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APPENDICES

APPENDIX A

DETAILS OF LYSOZYME, HYDROGENATED SOYBEAN PHOSPHATIDYLCHOLINE, CHOLESTEROL, D-MANNITOL AND GLYCINE

Lysozyme

Lysozyme, a globular protein, consists of a single chain polypeptide containing 129-amino acid residues divided into two domains, one predominantly α and the other β, cross-linked by four disulfide bridges (Figure 88). The hydrophilic groups tend to concentrate on the surface and the hydrophobic groups in the core (Rosenberger, 1996). The lysozyme may be isolated from a natural source, such as eggs, or synthesized by a bioprocess, such as fermentation.

Figure 88 Structure of lysozyme
(http://users.rcn.com/jkimball.ma.ultranet/BiologyPages/Lysozyme.gif)

Physicochemical Properties of Lysozyme (Product information of Sigma)

Appearance: White to off-white powder

Molecular weight: 14,307 based upon amino acid sequence and 14,400 by sedimentation

Isoelectric point: 11.35

pH optimum: The activity of lysozyme is a function of both pH and ionic strength. Lysozyme is active over a broad pH range (6.0-9.0). At pH 6.2, maximal

activity is observed over a wider range of ionic strengths (0.02-0.100) than at pH 9.2 (0.01-0.06). Sigma determines the activity of this product at pH 6.24.

Salts present: The salt content of this product is approximately 5% which is present as sodium acetate and sodium chloride buffer salts.

Inhibitors: Lysozyme is inhibited by indole derivatives (which bind to and distort the active site) and imidazole (formation of a chargetransfer complex). It is also inhibited by surface-active agents such as sodium dodecyl sulfate, sodium dodecanate, and dodecyl alcohol. Other compounds of these types will inhibit lysozyme provided that the carbon chain present is at least 12 or more carbons in length. Lysozyme is also inhibited by N-acetylglucosamine (NAG) and lactone analogs of peptidoglycan.

Substrates: Lysozyme hydrolyzes the β(1->4) glycosidic bond between N-acetylglucosamine and N-acetylmuramic acid in the polysaccharide backbone of peptidoglycan. It is effective in lysing bacteria by hydrolyzing the peptidoglycan which is present in bacterial cell walls. The substrate used in the Sigma enzyme assay for this product is *Micrococcus luteus* cells (ATCC 4698). Lysozyme will also hydrolyze chitin oligosaccharides.

Solubility / Solution stability: When this product is solubilized at 10 mg/ml in deionized water, a clear to slightly hazy colorless solution is observed. Solutions prepared in this way should be stable for at least 1 month when stored at 2-8 °C. Hen egg white lysozyme solution is inactivated irreversibly by heat treatment at neutral pH.

Unit definition: One unit will produce a ΔA450nm of 0.001 per min at pH 6.24 at 25 °C, using a suspension of *Micrococcus lysodeikticus* as substrate in a 2.6 ml reaction mixture (1 cm light path).

Therapeutic Activity of Lysozyme (Cantor and Shteyngart, 2004; 2008)

Lysozyme was studied for prevention and treatment of respiratory disorders by intratracheal administration either alone or in combination with other therapeutic agents. This may be because of the enhanced binding of lysozyme to elastic fibers in diseases involving damage to elastic fibers which may protect these fibers from

further injury. Applicable respiratory disorders include, but are not limited to, pulmonary emphysema, asthma, bronchitis, pneumonia, respiratory distress syndrome, bronchopulmonary dysplasia, interstitial fibrosis, cystic fibrosis, and neoplasia. The supplementation of known pulmonary therapeutic agents with lysozyme is designed to potentiate the activity of the therapeutic agent(s). For example, lysozyme might be added to an antibiotic to increase its ability to kill bacteria. Likewise, lysozyme might be added to surfactant to prolong its useful effects in the lung and to prevent infection. Administration of lysozyme may be performed by aerosol, which can be generated by a nebulizer or by instillation. The lysozyme may be administered alone or with a carrier such as saline solution, DMSO, and alcohol, or water. It may also be used as a vehicle for the intratracheal administration of drugs or other agents to the lung. The effective daily amount of lysozyme is from about 10 μg/kg to about 1 mg/kg of body weight.

Hydrogenated Soybean Phosphatidylcholine (Phospholipon® 90H)

Phospholipon® 90H (Figure 89) composed of hydrogenated PC 94.9 %, hydrogenated LPC 2.2 % and oil triglyceride 0.8 %. The product is biological decomposable and can be considered as non-toxic for human beings and animals.

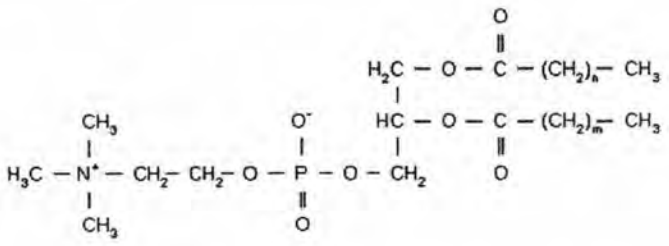

Figure 89 Structural formula of hydrogenated soybean Phosphatidylcholine (http://www.rxlist.com/cgi/generic/doxor.htm)

Properties of Phospholipon® 90H (Product information of Phospholipid GmbH)

Fatty acid composition: Approximately 85 % stearic acid and approximately 15 % palmitic acid

Appearance: White, crystalline powder

Molecular weight: 780

Bulk density: 400-500 kg/m^3

Phase transition temperature in hydrated form: Approximately 54 °C

Solubility: 10 % in chloroform/methanol = 2/1 v/v. Insoluble in triglycerides, alcohols and water. Dispersible in water.

pH: 6 ± 1 at 10 g/L (20 °C)

Storage: Product is hygroscopic. Keep container tightly closed and store in a dry place.

Cholesterol

Cholesterol (Figure 90) is from lanolin. It has a physiological role. It is the major sterol of the higher animals, and it is found in all body tissues, especially in the brain and spinal cord. It is also the main constituent of gallstones (Rowe, Sheskey, and Weller, 2003).

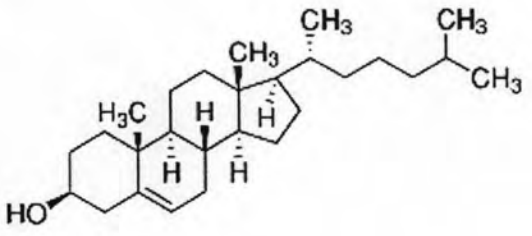

Figure 90 Structural formula of cholesterol
(http://www.sigmaaldrich.com/catalog/search/ProductDetail/FLUKA/26740)

Properties of Cholesterol (Product information of Fluka)

Appearance: White, crystalline powders
Molecular weight: 386.65
Optical activity: [α]20/D −36±2°, 2% in dioxane
Boiling point: 360 °C
Melting point: 147-150 °C
Density: 1.067 g/cm^3 at 25 °C
Solubility: Soluble in acetone, chloroform (1 in 4.5) and vegetable oils
Storage temperature: 2-8 °C, store in a well-closed container and protect from light

D-mannitol

Mannitol (Figure 91) is a non-reducing acyclic sugar commonly used in pharmaceutical formulations. Mannitol is known to exist primarily in 3 main polymorphic forms designated as α, β and δ forms that can be prepared by altering solution conditions and drying rates. The spectral features of these polymorphs have been discussed elsewhere (Burger et al., 2000). The commercially available mannitol mainly comes as the β-polymorph, which is also the thermodynamically stable form of mannitol at room temperature.

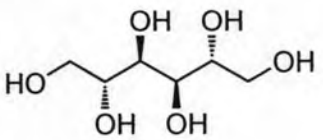

Figure 91 Structural formula of D-mannitol
(http://commons.wikimedia.org/wiki/Image:D-Mannitol_structure.svg)

Properties of D-mannitol (Rowe et al., 2003)

Appearance: White, crystalline powders
Molecular weight: 182.17
Melting point: 166-168 °C

Density (bulk): 0.430 g/ cm^3 for powder

Density (tapped): 0.734 g/ cm^3 for powder

Density (true): 1.514 g/ cm^3 for powder

Dissociation constant: pK_a = 13.5 at 18 °C

Flash point: 150 °C

Refractive index: 1.333

Solubility: Soluble in alkalis, ethanol 95 % (1:83), propan-2-ol (1:100), water (1:5.5); Practically insoluble in ether

Storage temperature: Store in a well-closed container in a cool and dry place

Glycine

Glycine (Figure 92) is one of polar and uncharged amino acids. It is the simplest amino acid. It has acid group as well as amino group which both groups act as a base. It is not optically active. It is nonessential amino acids for mammals.

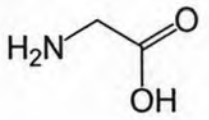

Figure 92 Structural formula of glycine
(http://commons.wikimedia.org/wiki/Image:Glycine.png)

Properties of Glycine (Maryadele et al., 2006)

Appearance: White, crystalline powders

Molecular weight: 75.07

Melting point: 245 °C (decompose)

Density (bulk): 1.6 g/ cm^3

Flash point: 145 °C

Solubility: Soluble in water (1:4), very slightly soluble in alcohol

Isoelectric point: 5.97

Storage temperature: Stored in a well-closed container in a cool, dry place

APPENDIX B

DATA OF CHARACTERIZATION OF THE SPRAY-DRIED POWDERS AND THE RECONSTITUTED LIPOSOMES

Table 66 Hydrolysis stability of hydrogenated phosphatidylcholine in various formulations

Experiment	Hydrolysis (%)					
	n1	n2	n3	n4	Mean	SD
HPC starting material (1)	4.09	4.17	3.94	3.89	4.02	0.13
HPC liposomes (2)	3.86	4.01	3.90	3.98	3.94	0.07
HPC/M 10:0 (3)	4.08	4.31	4.40	4.07	4.21	0.17
HPC/M 5:5 (4)	3.94	3.97	4.03	3.76	3.93	0.12

Table 67 Test of hydrolysis stability of hydrogenated phosphatidylcholine in various formulations

Test of Homogeneity of Variances

Hydrolysis

Levene Statistic	df1	df2	*P*-Value
2.586	3	12	0.102

ANOVA

Hydrolysis

Source of Variation	df	Sum of Squares	Mean Square	*F*	*P*-Value
Between Groups	3	0.215	0.072	4.572	0.023
Within Groups	12	0.188	0.016		
Total	15	0.403			

Table 67 (Continued)

Multiple Comparisons

Dependent Variable: Hydrolysis
Tukey HSD

(I) x	(J) x	Mean Difference (I-J)	SE	*P*-Value	95% Confidence Interval	
					Lower Bound	Upper Bound
1	2	0.08500	0.08854	0.774	-0.1779	0.3479
	3	-0.19250	0.08854	0.186	-0.4554	0.0704
	4	0.09750	0.08854	0.696	-0.1654	0.3604
2	3	-0.27750*	0.08854	0.038	-0.5404	-0.0146
	4	0.01250	0.08854	0.999	-0.2504	0.2754
3	4	0.29000*	0.08854	0.029	0.0271	0.5529

* The mean difference is significant at the 0.05 level.

Tukey HSD

x	N	Subset for alpha = 0.05	
		1	2
4	4	3.9250	
2	4	3.9375	
1	4	4.0225	4.0225
3	4		4.2150
P-Value		0.696	0.186

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

Table 68 Oxidation stability of hydrogenated phosphatidylcholine in various formulations

Experiment	Oxidation index					
	n1	**n2**	**n3**	**n4**	**Mean**	**SD**
HPC starting material	0.178	0.175	0.129	0.235	0.179	0.043
HPC liposomes	0.110	0.184	0.116	0.150	0.140	0.034
HPC/M 10:0	0.203	0.179	0.175	0.180	0.184	0.013
HPC/M 5:5	0.139	0.112	0.177	0.160	0.147	0.028

Table 69 Test of oxidation stability of hydrogenated phosphatidylcholine in various formulations

Test of Homogeneity of Variances

Oxidation

Levene Statistic	df1	df2	*P*-Value
0.922	3	12	0.460

ANOVA

Oxidation

Source of Variation	df	Sum of Squares	Mean Square	*F*	*P*-Value
Between Groups	3	0.006	0.002	1.999	0.168
Within Groups	12	0.012	0.001		
Total	15	0.018			

Table 70 Moisture content of the spray-dried powders prepared with different formulations

Formulation	Moisture content (%w/w)				
	n1	n2	n3	Mean	SD
HPC/M 10:0	6.37	7.07	6.59	6.68	0.36
HPC/M 9:1	3.47	3.81	3.69	3.65	0.17
HPC/M 7:3	4.06	3.51	3.63	3.73	0.29
HPC/M 5:5	4.10	3.67	3.74	3.84	0.23
HPC/M 3:7	1.76	1.79	1.57	1.71	0.12
HPC/M 1:9	0.76	0.83	0.80	0.80	0.03
HPC/M 0:10	0.40	0.42	0.34	0.39	0.04
HPC/M/G0.5	3.73	3.15	3.44	3.44	0.29
HPC/M/G1	3.03	3.09	3.66	3.26	0.35
HPC/M/G5	3.10	3.27	3.36	3.24	0.14
HPC/M/G10	2.82	2.77	2.80	2.80	0.03
Spray-dried LSZ	12.47	13.92	15.30	13.90	1.41
M/L	0.90	0.89	0.92	0.90	0.01
HPC/M/L	3.82	3.91	3.81	3.85	0.06
HPC/Chol9:1/M/L	3.67	4.13	3.54	3.78	0.31
HPC/Chol8:2/M/L	4.23	4.09	3.90	4.07	0.16
HPC/Chol7:3/M/L	3.01	3.01	2.70	2.91	0.18
DRV HPC	4.43	4.21	4.03	4.22	0.20
DRV HPC in M	3.98	3.84	3.38	3.73	0.32
DRV HPC/Chol8:2	5.06	4.98	4.73	4.92	0.17
DRV HPC/Chol8:2 in M	4.08	4.19	4.66	4.31	0.31

Table 71 Size characterization of the spray-dried powders prepared with different formulations

Formulation	$D_{0.1}$ (μm)	$D_{0.5}$ (μm)	$D_{0.9}$ (μm)	$D_{[4,3]}$ (μm)	Span
HPC/M 5:5	3.86	7.70	12.31	7.89	1.10
	3.93	7.78	12.36	7.96	1.09
	3.94	7.80	12.38	7.98	1.08
Average	3.91	7.76	12.35	7.94	1.09
SD	0.04	0.05	0.04	0.05	0.01
HPC/M 7:3	3.58	8.58	18.67	9.97	1.76
	3.45	8.26	18.06	9.63	1.77
	3.52	8.5	18.58	9.89	1.77
Average	3.52	8.45	18.44	9.83	1.77
SD	0.07	0.17	0.33	0.18	0.01
HPC/M 1:9	0.14	6.56	17.13	7.55	2.60
	0.14	6.61	17.27	7.61	2.60
	0.14	6.59	17.31	7.61	2.60
Average	0.14	6.59	17.24	7.59	2.60
SD	0.00	0.03	0.09	0.04	0.01
HPC/M 0:10	0.12	0.68	11.51	3.92	16.80
	0.12	0.66	11.29	3.84	16.94
	0.12	0.63	11.52	3.89	18.04
Average	0.12	0.66	11.44	3.88	17.26
SD	0.00	0.03	0.13	0.04	0.68
HPC/M/L	3.88	7.89	12.79	8.08	1.13
	3.90	7.91	12.79	8.10	1.12
	3.89	7.91	12.80	8.10	1.13
Average	3.89	7.90	12.79	8.09	1.13
SD	0.01	0.01	0.01	0.01	0.00
HPC/Chol8:2/M/L	2.34	6.38	12.96	7.10	1.66
	2.27	6.29	12.90	7.03	1.69
	2.35	6.41	13.05	7.14	1.67
Average	2.32	6.36	12.97	7.09	1.67
SD	0.04	0.06	0.08	0.06	0.01

Table 72 Entrapment efficiency of the reconstituted liposomes from the spray-dried lysozyme-loaded liposomal powders with various formulations in HBS at 37 °C

Formulation	Entrapment efficiency (μg LSZ/ mg lipid)					
	n1	n2	n3	n4	Mean	SD
HPC/M/L	6.71	6.91	6.39	6.94	6.74	0.25
HPC/Chol9:1/M/L	4.14	4.63	4.92	4.70	4.60	0.33
HPC/Chol8:2/M/L	16.41	15.77	13.36	13.95	14.81	1.36
HPC/Chol7:3/M/L	27.66	27.64	28.35	27.87	27.88	0.33
DRV HPC	6.69	6.45	5.65	7.46	6.56	0.75
DRV HPC in M	5.90	5.06	5.65	5.66	5.57	0.36
DRV HPC/Chol8:2	14.81	12.13	11.03	13.06	12.76	1.60
DRV HPC/Chol8:2 in M	11.59	13.35	11.99	11.62	12.14	0.82

Table 73 Entrapment efficiency of the reconstituted liposomes from the spray-dried lysozyme-loaded liposomal powders with HSPC and HSPC/Chol (8:2) in HBS at 60 °C

Formulation	Entrapment efficiency (μg LSZ/ mg lipid)					
	n1	n2	n3	n4	Mean	SD
HPC/M/L	38.64	36.92	37.03	37.14	37.43	0.81
HPC/Chol8:2/M/L	19.34	19.87	18.73	18.08	19.00	0.77

Table 74 Process yield of the spray-dried lysozyme-loaded liposomal powders for the central composite design

Exp.	Factor			Yield (%) in		Total yield (%)
	T (°C)	P (%)	C (%w/w)	Collector	Cyclone	
1	110	5	2.975	29.55	32.16	61.71
2	150	5	2.975	60.43	16.26	76.68
3	110	15	2.975	24.12	32.14	56.26
4	150	15	2.975	67.03	4.73	71.77
5	110	5	8.575	30.51	31.56	62.06
6	150	5	8.575	47.92	18.12	66.04
7	110	15	8.575	39.25	17.04	56.29
8	150	15	8.575	54.76	6.74	61.5
9	130	10	5.775	49.26	16.33	65.59
10	130	10	5.775	49.63	15.50	65.12
11	130	10	5.775	47.24	17.34	64.58
12	96	10	5.775	18.82	32.36	51.18
13	164	10	5.775	58.50	7.15	65.65
14	130	2	5.775	47.64	28.00	75.47
15	130	18	5.775	61.36	9.60	70.97
16	130	10	1.05	44.68	16.06	60.75
17	130	10	10.5	55.38	5.77	61.16

Table 75 Moisture content of the spray-dried lysozyme-loaded liposomal powders for the central composite design

Exp.	Factor			Moisture content (%w/w)				
	T (°C)	P (%)	C (%w/w)	n1	n2	n3	Mean	SD
1	110	5	2.975	3.58	3.36	3.44	3.46	0.11
2	150	5	2.975	3.48	3.33	3.35	3.39	0.08
3	110	15	2.975	3.17	3.08	3.12	3.12	0.05
4	150	15	2.975	3.03	3.04	3.08	3.05	0.03
5	110	5	8.575	3.28	3.31	3.29	3.29	0.02
6	150	5	8.575	2.59	2.45	2.26	2.43	0.17
7	110	15	8.575	3.47	3.36	3.38	3.40	0.06
8	150	15	8.575	3.62	3.63	3.64	3.63	0.01
9	130	10	5.775	2.94	2.91	3.18	3.01	0.15
10	130	10	5.775	2.81	3.04	3.15	3.00	0.17
11	130	10	5.775	2.93	2.79	3.16	2.96	0.19
12	96	10	5.775	4.39	4.12	4.03	4.18	0.19
13	164	10	5.775	3.01	2.92	2.98	2.97	0.05
14	130	2	5.775	2.76	2.69	2.86	2.77	0.09
15	130	18	5.775	3.21	3.35	3.27	3.28	0.07
16	130	10	1.05	3.11	3.00	3.30	3.14	0.15
17	130	10	10.5	3.26	3.16	3.14	3.19	0.06

Table 76 Size characterization of the spray-dried lysozyme-loaded liposomal powders for the central composite design

Experiment	$D_{0.1}$ (μm)	$D_{0.5}$ (μm)	$D_{0.9}$ (μm)	$D_{[4,3]}$ (μm)	Span
1	2.34	6.12	12.50	6.83	1.66
	2.31	6.07	12.47	6.79	1.67
	2.40	6.21	12.65	6.93	1.65
Average	2.35	6.13	12.54	6.85	1.66
SD	0.05	0.07	0.10	0.07	0.01
2	2.59	8.30	16.96	9.13	1.73
	2.67	8.40	17.03	9.21	1.71
	2.64	8.35	16.99	9.17	1.72
Average	2.63	8.35	16.99	9.17	1.72
SD	0.04	0.05	0.04	0.04	0.01
3	2.42	6.05	12.11	6.72	1.60
	2.41	5.99	12.02	6.67	1.60
	2.43	6.01	12.02	6.68	1.60
Average	2.42	6.02	12.05	6.69	1.60
SD	0.01	0.03	0.05	0.03	0.00
4	2.56	8.02	16.33	8.83	1.72
	2.58	8.03	16.33	8.84	1.71
	2.65	8.09	16.36	8.90	1.69
Average	2.60	8.05	16.34	8.86	1.71
SD	0.05	0.04	0.02	0.04	0.01
5	2.50	7.54	17.66	8.95	2.01
	2.58	7.81	18.16	9.22	1.99
	2.58	7.83	18.27	9.26	2.00
Average	2.55	7.73	18.03	9.14	2.00
SD	0.05	0.16	0.33	0.17	0.01
6	2.77	9.28	19.36	10.28	1.79
	2.63	9.06	19.19	10.10	1.83
	2.73	9.25	19.38	10.26	1.80
Average	2.71	9.20	19.31	10.21	1.81
SD	0.07	0.12	0.10	0.10	0.02

Table 76 (Continued)

Experiment	$D_{0.1}$ (μm)	$D_{0.5}$ (μm)	$D_{0.9}$ (μm)	$D_{[4,3]}$ (μm)	Span
7	2.80	8.65	18.16	9.69	1.78
	2.74	8.51	18.00	9.56	1.79
	2.84	8.66	18.13	9.69	1.77
Average	2.79	8.61	18.10	9.65	1.78
SD	0.05	0.08	0.09	0.08	0.01
8	2.70	9.79	19.94	10.66	1.76
	2.69	9.82	19.97	10.68	1.76
	2.68	9.81	19.99	10.67	1.76
Average	2.69	9.81	19.97	10.67	1.76
SD	0.01	0.02	0.03	0.01	0.00
9	2.97	8.39	17.01	9.27	1.67
	3.04	8.51	17.19	9.39	1.66
	3.05	8.52	17.19	9.39	1.66
Average	3.02	8.47	17.13	9.35	1.67
SD	0.04	0.07	0.10	0.07	0.01
10	2.83	8.33	17.23	9.30	1.73
	2.91	8.48	17.36	9.42	1.70
	2.80	8.37	17.32	9.33	1.73
Average	2.85	8.39	17.30	9.35	1.72
SD	0.06	0.08	0.07	0.06	0.02
11	2.92	8.63	17.71	9.59	1.71
	2.98	8.72	17.81	9.67	1.70
	3.02	8.81	17.91	9.75	1.69
Average	2.97	8.72	17.81	9.67	1.70
SD	0.05	0.09	0.10	0.08	0.01
12	2.08	4.76	9.30	5.26	1.52
	1.97	4.63	9.15	5.13	1.55
	2.03	4.69	9.22	5.19	1.53
Average	2.03	4.69	9.22	5.19	1.53
SD	0.06	0.07	0.08	0.07	0.02

Table 76 (Continued)

Experiment	$D_{0.1}$ (μm)	$D_{0.5}$ (μm)	$D_{0.9}$ (μm)	$D_{[4,3]}$ (μm)	Span
13	2.84	8.58	17.00	9.35	1.65
	2.87	8.62	17.03	9.39	1.64
	2.83	8.51	16.95	9.30	1.66
Average	2.85	8.57	16.99	9.35	1.65
SD	0.02	0.06	0.04	0.05	0.01
14	2.88	8.66	17.61	9.59	1.70
	2.93	8.86	17.83	9.76	1.68
	2.80	8.57	17.47	9.47	1.71
Average	2.87	8.70	17.64	9.61	1.70
SD	0.07	0.15	0.18	0.15	0.02
15	2.61	8.50	17.43	9.37	1.74
	2.66	8.57	17.49	9.43	1.73
	2.71	8.63	17.57	9.50	1.72
Average	2.66	8.57	17.50	9.43	1.73
SD	0.05	0.07	0.07	0.07	0.01
16	2.25	6.13	12.19	6.74	1.62
	2.27	6.15	12.19	6.75	1.61
	2.33	6.23	12.28	6.83	1.60
Average	2.28	6.17	12.22	6.77	1.61
SD	0.04	0.05	0.05	0.05	0.01
17	3.12	10.05	20.78	11.14	1.76
	3.20	10.34	21.15	11.40	1.74
	3.08	10.13	20.95	11.21	1.76
Average	3.13	10.17	20.96	11.25	1.75
SD	0.06	0.15	0.19	0.13	0.01

Table 77 Entrapment efficiency of the reconstituted liposomes from the spray-dried lysozyme-loaded liposomal powders in HBS at 37 °C for the central composite design

Experiment	Entrapment efficiency (μg LSZ/ mg lipid)					
	n1	n2	n3	n4	Mean	SD
1	14.27	13.15	11.90	14.36	13.42	1.15
2	8.09	7.30	7.52	7.65	7.64	0.33
3	15.34	12.65	18.02	17.39	15.85	2.42
4	11.14	9.81	11.69	10.89	10.88	0.79
5	14.96	15.16	12.76	14.29	14.29	1.09
6	9.54	8.95	8.52	9.00	9.00	0.42
7	14.31	15.01	14.51	16.37	15.05	0.93
8	11.11	8.58	10.06	9.94	9.92	1.04
9	13.46	13.69	14.14	13.91	13.80	0.29
10	12.27	11.36	13.63	13.55	12.70	1.09
11	15.54	12.99	12.46	12.73	13.43	1.42
12	15.93	16.15	16.60	15.41	16.02	0.49
13	6.11	6.18	7.85	8.36	7.13	1.15
14	8.79	9.45	9.05	9.05	9.09	0.27
15	13.64	11.49	10.51	12.30	11.98	1.32
16	14.87	14.30	14.60	14.34	14.53	0.27
17	13.34	13.22	15.26	15.38	14.30	1.18

Table 78 Properties of the spray-dried lysozyme-loaded liposomal powders prepared with optimized condition no. 1 (T = 110 °C, P = 5 % and C = 2.975 %w/w)

Properties	n	Batch 1	Batch 2	Batch 3
EE (μg LSZ/ mg lipid)	1	14.25	13.71	14.26
	2	14.07	13.55	13.12
	3	12.19	14.17	14.66
	4	15.53	14.01	12.49
	Mean	14.01	13.86	13.63
	SD	1.37	0.28	1.00
Moisture content (%w/w)	1	3.27	3.15	3.16
	2	3.16	3.27	3.24
	3	3.25	3.19	3.09
	Mean	3.23	3.20	3.16
	SD	0.06	0.06	0.08
Remaining activity of LSZ (%) (n = 4)	1	94.78	96.05	97.96
	2	111.07	101.41	104.61
	3	98.77	107.22	100.28
	4	104.35	100.54	109.38
	Mean	102.24	101.30	103.06
	SD	7.08	4.59	5.03

Table 79 Size characterization of the spray-dried lysozyme-loaded liposomal powders prepared with optimized condition no. 1

Batch	$D_{0.1}$ (μm)	$D_{0.5}$ (μm)	$D_{0.9}$ (μm)	$D_{[4,3]}$ (μm)	Span
1	2.01	6.15	13.20	6.97	1.82
	2.01	6.13	13.15	6.95	1.82
	2.00	6.12	13.14	6.94	1.82
Average	2.01	6.13	13.16	6.95	1.82
SD	0.01	0.02	0.03	0.02	0.00
2	1.91	6.15	13.20	6.94	1.84
	1.91	6.13	13.17	6.93	1.84
	1.91	6.16	13.21	6.95	1.83
Average	1.91	6.15	13.19	6.94	1.84
SD	0.00	0.02	0.02	0.01	0.00
3	1.87	5.71	12.28	6.46	1.82
	1.88	5.71	12.29	6.46	1.82
	1.87	5.70	12.27	6.45	1.82
Average	1.87	5.71	12.28	6.46	1.82
SD	0.01	0.01	0.01	0.01	0.00

Table 80 Size characterization of the reconstituted liposomes from the spray-dried lysozyme-loaded liposomal powders prepared with optimized condition no. 1

Batch	$D_{0.1}$ (μm)	$D_{0.5}$ (μm)	$D_{0.9}$ (μm)	$D_{[4,3]}$ (μm)	Span
1	2.54	4.28	7.20	4.64	1.09
	2.55	4.29	7.19	4.64	1.08
	2.56	4.30	7.19	4.64	1.08
Average	2.55	4.29	7.19	4.64	1.08
SD	0.01	0.01	0.01	0.00	0.00
2	2.56	4.36	7.43	4.74	1.12
	2.57	4.36	7.41	4.74	1.11
	2.57	4.36	7.41	4.74	1.11
Average	2.57	4.36	7.41	4.74	1.11
SD	0.01	0.00	0.01	0.00	0.00
3	2.55	4.32	7.30	4.68	1.10
	2.56	4.32	7.28	4.68	1.09
	2.56	4.33	7.27	4.68	1.09
Average	2.56	4.32	7.28	4.68	1.09
SD	0.01	0.00	0.01	0.00	0.00

VITA

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