHS1
CenterX	317.331	Integration	2465836	1685249	2077472
CenterY	317.331	Average	9.406	6.429	7.925
Area	404373.897	Max	4095	4095	4095
Perimeter	2543.616	Min	0	0	0
LengthL	635.904	Range	4095	4095	4095
LengthS	635.904	StdDev	159.81	144.56	162.498
		3StdDev	479.43	433.68	487.493

Table 6.14 Fluorescent intensity of 1C PLGA particles incubated porcine nasal tissue for 90 minutes

Measure	ROI MaxSize	Statistics	CHS1	CHS1	CHS1
CenterX	317.331	Integration	2261194	889167	1531857
CenterY	317.331	Average	8.626	3.392	5.844
Area	404373.897	Max	4095	4095	4095
Perimeter	2543.616	Min	0	0	0
LengthL	635.904	Range	4095	4095	4095
LengthS	635.904	StdDev	169.492	78.32	51.821
		3StdDev	508.477	234.961	155.464

Table 6.15 Fluorescent intensity of 1C PLGA particles

Measure	ROI MaxSize	Statistics	CHS1	CHS1	CHS1
CenterX	317.331	Integration	8517165	16115662	33684767
CenterY	317.331	Average	32.49	61.476	128.497
Area	404373.897	Max	4095	4095	4095
Perimeter	2543.616	Min	0	0	0
LengthL	635.904	Range	4095	4095	4095
LengthS	635.904	StdDev	135.801	165.513	196.106
		3StdDev	407.402	496.539	588.318

Table 6.16 Fluorescent intensity of 1A PLGA particles incubated porcine nasal tissue for 20 minutes

Measure	ROI MaxSize	Statistics	CHS1	CHS1	CHS1
CenterX	317.331	Integration	2108932	904062	1521643
CenterY	317.331	Average	8.045	3.449	5.805
Area	404373.897	Max	4095	4095	4095
Perimeter	2543.616	Min	0	0	0
LengthL	635.904	Range	4095	4095	4095
LengthS	635.904	StdDev	145.573	94.243	107.857
		3StdDev	436.72	282.73	323.57

Table 6.17 Fluorescent intensity of 1A PLGA particles incubated porcine nasal tissue for 90 minutes

Measure	ROI MaxSize	Statistics	CHS1	CHS1	CHS1
CenterX	317.331	Integration	1063680	289625	1343822
CenterY	317.331	Average	4.058	1.105	5.126
Area	404373.897	Max	4095	4095	4095
Perimeter	2543.616	Min	0	0	0
LengthL	635.904	Range	4095	4095	4095
LengthS	635.904	StdDev	118.86	55.813	129.532
		3StdDev	356.579	167.44	388.597

Table 6.18 Fluorescent intensity of 1A PLGA particles

Measure	ROI MaxSize	Statistics	CHS1	CHS1	CHS1
CenterX	317.331	Integration	3669758	2985383	2818946
CenterY	317.331	Average	13.999	11.388	10.753
Area	404373.897	Max	4095	4095	4095
Perimeter	2543.616	Min	0	0	0
LengthL	635.904	Range	4095	4095	4095
LengthS	635.904	StdDev	139.848	157.698	131.559
		3StdDev	419.544	473.093	394.676

Adhesion study (Figure 4.4)

Table 6.19 Percentage of washed particles

Time (minutes)	%Cumulative washed particles (SD)				
	1 μ m	5 μ m	15 μ m	1A*	1C*
0	0	0	0	0	0
5	67.01(7.54)	19.35(4.20)	1.62(6.00)	11.81(3.00)	2.93(4.00)
10	92.19(2.40)	48.56(4.23)	26.95(3.00)	30.18(7.01)	8.3(1.50)
30	103.17(5.02)	57.82(4.22)	40.68(8.60)	40.15(1.20)	31.28(7.00)

1A* : 1 μ m PLGA conjugated 0.75% Al(OH)₃ particle

1C* : 1 μ m PLGA conjugated CS particle

Cytotoxicity study (Figure 4.6)

Table 6.20 Percentage of cells viability

Dose (μg)	% Cell availability (SD)				
	1 μm	5 μm	15 μm	1A*	1C*
100	124.66(2.13)	112.69(4.11)	74.75(0.06)	117.46(2.06)	92.35(2.05)
300	118.3(2.06)	95.09(3.33)	72.87(6.16)	87.88(1.08)	68.3(2.12)
600	107.22(8.62)	74.31(2.08)	62.34(2.07)	89.18(7.34)	65.51(5.15)

1A* : 1 μm PLGA conjugated 0.75% Al(OH)₃ particle1C* : 1 μm PLGA conjugated CS particle

Permeation study (Figure 4.7)

Table 6.21 Percentage of cumulative permeation of particles

Time (hours)	% Cumulative permeation of particles (SD)				
	1 μm	5 μm	15 μm	1A*	1C*
0	0	0	0	0	0
0.5	7.75(5.57)	11.31(7.57)	8.20(1.10)	20.44(2.65)	22.61(6.11)
1	15.21(2.08)	19.61(4.50)	16.28(2.08)	28.84(1.53)	34.56(1.73)
2	21.34(1.73)	30.17(3.50)	27.50(0.70)	37.23(0.50)	45.36(3.05)
4	31.22(6.00)	41.29(4.00)	38.07(4.50)	48.26(5.03)	70.86(8.70)

1A* : 1 μm PLGA conjugated 0.75% Al(OH)₃ particle1C* : 1 μm PLGA conjugated CS particle

Table 6.22 Percentage of cumulative permeation of JE released from particles

Time (hours)	% Cumulative permeation of JE released from particles (SD)				
	1 μ m	5 μ m	15 μ m	1A*	1C*
0	0	0	0	0	0
0.5	1.23(2.50)	0.6(0.15)	0.56(0.68)	0.6(0.40)	0.8(0.90)
1	1.83(1.25)	1.92(0.73)	1.67(0.24)	1.33(0.70)	2.36(1.10)
2	4.66(0.75)	3.53(0.60)	3.51(0.43)	2.07(1.10)	4.19(0.30)
4	7.4(0.15)	5.3(0.20)	5.66(0.80)	2.85(1.50)	5.96(1.10)

1A* : 1 μ m PLGA conjugated 0.75% Al(OH)₃ particle

1C* : 1 μ m PLGA conjugated CS particle

APPENDIX C

**In vivo evaluation of Al(OH)₃ and chitosan conjugated PLGA microparticles as
nasal Japanese encephalitis vaccine carrier**

Table 6.23 Molar ellipticity of JE, Purified JE and Purified JEx4

Wavelength[nm]	JE	Purified JE x4	Purified JE
250	-0.73772	-2.50359	-1.61108
49.5	-0.8205	-2.455242	-1.65812
249	-0.80555	-2.61237	-1.76918
248.5	-0.79517	-2.750187	-1.80835
248	-0.85137	-2.834403	-1.8729
247.5	-0.92704	-2.965208	-1.87642
247	-1.10733	-3.050225	-2.03877
246.5	-1.08575	-3.173553	-2.13873
246	-1.14239	-3.28222	-2.20205
245.5	-1.13416	-3.29272	-2.27144
245	-1.26181	-3.455673	-2.32455
244.5	-1.31472	-3.617882	-2.37191
244	-1.476	-3.82938	-2.39687
243.5	-1.57967	-4.101475	-2.36247
243	-1.61762	-4.294673	-2.44986
242.5	-1.59196	-4.614283	-2.56616
242	-1.62897	-4.881875	-2.6918
241.5	-1.67021	-5.119845	-2.7408
241	-1.76635	-5.289203	-2.75044
240.5	-1.87722	-5.370607	-2.79883
240	-1.95139	-5.46566	-2.79989
239.5	-2.07264	-5.66711	-2.86364
239	-2.16524	-5.890267	-2.98999
238.5	-2.32851	-6.1665	-3.03936
238	-2.35168	-6.427133	-3.0886
237.5	-2.48987	-6.754442	-3.24434
237	-2.69878	-7.046118	-3.31519
236.5	-2.79151	-7.42293	-3.50603
236	-2.94609	-7.704273	-3.50765
235.5	-3.1316	-7.846015	-3.64777
235	-3.34026	-8.053738	-3.69114
234.5	-3.62952	-8.378665	-3.74298
234	-3.90954	-8.7685	-3.71479
233.5	-4.17261	-9.18509	-3.86986
233	-4.53082	-9.610573	-4.0434
232.5	-4.87898	-9.887295	-4.22749
232	-5.13177	-10.136738	-4.4005
231.5	-5.48776	-10.509075	-4.52091
231	-5.83021	-10.82515	-4.57157
230.5	-6.09743	-11.311525	-4.67552
230	-6.41766	-11.65425	-4.77201
229.5	-6.71643	-11.916525	-4.90762
229	-7.06549	-12.061951	-4.95493
228.5	-7.35806	-11.91315	-4.87533
228	-7.66709	-11.83805	-4.85481

227.5	-7.87495	-11.998625	-4.75668
227	-8.24857	-12.183975	-4.70925
226.5	-8.48102	-12.389976	-4.74226
226	-8.79635	-12.183175	-4.74989
225.5	-9.15222	-11.91675	-4.77693
225	-9.3876	-11.814325	-4.68071
224.5	-9.63248	-11.720401	-4.45036
224	-9.4752	-11.822326	-4.20867
223.5	-9.26715	-11.4622	-4.01565
223	-9.06503	-11.15665	-3.98215
222.5	-9.19508	-10.695188	-3.94896
222	-9.2006	-10.291147	-3.91518
221.5	-9.14576	-9.898143	-3.75741
221	-9.1244	-9.765987	-3.60396
220.5	-9.06464	-9.751215	-3.51235
220	-9.09511	-9.688125	-3.27552
219.5	-9.11484	-9.213898	-2.97047
219	-9.11067	-8.729193	-2.84398
218.5	-9.05352	-8.251307	-2.70938
218	-8.98437	-8.011723	-2.6019
217.5	-8.87067	-7.684613	-2.57592
217	-8.76355	-7.444452	-2.36865
216.5	-8.73831	-7.40324	-2.10137
216	-8.67464	-7.064888	-2.12683
215.5	-8.58705	-6.781842	-2.00698
215	-8.62745	-6.407615	-1.85341
214.5	-8.61331	-6.162172	-1.67745
214	-8.95675	-5.823873	-1.46562
213.5	-8.88964	-5.648265	-1.44553
213	-9.05676	-5.56842	-1.30148
212.5	-9.40618	-5.418625	-1.20301
212	-9.60864	-4.844023	-1.41812
211.5	-9.65184	-4.68504	-1.53461
211	-9.87134	-4.82853	-1.56306
210.5	-10.2179	-5.101928	-1.48173
210	-10.9326	-6.157533	-1.10335
209.5	-10.8981	-6.269877	-1.00391
209	-11.1002	-5.940053	-0.92955
208.5	-11.0466	-5.471952	-1.01915
208	-10.8054	-4.798165	-0.86398
207.5	-10.8892	-4.63001	-0.75752
207	-10.8116	-4.201358	-0.59843
206.5	-10.8663	-3.678668	0.01243
206	-10.6995	-2.828569	0.116265
205.5	-10.7645	-2.727192	0.313569
205	-10.5796	-2.193362	0.550939
204.5	-9.1237	-1.464414	0.542685
204	-7.66421	-0.557582	1.007042
203.5	-6.0186	1.485178	1.359529
203	-4.75171	1.769918	1.662596
202.5	-5.3617	2.554803	1.928558
202	-6.26803	3.741115	1.826419
201.5	-3.61393	5.307095	2.375278
201	-2.65648	6.459989	3.041148
200.5	1.373507	8.884203	3.951898
200	6.491048	9.443945	4.41459

Table 6.24 Molar ellipticity of 1 μ m, 5 μ m, 15 μ m and JE with DCM

Wavelength[nm]	1 μ m	5 μ m	15 μ m	JE:DCM
250	-0.60636	-0.69789	-0.51994	-1.64818
249.5	-0.62884	-0.68278	-0.56713	-1.52383
249	-0.8173	-0.71524	-0.65005	-1.69957
248.5	-0.90908	-0.85338	-0.60805	-1.67644
248	-1.04959	-0.88177	-0.65138	-1.67402
247.5	-1.03852	-0.95643	-0.68092	-1.58593
247	-1.0232	-0.99583	-0.75985	-1.84865
246.5	-0.94765	-1.1471	-0.9147	-2.06283
246	-0.99481	-1.25351	-0.93243	-2.3553
245.5	-1.11283	-1.35832	-1.02651	-2.33824
245	-1.2367	-1.47218	-1.09999	-2.3871
244.5	-1.36011	-1.57841	-1.20716	-2.44144
244	-1.40478	-1.61222	-1.3644	-2.62282
243.5	-1.52166	-1.70448	-1.58949	-2.72443
243	-1.6314	-1.74392	-1.66274	-2.97414
242.5	-1.72531	-1.94253	-1.7166	-3.19586
242	-1.79355	-2.13813	-1.80426	-3.50081
241.5	-1.90482	-2.20924	-1.9169	-3.76509
241	-1.96892	-2.33392	-2.03101	-4.02182
240.5	-2.07747	-2.32531	-2.22244	-4.25509
240	-2.15639	-2.48377	-2.38056	-4.60813
239.5	-2.25848	-2.64404	-2.59511	-4.76284
239	-2.39814	-2.77995	-2.74531	-4.99271
238.5	-2.51648	-2.95992	-2.96234	-5.0697
238	-2.73976	-3.09425	-3.15554	-5.44832
237.5	-2.93965	-3.15439	-3.37835	-5.73292
237	-3.09181	-3.35555	-3.6195	-6.03902
236.5	-3.2978	-3.61814	-3.66152	-6.38798
236	-3.54395	-3.88335	-3.87952	-6.42384
235.5	-3.82057	-4.10517	-4.06129	-6.74457
235	-3.96715	-4.35441	-4.37577	-6.82623
234.5	-4.20515	-4.6171	-4.83	-7.12562
234	-4.35419	-4.87664	-5.07509	-7.45005
233.5	-4.7751	-5.26886	-5.32728	-7.6993
233	-5.07372	-5.7385	-5.6346	-7.95668
232.5	-5.38789	-6.19123	-5.89355	-8.04854
232	-5.65227	-6.51566	-6.3538	-8.33992
231.5	-5.98631	-6.90316	-6.77559	-8.64645
231	-6.33115	-7.19417	-7.20972	-8.80499
230.5	-6.73811	-7.54613	-7.6884	-8.9848
230	-7.09932	-7.91935	-8.0312	-9.36554
229.5	-7.42152	-8.20518	-8.39725	-9.51196
229	-7.7202	-8.5734	-8.66661	-9.46335
228.5	-7.93339	-9.09693	-8.98285	-9.40561
228	-8.31138	-9.58774	-9.40879	-9.2865
227.5	-8.56425	-9.92663	-9.79207	-9.16807
227	-8.87993	-10.234	-10.0378	-9.28703
226.5	-9.13791	-10.2544	-10.3925	-9.256
226	-9.39125	-10.59	-10.687	-9.50217
225.5	-9.60644	-10.7247	-11.268	-9.06504

225	-9.70557	-10.7831	-11.7497	-9.02445
224.5	-9.73591	-10.9674	-11.8319	-8.80267
224	-9.88425	-10.9685	-11.6651	-9.02305
223.5	-9.99276	-11.0051	-11.5258	-8.76795
223	-10.2234	-11.0438	-11.3369	-8.93544
222.5	-10.1631	-11.0157	-10.9466	-8.61549
222	-9.69052	-11.0005	-10.9647	-8.61516
221.5	-9.3649	-11.0435	-10.982	-8.15049
221	-9.15599	-10.5855	-11.0474	-8.07702
220.5	-9.15168	-10.4908	-11.1632	-7.54715
220	-9.38193	-10.1632	-11.1713	-7.16213
219.5	-9.31561	-10.1597	-11.2609	-6.59938
219	-9.20578	-10.088	-10.8568	-6.622
218.5	-8.8853	-10.2335	-10.2817	-6.65546
218	-9.00035	-10.1049	-10.3764	-6.85961
217.5	-9.04159	-10.348	-10.0954	-6.68281
217	-8.70108	-10.417	-10.0761	-7.53864
216.5	-8.69465	-10.5753	-9.82295	-7.12017
216	-8.64929	-10.3328	-9.84095	-7.31143
215.5	-8.80848	-10.3972	-9.9995	-7.35695
215	-9.09492	-10.0517	-10.4784	-7.42984
214.5	-9.14068	-10.0696	-10.236	-7.64011
214	-8.81229	-9.942	-10.4302	-8.61876
213.5	-8.49273	-10.1051	-10.1831	-9.5554
213	-8.87822	-9.96997	-9.88182	-9.22262
212.5	-9.02471	-10.1227	-10.0161	-9.27849
212	-9.39241	-10.7104	-10.5289	-8.32793
211.5	-9.05	-11.214	-10.6636	-7.94633
211	-9.5641	-11.7352	-11.9834	-9.10509
210.5	-10.4033	-12.0929	-11.8543	-9.74049
210	-10.7137	-12.9411	-13.2049	-10.925
209.5	-10.9059	-13.4287	-14.4168	-11.986
209	-10.5904	-15.3272	-14.7564	-12.3139
208.5	-12.084	-15.5572	-15.2759	-11.8689
208	-12.9414	-16.3691	-14.6068	-14.4687
207.5	-12.8634	-15.0126	-13.6218	-12.5322
207	-11.8316	-12.6622	-14.2943	-12.4515
206.5	-10.1601	-12.0152	-15.513	-11.6869
206	-11.0989	-13.6521	-16.909	-8.01947
205.5	-12.7212	-14.173	-14.332	-5.08175
205	-17.1824	-14.8544	-11.9609	-13.1118
204.5	-17.9869	-13.9772	-9.24821	-19.0599
204	-15.6523	-9.02301	-5.78196	-14.2589
203.5	-12.1063	-6.77121	-6.60874	-5.85903
203	-0.56768	-2.69875	-2.63217	-10.3949
202.5	0.740984	-6.05711	-0.91243	-12.461
202	6.2623	-12.7403	2.614322	-8.94392
201.5	12.12505	-15.994	-0.6069	-0.73841
201	12.79948	-6.62498	-2.80643	10.57003
200.5	9.623289	7.346095	-6.91616	13.95227
200	-4.98803	16.37745	3.95728	16.8086

Table 6.25 Molar ellipticity of 1 μm , 1C μm , 1A μm particles

Wavelength[nm]	1 μm	1C μm	1A μm
250	-0.60636	-0.94777	-0.71981
249.5	-0.62884	-0.99661	-0.73982
249	-0.8173	-1.06103	-0.69606
248.5	-0.90908	-1.12392	-0.7292
248	-1.04959	-1.11441	-0.78556
247.5	-1.03852	-1.21744	-0.7868
247	-1.0232	-1.3833	-0.78117
246.5	-0.94765	-1.58216	-0.78053
246	-0.99481	-1.76378	-0.75768
245.5	-1.11283	-1.93042	-0.71654
245	-1.2367	-2.02262	-0.71388
244.5	-1.36011	-2.24233	-0.68428
244	-1.40478	-2.36902	-0.7267
243.5	-1.52166	-2.59244	-0.76583
243	-1.6314	-2.79223	-0.67311
242.5	-1.72531	-2.9227	-0.71315
242	-1.79355	-3.12573	-0.65521
241.5	-1.90482	-3.33779	-0.64811
241	-1.96892	-3.65168	-0.68996
240.5	-2.07747	-4.00827	-0.50249
240	-2.15639	-4.17399	-0.44331
239.5	-2.25848	-4.43578	-0.36062
239	-2.39814	-4.65071	-0.26682
238.5	-2.51648	-5.09079	-0.22766
238	-2.73976	-5.50758	-0.07221
237.5	-2.93965	-5.90467	-0.0597
237	-3.09181	-6.27697	-0.02689
236.5	-3.2978	-6.67054	0.083883
236	-3.54395	-7.0714	0.135439
235.5	-3.82057	-7.54721	0.305993
235	-3.96715	-8.06197	0.344537
234.5	-4.20515	-8.64538	0.359691
234	-4.35419	-9.35758	0.3253
233.5	-4.7751	-10.0619	0.286897
233	-5.07372	-10.703	0.215962
232.5	-5.38789	-11.3821	0.289753
232	-5.65227	-12.3193	0.158985
231.5	-5.98631	-13.1841	0.14845
231	-6.33115	-14.0368	0.123574
230.5	-6.73811	-14.8676	0.175932
230	-7.09932	-15.7095	0.276752
229.5	-7.42152	-16.3252	0.312236
229	-7.7202	-17.1507	0.343352
228.5	-7.93339	-17.8669	0.298455
228	-8.31138	-18.6357	0.345796
227.5	-8.56425	-19.3227	0.353857
227	-8.87993	-20.0083	0.323682
226.5	-9.13791	-20.7993	0.263488
226	-9.39125	-21.6721	0.279177
225.5	-9.60644	-22.3873	0.261449

225	-9.70557	-23.0728	0.329057
224.5	-9.73591	-23.6075	0.309655
224	-9.88425	-23.9966	0.339927
223.5	-9.99276	-24.6647	0.360972
223	-10.2234	-25.1739	0.463379
222.5	-10.1631	-25.7317	0.432135
222	-9.69052	-26.2435	0.510483
221.5	-9.3649	-26.6106	0.51559
221	-9.15599	-27.1181	0.474667
220.5	-9.15168	-27.5575	0.635615
220	-9.38193	-27.8189	0.74352
219.5	-9.31561	-28.2418	0.77509
219	-9.20578	-28.4808	0.805424
218.5	-8.8853	-28.543	0.740042
218	-9.00035	-28.9648	0.773931
217.5	-9.04159	-29.4562	0.897443
217	-8.70108	-29.4964	0.862049
216.5	-8.69465	-30.0165	0.69919
216	-8.64929	-29.8677	0.681152
215.5	-8.80848	-30.0669	0.657573
215	-9.09492	-30.9547	0.7046
214.5	-9.14068	-31.5845	0.776432
214	-8.81229	-32.6563	0.537665
213.5	-8.49273	-32.8928	0.590117
213	-8.87822	-32.5784	0.789539
212.5	-9.02471	-32.5302	0.84683
212	-9.39241	-32.3946	0.819787
211.5	-9.05	-33.3795	0.579
211	-9.5641	-33.9458	0.300045
210.5	-10.4033	-34.5335	0.191834
210	-10.7137	-36.1081	0.192351
209.5	-10.9059	-36.0256	0.18899
209	-10.5904	-35.463	0.161538
208.5	-12.084	-33.1974	0.023688
208	-12.9414	-31.9551	0.044085
207.5	-12.8634	-33.338	-0.09274
207	-11.8316	-33.7528	-0.04017
206.5	-10.1601	-29.1097	-4.62E-05
206	-11.0989	-25.9229	0.187752
205.5	-12.7212	-15.5478	0.410833
205	-17.1824	-19.3393	0.457455
204.5	-17.9869	-2.97338	0.289942
204	-15.6523	7.12923	0.336207
203.5	-12.1063	20.4837	0.232803
203	-0.56768	16.38441	0.228037
202.5	0.740984	6.240478	-0.07982
202	6.2623	-3.00833	0.019604
201.5	12.12505	-6.95267	0.42167
201	12.79948	-9.56131	0.863078
200.5	9.623289	-12.6172	1.344352
200	-4.98803	-14.5908	1.635855

Table 6.26 IgA antibody response of different doses of vaccine

Weeks	Dose of vaccine, μg (SD)		
	10 μg	40 μg	80 μg
0	0	0	0
2	77.17(56.05)	61.99(41.45)	92.99(84.58)
4	102.06(22.88)	79.44(45.59)	66.85(10.95)
6	67.04(11.12)	112.47(22.86)	115.82(45.16)
8	ND	ND	ND

Table 6.27 IgA antibody response of different sizes of particles encapsulated vaccine

Weeks	Size of particles encapsulated vaccine, μm (SD)		
	1 μm	5 μm	15 μm
0	0	0	0
2	9.63(58.27)	72.70(49.18)	0
4	31.94(30.56)	89.36(34.59)	10.36(11.92)
6	21.85(36.40)	204.78(237.26)	32.09(9.93)
8	57.21(30.29)	198.91(47.21)	46.38(12.00)

Table 6.28 IgA antibody response of different conjugated particles encapsulated vaccine

Weeks	Conjugated particles encapsulated vaccine, μm (SD)			
	1C*	1A*	PBS	Sc
0	0	0	0	0
2	26.07(10.87)	11.50(79.49)	2.28(25.68)	53.83(30.51)
4	65.77(110.95)	98.15(229.52)	0(13.39)	23.25(10.35)
6	69.36(119.88)	341.47(651.01)	36.57(39.29)	139.53(30.79)
8	328.16(650.92)	114.75(292.96)	ND	ND

1A* : $1\mu\text{m}$ PLGA conjugated 0.75% $\text{Al}(\text{OH})_3$ particle

1C* : $1\mu\text{m}$ PLGA conjugated CS particle

Sc : Subcutaneous injection

Table 6.29 IgG antibody response of different doses of vaccine

Weeks	Dose of vaccine, μg (SD)		
	10 μg	40 μg	80 μg
0	0	0	0
2	514.34(1540.78)	472.09(2723.07)	879.89(2260.16)
4	2368.01(2882.69)	1920.28(2220.97)	2623.92(1785.35)
6	2364.18(3115.94)	3110.68(1197.55)	925.12(960.50)
8	ND	ND	ND

Table 6.30 IgG antibody response of different sizes of particles encapsulated vaccine

Weeks	Size of particles encapsulated vaccine, μm (SD)		
	1 μm	5 μm	15 μm
0	0	0	0
2	2380.36(1292.17)	1213.52(261.39)	577.3(448.56)
4	9130.25(4084.82)	6048.63(4003.74)	3631.24(3940.64)
6	2755.52(1918.64)	5018.66(4990.67)	1348.62(1091.33)
8	16187.89(10684.36)	4804.58(801.81)	4039.67(852.81)

Table 6.31 IgG antibody response of different conjugated particles encapsulated vaccine

Weeks	Conjugated particles encapsulated vaccine, μm (SD)			
	1C*	1A*	PBS	Sc
0	0	0	0	0
2	4537.45(2239.09)	4104.23(5486.33)	618.38(1233.63)	24735.69(0)
4	8171.67(5995.38)	6124.21(4946.59)	468.31(556.08)	50335.69(0)
6	12607.88(8805.69)	12820.12(7785.94)	176.84(832.67)	50335.69(0)
8	19683.42(8226.69)	10313.45(2309.4)	ND	ND

1A* : 1 μm PLGA conjugated 0.75% Al(OH)₃ particle

1C* : 1 μm PLGA conjugated CS particle

Sc : Subcutaneous injection

BIOGRAPHY

Miss Amolnat Tunsirikongkon was born on December 27th, 1979 in Bangkok, Thailand. She received her Bachelor of Science in Pharmacy in 2003 from Faculty of Pharmaceutical Sciences, Chulalongkorn University, Bangkok, Thailand. She also received the Master degree in 2006 from Mahidol University, Bangkok, Thailand in the field of Pharmaceutics. After her graduation in 2006, she has continued her study for the Doctoral degree program at Chulalongkorn University in the field of Pharmaceutics.

