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APPENDICES

Appendix A

Details of Statistical Analysis of Model Equations

Response: Size

Source	Sum of Squares	DF	Mean Square	F-value	Prob>F
Mean	4.52E+005	1	4.52E+005		
Linear	1,603.09	3	534.36	4.06	0.0285
2FI	975.78	3	325.26	4.14	0.0343
Quadratic	788.48	3	262.83	27.54	0.0001
Cubic	15.94	4	3.99	0.26	0.8874
Residual	60.40	4	15.10		
Total	4.56E+005	18	25308.46		

***p*-value of Regression Coefficient (α=0.05)**

Regression Coefficient	*p*-value
a	<0.0001
b	<0.0001
c	0.0013
a^2	0.0208
b^2	0.0002
c^2	0.0106
ab	0.0001
bc	<0.0001

Response: PI

Source	Sum of Squares	DF	Mean Square	F-value	Prob>F
Mean	1.36	1	1.36		
Linear	4.03E-003	3	1.34E-003	0.77	0.5281
2FI	0.011	3	3.54E-003	2.84	0.0867
Quadratic	0.012	3	4.07E-003	21.49	0.0003
Cubic	2.17E-004	4	5.42E-005	0.17	0.9443
Residual	1.30E-003	4	3.24E-004		
Total	1.39	18	0.08		

***p*-value of Regression Coefficient (α=0.05)**

Regression Coefficient	*p*-value
a	0.0478
b	0.0019
c	0.0498
a^2	0.0415
b^2	<0.0001
c^2	0.0118
ab	0.0088
ac	0.0008
bc	0.0031

Response: Zeta potential

Source	Sum of Squares	DF	Mean Square	F-value	Prob>F
Mean	37753.86	1	37753.86		
Linear	2649.08	3	883.03	10.05	0.0009
2FI	534.33	3	178.11	2.82	0.0884
Quadratic	691.94	3	230.65	561.23	<0.0001
Cubic	0.46	4	0.11	0.16	0.9477
Residual	2.83	4	0.71		
Total	41632.49	18	2312.92		

***p*-value of Regression Coefficient (α=0.05)**

Regression Coefficient	*p*-value
a	<0.0001
b	<0.0001
c	0.0040
a^2	0.0020
b^2	<0.0001
c^2	<0.0001
ab	<0.0001
ac	<0.0001
bc	0.0002

Appendix B

Ingredients of Reagents for ELISA

0.05M Carbonate-bicarbonate buffer (pH 9.6)

Sodium carbonate	0.8 g
Sodium hydrogen carbonate	1.5 g
Purified water added up to make	500 ml

Phosphate buffer saline pH 7.4 with 0.05% Tween 20 (PBS-T)

Sodium chloride	8.0 g
Potassium dihydrogen phosphate	0.2 g
Disodium hydrogen phosphate	2.9 g
Potassium chloride	0.2 g
Thimerosol	0.1 g
Tween 20	0.5 ml
Purified water added up to make	1,000 ml

3% Gelatin in PBS-T

Gelatin	3.0 g
PBS-T added up to make	100 ml

Citrate-phosphate buffer pH 5.0

Citric acid (monohydrate)	10.30 g
Sodium hydrogen phosphate	18.16 g
30% Hydrogen peroxide	1.00 g
Purified water added up to make	1,000 ml

1% Gelatin in PBS-T

Gelatin	1.0 g
PBS-T added up to make	100 ml

4 N Sulfuric acid

98% Sulfuric acid	54.4 g
Purified water added up to make	500 ml

Appendix C

Physical Stability Study of SLN and CSN

Particle size (nm) of SLN and CSN during 60-day storage (mean±SD)

Formulation	Day 1	Day 7	Day 14	Day 30	Day 60
SLN9	160±2	163±3	163±2	165±1	167±4
SLN10	179±1	179±3	179±3	178±2	180±4
SLN11	142±1	142±4	142±3	148±2	153±3
SLN12	163±1	163±2	163±2	163±3	165±4
SLN13	161±1	162±2	163±4	163±3	164±3
SLN14	154±2	154±2	154±2	154±1	156±5
SLN15	166±1	166±2	166±1	165±2	168±2
CSN2	258±1	259±1	259±1	261±1	269±1

PI of SLN and CSN during 60-day storage (mean±SD)

Formulation	Day 1	Day 7	Day 14	Day 30	Day 60
SLN9	0.27±0.01	0.27±0.01	0.27±0.00	0.28±0.01	0.28±0.01
SLN10	0.24±0.01	0.25±0.00	0.25±0.00	0.26±0.01	0.25±0.01
SLN11	0.32±0.01	0.32±0.00	0.33±0.00	0.32±0.00	0.33±0.01
SLN12	0.36±0.01	0.36±0.00	0.37±0.01	0.37±0.01	0.37±0.00
SLN13	0.25±0.01	0.26±0.01	0.26±0.00	0.26±0.00	0.26±0.01
SLN14	0.24±0.00	0.25±0.00	0.25±0.01	0.25±0.00	0.25±0.01
SLN15	0.24±0.00	0.25±0.00	0.25±0.00	0.25±0.00	0.25±0.00
CSN2	0.28±0.00	0.29±0.00	0.29±0.00	0.30±0.01	0.35±0.01

Zeta potential (mV) of SLN and CSN during 60-day storage (mean±SD)

Formulation	Day 1	Day 7	Day 14	Day 30	Day 60
SLN9	58.21±1	59.01±0	58.33±1	59.34±0	54.44±1
SLN10	48.56±1	48.77±1	48.66±1	49.12±1	49.89±1
SLN11	21.51±0	22.55±0	21.78±1	19.81±0	19.49±0
SLN12	52.31±1	52.55±1	52.87±1	53.01±1	53.12±0
SLN13	55.72±1	55.82±2	56.12±1	56.39±0	57.31±1
SLN14	53.98±1	53.88±1	52.67±0	53.81±0	53.69±1
SLN15	50.12±1	50.18±1	50.33±1	51.21±0	51.11±0
CSN2	28.25±0	27.70±1	27.31±1	25.12±0	21.67±0

Appendix D

Powder X-Ray Diffraction Pattern of Bulk Materials, SLN10, SLN12, and SLN14

Cetylpalmitate

Angle (2θ°)	d-value (Å)	Intensity count	% Intensity
6.04	14.61	57.6	23.9
6.80	12.99	241.0	100.0
10.12	8.73	17.4	7.2
11.40	7.76	91.6	38.0
15.92	5.56	19.5	8.1
21.65	4.10	15.1	6.3
23.84	3.73	14.3	5.9
39.32	2.29	32.7	13.6
41.73	2.16	16.1	6.7

DDAB

Angle (2θ°)	d-value (Å)	Intensity count	% Intensity
7.35	12.01	1308.0	89.3
11.07	7.99	1374.0	93.8
14.83	5.97	1465.0	100.0

Cholesterol

Angle (2θ°)	d-value (Å)	Intensity count	% Intensity
5.13	17.23	25549.0	100.0
6.18	14.29	8287.0	32.4
6.96	12.70	3075.0	12.0
7.58	11.65	3756.0	14.7
8.49	10.40	2578.0	10.1
8.95	9.87	2276.0	8.9
9.39	9.41	4374.0	17.1
10.45	8.46	7190.0	28.1
11.16	7.92	4375.0	17.1
11.55	7.66	4389.0	17.2
12.47	7.09	6535.0	25.6
13.00	6.81	7887.0	30.9
14.03	6.31	17946.0	70.2
15.25	5.80	24973.0	97.7
16.05	5.52	6838.0	26.8
16.80	5.27	20444.0	80.0
17.21	5.15	18737.0	73.3
17.99	4.93	17517.0	68.6
18.77	4.72	6142.0	24.0
19.31	4.59	5735.0	22.4
20.07	4.42	3132.0	12.3
20.50	4.33	3792.0	14.8
21.13	4.20	4970.0	19.5
21.48	4.13	4149.0	16.2
22.14	4.01	3294.0	12.9
23.45	3.79	3414.0	13.4
24.75	3.59	2742.0	10.7
25.14	3.54	3093.0	12.1

Cholesterol (continued)

Angle (2θ°)	d-value (Å)	Intensity count	% Intensity
26.20	3.40	3010.0	11.8
28.18	3.16	2032.0	8.0
30.36	2.94	1794.0	7.0
32.56	2.75	1626.0	6.4
33.57	2.67	1504.0	5.9
34.18	2.62	1543.0	6.0
39.79	2.26	1544.0	6.0
42.27	2.14	1707.0	6.7

SLN10

Angle (2θ°)	d-value (Å)	Intensity count	% Intensity
6.08	14.52	30.6	6.4
6.80	12.99	476.0	100.0
11.35	7.79	131.0	27.5
15.92	5.56	40.9	8.6
39.32	2.29	32.3	6.8
41.72	2.16	25.7	5.4

SLN12

Angle (2θ°)	d-value (Å)	Intensity count	% Intensity
6.08	14.53	37.3	8.2
6.80	12.99	457.0	100.0
11.32	7.81	118.0	25.9
15.92	5.56	37.0	8.1
39.39	2.29	30.5	6.7
41.72	2.16	19.4	4.3

SLN14

Angle (2θ°)	d-value (Å)	Intensity count	% Intensity
6.05	14.60	55.9	8.8
6.80	12.99	637.0	100.0
11.32	7.81	202.0	31.6
15.91	5.57	56.1	8.8
39.32	2.29	57.4	9.0
41.72	2.16	29.4	4.6

Appendix E

In Vitro Cytotoxicity Test

%Cell viability of HeLa cells at varying concentrations of SLN (mean±SD)

Concentration of SLN (μg/ml)	SLN13	SLN14	SLN15
100	98.03±1.42	98.13±1.88	95.50±3.93
200	74.33±3.87	77.52±6.60	73.54±5.09
300	12.51±2.44	14.03±1.13	13.04±1.00
400	8.38±1.06	9.00±0.90	8.52±0.73
500	1.20±0.14	1.12±0.14	1.31±0.38
1,000	0.86±0.08	0.99±0.11	1.01±0.09
5,000	0.63±0.05	0.86±0.23	0.83±0.10
10,000	0.44±0.07	0.84±0.13	0.84±0.10

%Cell viability of HeLa cells at varying concentrations of DDAB (mean±SD)

Concentration of DDAB (concentration of SLN) (μg/ml)	% Cell viability
0.64 (100)	99.13±2.22
1.28 (200)	80.13±6.27
1.92 (300)	44.54±4.93
2.56 (400)	29.48±7.03
3.2 (500)	25.33±3.63
6.4 (1,000)	20.08±5.31
32 (5,000)	9.50±2.75
64 (10,000)	5.02±1.37

%Cell viability of HeLa cells at varying concentrations of CSN (mean±SD)

Concentration of CSN (μg/ml)	% Cell viability
100	100.62±6.72
500	100.60±5.00
1,000	98.83±4.69
5,000	98.54±4.19
10,000	97.36±6.01

Appendix F

Mice Immunization Study

Mean IgG titers against gag protein in mice serum determined at day 6 after the second and the third pHIS-HIV-hugag immunization (mean±SD)

Formulation	2nd immunization		3rd immunization	
	Intradermal injection	Topical application	Intradermal injection	Topical application
DOPE-SLN-pHIS-HIV-hugag	700±273.86	82±40.25	900±223.61	340±219.09
Chol-SLN-pHIS-HIV-hugag	520±319.37	64±49.30	700±273.86	180±178.89
CSN-pHIS-HIV-hugag	260±219.09	46±49.30	320±249.00	80±44.7
Naked-pHIS-HIV-hugag	28±40.25	8±4.47	82±40.25	26±41.59

VITA

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He worked as a Pharmacist in Dispensing Unit in 1998 and a Head of Pharmacy Compounding Unit, Department of Pharmacy, Thammasart Chalermprakiat Hospital, Thammasart University in 1999-2004. He has been working as a Lecturer in Department of Pharmaceutical Technology, Faculty of Pharmacy, Rangsit University since 2004.