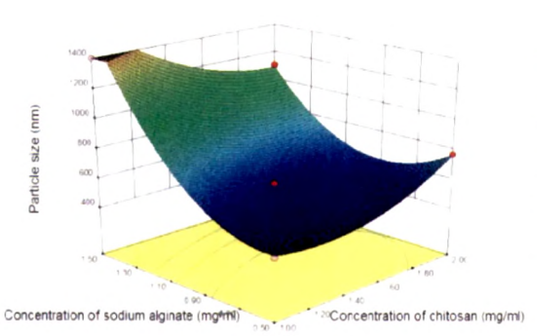
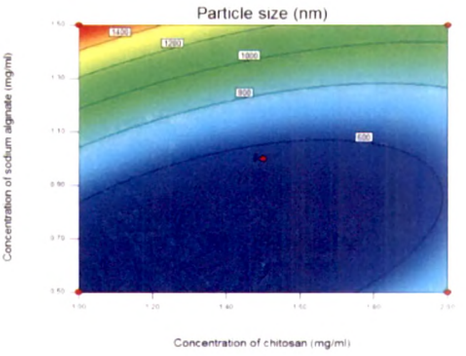
Chapter 5

Preparation and Optimization of Chitosan and Modified chitosan Nanoparticles





5.1. Introduction

Nanotechnology is an area of science devoted to the manipulation of atoms and molecules leading to the construction of structures in the nanometer scale size range (100nm to 1000nm), which retain unique properties and carrying drug in encapsulated or adsorbed form are a promising tool for the site specific and enhanced drug delivery. Polymeric nanoparticles in particulate systems have certain advantages over lipid-based formulations.

a. Possible drug targeting and controlled delivery
b. Increased drug stability
c. Biodegradability and Biocompatibility
d. Avoidance of use of organic solvents during manufacturing
e. Easy large scale production,
f. Non toxic.

Intranasal mucoadhesive nanoparticulate systems improve mucosal absorption, as they strongly attach to the mucosa and increase the viscosity of mucin. Among the various bioadhesive materials that have been proposed for nasal delivery of drugs such as chitosan, trimethyl chitosan and thiolated chitosan have received particular interest. However, a more systematic method for preparation of nanoparticles is being enabled by the application of Ionotropic gelation method. (Worawan et al., 2008, Dong-Won et al., 2006, Carmen et al., 2009).

Table: 5.1. Materials and Equipments

Material	Source
Water (distilled)	Prepared in laboratory by distillation
Chitosan low molecular weight and medium molecular weight	Sigma Aldrich, Bangalore, India
Sodium alginate	National chemical, Mumbai, India
Sodium deoxycholate	Sigma Aldrich, Bangalore, India
Glacial acetic acid, sodium hydroxide, hydrochloric acid	S.D. Fine chemicals, Mumbai, India
Tizanidine HCl	Gift sample from Endoc Pharma, Rajkot, India
Cyclobenzaprine HCl	Gift sample from Ranbaxy, Gurgaon, India
Sodium iodide and Methyl iodide	Spectrochem, Mumbai, India

Thioglycollic acid and Acetonitrile (HPLC grade)	Merck, Mumbai, India
Equipments	**Make**
Calibrated pipettes of 1.0 ml, 5.0 ml and 10.0 ml, volumetric flasks of 10 ml, 25 ml, 50 ml and 100 ml capacity, Funnels (i.d. 5.0 cm), beakers (250 ml) and other requisite glasswares	Schott & Corning (India) Ltd., Mumbai
Analytical balance	AX 120, EL 8300, Shimadzu Corp., Japan
pH meter	Pico^{+} Labindia, Mumbai, India
Cyclomixer, magnetic stirrer	Remi Scientific Equipments, Mumbai
Cooling Centrifuge	3K 30, Sigma Laboratory centrifuge, Osterode, GmBH.
Lyophilizer	DW1, 0-60E, Heto Drywinner, Denmark
UV-Visible Spectrophotometer	Shimadzu UV-1601, Japan
Particle and Zeta size Analyzer	Malvern zeta sizer NanoZS, U.K.
Transmission electron microscopy	Morgagni, Philips, Netherlands
^{1}H-NMR	av300, Bruker, UK
HPLC system	LC 20-AT prominence, Shimadzu Corp., Japan

5.2. Methods

5.2.1. Preparation of Tizanidine HCl (TZ) and Cyclobenzaprine HCl (CBZ) loaded chitosan, thiolated chitosan nanoparticles (TC) and trimethyl chitosan (TMC) nanoparticles

Particles from chitosan with difference molecular weight were prepared using Ionotropic gelation of chitosan with sodium alginate (SA). Briefly, chitosan was dissolved in 1% acetic acid aqueous solution. After stirring overnight the drug was added in chitosan solution. Sodium alginate solution was added dropwise to above chitosan solution under moderate stirring. Following stirring for 20 minutes, the particle suspension was centrifuged at 4°C at 18,000 rpm for 30 minutes. The particles were washed and subjected to characterize for zeta potential and particle size. The supernatant was analyzed for entrapment efficiency using U.V spectroscopy (Worawan et al., 2008).

Particles from trimethyl chitosan (TMC) and thiolated chitosan (TC) with difference molecular weight were prepared by Ionotropic gelation of TMC and TC using sodium

alginate with slight modification in above method. TMC and TC were dissolved in distilled water. Drug solution was incubated with sodium deoxycholate (SDC) for 30 seconds. The resulting complex was added to TMC/TC solution. The addition of sodium alginate solution to above mixture with stirring led to the immediate formation of nanoparticles. The particle suspension was centrifuged at 4°C at 18,000 rpm for 30 minutes. The particles were washed and subjected to characterize for zeta potential and particle size. The supernatant was analyzed for entrapment efficiency using U.V spectroscopy. The formulations were optimized to achieve maximum drug entrapment with optimum size by varying drug: polymer ratio and the optimized batch was selected on basis of percentage drug entrapment. (Dong-Won et al., 2006, Carmen et al., 2009).

5.2.2 Optimization of Formulation Parameters for chitosan Nanoparticles

Quantitative aspects of the effects and relationships among various formulation parameters of high therapeutics payload nanoparticles produced by Ionotropic gelation method are investigated using Response Surface Methodology (RSM).

To study this, we performed, "Box-Behnken" design (BBD) on three critical formulation factors known to affect their results. The BBD is a popular template for RSM because it requires only three-levels of each formulation factor and only a fraction of all the possible combinations. In this design, the experimental region is assumed to be a cube, and experiments are performed at points corresponding to midpoint of each edge and replicated experiments at the centre of this multidimensional cube.

This design is suitable for exploring quadratic response surfaces and constructing second-order polynomial models. The complete design consisted of 15 experimental points that included twelve factor points and three replications at the centre point. The non-linear quadratic model generated by the design is as follow:

$$Y= \beta_0+ \beta_1A+ \beta_2B+ \beta_3C+ \beta_{12} AB+ \beta_{13}AC+ \beta_{23}BC+ \beta_{11}A^2+ \beta_{22}B^2+ \beta_{33}C^2$$

------ Equation [5.1]

Where, Y is the measured response (dependant variable) associated with each factor-level combination; expressed in terms of size and entrapment efficiency of the nanoparticles, β_0 is an intercept, β_1, β_2, β_3, β_{12}, β_{13}, β_{23}, β_{11}, β_{22} and β_{33} are the regression coefficients. A, B and C are the (independent factors studied) concentration of chitosan (mg/ml), concentration of sodium alginate (mg/ml), and concentration of

drug (mg/ml) respectively. The independent factors and the dependent variable used in the design are listed in Table 5.2 for low and medium molecular weight chitosan. The Design Expert (Version 7.1, State Ease Inc, USA) program was used for design of experiment and analysis of this second-order model and for drawing of three dimensional response surface and contour plots.

Table: 5.2.Variables in Box Behnken Design

X_i	Independent variables	Units	Coded values			Response (Y1)	Response (Y2)
			-1	0	1		
A	Concentration of chitosan	mg/ml	1	1.5	2	Particle size in nm	Entrapment Efficiency in Percentage
B	Concentration of sodium alginate	mg/ml	0.5	1	1.5		
C	Concentration of Drug	mg/ml	1	2.5	4		

Table: 5.3. Matrix of Box Behnken Design, Particle size response and Entrapment Efficiency of each experimental run for LMC-TZ NPs

Std	Run	Concentration of chitosan (mg/ml) Factor: A	Concentration of Sodium alginate (mg/ml) Factor: B	Concentration of drug (mg/ml) Factor: C	Particle size (nm)	Predicted particle size (nm)	Entrapment Efficiency (% EE)	Predicted Entrapment Efficiency (% EE)
8	1	2	1	4	705.6±10	710.9	20.18±2.5	20.35
4	2	2	1.5	2.5	1105.4±34	1090.6	25.41±2.1	25.09
11	3	1.5	0.5	4	519.7±12	515.7	17.76±1.0	17.66
***8**	**4**	**1**	**0.5**	**2.5**	**479.1±15**	**493.5**	**20.40±1.8**	**20.71**
3	5	1	1.5	2.5	1607.0±44	1607.7	27.90±4.2	27.97
14	6	1.5	1	2.5	568.0±12	557.3	18.89±2.5	19.21
13	7	1.5	1	2.5	548.7±15	557.3	19.34±2.3	19.21
10	8	1.5	1.5	1	1119.7±35	1124.0	17.50±1.1	17.60
2	9	2	0.5	2.5	768.5±32	767.4	24.19±3.1	24.11
6	10	2	1	1	536.2±22	546.9	16.19±1.5	16.40
9	11	1.5	0.5	1	489.9±11	480.4	16.99±1.1	16.85
15	12	1.5	1	2.5	555.9±14	557.3	19.40±3.1	19.21
5	13	1	1	1	726.9±18	722.0	16.08±1.3	15.90
12	14	1.5	1.5	4	1299.8±33	1309.6	25.00±2.5	25.14
7	15	1	1	4	789.4±21	779.0	20.54±1.9	20.32

(Mean ± S.D., $n = 3$)
LMC-TZ NPs- Tizanidine HCl loaded low molecular weight chitosan NPs
*Ratio of chitosan solution to sodium alginate solution was taken 2:1

Table: 5.4. Matrix of Box Behnken Design, Particle size response and Entrapment Efficiency of each experimental run for LMC-CBZ NPs

Std	Run	Concentration of chitosan (mg/ml) Factor: A	Concentration of Sodium alginate (mg/ml) Factor: B	Concentration of drug (mg/ml) Factor: C	Particle size (nm)	Predicted particle size (nm)	Entrapment Efficiency (% EE)	Predicted Entrapment Efficiency (% EE)
8	1	2	1	4	699.6±11	670.2	19.69±1.1	19.17
4	2	2	1.5	2.5	1023.4±14	1025.6	25.10±1.3	25.63
11	3	1.5	0.5	4	599.7±10	567.6	17.89±2.1	18.41
***1**	**4**	**1**	**0.5**	**2.5**	**478.9±09**	**476.6**	**25.40±3.2**	**24.86**
3	5	1	1.5	2.5	1588.5±33	1526.9	28.00±4.1	28.00
14	6	1.5	1	2.5	666.6±21	642.0	18.99±1.3	19.34
13	7	1.5	1	2.5	650.7±26	642.0	19.44±1.5	19.34
10	8	1.5	1.5	1	1110.3±40	1142.4	17.44±2.1	16.91
2	9	2	0.5	2.5	708.9±31	770.5	24.20±3.4	24.20
6	10	2	1	1	589.7±12	555.3	15.43±0.9	15.42
9	11	1.5	0.5	1	512.5±17	485.4	18.36±1.8	18.36
15	12	1.5	1	2.5	608.6±14	642.0	19.60±1.4	19.34
5	13	1	1	1	666.5±19	695.9	16.39±2.2	16.91
12	14	1.5	1.5	4	1189.0±23	1216.1	24.43±2.8	24.42
7	15	1	1	4	702.6±20	737.06	20.70±1.5	20.71

(Mean ± S.D., $n = 3$)

LMC-CBZ NPs- Cyclobenzaprine HCl loaded low molecular weight chitosan NPs

*Ratio of chitosan solution to sodium alginate solution was taken 2:1

Table: 5.5. Matrix of Box Behnken Design, Particle size response and Entrapment Efficiency of each experimental run for MMC-TZ NPs

Std	Run	Concentration of chitosan (mg/ml) Factor: A	Concentration of Sodium alginate (mg/ml) Factor: B	Concentration of drug (mg/ml) Factor: C	Particle size (nm)	Predicted particle size (nm)	Entrapment Efficiency (% EE)	Predicted Entrapment Efficiency (% EE)
8	1	2	1	4	769.7±22	767.8	20.34±1.9	20.43
4	2	2	1.5	2.5	1145.7±33	1131.4	24.34±1.2	24.71
11	3	1.5	0.5	4	630.5±23	619.3	17.56±1.6	18.05
***1**	**4**	**1**	**0.5**	**2.5**	**602.1±19**	**616.3**	**23.56±2.7**	**23.18**
3	5	1	1.5	2.5	1688.7±29	1675.6	27.65±3.4	28.24
14	6	1.5	1	2.5	699.4±21	685.9	19.87±2.6	20.48
13	7	1.5	1	2.5	679.4±14	685.9	20.79±2.1	20.48
10	8	1.5	1.5	1	1123.6±27	1134.8	18.65±2.4	18.15
2	9	2	0.5	2.5	895.7±21	908.8	23.99±3.1	23.40
6	10	2	1	1	599.4±13	602.4	16.90±.1.5	17.02
9	11	1.5	0.5	1	575.3±16	559.1	17.18±1.1	17.65
15	12	1.5	1	2.5	678.9±19	685.9	20.78±1.9	20.48
5	13	1	1	1	766.3±15	768.2	19.12±1.2	19.02
12	14	1.5	1.5	4	1309.4±22	1325.5	24.38±2.9	23.91
7	15	1	1	4	856.7±24	853.7	21.89±2.3	21.76

(Mean ± S.D., $n = 3$)

MMC-TZ NPs- Tizanidine HCl loaded medium molecular weight chitosan NPs

*Ratio of chitosan solution to sodium alginate solution was taken 2:1

Table: 5.6. Matrix of Box Behnken Design, Particle size response and Entrapment Efficiency of each experimental run for MMC-CBZ NPs

Std	Run	Concentration of chitosan (mg/ml) Factor: A	Concentration of Sodium alginate (mg/ml) Factor: B	Concentration of drug (mg/ml) Factor: C	Particle size (nm)	Predicted particle size (nm)	Entrapment Efficiency (% EE)	Predicted Entrapment Efficiency (% EE)
8	1	2	1	4	766.3±22	783.5	20.78±1.2	20.79
4	2	2	1.5	2.5	1287.8±34	1269.1	24.89±1.9	25.18
11	3	1.5	0.5	4	634.7±19	597.8	17.98±1.3	18.54
***1**	**4**	**1**	**0.5**	**2.5**	**643.1±17**	**661.8**	**24.00±2.1**	**23.71**
3	5	1	1.5	2.5	1754.3±29	1734.7	27.76±2.9	28.33
14	6	1.5	1	2.5	721.3±23	697.1	19.67±2.3	20.18
13	7	1.5	1	2.5	714.5±20	697.1	20.99±1.7	20.18
10	8	1.5	1.5	1	1167.5±26	1204.3	18.78±1.3	18.21
2	9	2	0.5	2.5	923.9±28	943.5	23.56±3.1	22.98
6	10	2	1	1	705.9±31	687.9	16.56±1.1	16.83
9	11	1.5	0.5	1	599.7±17	598.2	16.90±1.8	17.20
15	12	1.5	1	2.5	655.4±14	697.1	19.89±2.2	20.18
5	13	1	1	1	799.8±12	782.7	18.99±2.8	18.99
12	14	1.5	1.5	4	1388.6±38	1390.1	24.66±3.1	24.36
7	15	1	1	4	854.5±16	872.6	22.78±2.6	22.51

(Mean ± S.D., $n = 3$)

MMC-CBZ NPs-Cyclobenzaprine HCl loaded medium molecular weight chitosan NPs

*Ratio of chitosan solution to sodium alginate solution was taken 2:1

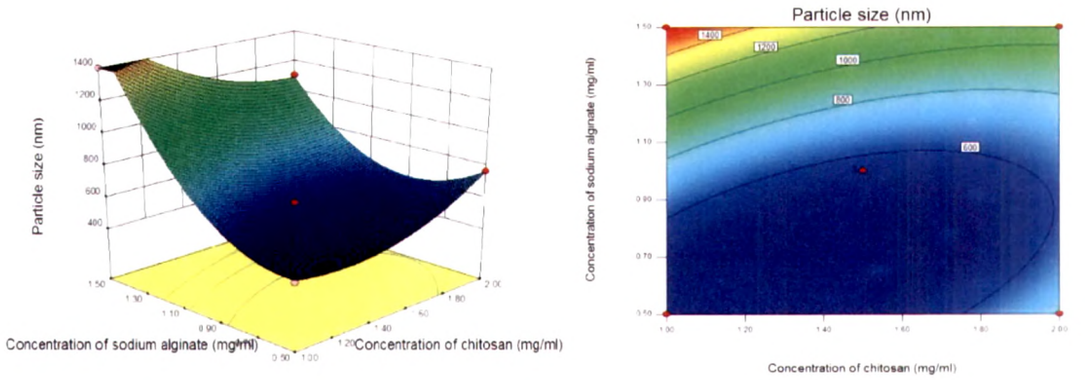
In order to optimize chitosan to sodium alginate solution ratio in each formulations, nanoparticles were formulated using chitosan: sodium alginate solution with different ratio (2:1, 3:1, 4:1) and the Factor A, B, C concentration was kept constant (Wang et al., 2008). Moreover impact of chitosan solution pH (4 and 5) and sodium alginate pH (8, 9, 10 and 11) was seen on the effect of particle size and drug entrapment on optimal formulations (Sadeghi et al., 2008) (Table: 5.7).

Table: 5.7. Effect of pH on optimal batch

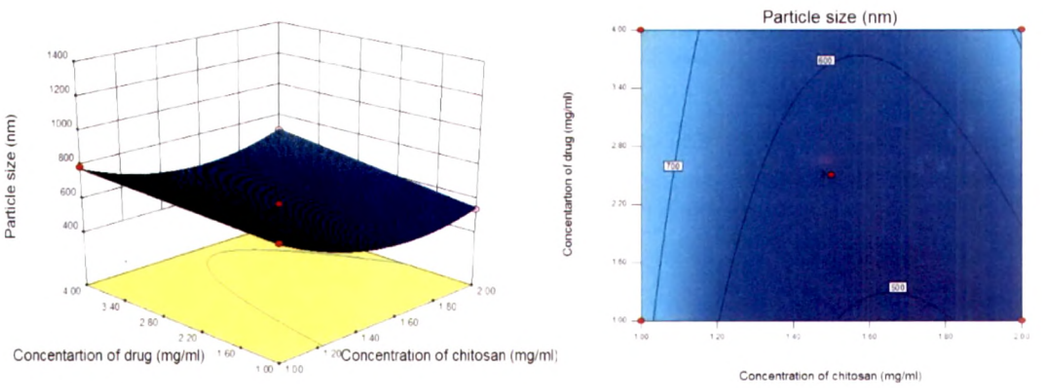
Formulations	Concentration of	Concentration of SA	Concentration of drug	pH of Factor: A	pH of Factor :B	Particle Size	Entrapment
LMC-TZ NPs	1	0.5	2.5	4	8	468.5±11.1	20.52±1.8
	1	0.5	2.5	4	9	479.3±13.3	28.35±2.7
	1	0.5	2.5	4	10	486.1±13.4	34.34±3.2
	1	0.5	2.5	4	11	466.1±1.9	42.65±4.2
	1	0.5	2.5	5	8	481.6±12.1	37.94±3.9
	1	**0.5**	**2.5**	**5**	**9**	**473.5± 13.5**	**44.69±3.5**
	1	0.5	2.5	5	10	490.3±11.8	39.38±3.3
	1	0.5	2.5	5	11	452.2±20.2	35.67±4.2
LMC-CBZ NPs	1	0.5	2.5	4	8	435.2±18.4	65.13±3.7
	1	0.5	2.5	4	9	468.3±12.8	60.84±5.3
	1	0.5	2.5	4	10	477.5±16.3	61.32±4.9
	1	0.5	2.5	4	11	461.4±13.2	57.03±5.6
	1	0.5	2.5	5	8	434.9±14.9	60.25±4.6
	1	**0.5**	**2.5**	**5**	**9**	**472.6±12.7**	**62.27±6.1**
	1	0.5	2.5	5	10	431.5±20.1	58.94±5.9
	1	0.5	2.5	5	11	364.0±17.8	55.84±4.7
MMC-TZ NPs	1	0.5	2.5	4	8	593.7±14.6	31.80±3.9
	1	0.5	2.5	4	9	645.6±13.3	41.70±4.3
	1	0.5	2.5	4	10	681.4±14.3	43.57±4.1
	1	0.5	2.5	4	11	571.9±10.9	40.43±3.8
	1	0.5	2.5	5	8	583.1±11.9	36.45±3.5
	1	**0.5**	**2.5**	**5**	**9**	**598.7±18**	**46.65± 2.4**
	1	0.5	2.5	5	10	576.4±16.7	39.82±3.2
	1	0.5	2.5	5	11	542.9±18.3	29.63±2.7
MMC-CBZ NPs	1	0.5	2.5	4	8	595.5±15.5	46.04±4.1
	1	0.5	2.5	4	9	643.8±19.6	53.34±5.8
	1	0.5	2.5	4	10	526.2±19.9	51.28±4.9
	1	0.5	2.5	4	11	504.4±21.3	44.61±4.4
	1	0.5	2.5	5	8	566.4±23.1	55.41±5.6
	1	**0.5**	**2.5**	**5**	**9**	**628.2±13.1**	**64.14±6.4**
	1	0.5	2.5	5	10	648.4±21.3	59.06±4.9
	1	0.5	2.5	5	11	578.3±18.9	45.41±4.5

*Ratio of chitosan solution to sodium alginate solution was taken 2:1.

(a)



(b)



(c)

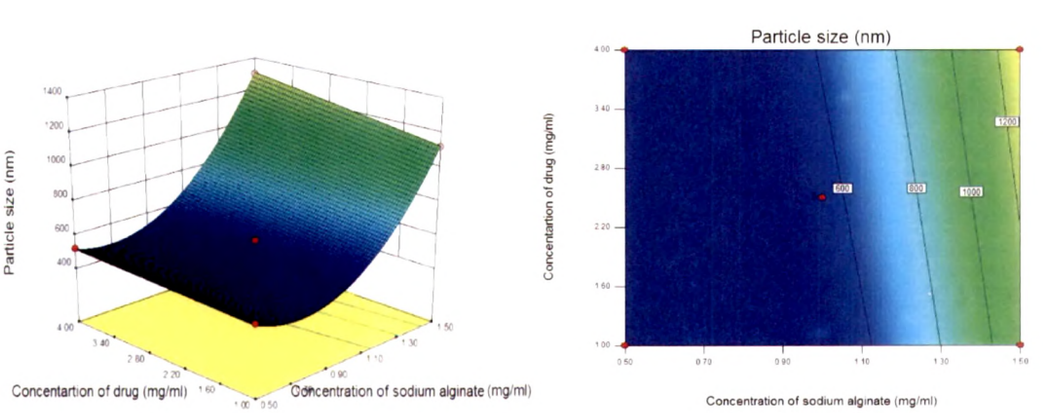


Figure: 5.1. Response surface and contour plots of a) the effects of Concentration of chitosan (A) and Concentration of sodium alginate (B), b) effects of Concentration of chitosan (A) and Concentration of drug (C) and c) Concentration of sodium alginate (B) and Concentration of Drug (C) on the particle size of the LMC-TZ NPs

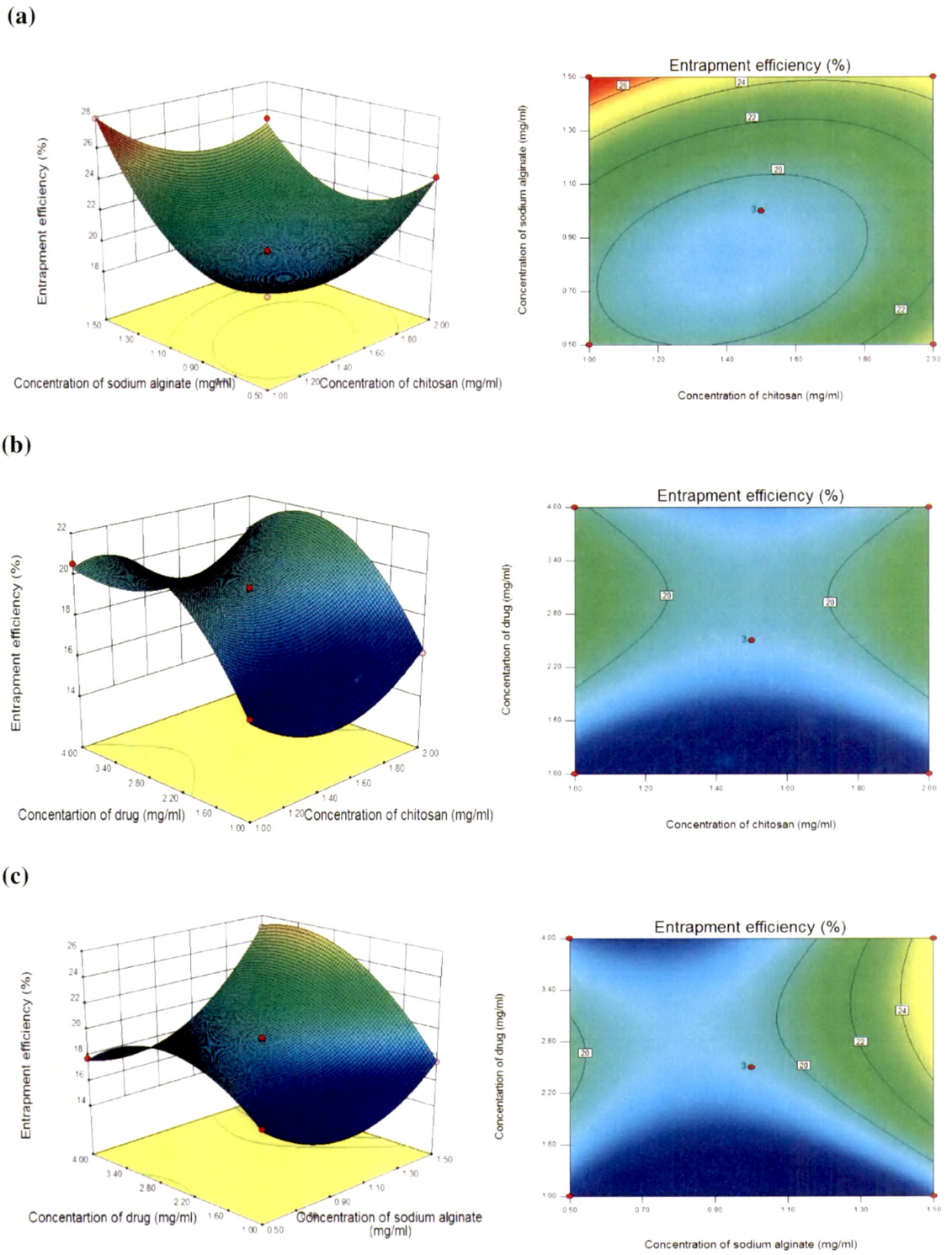
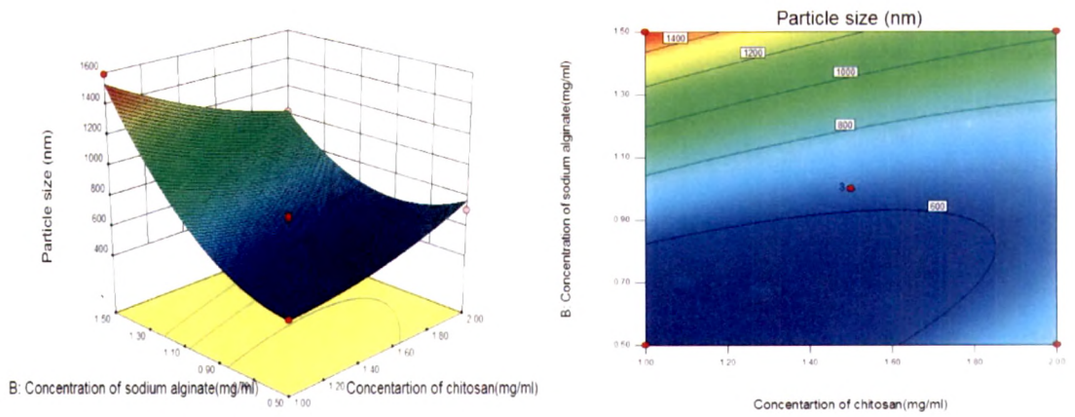
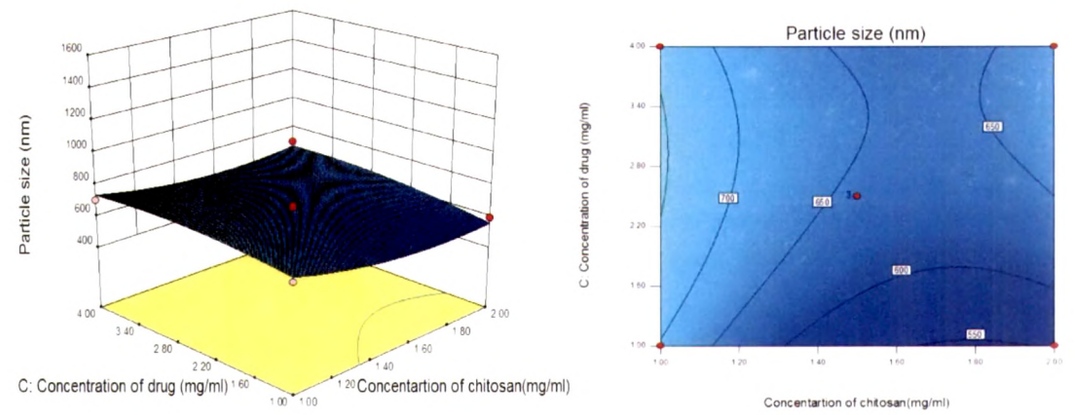


Figure: 5.2. Response surface and contour plots of a) the effects of Concentration of chitosan (A) and Concentration of sodium alginate (B), b) effects of Concentration of chitosan (A) and Concentration of drug (C) and c) Concentration of sodium alginate (B) and Concentration of Drug (C) on the entrapment efficiency of the LMC-TZ NPs

(a)



(b)



(c)

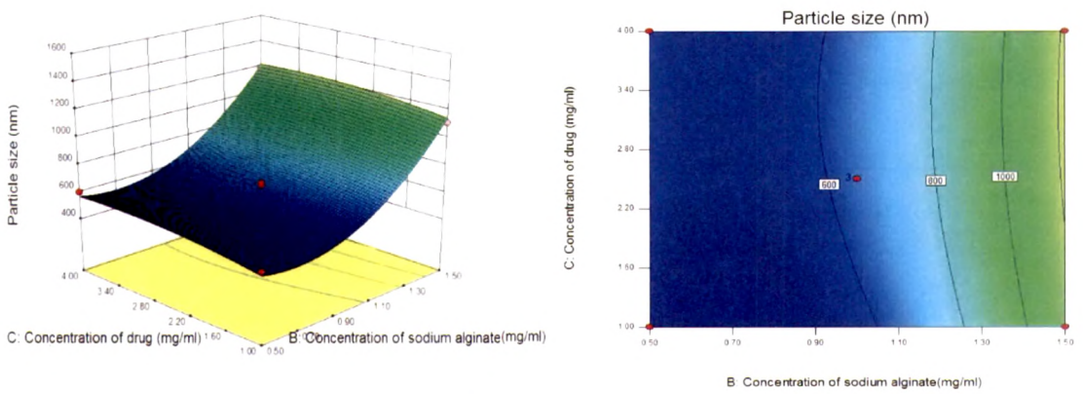
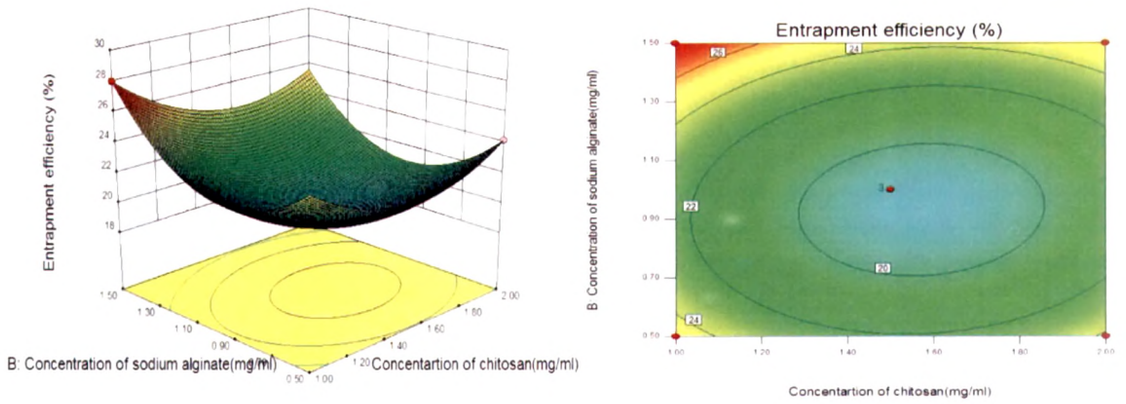
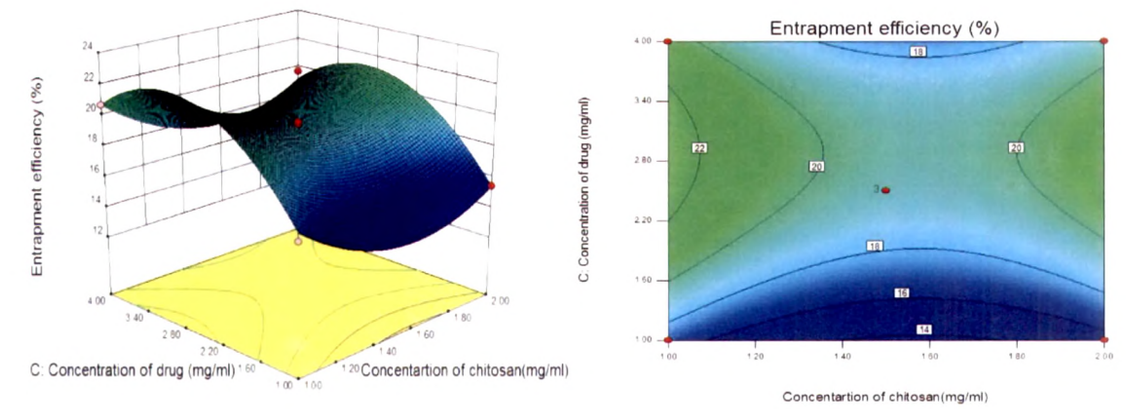


Figure: 5.3. Response surface and contour plots of a) the effects of Concentration of chitosan (A) and Concentration of sodium alginate (B), b) effects of Concentration of chitosan (A) and Concentration of drug (C) and c) Concentration of sodium alginate (B) and Concentration of Drug (C) on the particle size of the LMC-CBZ NPs

(a)



(b)



(c)

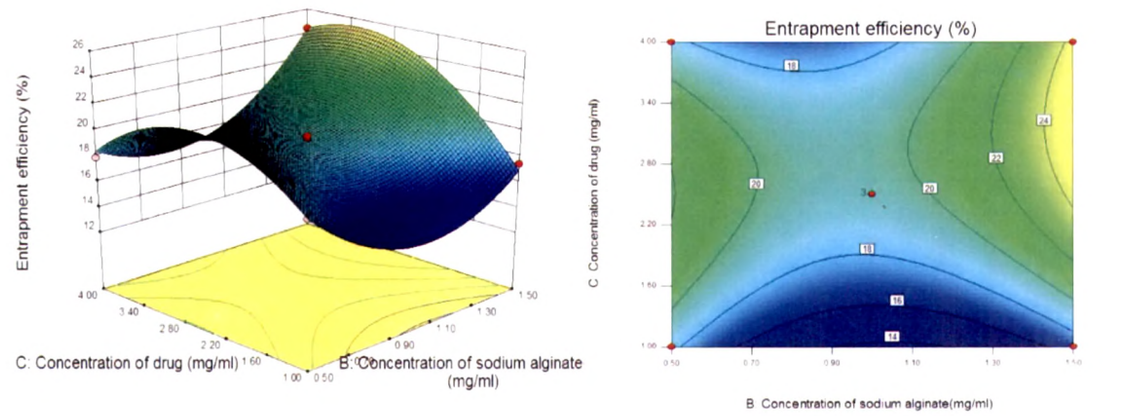
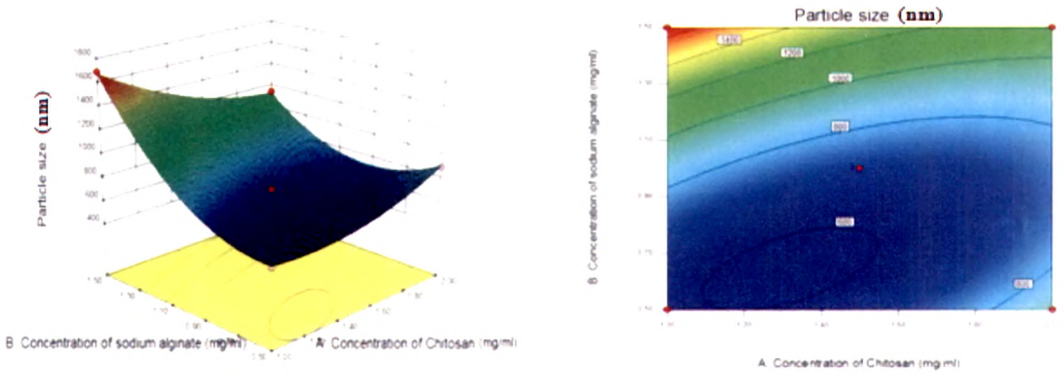
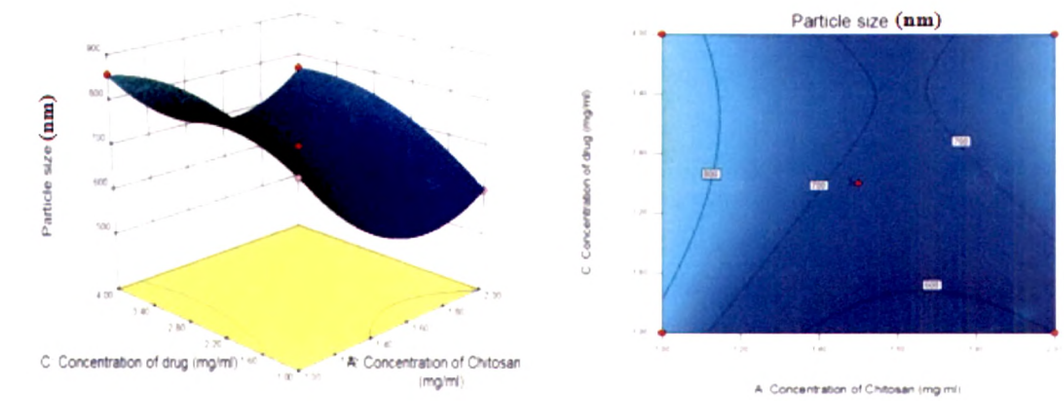


Figure: 5.4. Response surface and contour plots of a) the effects of Concentration of chitosan (A) and Concentration of sodium alginate (B), b) effects of Concentration of chitosan (A) and Concentration of drug (C) and c) Concentration of sodium alginate (B) and Concentration of Drug (C) on the entrapment efficiency of the LMC-CBZ NPs

(a)



(b)



(c)

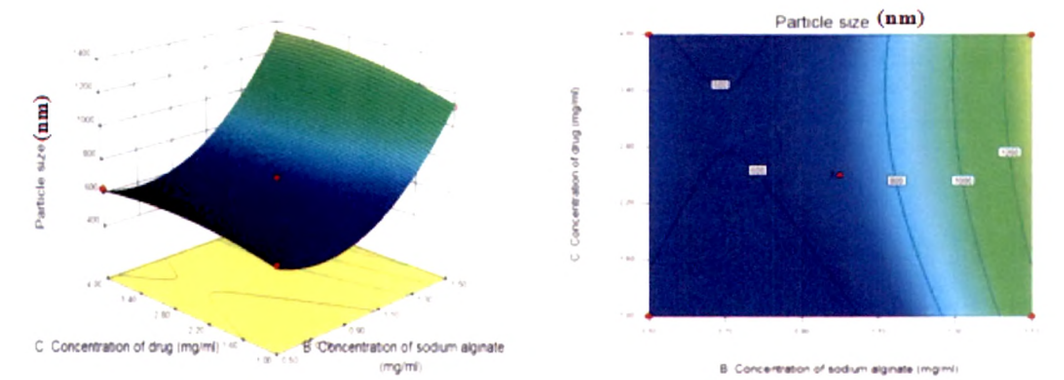
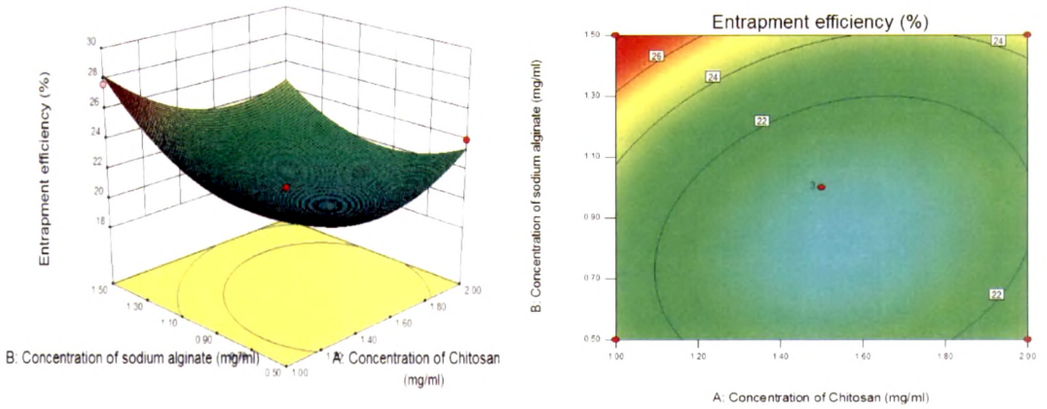
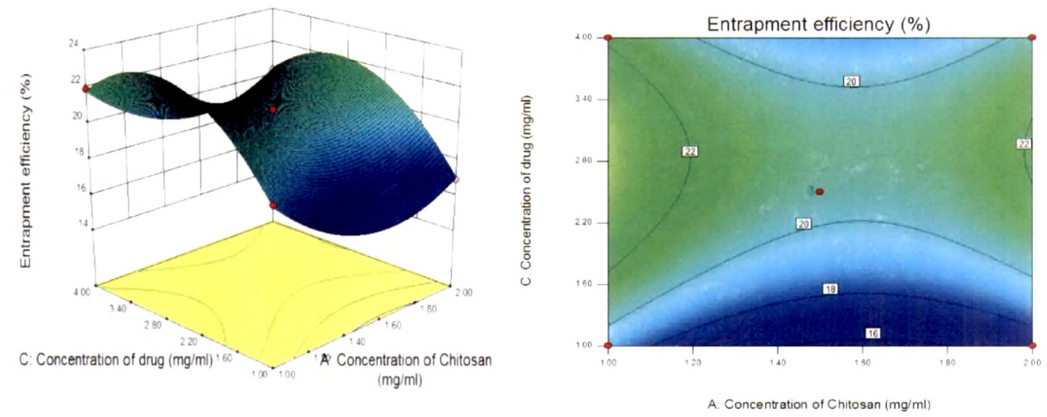


Figure: 5.5. Response surface and contour plots of a) the effects of Concentration of chitosan (A) and Concentration of sodium alginate (B), b) effects of Concentration of chitosan (A) and Concentration of drug (C) and c) Concentration of sodium alginate (B) and Concentration of Drug (C) on the particle size of the MMC-TZ NPs

(a)



(b)



(c)

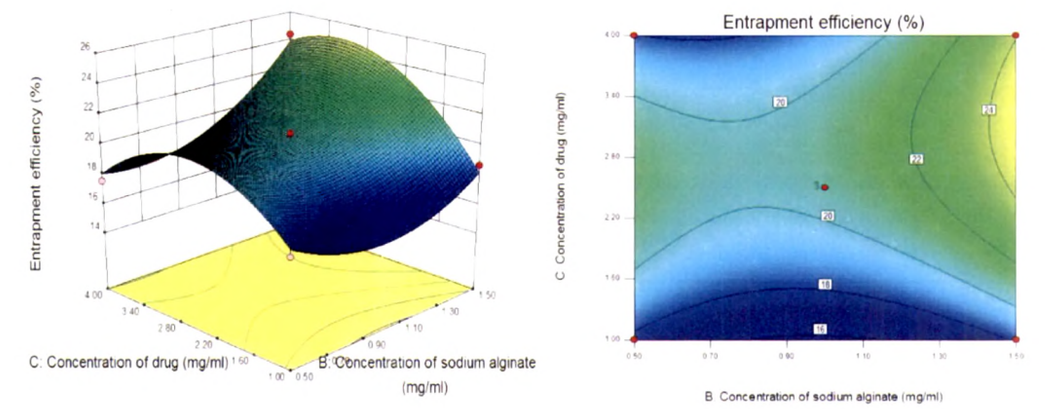
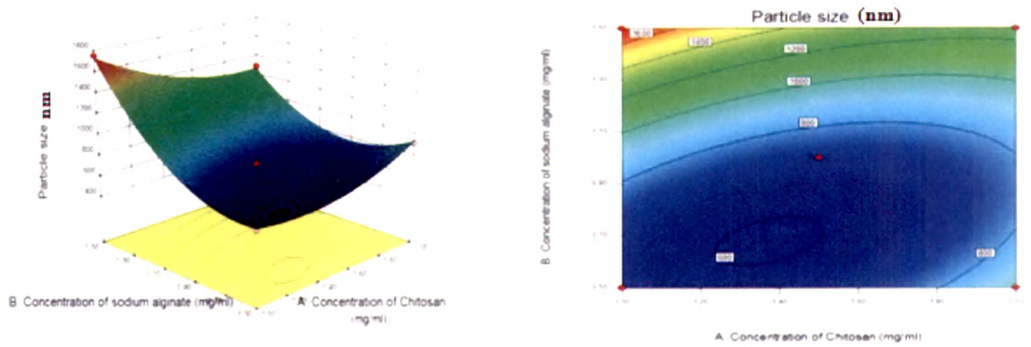
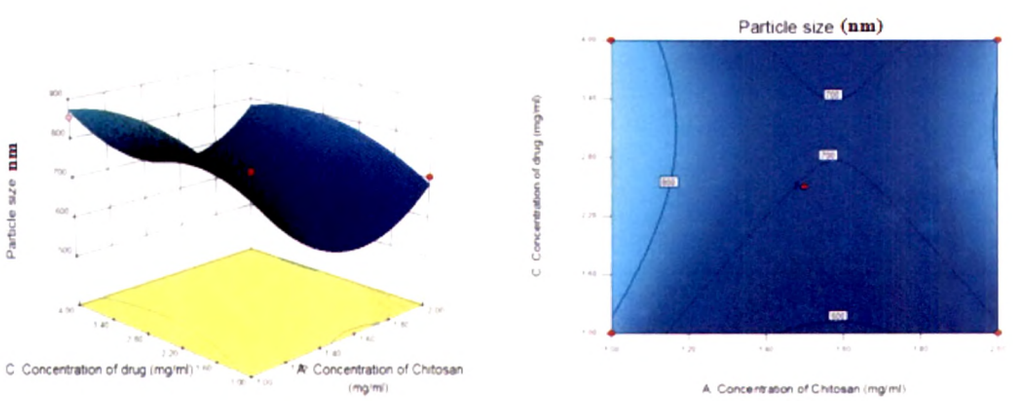


Figure: 5.6. Response surface and contour plots of a) the effects of Concentration of chitosan (A) and Concentration of sodium alginate (B), b) effects of Concentration of chitosan (A) and Concentration of drug (C) and c) Concentration of sodium alginate (B) and Concentration of Drug (C) on the entrapment efficiency of the MMC-TZ NPs

(a)



(b)



(c)

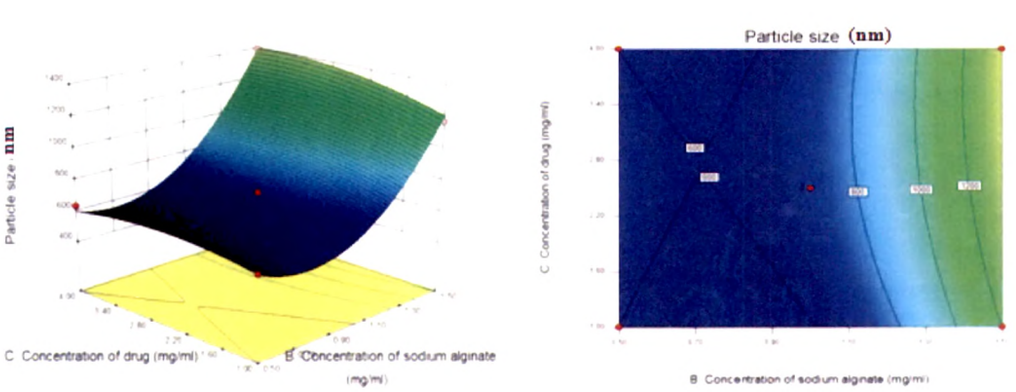
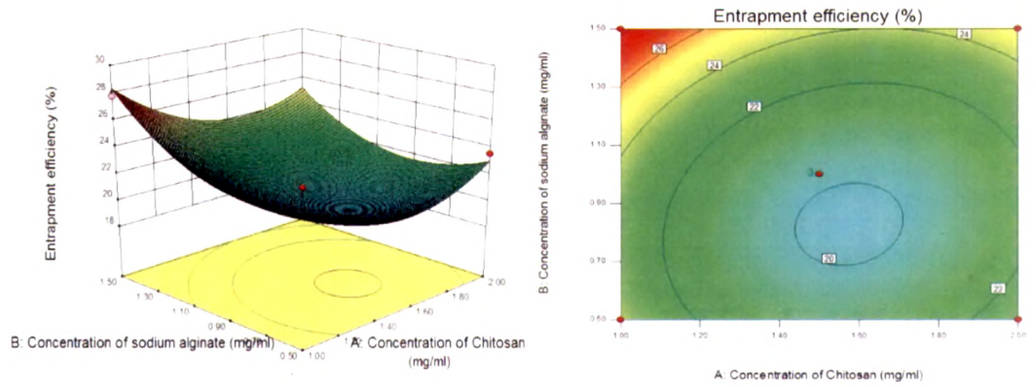
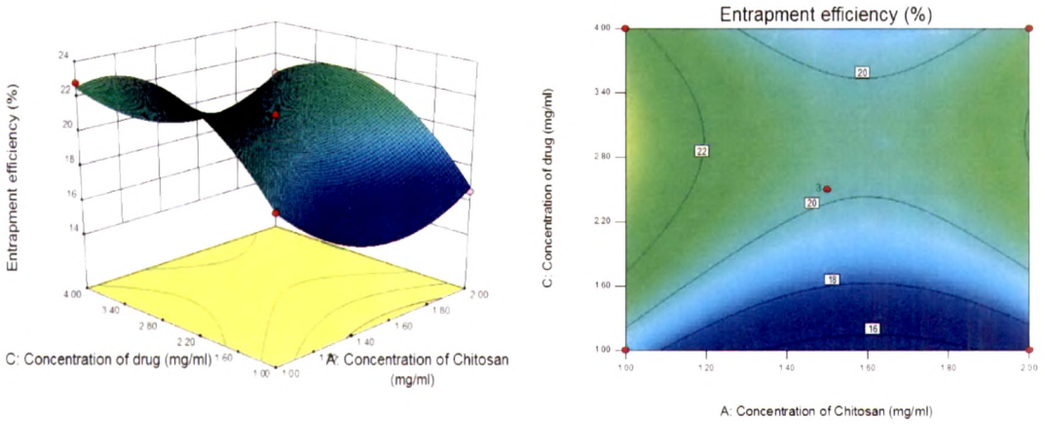


Figure: 5.7. Response surface and contour plots of a) the effects of Concentration of chitosan (A) and Concentration of sodium alginate (B), b) effects of Concentration of chitosan (A) and Concentration of drug (C) and c) Concentration of sodium alginate (B) and Concentration of Drug (C) on the particle size of the MMC-CBZ NPs

(a)



(b)



(c)

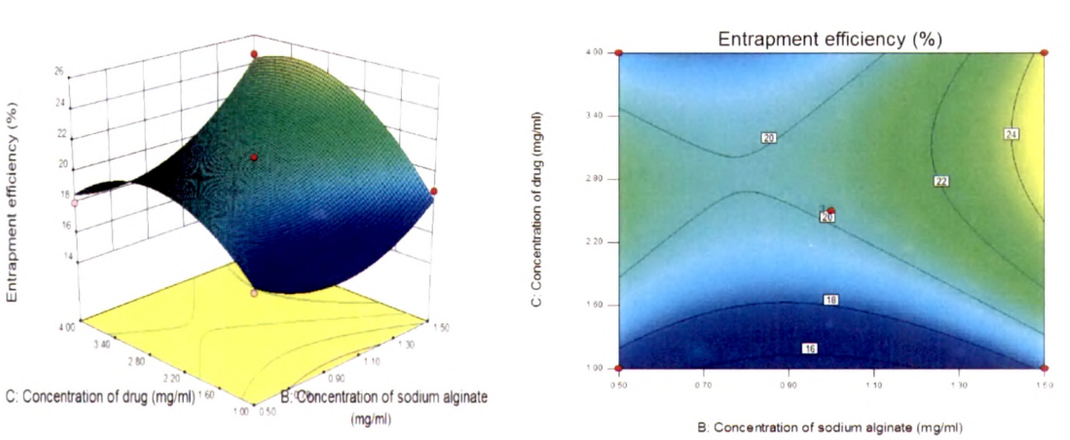


Figure: 5.8. Response surface and contour plots of a) the effects of Concentration of chitosan (A) and Concentration of sodium alginate (B), b) effects of Concentration of chitosan (A) and Concentration of drug (C) and c) Concentration of sodium alginate (B) and Concentration of Drug (C) on the entrapment efficiency of the MMC-CBZ NPs

Data processing:

Nanoparticles were prepared by Ionotropic gelation method using chitosan/Sodium alginate/drug. Formulation parameters, such as concentration of chitosan, concentration of sodium alginate and concentration of drug found to have significant influence on preparation of nanoparticles. Hence, formulation parameters were optimized and kept unaltered in subsequent experiments. In order to optimize formulation parameters, a Box Behnken Design (BBD) of RSM was used. Fifteen batches of nanoparticles were prepared by Ionotropic gelation method using a 3-factor, 3-level BBD varying three independent variables (Concentration of chitosan (mg/ml) (A), Concentration of sodium alginate (mg/ml) (B) and concentration of drug (mg/ml) (C) of the formulation according to Table: 5.2. The influence of these variables on observed response (Y, particle size and entrapment efficiency) is recorded in Table: 5.3 to Table: 5.6 for each formulation. Each batch of nanoparticles was prepared three times and was evaluated for particle size and entrapment efficiency.

Polynomial equation for each formulation:

LMC-TZ NPs- The maximum response as particle size was 1607nm and minimum response was 479.1nm. The mathematical relationship for the same in terms of a polynomial equation relating the response Y and independent variables was: (Y1)=+557.59-60.83 * A+359.35 * B+55.24 * C-197.74 * A * B+26.75 * A * C+37.58 * B * C+132.35 * A^2+300.09 * B^2-0.37 * C^2 and (Y2) =+19.21+0.13 * A+2.06 * B+2.09 * C-1.57 * A * B-0.12 * A *C +1.68 * B * C+2.10 * A^2+3.16 * B^2-3.06 * C^2 for particle size and entrapment efficiency respectively.

…….. Equation [5.2] & [5.3].

LMC-CBZ NPs- The maximum response as particle size was 1588.5nm and minimum response was 478.9 nm. The mathematical relationship for the same in terms of a polynomial equation relating the response Y and independent variables was :(Y1) =+642.02-51.85 * A+326.38 * B+38.99 * C-198.79 * A * B+18.43 * A * C-2.13 * B * C+59.84 * A^2+248.08 * B^2-37.20 * C^2 and (Y2) =+19.34-0.76 * A+1.14 * B+1.89 * C-0.43 * A * B-0.010 * A * C+1.86 * B * C+2.43 * A^2+3.90 * B^2-3.72 * C^2 for particle size and entrapment efficiency respectively.

…….. Equation [5.4] & [5.5].

MMC-TZ NPs- The maximum response as particle size was 1688.7nm and minimum response was 575.32nm. The mathematical relationship for the same in terms of a polynomial equation relating the response Y and independent variables was :(Y1) =+685.93-62.90 * A+320.47 * B+62.73 * C-209.17 * A * B+19.97 * A * C+32.64 * B * C+117.73 * A^2+279.41 * B^2-55.59 * $C^2$and (Y2) =+20.49-0.83 * A+1.59 * B+1.54 * C-0.94 * A * B+0.17 * A * C+1.34 * B * C+2.26 * A^2+2.14 * B^2-3.18 * C^2 for particle size and entrapment efficiency respectively.

..... Equation [5.6] & [5.7].

MMC-CBZ NPs- The maximum response as particle size was 1754.34nm and minimum response was 599.78nm. The mathematical relationship for the same in terms of a polynomial equation relating the response Y and independent variables was: (Y1) = +697.11-45.97* A+349.61 * B+46.38 * C-186.81 * A * B+1.42 * A * C+46.54* B * C+144.62* A^2+310.58 * B^2-60.04 * C^2 and (Y2) =+20.18-0.97 * A+1.71 * B+1.87 * C-0.61 * A * B+0.11 * A * C+1.20 * B * C+2.54 * A^2+2.34 * B^2-2.94 * C^2 for particle size and entrapment efficiency respectively.

..... Equation [5.8] & [5.9]

Equation expresses the quantitative effect of the individual formulation components (A, B, and C) and combination there of on the response (Y) in terms of interaction coefficients. The values of the coefficients A to C are related to the effect of these variables on the response (Y). Coefficients with more than one factor term and those with higher order terms represent interaction terms and quadratic relationships respectively. A positive and negative signs suggest a positive and negative effect on response respectively. The theoretical (predicted) values and the observed values were in reasonably good agreement as seen from Table: 5.3 to Table: 5.6 for each formulation. The significance of the ratio of mean square variation due to regression and residual error was tested using analysis of variance (ANOVA). It was observed that the best-fitted models were quadratic. The values of R^2, adjusted R^2, predicted R^2, Degree of freedom (Df), Sum of squares(SS) and Mean square(MS) are given in Table: 5.8 to Table: 5.11 for each formulation, along with the regression equation generated for each response. The results of ANOVA in Table: 5.8 to Table: 5.11 for the dependent variables demonstrate that the model was significant for both the response variables.

Table: 5.8. Analysis of Variance for BBD Model of LMC-TZ NPs

Parameters		Df	SS	MS	R^2	AdjR^2	PredR^2	F	Lack of fit
Particle size	Model	9	1.632E+006	1.814E+005	0.9993	0.9982	0.9907	0.0001	0.2431
	Residual	5	1111.91	222.38	-	-	-	-	-
Entrapment efficiency	Model	9	184.02	20.45	0.9968	0.9911	0.9611	0.0001	0.380
	Residual	5	0.59	0.12	-	-	-	-	-

*Df- Degree of freedom; SS- Sum of squares; MS-Mean square; F- Significance
AdjR^2 -Adjusted R^2; PredR^2- Predicted R^2

Table: 5.9. Analysis of Variance for BBD Model of LMC-CBZ NPs

Parameters		Df	SS	MS	R^2	AdjR^2	PredR^2	F	Lack of fit
Particle size	Model	9	1.292E+006	1.435E+005	0.9870	0.9636	0.8109	0.0003	0.1545
	Residual	5	17016.60	3403.32	-	-	-	-	-
Entrapment efficiency	Model	9	196.15	21.79	0.9906	0.9736	0.8633	0.0002	0.1566
	Residual	5	1.86	0.37	-	-	-	-	-

*Df- Degree of freedom; SS- Sum of squares; MS-Mean square; F- Significance
AdjR^2 -Adjusted R^2; PredR^2- Predicted R^2

Table: 5.10. Analysis of Variance for BBD Model of MMC-TZ NPs

Parameters		Df	SS	MS	R^2	AdjR^2	PredR^2	F	Lack of fit
Particle size	Model	9	1.415E+006	1.572E+005	0.9987	0.9964	0.9821	0.0001	0.2174
	Residual	5	1818.47	363.69	-	-	-	-	-
Entrapment efficiency	Model	9	134.43	14.94	0.9815	0.9483	0.7614	0.0008	0.3173
	Residual	5	2.53	0.51	-	-	-	-	-

*Df- Degree of freedom; SS- Sum of squares; MS-Mean square; F- Significance
AdjR^2 -Adjusted R^2; PredR^2- Predicted R^2

Table: 5.11. Analysis of Variance for BBD Model of MMC-CBZ NPs

Parameters		Df	SS	MS	R^2	AdjR^2	PredR^2	F	Lack of fit
Particle size	Model	9	1.602E+006	1.781E+005	0.9950	0.9860	0.9423	0.0001	0.4466
	Residual	5	8064.99	1613.00	-	-	-	-	-
Entrapment efficiency	Model	9	147.15	16.35	0.9813	0.9476	0.7922	0.0009	0.4836
	Residual	5	2.81	0.56	-	-	-	-	-

*Df- Degree of freedom; SS- Sum of squares; MS-Mean square; F- Significance
AdjR^2 -Adjusted R^2; PredR^2- Predicted R^2

The ANOVA indicated a significant ($P < 0.05$) effect of factors on response. Lack of fit was not significant and regression was highly significant for all formulations. The Adjusted R-square and Predicted R-square should be within approximately 0.20 of each other to be in reasonable agreement. The "Pred R-Square" is in reasonable agreement with the "Adj R-Square" of all the formulations, which is very good for chosen factorial model. So it was concluded that the second-order model adequately approximated the true surface.

Over the past 30 years, chitosan NPs preparation technique has been developed based on chitosan microparticles technology. There are at least four methods available: ionotropic gelation, microemulsion, emulsification solvent diffusion and polyelectrolyte complex. The most widely developed methods are ionotropic gelation and self assemble polyelectrolytes. These methods offer many advantages such as simple and mild preparation method without the use of organic solvent or high shear force. Thus, they would be applicable to a broad categories of drugs including macromolecules which notorious as labile drugs. In general, the factors found to affect nanoparticles formation including particle size and surface charge, are molecular weight and degree of deacetylation of chitosan. The entrapment efficiency is found to be dependent on the pKa and solubility of entrapped drugs. The drug is found to be associated with chitosan via electrostatic interaction, hydrogen bonding, and hydrophobic interaction (Tiyaboonchai et al., 2003).

Chitosans with a high degree of deacetylation (DD) (85 to 99%) promote drug absorption at both low and high molecular weights but also show clear dose-dependent toxicity. On the other hand, chitosans with DDs of 51 to 65% only increase the absorption of drugs with high molecular weights and display low toxicity. Chitosan-based formulations can greatly improve the absorption of drugs from the nasal cavity, and products for the treatment of migraine and cancer pain have reached Phase II clinical evaluation (Illum et al., 2001). A Japanese patent reported that a nasal composition containing salmon calcitonin and chitosan (particle size 30-60 μm) was administered to normal human subjects to determine pharmacokinetics, and good bioavailability was obtained. A nasal solution formulation of chitosan greatly enhanced the absorption of insulin across the nasal mucosa of rat and sheep. This effect was concentration dependent, with the optimal efficacy obtained for concentrations > 0.2% and 0.5% in rats and sheep, respectively (Illum et al., 1994).

5.2.3. Optimization of Formulation Parameters for Thiolated Chitosan (TC) Nanoparticles

Quantitative aspects of the effects and relationships among various formulation parameters of high therapeutics payload nanoparticles produced by Ionotropic gelation method are investigated using Response Surface Methodology (RSM).

To study this, we performed, "Box-Behnken" design (BBD) on four critical formulation factors known to affect their results. The BBD is a popular template for RSM because it requires only three-levels of each formulation factor and only a fraction of all the possible combinations. In this design, the experimental region is assumed to be a cube, and experiments are performed at points corresponding to midpoint of each edge and replicated experiments at the centre of this multidimensional cube.

This design is suitable for exploring quadratic response surfaces and constructing second-order polynomial models. The complete design consisted of 28 experimental points that included twenty five factor points and three replications at the centre point. The non-linear quadratic model generated by the design is as follow:

$$Y = \beta_0 + \beta_1 A + \beta_2 B + \beta_3 C + \beta_4 D + \beta_{12} AB + \beta_{13} AC + \beta_{14} A D + \beta_{23} B C + \beta_{24} BD + \beta_{34} CD + \beta_{11} A^2 + \beta_{22} B^2 + \beta_{33} C^2 + \beta_{44} D^2$$

------ Equation [5.10]

Where, Y is the measured response (dependant variable) associated with each factor-level combination; expressed in terms of size and entrapment efficiency of the nanoparticles, β_0 is an intercept, β_1, β_2, β_3, β_4, β_{12}, β_{13}, β_{14}, β_{23}, β_{24}, β_{34}, β_{11}, β_{22}, β_{33}, β_{44} are the regression coefficients. A, B, C and D are the (independent factors studied) concentration of thiolated chitosan (mg/ml), concentration of sodium alginate (mg/ml), concentration of drug (mg/ml) and concentration of sodium deoxycholate (mg/ml) respectively. The independent factors and the dependent variable used in the design are listed in Table 5.12 for low and medium molecular weight thiolated chitosan. The Design Expert (Version 7.1, State Ease Inc, USA) program was used for design of experiment and analysis of this second-order model and for drawing of three dimensional response surface and contour plots.

Table: 5.12.Variables in Box Behnken Design

X_i	**Independent variables**	**Units**	**Coded values**			**Response (Y1)**	**Response (Y2)**
			-1	0	1		
A	Concentration of thiolated chitosan	mg/ml	1	2	3	Particle size in nm	Entrapment Efficiency in Percentage
B	Concentration of sodium alginate	mg/ml	0.25	0.5	0.75		
C	Concentration of Drug	mg/ml	1	2	3		
D	Concentration of sodium deoxycholate	mg/ml	5	10	15		

Table: 5.13. Matrix of Box Behnken Design, Particle size response and Entrapment Efficiency of each experimental run for LMTC-TZ NPs

Std	Run	Concentration of TC(mg/ml) Factor :A	Concentration of SA (mg/ml) Factor :B	Concentration of drug mg/ml) Factor :C	Concentration of SDC(mg/ml) Factor:D	Particle size (nm)	Predicted particle size (nm)	Entrapment Efficiency (% EE)	Predicted Entrapment Efficiency (% EE)
21	1	2	0.25	2	5	388.7±11.2	380.4	46.34±2.4	44.59
***25**	**2**	**2**	**0.5**	**2**	**10**	**262.5±10.1**	**265.1**	**68.8±3.7**	**69.19**
18	3	3	0.5	1	10	354.8±9.8	355.4	66.98±5.6	67.78
27	4	2	0.5	2	10	259.6±8.5	265.1	66.32±6.7	69.19
14	5	2	0.75	1	10	400.7±13.4	401.3	69.43±3.8	65.88
20	6	3	0.5	3	10	378.1±11.8	378.8	65.22±2.7	62.97
24	7	2	0.75	2	15	299.5±10.5	315.1	64.90±2.5	64.04
3	8	1	0.75	2	10	391.1±16.8	387.0	62.54±1.9	63.87
9	9	1	0.5	2	5	455.7±19.8	451.6	48.93±3.2	48.62
26	10	2	0.5	2	10	267.9±12.9	265.1	69.76±4.1	69.19
6	11	2	0.5	3	5	373.5±15.8	381.5	44.23±5.2	43.93
28	12	2	0.5	2	10	270.3±17.2	265.1	71.88±3.7	69.19
15	13	2	0.25	3	10	355.5±20.9	348.3	51.44±3.8	60.00
17	14	1	0.5	1	10	330.4±14.5	337.0	62.89±3.2	62.53
4	15	3	0.75	2	10	408.7±18.7	403.2	67.43±4.5	67.82
13	16	2	0.25	1	10	250.5±11.8	247.6	53.21±4.7	58.80
16	17	2	0.75	3	10	299.6±17.8	295.9	60.59±1.9	60.01
10	18	3	0.5	2	5	423.1±19.0	426.3	49.34±3.9	48.82
22	19	2	0.75	2	5	456.7±16.8	454.0	43.98±4.9	47.22
23	20	2	0.25	2	15	277.5±15.7	287.5	65.45±4.4	59.60
2	21	3	0.25	2	10	377.1±13.2	380.6	66.87±5.5	63.11
5	22	2	0.5	1	5	399.5±20.1	403.3	48.09±5.2	47.70
12	23	3	0.5	2	15	382.3±10.6	379.8	61.99±6.4	67.31
1	24	1	0.25	2	10	303.6±9.4	308.5	64.32±3.5	61.50
8	25	2	0.5	3	15	289.6±11.2	285.0	63.32±3.9	61.28
11	26	1	0.5	2	15	276.0±12.1	266.2	56.43±4.3	61.96
19	27	1	0.5	3	10	302.1±14.7	308.8	66.09±1.7	62.67
7	28	2	0.5	1	15	276.6±11.9	267.9	64.32±5.8	62.19

(Mean ± S.D., *n* = 3) ; LMTC-TZ NPs- Tizanidine HCl loaded low molecular weight thiolated chitosan NPs

*Ratio of thiolated chitosan: sodium alginate: drug: sodium deoxycholate solution was taken **7:1:1:1**

Table: 5.14. Matrix of Box Behnken Design, Particle size response and Entrapment Efficiency of each experimental run for LMTC-CBZ NPs

Std	Run	Concentration of TC(mg/ml) Factor :A	Concentration of SA (mg/ml) Factor :B	Concentration of drug mg/ml) Factor :C	Concentration of SDC(mg/ml) Factor:D	Particle size (nm)	Predicted particle size (nm)	Entrapment °Efficiency (% EE)	Predicted Entrapment Efficiency (% EE)
21	1	2	0.25	2	5	380.4±10.3	380.5	48.65±2.3	49.16
***25**	**2**	**2**	**0.5**	**2**	**10**	**272.1±11.5**	**270.6**	**70.45±4.3**	**68.54**
18	3	3	0.5	1	10	364.0±12.3	363.4	67.43±5.6	68.28
27	4	2	0.5	2	10	271.0±14.5	270.6	68.11±7.8	68.54
14	5	2	0.75	1	10	403.1±13.6	401.5	68.87±8.5	69.70
20	6	3	0.5	3	10	388.1±15.6	386.5	67.12±5.6	67.65
24	7	2	0.75	2	15	318.6±16.7	317.6	63.87±3.4	63.41
3	8	1	0.75	2	10	350.4±18	352.1	63.87±2.6	62.68
9	9	1	0.5	2	5	467.7±4.0	466.7	49.09±6.7	50.08
26	10	2	0.5	2	10	270.1±19.4	270.6	68.06±4.3	68.54
6	11	2	0.5	3	5	395.6±16.7	396.9	54.16±3.6	53.25
28	12	2	0.5	2	10	269.4±13.6	270.6	67.54±4.1	68.54
15	13	2	0.25	3	10	342.4±17.8	341.8	69.19±2.9	70.70
17	14	1	0.5	1	10	342.3±19.5	343.0	64.32±4.6	63.84
4	15	3	0.75	2	10	421.2±13.7	423.8	69.11±6.1	68.01
13	16	2	0.25	1	10	252.3±15.7	250.1	55.67±5.9	57.71
16	17	2	0.75	3	10	298.7±11.9	298.7	61.09±5.2	61.40
10	18	3	0.5	2	5	433.8±18.4	433.1	50.98±3.7	50.55
22	19	2	0.75	2	5	466.2±17.3	464.4	45.18±2.5	46.75
23	20	2	0.25	2	15	292.3±14.3	293.2	59.83±3.7	58.31
2	21	3	0.25	2	10	345.5±12.3	346.9	64.04±4.3	62.80
5	22	2	0.5	1	5	425.3±9.8	427.3	49.54±4.1	47.78
12	23	3	0.5	2	15	399.9±11.5	398.7	63.08±2.6	64.44
1	24	1	0.25	2	10	320.2±21.1	320.6	66.54±4.3	65.21
8	25	2	0.5	3	15	303.6±12.5	304.7	63.70±2.9	63.03
11	26	1	0.5	2	15	268.5±16.8	267.0	59.21±5.2	61.99
19	27	1	0.5	3	10	309.2±19.1	308.9	69.97±1.3	69.17
7	28	2	0.5	1	15	283.6±11.4	285.3	65.32±4.3	63.80

(Mean ± S.D., *n* = 3) ; LMTC-CBZ NPs- Cyclobenzaprine HCl loaded low molecular weight thiolated chitosan NPs

*Ratio of thiolated chitosan: sodium alginate: drug: sodium deoxycholate solution was taken **7:1:1:1**

Table: 5.15. Matrix of Box Behnken Design, Particle size response and Entrapment Efficiency of each experimental run for MMTC-TZ NPs

Std	Run	Concentration of TC(mg/ml) Factor :A	Concentration of SA (mg/ml) Factor :B	Concentration of drug mg/ml) Factor :C	Concentration of SDC(mg/ml) Factor:D	Particle size (nm)	Predicted particle size (nm)	Entrapment Efficiency (% EE)	Predicted Entrapment Efficiency (% EE)
21	1	2	0.25	2	5	397.2±13.4	395.2	45.67±2.3	45.53
***25**	**2**	**2**	**0.5**	**2**	**10**	**264.9±12.7**	**266.4**	**72.20±4.5**	**70.83**
18	3	3	0.5	1	10	361.0±17.8	358.3	67.32±1.5	69.90
27	4	2	0.5	2	10	269.2±13.6	266.4	68.45±3.8	70.83
14	5	2	0.75	1	10	422.7±14.6	419.9	70.90±5.1	68.17
20	6	3	0.5	3	10	381.8±18.5	383.9	66.54±3.4	66.19
24	7	2	0.75	2	15	333.9±15.3	339.2	64.76±5.7	65.12
3	8	1	0.75	2	10	395.1±11.3	397.8	63.59±3.1	64.96
9	9	1	0.5	2	5	434.9±17.8	430.7	49.45±5.2	49.00
26	10	2	0.5	2	10	268.1±19.3	266.4	70.11±6.1	70.83
6	11	2	0.5	3	5	387.1±11.3	391.9	45.65±2.8	45.76
28	12	2	0.5	2	10	263.5±10.9	266.4	72.56±5.3	70.83
15	13	2	0.25	3	10	362.1±8.9	358.1	61.12±5.2	64.81
17	14	1	0.5	1	10	337.8±22.9	339.0	64.89±4.3	65.46
4	15	3	0.75	2	10	415.6±12.5	419.7	69.87±3.6	69.53
13	16	2	0.25	1	10	264.9±15.6	268.1	60.05±2.4	61.84
16	17	2	0.75	3	10	341.8±16.3	331.8	62.51±3.8	61.68
10	18	3	0.5	2	5	440.9±14.6	439.2	50.56±5.2	49.80
22	19	2	0.75	2	5	464.7±17.5	465.3	45.77±4.5	47.91
23	20	2	0.25	2	15	281.1±14.6	283.7	66.23±5.1	64.30
2	21	3	0.25	2	10	378.2±11.4	378.9	68.41±2.9	65.84
5	22	2	0.5	1	5	409.7±13.5	412.2	49.32±3.9	48.40
12	23	3	0.5	2	15	358.3±18.5	355.8	68.06±4.3	69.47
1	24	1	0.25	2	10	313.7±11.1	313.0	66.31±4.6	65.45
8	25	2	0.5	3	15	293.4±17.8	294.4	64.91±3.2	64.63
11	26	1	0.5	2	15	281.5±19.5	276.4	63.59±3.9	65.31
19	27	1	0.5	3	10	309.4±23.2	315.4	68.02±4.3	65.66
7	28	2	0.5	1	15	273.4±16.4	272.1	66.81±4.9	65.50

(Mean ± S.D., *n* = 3); MMTC-TZ NPs- Tizanidine HCl loaded medium molecular weight thiolated chitosan NPs

*Ratio of thiolated chitosan: sodium alginate: drug: sodium deoxycholate solution was taken **7:1:1:1**

Table: 5.16. Matrix of Box Behnken Design, Particle size response and Entrapment Efficiency of each experimental run for MMTC-CBZ NPs

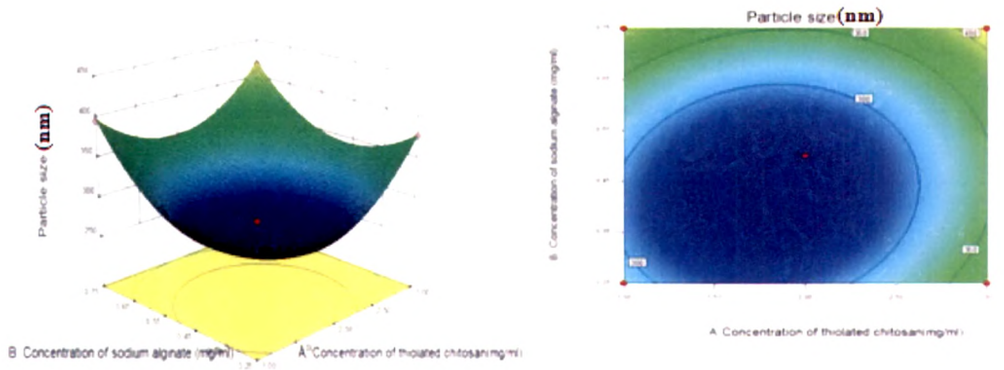
Std	Run	Concentration of TC(mg/ml) Factor :A	Concentration of SA (mg/ml) Factor :B	Concentration of drug mg/ml) Factor :C	Concentration of SDC(mg/ml) Factor:D	Particle size (nm)	Predicted particle size (nm)	Entrapment Efficiency (% EE)	Predicted Entrapment Efficiency (% EE)
21	1	2	0.25	2	5	399.8±12.5	392.9	50.33±2.1	50.40
***25**	**2**	**2**	**0.5**	**2**	**10**	**282.9±15.6**	**280.9**	**80.20±3.2**	**79.95**
18	3	3	0.5	1	10	374.5±20.1	375.0	69.12±4.3	70.11
27	4	2	0.5	2	10	275.9±12.5	280.9	77.98±3.6	79.95
14	5	2	0.75	1	10	401.2±14.6	401.3	70.08±3.5	69.96
20	6	3	0.5	3	10	397.4±13.6	395.0	68.11±4.3	70.07
24	7	2	0.75	2	15	313.4±17.5	322.7	64.34±5.1	65.03
3	8	1	0.75	2	10	370.9±14.6	367.0	66.12±4.7	65.69
9	9	1	0.5	2	5	477.9±11.5	481.1	50.44±4.1	50.52
26	10	2	0.5	2	10	279.6±18.9	280.9	81.76±3.6	79.95
6	11	2	0.5	3	5	395.9±12.3	402.0	55.43±5.2	53.60
28	12	2	0.5	2	10	285.4±11.5	280.9	79.87±2.9	79.95
15	13	2	0.25	3	10	351.7±17.2	348.4	71.31±1.9	73.22
17	14	1	0.5	1	10	341.9±18.1	346.6	67.98±2.7	66.78
4	15	3	0.75	2	10	425.8±21.8	427.5	71.12±4.3	68.57
13	16	2	0.25	1	10	258.6±11.2	255.3	61.76±3.9	63.54
16	17	2	0.75	3	10	301.0±10.3	301.1	64.21±5.3	64.21
10	18	3	0.5	2	5	441.0±19.9	442.9	52.76±6.1	52.76
22	19	2	0.75	2	5	473.5±20.3	466.2	47.11±3.7	49.49
23	20	2	0.25	2	15	287.6±9.9	297.3	68.34±5.4	66.71
2	21	3	0.25	2	10	365.1±12.4	369.7	70.44±4.5	68.30
5	22	2	0.5	1	5	426.8±18.7	429.9	50.33±3.7	49.60
12	23	3	0.5	2	15	419.9±17.4	413.5	66.07±2.5	67.77
1	24	1	0.25	2	10	327.0±18.3	326.2	68.55±3.6	68.53
8	25	2	0.5	3	15	309.2±11.5	306.8	69.33±5.4	67.49
11	26	1	0.5	2	15	276.4±11.1	271.4	65.58±4.9	67.36
19	27	1	0.5	3	10	317.7±15.6	319.5	70.99±3.6	70.75
7	28	2	0.5	1	15	291.3±13.9	286.0	68.31±3.2	67.57

(Mean ± S.D., *n* = 3); MMTC-TZ NPs- Cyclobenzaprine HCl loaded medium molecular weight thiolated chitosan NPs

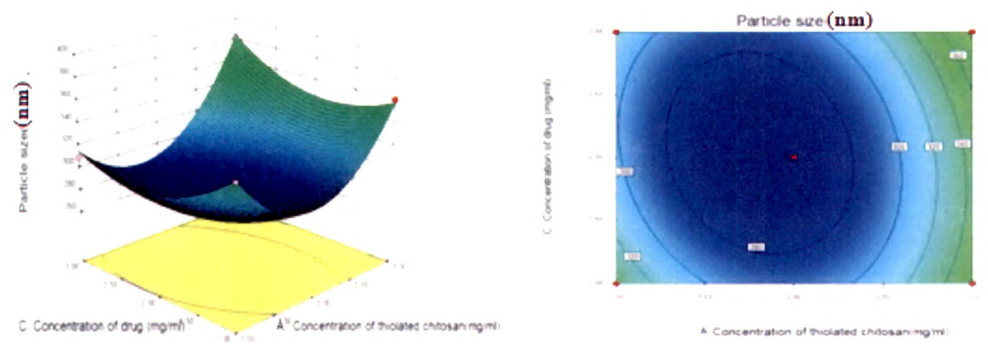
*Ratio of thiolated chitosan: sodium alginate: drug: sodium deoxycholate solution was taken **7:1:1:1**

In order to optimize thiolated chitosan: sodium alginate: drug: sodium deoxycholate solution ratio in each formulations, nanoparticles were formulated using thiolated chitosan: sodium alginate: drug: sodium deoxycholate solution with different ratio **7:1:1:1,** 4:2:2:2, 5.5:1.5:1.5:1.5 and the Factor A, B, C and D concentration was kept constant (Wang et al., 2008).

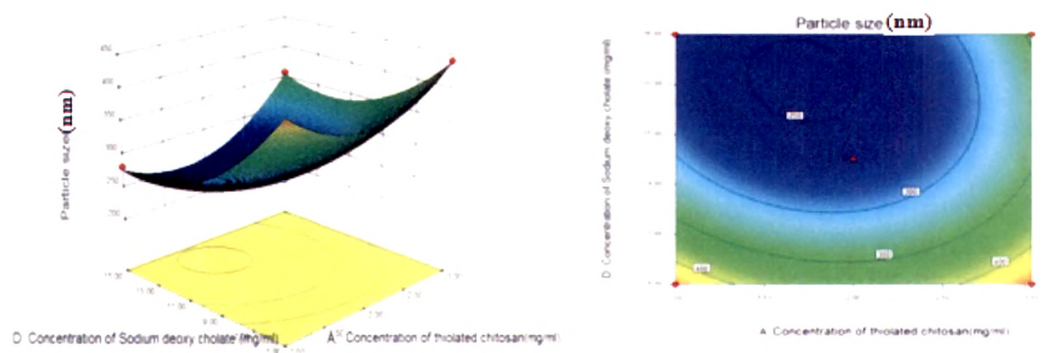
(a)


Particle size (nm)
B: Concentration of sodium alginate (mg/ml)
A: Concentration of thiolated chitosan (mg/ml)
Particle size (nm)
B: Concentration of sodium alginate (mg/ml)
A: Concentration of thiolated chitosan (mg/ml)

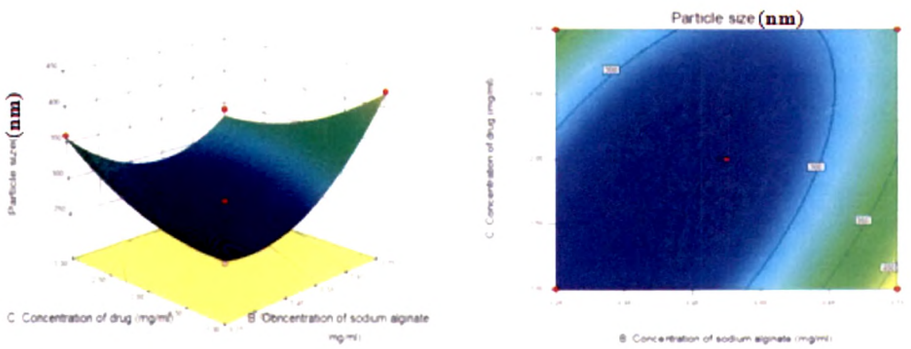
(b)


Particle size (nm)
C: Concentration of drug (mg/ml)
A: Concentration of thiolated chitosan (mg/ml)
Particle size (nm)
C: Concentration of drug (mg/ml)
A: Concentration of thiolated chitosan (mg/ml)

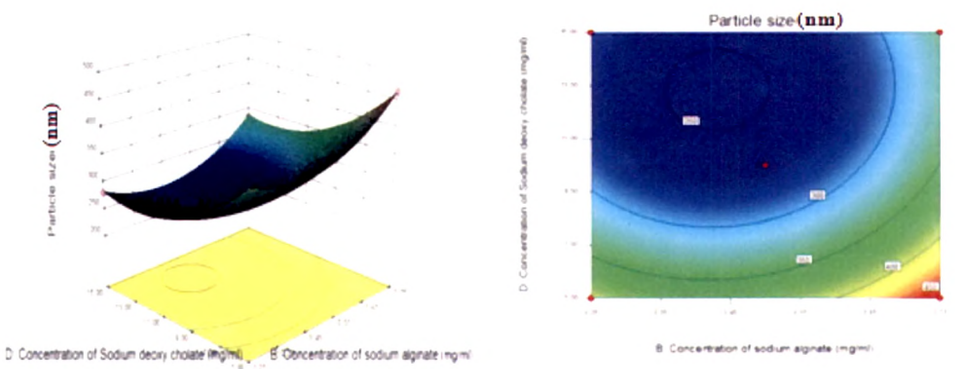
(c)


Particle size (nm)
D: Concentration of Sodium deoxy cholate (mg/ml)
A: Concentration of thiolated chitosan (mg/ml)
Particle size (nm)
D: Concentration of Sodium deoxy cholate (mg/ml)
A: Concentration of thiolated chitosan (mg/ml)

(d)



(e)



(f)

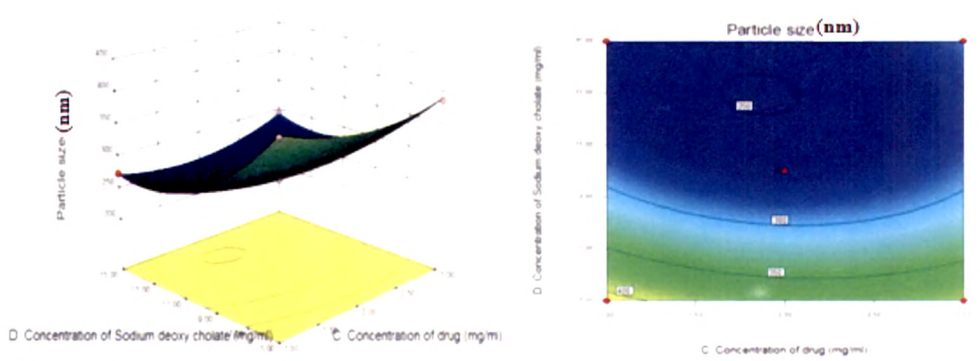
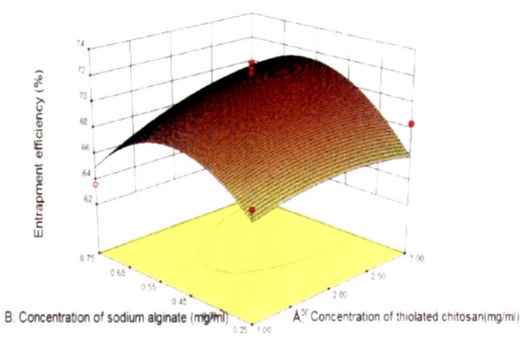
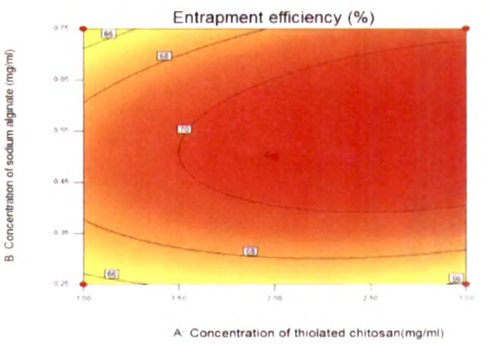


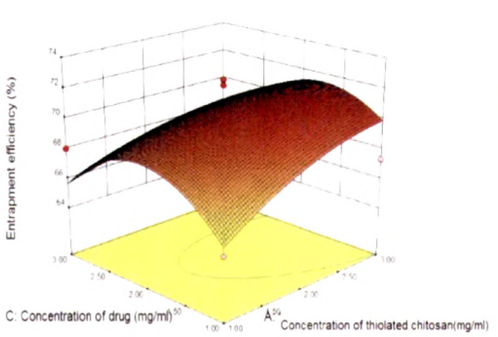
Figure: 5.9. Response surface and contour plots of a) the effects of Concentration of thiolated chitosan (A) and Concentration of sodium alginate (B) b) effects of Concentration of thiolated chitosan (A) and Concentration of drug (C) c) Concentration of thiolated chitosan (A) and concentration of sodium deoxycholate (D) d) Concentration of sodium alginate (B) and Concentration of Drug (C) e) Concentration of sodium alginate (B) and concentration of sodium deoxycholate (D) f) Concentration of drug (C) and concentration of sodium deoxycholate (D) on the particle size of the LMTC-TZ NPs

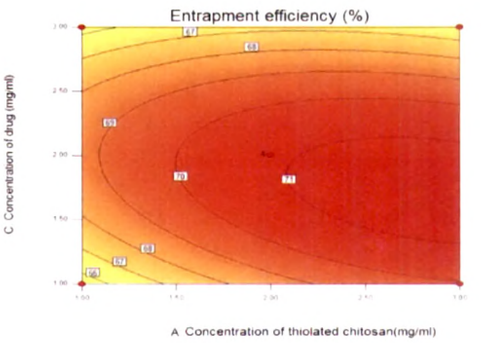
(a)


Entrapment efficiency (%)
B: Concentration of sodium alginate (mg/ml)
A: Concentration of thiolated chitosan(mg/ml)

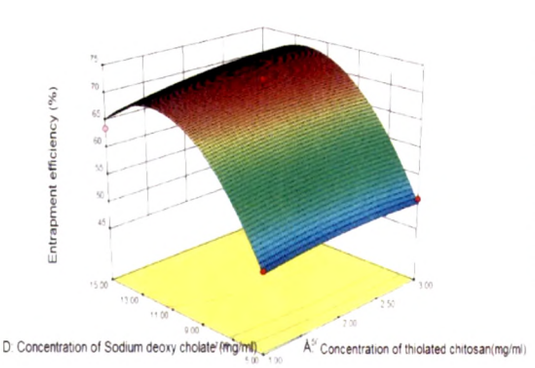

Entrapment efficiency (%)
B: Concentration of sodium alginate (mg/ml)
A: Concentration of thiolated chitosan(mg/ml)

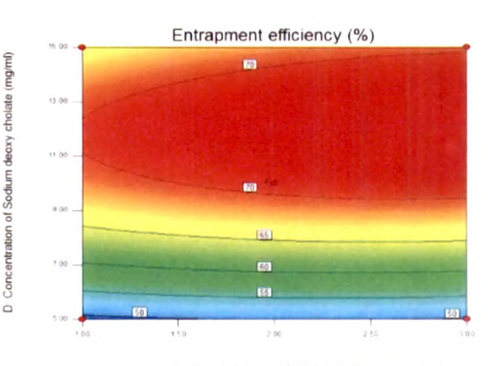
(b)


Entrapment efficiency (%)
C: Concentration of drug (mg/ml)
A: Concentration of thiolated chitosan(mg/ml)

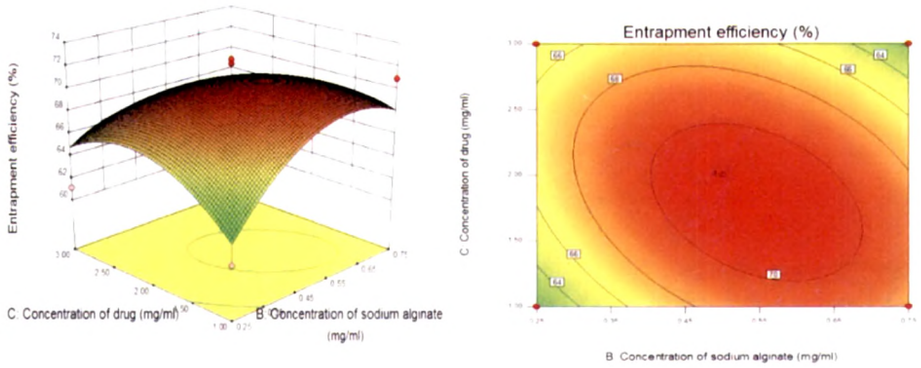

Entrapment efficiency (%)
C: Concentration of drug (mg/ml)
A: Concentration of thiolated chitosan(mg/ml)

(c)

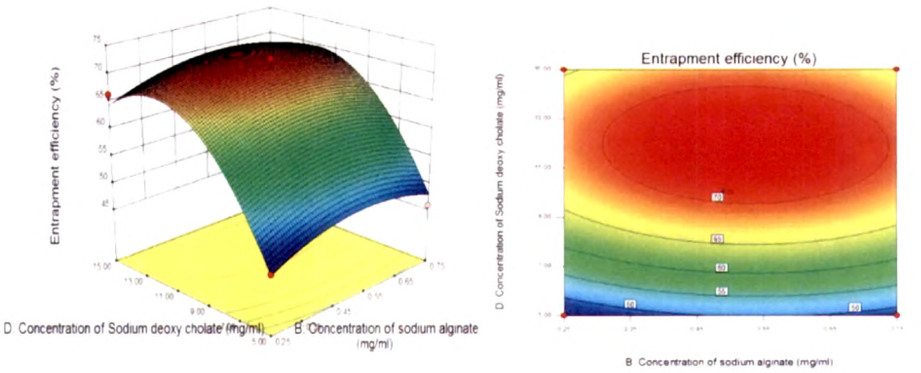

Entrapment efficiency (%)
D: Concentration of Sodium deoxy cholate (mg/ml)
A: Concentration of thiolated chitosan(mg/ml)


Entrapment efficiency (%)
D: Concentration of Sodium deoxy cholate (mg/ml)
A: Concentration of thiolated chitosan(mg/ml)

(d)



(e)



(f)

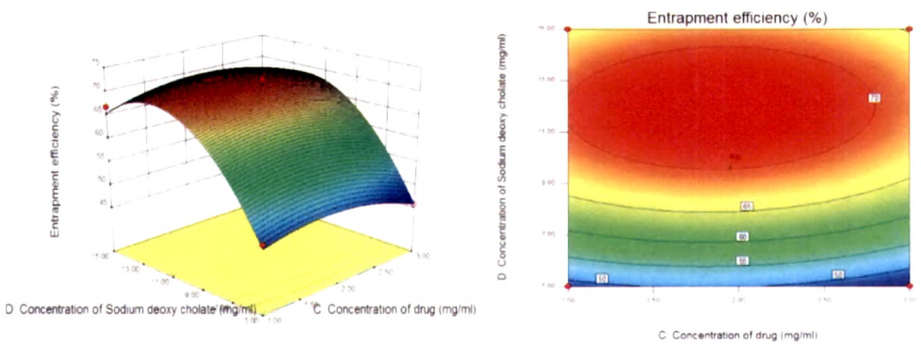
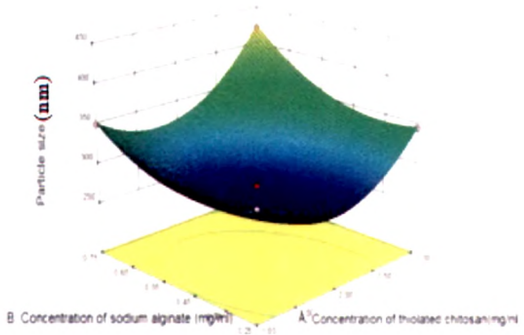
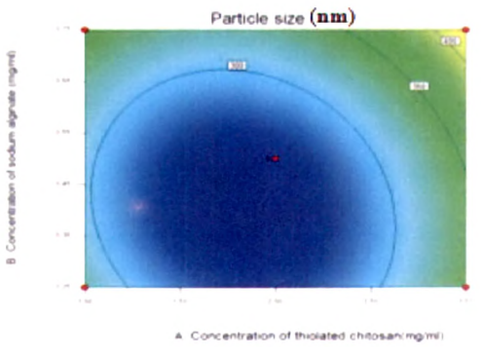


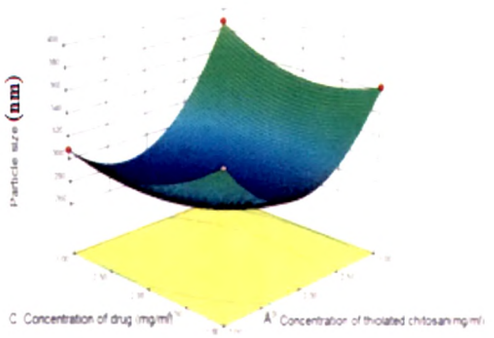
Figure: 5.10. Response surface and contour plots of a) the effects of Concentration of thiolated chitosan (A) and Concentration of sodium alginate (B) b) effects of Concentration of thiolated chitosan (A) and Concentration of drug (C) c) Concentration of thiolated chitosan (A) and concentration of sodium deoxycholate (D) d) Concentration of sodium alginate (B) and Concentration of Drug (C) e) Concentration of sodium alginate (B) and concentration of sodium deoxycholate (D) f) Concentration of drug (C) and concentration of sodium deoxycholate (D) on the entrapment efficiency of the LMTC-TZ NPs

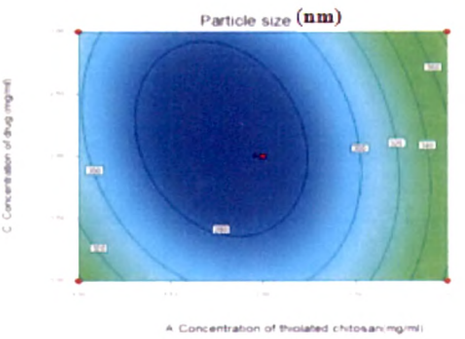
(a)


Particle size (nm)
B: Concentration of sodium alginate (mg/ml)
A: Concentration of thiolated chitosan(mg/ml)

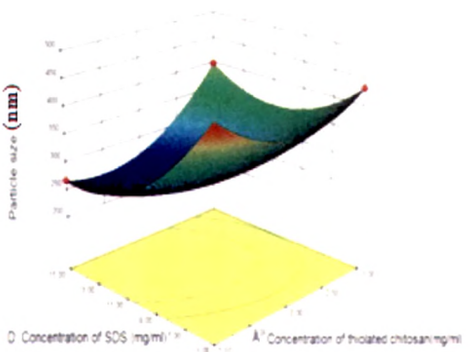

Particle size (nm)
B: Concentration of sodium alginate (mg/ml)
A: Concentration of thiolated chitosan(mg/ml)

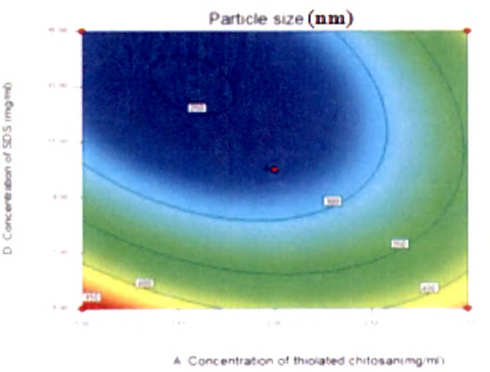
(b)


Particle size (nm)
C: Concentration of drug (mg/ml)
A: Concentration of thiolated chitosan(mg/ml)

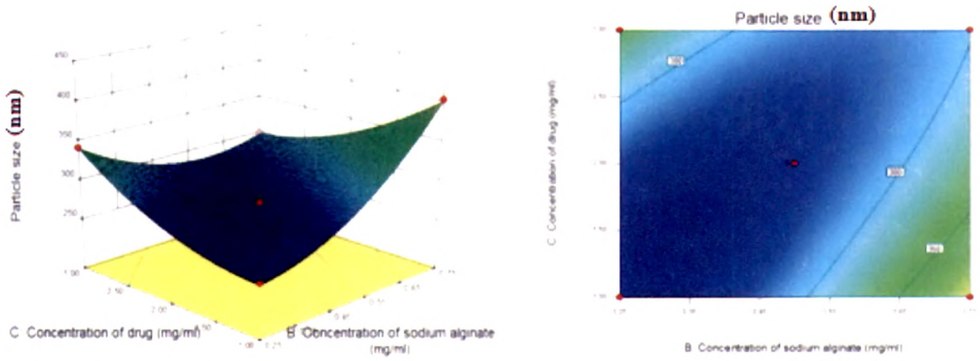

Particle size (nm)
C: Concentration of drug (mg/ml)
A: Concentration of thiolated chitosan(mg/ml)

(c)

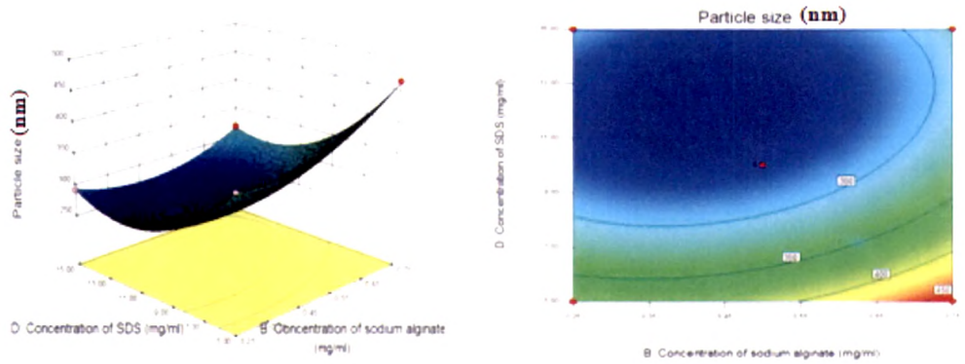

Particle size (nm)
D: Concentration of SDS (mg/ml)
A: Concentration of thiolated chitosan(mg/ml)


Particle size (nm)
D: Concentration of SDS (mg/ml)
A: Concentration of thiolated chitosan(mg/ml)

(d)



(e)



(f)

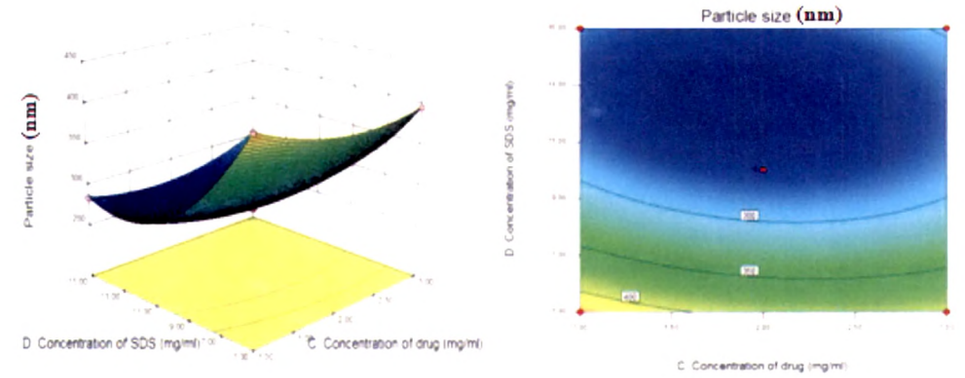
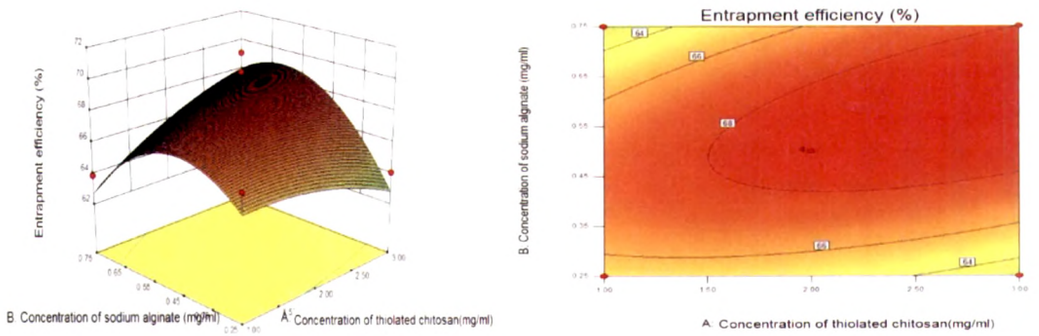
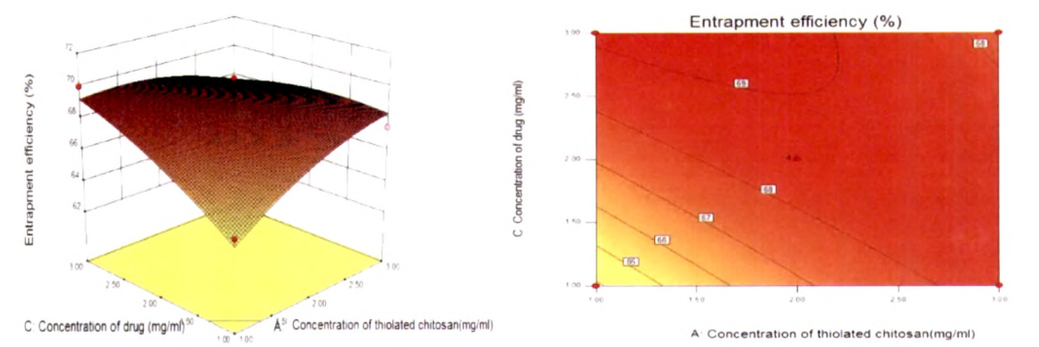


Figure: 5.11. Response surface and contour plots of a) the effects of Concentration of thiolated chitosan (A) and Concentration of sodium alginate (B) b) effects of Concentration of thiolated chitosan (A) and Concentration of drug (C) c) Concentration of thiolated chitosan (A) and concentration of sodium deoxycholate (D) d) Concentration of sodium alginate (B) and Concentration of Drug (C) e) Concentration of sodium alginate (B) and concentration of sodium deoxycholate (D) f) Concentration of drug (C) and concentration of sodium deoxycholate (D)on the particle size of the LMTC-CBZ NPs

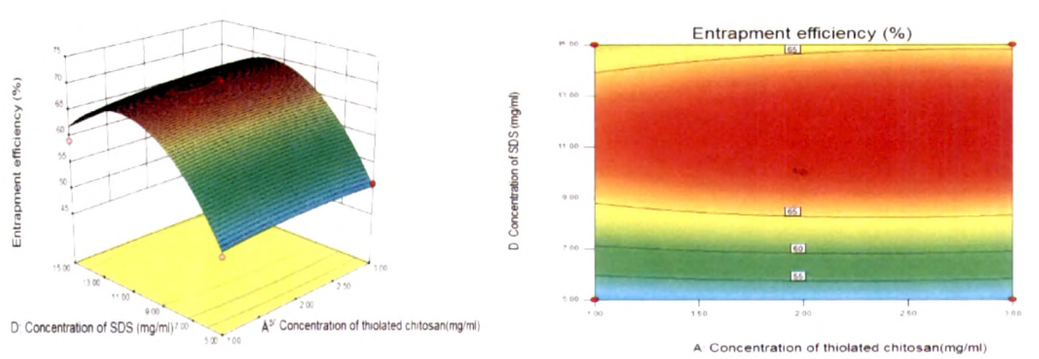
(a)


Entrapment efficiency (%)
Entrapment efficiency (%)
B: Concentration of sodium alginate (mg/ml)
A: Concentration of thiolated chitosan(mg/ml)
Entrapment efficiency (%)
B: Concentration of sodium alginate (mg/ml)
A: Concentration of thiolated chitosan(mg/ml)

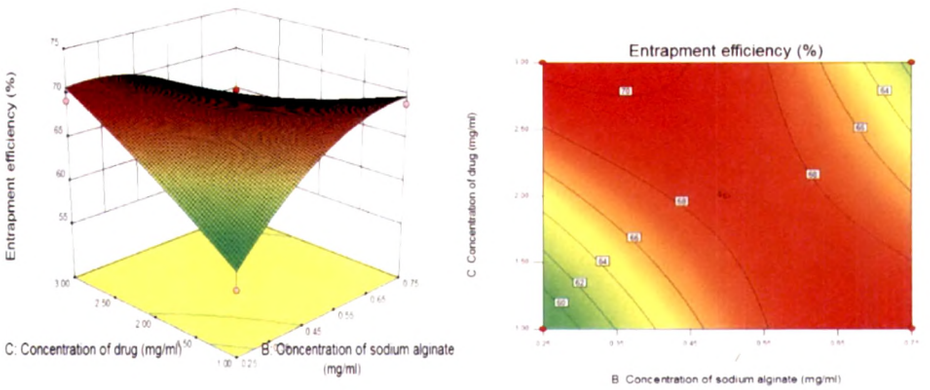
(b)


Entrapment efficiency (%)
Entrapment efficiency (%)
C: Concentration of drug (mg/ml)
A: Concentration of thiolated chitosan(mg/ml)
Entrapment efficiency (%)
C: Concentration of drug (mg/ml)
A: Concentration of thiolated chitosan(mg/ml)

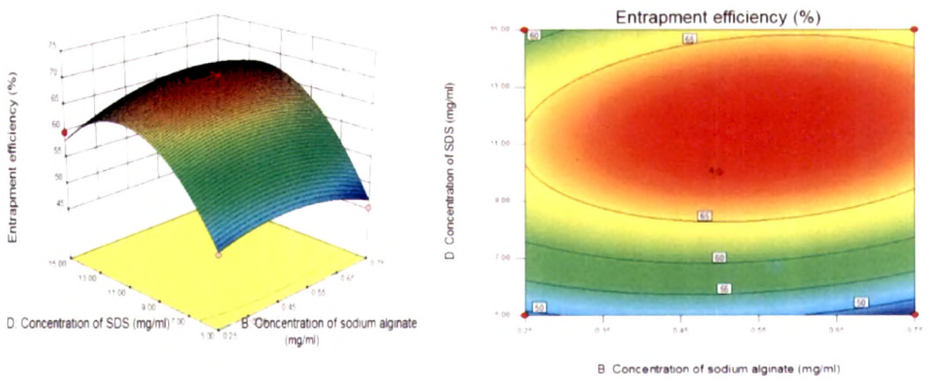
(c)


Entrapment efficiency (%)
Entrapment efficiency (%)
D: Concentration of SDS (mg/ml)
A: Concentration of thiolated chitosan(mg/ml)
Entrapment efficiency (%)
D: Concentration of SDS (mg/ml)
A: Concentration of thiolated chitosan(mg/ml)

(d)



(e)



(f)

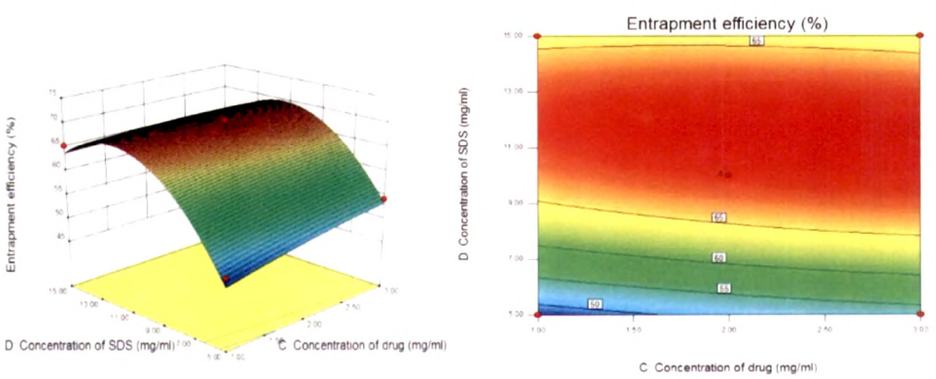
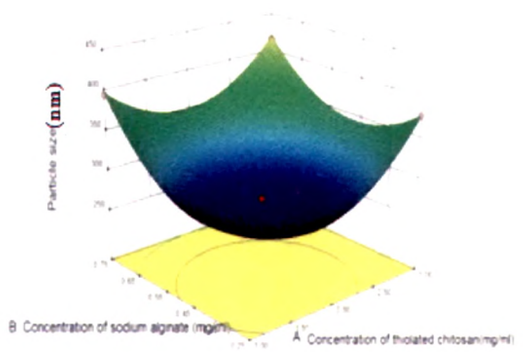
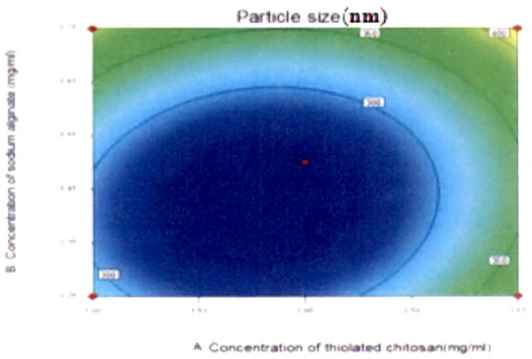


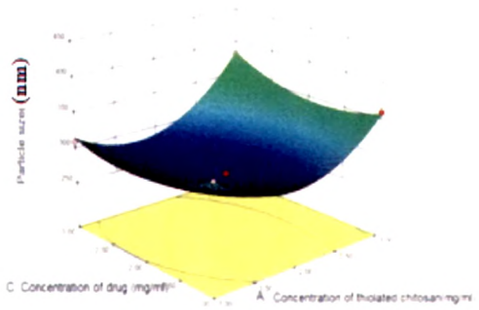
Figure: 5.12. Response surface and contour plots of a) the effects of Concentration of thiolated chitosan (A) and Concentration of sodium alginate (B) b) effects of Concentration of thiolated chitosan (A) and Concentration of drug (C) c) Concentration of thiolated chitosan (A) and concentration of sodium deoxycholate (D) d) Concentration of sodium alginate (B) and Concentration of Drug (C) e) Concentration of sodium alginate (B) and concentration of sodium deoxycholate (D) f) Concentration of drug (C) and concentration of sodium deoxycholate (D)on the entrapment efficiency of the LMTC-CBZ NPs

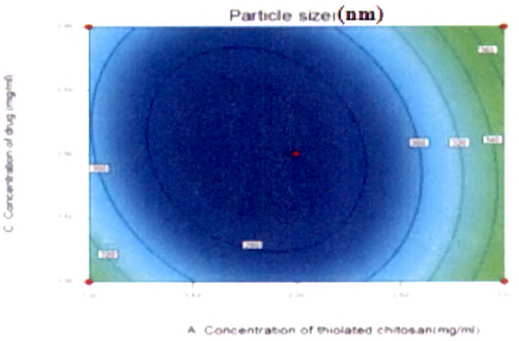
(a)


Particle size(nm)
B: Concentration of sodium alginate (mg/ml)
A: Concentration of thiolated chitosan(mg/ml)

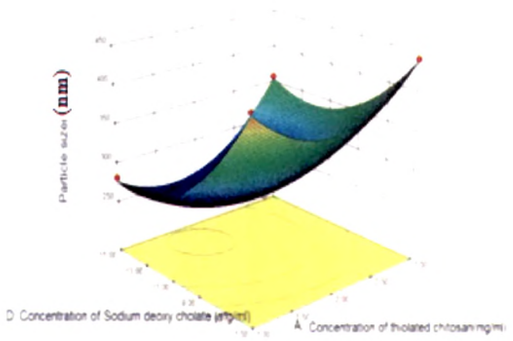

Particle size(nm)
B: Concentration of sodium alginate (mg/ml)
A: Concentration of thiolated chitosan(mg/ml)

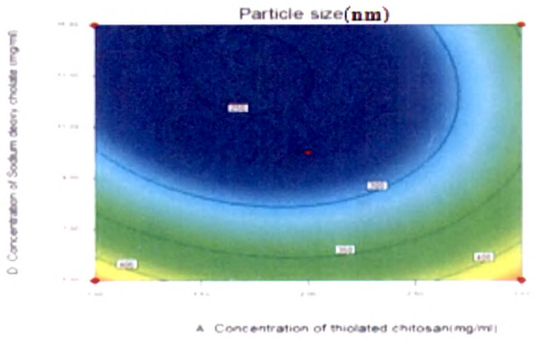
(b)


Particle size(nm)
C: Concentration of drug (mg/ml)
A: Concentration of thiolated chitosan(mg/ml)

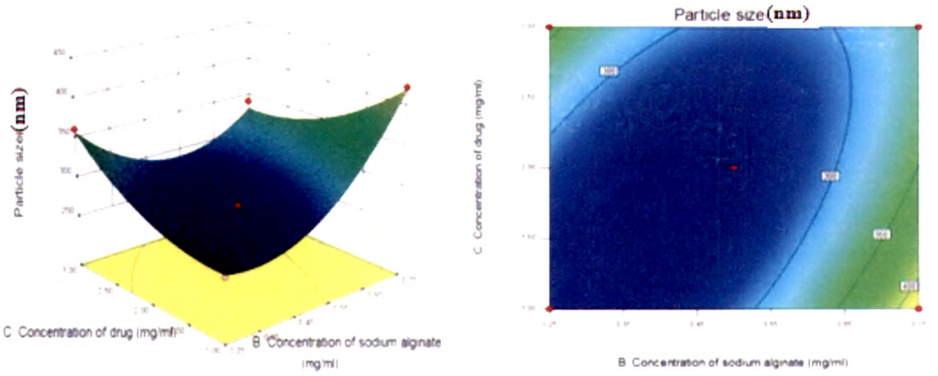

Particle size(nm)
C: Concentration of drug (mg/ml)
A: Concentration of thiolated chitosan(mg/ml)

(c)

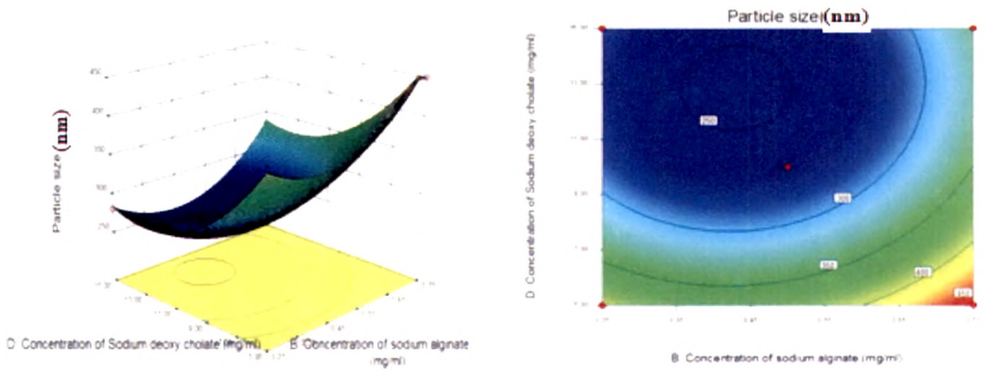

Particle size(nm)
D: Concentration of Sodium deoxy cholate (mg/ml)
A: Concentration of thiolated chitosan(mg/ml)


Particle size(nm)
D: Concentration of Sodium deoxy cholate (mg/ml)
A: Concentration of thiolated chitosan(mg/ml)

(d)



(e)



(f)

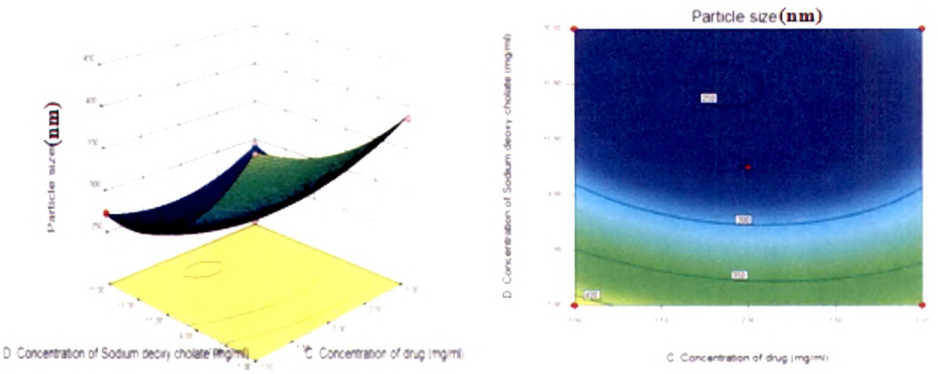
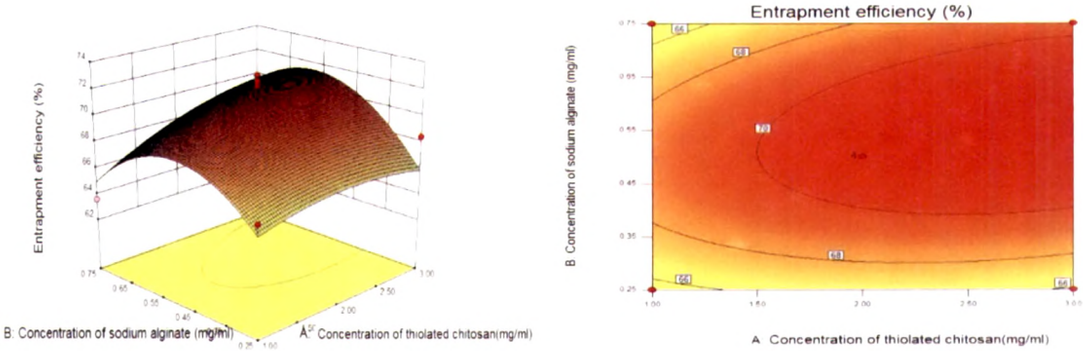
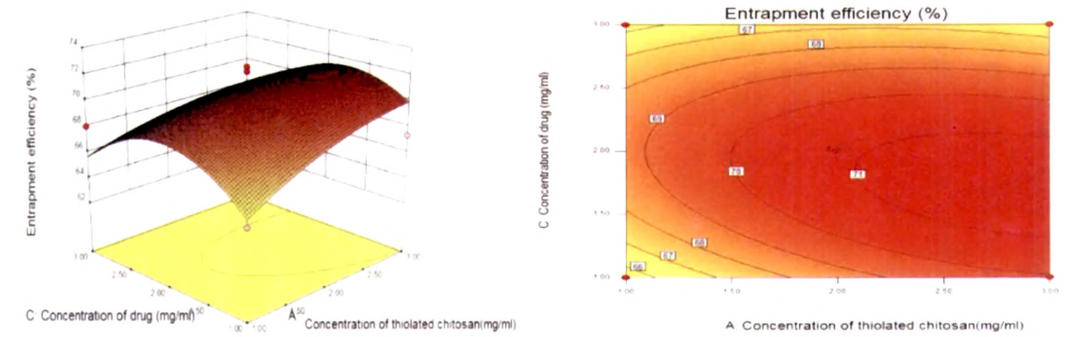


Figure: 5.13. Response surface and contour plots of a) the effects of Concentration of thiolated chitosan (A) and Concentration of sodium alginate (B) b) effects of Concentration of thiolated chitosan (A) and Concentration of drug (C) c) Concentration of thiolated chitosan (A) and concentration of sodium deoxycholate (D) d) Concentration of sodium alginate (B) and Concentration of Drug (C) e) Concentration of sodium alginate (B) and concentration of sodium deoxycholate (D) f) Concentration of drug (C) and concentration of sodium deoxycholate (D) on the particle size of the MMTC-TZ NP

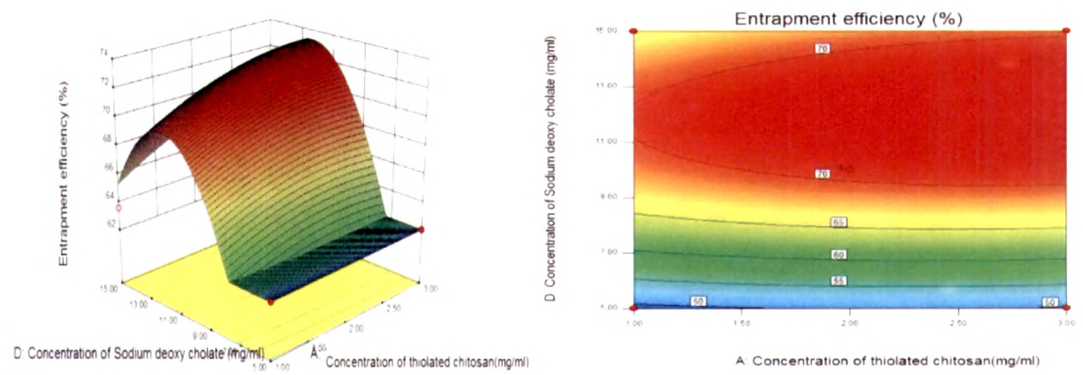
(a)


Entrapment efficiency (%)
Entrapment efficiency (%)
B: Concentration of sodium alginate (mg/ml)
A: Concentration of thiolated chitosan(mg/ml)
Entrapment efficiency (%)
B: Concentration of sodium alginate (mg/ml)
A: Concentration of thiolated chitosan(mg/ml)

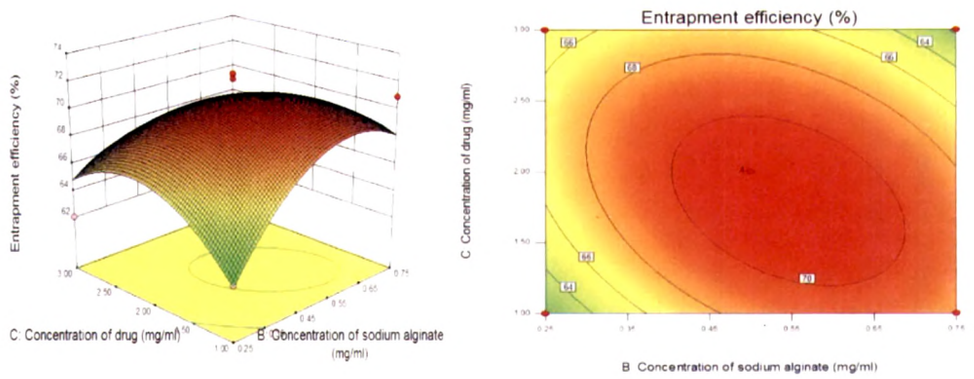
(b)


Entrapment efficiency (%)
C: Concentration of drug (mg/ml)
A: Concentration of thiolated chitosan(mg/ml)
Entrapment efficiency (%)
C: Concentration of drug (mg/ml)
A: Concentration of thiolated chitosan(mg/ml)

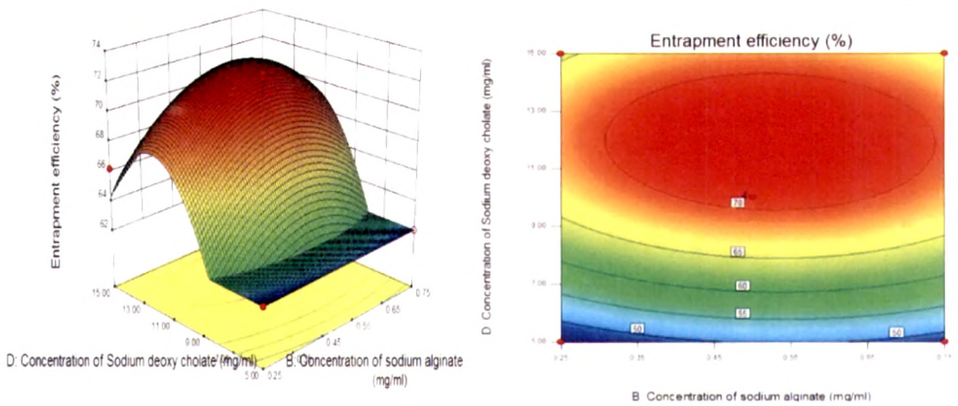
(c)


Entrapment efficiency (%)
D: Concentration of Sodium deoxy cholate (mg/ml)
A: Concentration of thiolated chitosan(mg/ml)
Entrapment efficiency (%)
D: Concentration of Sodium deoxy cholate (mg/ml)
A: Concentration of thiolated chitosan(mg/ml)

(d)



(e)



(f)

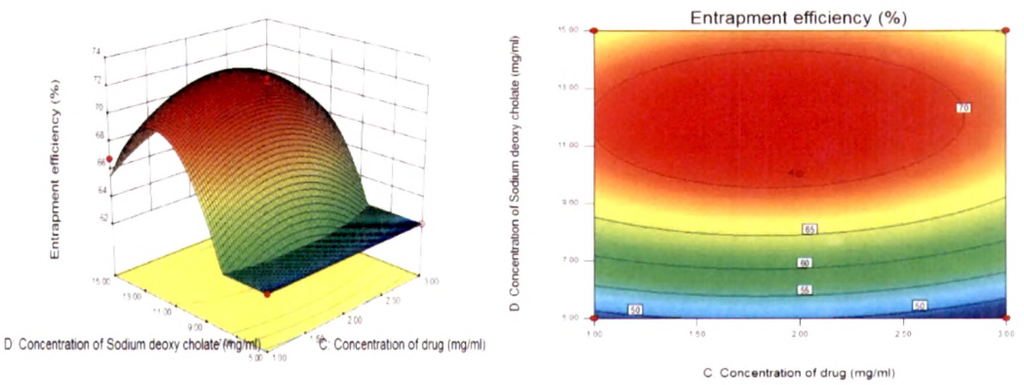
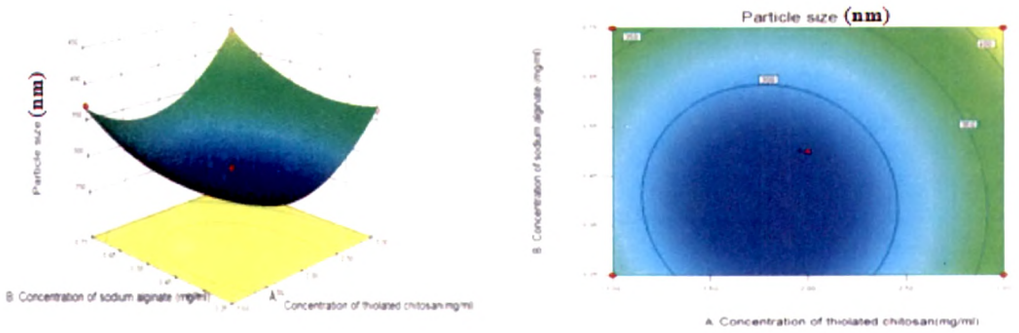
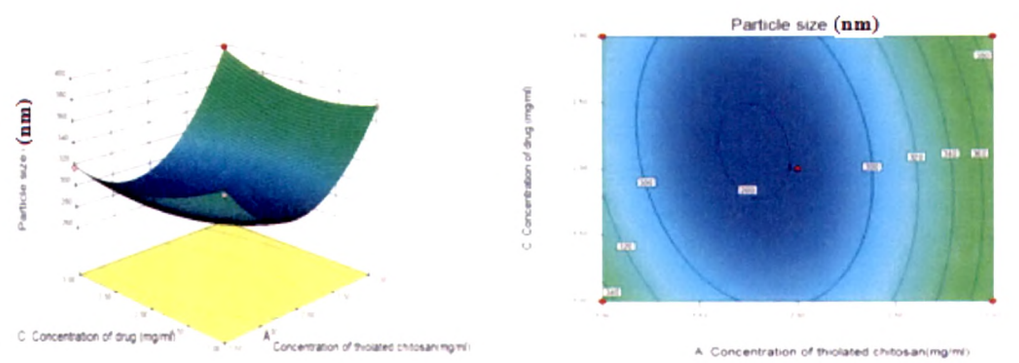


Figure: 5.14. Response surface and contour plots of a) the effects of Concentration of thiolated chitosan (A) and Concentration of sodium alginate (B) b) effects of Concentration of thiolated chitosan (A) and Concentration of drug (C) c) Concentration of thiolated chitosan (A) and concentration of sodium deoxycholate (D) d) Concentration of sodium alginate (B) and Concentration of Drug (C) e) Concentration of sodium alginate (B) and concentration of sodium deoxycholate (D) f) Concentration of drug (C) and concentration of sodium deoxycholate (D) on the entrapment efficiency of the MMTC-TZ NPs

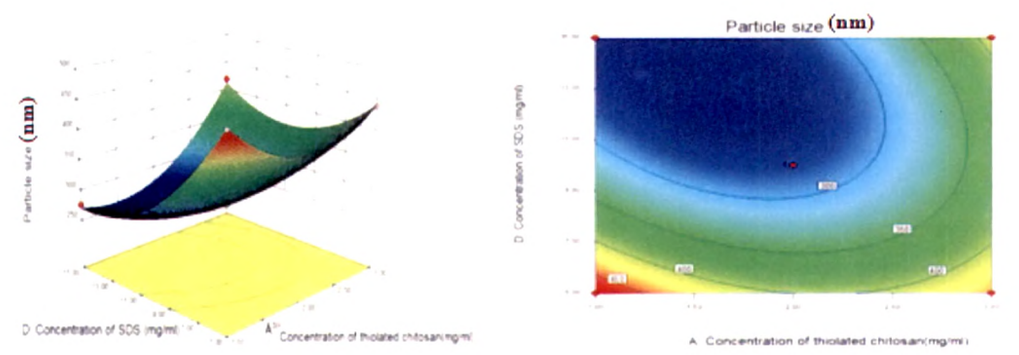
(a)


Particle size (nm)
Particle size (nm)
B: Concentration of sodium alginate (mg/ml)
A: Concentration of thiolated chitosan(mg/ml)
Particle size (nm)
B: Concentration of sodium alginate (mg/ml)
A: Concentration of thiolated chitosan(mg/ml)

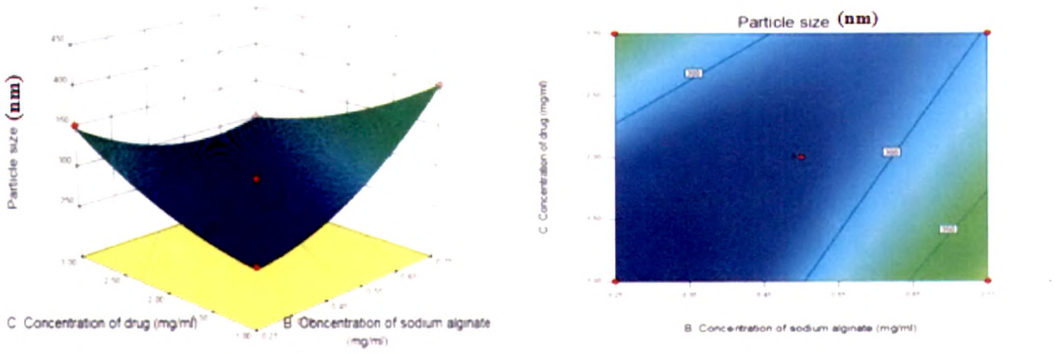
(b)


Particle size (nm)
Particle size (nm)
C: Concentration of drug (mg/ml)
A: Concentration of thiolated chitosan(mg/ml)
Particle size (nm)
C: Concentration of drug (mg/ml)
A: Concentration of thiolated chitosan(mg/ml)

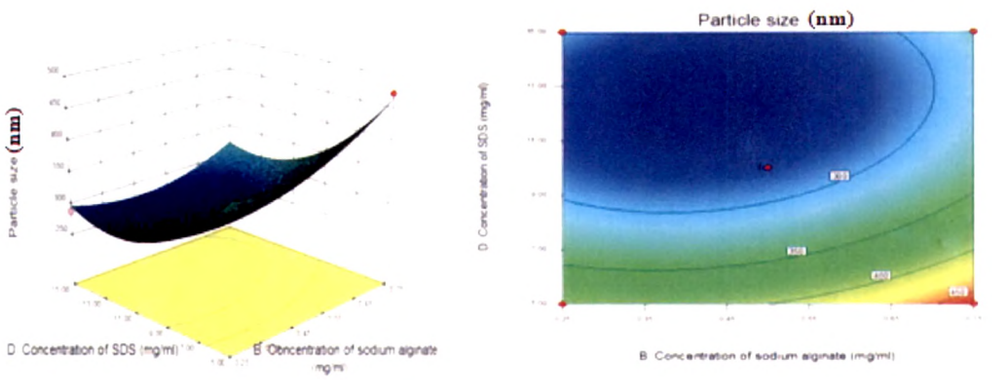
(c)


Particle size (nm)
Particle size (nm)
D: Concentration of SDS (mg/ml)
A: Concentration of thiolated chitosan(mg/ml)
Particle size (nm)
D: Concentration of SDS (mg/ml)
A: Concentration of thiolated chitosan(mg/ml)

(d)



(e)



(f)

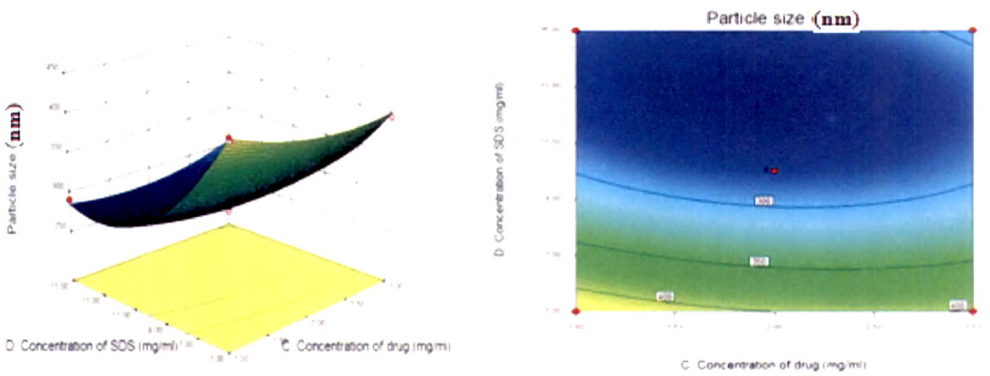
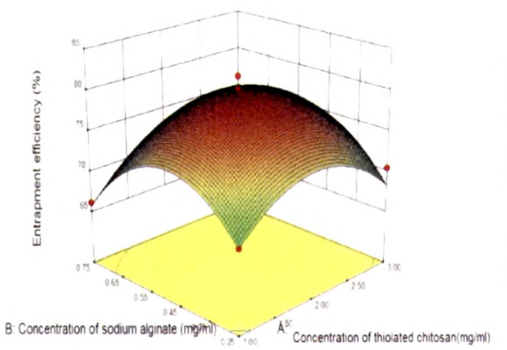
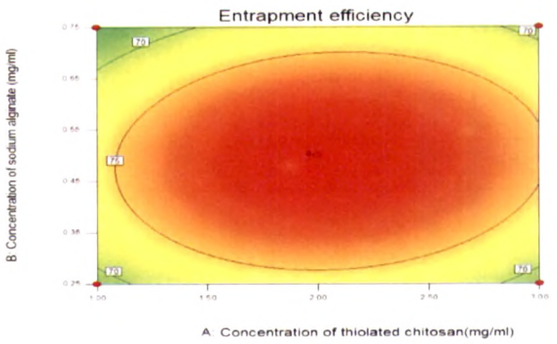


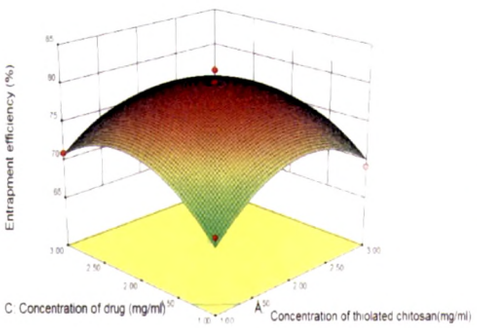
Figure: 5.15. Response surface and contour plots of a) the effects of Concentration of thiolated chitosan (A) and Concentration of sodium alginate (B) b) effects of Concentration of thiolated chitosan (A) and Concentration of drug (C) c) Concentration of thiolated chitosan (A) and concentration of sodium deoxycholate (D) d) Concentration of sodium alginate (B) and Concentration of Drug (C) e) Concentration of sodium alginate (B) and concentration of sodium deoxycholate (D) f) Concentration of drug (C) and concentration of sodium deoxycholate (D) on the particle size of the MMTC-CBZ NPs

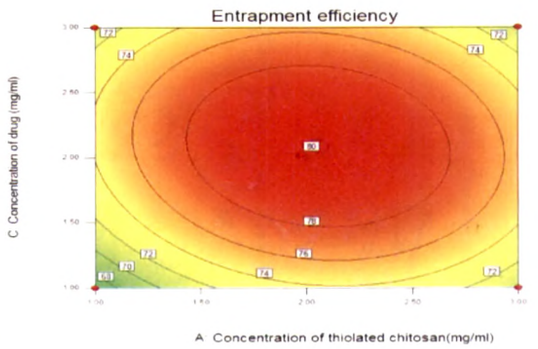
(a)


Entrapment efficiency (%)
B: Concentration of sodium alginate (mg/ml)
A: Concentration of thiolated chitosan(mg/ml)

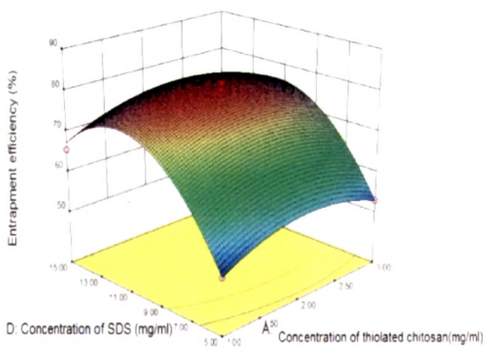

Entrapment efficiency
B: Concentration of sodium alginate (mg/ml)
A: Concentration of thiolated chitosan(mg/ml)

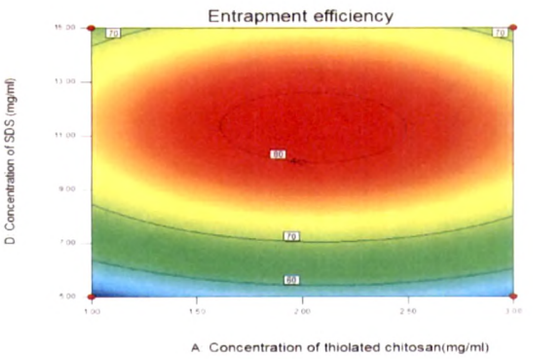
(b)


Entrapment efficiency (%)
C: Concentration of drug (mg/ml)
A: Concentration of thiolated chitosan(mg/ml)

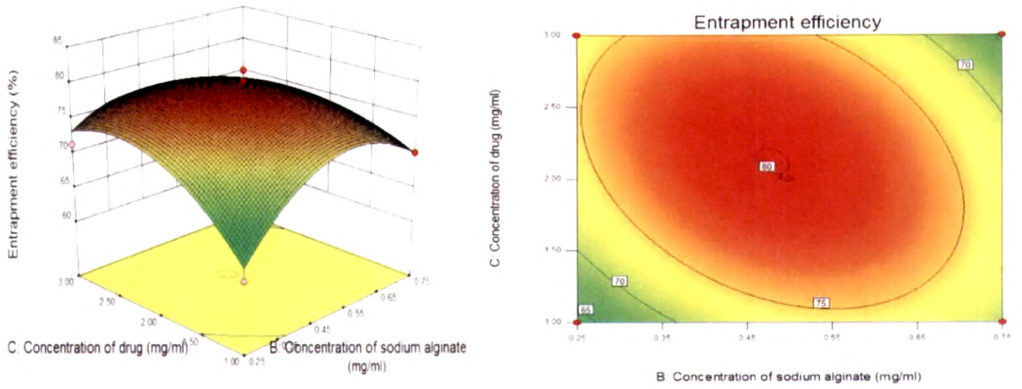

Entrapment efficiency
C: Concentration of drug (mg/ml)
A: Concentration of thiolated chitosan(mg/ml)

(c)

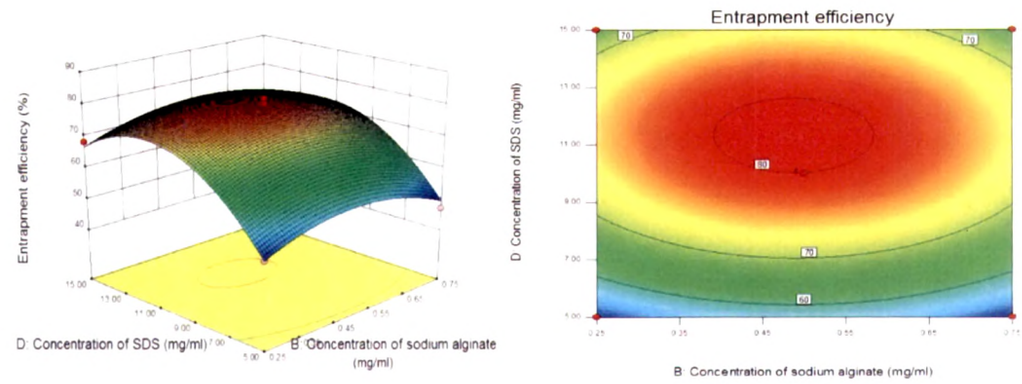

Entrapment efficiency (%)
D: Concentration of SDS (mg/ml)
A: Concentration of thiolated chitosan(mg/ml)


Entrapment efficiency
D: Concentration of SDS (mg/ml)
A: Concentration of thiolated chitosan(mg/ml)

(d)



(e)



(f)

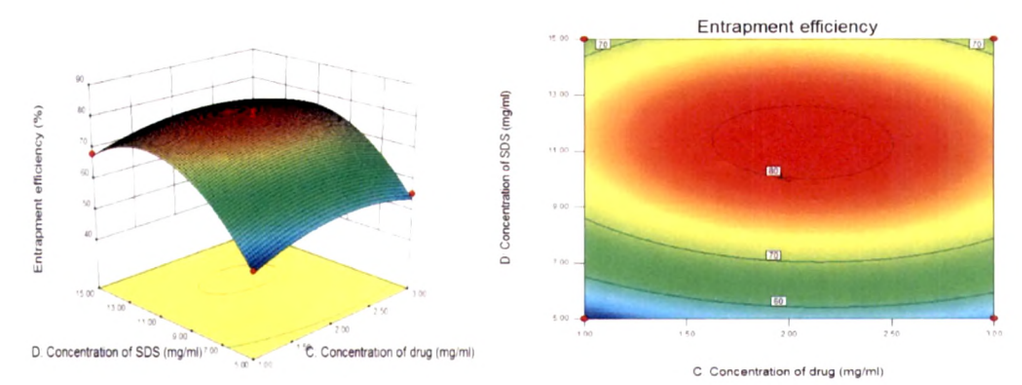


Figure: 5.16. Response surface and contour plots of a) the effects of Concentration of thiolated chitosan (A) and Concentration of sodium alginate (B) b) effects of Concentration of thiolated chitosan (A) and Concentration of drug (C) c) Concentration of thiolated chitosan (A) and concentration of sodium deoxycholate (D) d) Concentration of sodium alginate (B) and Concentration of Drug (C) e) Concentration of sodium alginate (B) and concentration of sodium deoxycholate (D) f) Concentration of drug (C) and concentration of sodium deoxycholate (D) on the entrapment efficiency of the MMTC-CBZ NPs

Data processing:

Thiolated chitosan nanoparticles were prepared by Ionotropic gelation with slight modification using thiolated chitosan/sodium alginate/drug/sodium deoxycholate. Formulation parameters, such as concentration of thiolated chitosan, concentration of sodium alginate, concentration of drug and concentration of sodium deoxycholate found to have significant influence on preparation of nanoparticles. Hence, formulation parameters were optimized and kept unaltered in subsequent experiments. In order to optimize formulation parameters, a Box Behnken Design (BBD) of RSM was used. Twenty eight batches of nanoparticles for each were prepared by Ionotropic gelation method using a 4-factor, 3-level BBD varying four independent variables (Concentration of thiolated chitosan (mg/ml) (A), Concentration of sodium alginate (mg/ml) (B) and concentration of drug (mg/ml) (C) and concentration of sodium deoxycholate (D) of the formulation according to Table: 5.12. The influence of these variables on observed response (Y, particle size and entrapment efficiency) is recorded in Table: 5.13 to Table: 5.16 for each formulation. Each batch of nanoparticles was prepared three times and was evaluated for particle size and entrapment efficiency.

Polynomial equation for each formulation:

LMTC-TZ NPs- The maximum response as particle size was 456.78nm and minimum response was 250.56nm. The mathematical relationship for the same in terms of a polynomial equation relating the response Y and independent variables was: (Y1) =+265.12+22.09 * A+25.29 * B-1.18 * C-57.97 * D-13.96 +* A * B +12.89 * A * C +72 * A * D-51.52 * B * C-11.50 * B * D+9.74 * C * D+63.22 * A^2+41.51 * B^2+16.70* C^2+52.68 * D^2 and (Y2) =+69.19+1.39* A+1.77 * B-1.17 * C+7.96 * D+0.59 * A * B-1.24 * A * C+1.29 * A * D-1.77 * B * C+0.45 * B * D+0.72 * C * D-1.15 * A^2-3.96* B^2-4.05 * C^2-1.36 * D^2 for particle size and entrapment efficiency respectively. ..... Equation [5.11] & [5.12]

LMTC-CBZ NPs- The maximum response as particle size was 467.79nm and minimum response was 252.34nm. The mathematical relationship for the same in terms of a polynomial equation relating the response Y and independent variables was :(Y1) =+270.70+24.52 * A+27.09 * B-2.75 * C-58.55 * D+11.36* A * B+14.29* A * C+41.33 * A * D-48.63 * B * C-14.86 * B * D+12.43* C * D+58.82 * A^2+31.38 * B^2+21.00 * C^2+61.91 * D^2 and (Y2) =+68.54+0.73* A+0.67* B+1.17 * C+6.45 *

D+1.94* A * B-1.49 * A* C+0.50 * A * D-5.32 * B * C+1.88* B * D-1.56 * C * D-0.75 * A^2-3.11 * B^2-0.55 * C^2-11.02 * D^2 for particle size and entrapment efficiency respectively. ..... Equation [5.13] & [5.14]

MMTC-TZ NPs- The maximum response as particle size was 464.71nm and minimum response was 263.55nm. The mathematical relationship for the same in terms of a polynomial equation relating the response Y and independent variables was :(Y1) =+266.45+21.95 * A+31.39 * B+0.49 * C-59.42* D-11.00* A * B+12.30 * A* C+17.71* A * D-44.52 * B * C-3.67 * B * D+10.67 * C * D+57.82 * A^2+53.16 * B^2+24.92 * C^2+51.30* D^2 and (Y2) =+70.83+1.24* A+0.80* B-0.88* C+9.00* D+1.05* A * B-0.98* A *C+0.84 * A * D-2.37* B * C-0.39* B * D+0.44* C * D-0.85 * A^2-3.53* B^2-3.17 * C^2-11.58 * D^2 for particle size and entrapment efficiency respectively. ..... Equation [5.15] & [5.16]

MMTC-CBZ NPs- The maximum response as particle size was 477.98nm and minimum response was 258.62nm. The mathematical relationship for the same in terms of a polynomial equation relating the response Y and independent variables was: (Y1) =+281.00+25.99* A+24.66* B-1.77* C-59.78* D+4.23* A * B+11.75* A * C+45.09* A * D-48.33* B * C-11.97* B * D+12.20* C * D+62.07* A^2+29.58* B^2+16.02 *C^2+59.22* D^2 and (Y2) =+79.95+0.66* A-0.65 * B+0.98* C+7.96* D+0.78* A * B-1.00* A *C-0.46* A * D-3.86* B * C-0.19* B * D-1.02* C * D-5.24 * A^2-6.94 * B^2-5.28* C^2-15.10 * D^2 for particle size and entrapment efficiency respectively. ..... Equation [5.17] & [5.18]

Equation expresses the quantitative effect of the individual formulation components (A, B, C and D) and combination there of on the response (Y) in terms of interaction coefficients. The values of the coefficients A to D are related to the effect of these variables on the response (Y). Coefficients with more than one factor term and those with higher order terms represent interaction terms and quadratic relationships respectively. A positive and negative signs suggest a positive and negative effect on response respectively. The theoretical (predicted) values and the observed values were in reasonably good agreement as seen from Table: 5.13 to Table: 5.16 for each formulation. The significance of the ratio of mean square variation due to regression and residual error was tested using analysis of variance (ANOVA). It was observed that the best-fitted models were quadratic. The values of R^2, adjusted R^2, predicted R^2, Degree of freedom (Df), Sum of squares(SS) and Mean square(MS) are given in

Table: 5.17 to Table: 5.20 for each formulation, along with the regression equation generated for each response. The results of ANOVA in Table: 5.17 to Table: 5.20 for the dependent variables demonstrate that the model was significant for both the response variables.

Table: 5.17. Analysis of Variance for BBD Model of LMTC-TZ NPs

Parameters		**Df**	**SS**	**MS**	**R^2**	**AdjR^2**	**PredR^2**	**F**	**Lack of fit**
Particle size	Model	14	1.072E+005	7658.14	0.9904	0.9800	0.9471	0.0001	0.1383
	Residual	13	1044.07	80.31	-	-	-	-	-
Entrapment efficiency	Model	14	1685.19	120.37	0.8524	0.6934	0.1819	0.0022	0.1010
	Residual	13	291.83	22.45	-	-	-	-	-

*Df- Degree of freedom; SS- Sum of squares; MS-Mean square; F- Significance
AdjR^2 -Adjusted $R^{2;}$ PredR^2- Predicted R^2

Table: 5.18. Analysis of Variance for BBD Model of LMTC-CBZ NPs

Parameters		**Df**	**SS**	**MS**	**R^2**	**AdjR^2**	**PredR^2**	**F**	**Lack of fit**
Particle size	Model	14	1.119E+005	7990.45	0.9996	0.9991	0.9977	0.0001	0.1807
	Residual	13	46.79	3.60	-	-	-	-	-
Entrapment efficiency	Model	14	1473.46	105.25	0.9718	0.9414	0.8508	0.0001	0.2756
	Residual	13	42.77	3.29	-	-	-	-	-

*Df- Degree of freedom; SS- Sum of squares; MS-Mean square; F- Significance
AdjR^2 -Adjusted $R^{2;}$ PredR^2- Predicted R^2

Table: 5.19. Analysis of Variance for BBD Model of MMTC-TZ NPs

Parameters		**Df**	**SS**	**MS**	**R^2**	**AdjR^2**	**PredR^2**	**F**	**Lack of fit**
Particle size	Model	14	1.061E+005	7577.02	0.9967	0.9931	0.9817	0.0001	0.1157
	Residual	13	353.28	27.18	-	-	-	-	-
Entrapment efficiency	Model	14	1881.61	134.40	0.9618	0.9206	0.8022	0.0001	0.3573
	Residual	13	74.82	5.76	-	-	-	-	-

*Df- Degree of freedom; SS- Sum of squares; MS-Mean square; F- Significance
AdjR^2 -Adjusted $R^{2;}$ PredR^2- Predicted R^2

Table: 5.20. Analysis of Variance for BBD Model of MMTC-CBZ NPs

Parameters		Df	SS	MS	R^2	AdjR^2	PredR^2	F	Lack of fit
Particle size	Model	14	1.137E+005	8120.57	0.9949	0.9894	0.9724	0.0001	0.1855
	Residual	13	582.42	44.80	-	-	-	-	-
Entrapment efficiency	Model	14	2294.95	163.92	0.9769	0.9520	0.8790	0.0001	0.3164
	Residual	13	54.35	4.18	-	-	-	-	-

*Df- Degree of freedom; SS- Sum of squares; MS-Mean square; F- Significance
AdjR^2 -Adjusted R^2; PredR^2- Predicted R^2

The ANOVA indicated a significant ($P < 0.05$) effect of factors on response. Lack of fit was not significant and regression was strongly significant for all formulations. The Adjusted R-square and Predicted R-square should be within approximately 0.20 of each other to be in reasonable agreement. The "Pred R-Square" is in reasonable agreement with the "Adj R-Square" of all the formulations, which is very good for chosen factorial model. So it was concluded that the second-order model adequately approximated the true surface.

5.2.4 Optimization of Formulation Parameters for trimethyl chitosan Nanoparticles

Quantitative aspects of the effects and relationships among various formulation parameters of high therapeutics payload nanoparticles produced by Ionotropic gelation method are investigated using Response Surface Methodology (RSM).

To study this, we performed, "Box-Behnken" design (BBD) on four critical formulation factors known to affect their results. The BBD is a popular template for RSM because it requires only three-levels of each formulation factor and only a fraction of all the possible combinations. In this design, the experimental region is assumed to be a cube, and experiments are performed at points corresponding to midpoint of each edge and replicated experiments at the centre of this multidimensional cube.

This design is suitable for exploring quadratic response surfaces and constructing second-order polynomial models. The complete design consisted of 29 experimental points that included twenty six factor points and three replications at the centre point. The non-linear quadratic model generated by the design is as follow:

$$Y = \beta_0 + \beta_1 A + \beta_2 B + \beta_3 C + \beta_4 D + \beta_{12} AB + \beta_{13} AC + \beta_{14} A D + \beta_{23} B C + \beta_{24} BD + \beta_{34} CD + \beta_{11} A^2 + \beta_{22} B^2 + \beta_{33} C^2 + \beta_{44} D^2$$

------ Equation [5.19]

Where, Y is the measured response (dependant variable) associated with each factor-level combination; expressed in terms of size and entrapment efficiency± of the nanoparticles, β_0 is an intercept, β_1, β_2, β_3, β_4, β_{12}, β_{13}, β_{14}, β_{23}, β_{24}, β_{34}, β_{11}, β_{22}, β_{33}, β_{44} are the regression coefficients. A, B, C and D are the (independent factors studied) concentration of trimethyl chitosan (mg/ml), concentration of sodium alginate (mg/ml), concentration of drug (mg/ml) and concentration of sodium deoxycholate (mg/ml) respectively. The independent factors and the dependent variable used in the design are listed in Table 5.21 for low and medium molecular weight trimethyl chitosan. The Design Expert (Version 7.1, State Ease Inc, USA) program was used for design of experiment and analysis of this second-order model and for drawing of three dimensional response surface and contour plots.

Table: 5.21.Variables in Box Behnken Design

X_i	**Independent variables**	**Units**	**Coded values**			**Response (Y1)**	**Response (Y2)**
			-1	0	1		
A	Concentration of trimethyl chitosan	mg/ml	1	1.5	2	Particle size in nm	Entrapment Efficiency in Percentage
B	Concentration of sodium alginate	mg/ml	0.5	1.25	2		
C	Concentration of Drug	mg/ml	1	2	3		
D	Concentration of sodium deoxycholate	mg/ml	5	10	15		

Table: 5.22. Matrix of Box Behnken Design, Particle size response and Entrapment Efficiency of each experimental run for LMTMC-TZ NPs

Std	Run	Concentration of TMC (mg/ml) Factor :A	Concentration of SA (mg/ml) Factor :B	Concentration of drug(mg/ml) Factor :C	Concentration of SDC(mg/ml) Factor: D	Particle size (nm)	Predicted particle size (nm)	Entrapment Efficiency (% EE)	Predicted Entrapment Efficiency (% EE)
*3	1	1	2	2	10	168.0±10.2	192.1	66.90±1.2	59.61
8	2	1.5	1.25	3	15	320.6±13.4	330.3	55.67±2.3	55.98
1	3	1	0.5	2	10	312.6±12.4	328.7	60.57±3.5	59.30
26	4	1.5	1.25	2	10	362.7±14.5	371.1	59.43±4.6	55.92
2	5	2	0.5	2	10	367.8±13.6	362.7	48.98±1.5	49.57
18	6	2	1.25	1	10	445.8±15.4	449.0	52.78±2.4	55.20
15	7	1.5	0.5	3	10	334.8±16.4	329.3	44.78±3.6	47.89
29	8	1.5	1.25	2	10	354.5±13.5	371.1	51.23±4.5	55.92
22	9	1.5	2	2	5	443.8±17.5	447.2	43.34±4.2	41.70
17	10	1	1.25	1	10	323.8±12.5	333.6	46.98±5.4	50.48
7	11	1.5	1.25	1	15	343.9±18.6	400.1	41.23±6.3	41.39
9	12	1	1.25	2	5	444.8±13.2	416.5	34.78±1.9	43.13
6	13	1.5	1.25	3	5	513.8±19.2	476.6	33.89±2.6	27.03
16	14	1.5	2	3	10	313.6±13.2	350.0	39.67±3.9	49.99
10	15	2	1.25	2	5	488.0±15.3	487.7	34.67±4.8	42.24
27	16	1.5	1.25	2	10	412.6±16.4	371.1	52.23±3.2	55.92
12	17	2	1.25	2	15	412.8±12.4	417.3	48.56±4.5	47.37
20	18	2	1.25	3	10	415.8±18.9	410.8	44.35±3.1	40.35
21	19	1.5	0.5	2	5	402.7±19.8	456.0	42.11±2.5	41.66
13	20	1.5	0.5	1	10	503.7±21.9	443.5	53.23±3.7	50.07
23	21	1.5	0.5	2	15	360.6±14.6	362.0	49.21±4.6	50.35
5	22	1.5	1.25	1	5	499.7±17.8	509.0	54.43±2.1	47.42
24	23	1.5	2	2	15	334.6±12.3	286.1	55.98±2.2	55.94
4	24	2	2	2	10	411.9±15.6	414.7	60.32±2.9	54.89
28	25	1.5	1.25	2	10	368.9±16.4	371.1	59.43±4.3	55.92
14	26	1.5	2	1	10	356.4±13.5	338.1	49.56±4.3	53.61
19	27	1	1.25	3	10	267.9±11.9	269.5	62.45±3.5	59.53
11	28	1	1.25	2	15	255.3±8.9	231.8	61.34±3.1	60.93
25	29	1.5	1.25	2	10	356.9±10.6	371.1	57.32±2.4	55.92

(Mean ± S.D., $n = 3$)

LMTMC-TZ NPs- Tizanidine HCl loaded low molecular weight trimethyl chitosan NPs

*Ratio of trimethyl chitosan: sodium alginate: drug: sodium deoxycholate solution was taken 7.9:0.7:0.7:0.7

Table: 5.23. Matrix of Box Behnken Design, Particle size response and Entrapment Efficiency of each experimental run for LMTMC-CBZ NPs

Std	Run	Concentration of TMC (mg/ml) Factor :A	Concentration of SA (mg/ml) Factor :B	Concentration of drug(mg/ml) Factor :C	Concentration of SDC(mg/ml) Factor: D	Particle size (nm)	Predicted particle size (nm)	Entrapment Efficiency (% EE)	Predicted Entrapment Efficiency (% EE)
***3**	**1**	**1**	**2**	**2**	**10**	**184.6±13.9**	**207.9**	**71.75±5.4**	**63.00**
8	2	1.5	1.25	3	15	340.5±12.3	345.6	58.56±5.6	58.42
1	3	1	0.5	2	10	333.7±11.5	348.0	62.65±4.3	61.39
26	4	1.5	1.25	2	10	378.9±13.5	387.3	60.32±3.5	58.23
2	5	2	0.5	2	10	389.4±14.3	373.7	52.12±1.2	54.75
18	6	2	1.25	1	10	443.5±14.3	449.1	57.89±5.3	60.32
15	7	1.5	0.5	3	10	343.7±12.4	347.5	49.54±2.5	50.91
29	8	1.5	1.25	2	10	366.4±15.6	387.3	54.32±3.2	58.23
22	9	1.5	2	2	5	456.8±17.5	467.4	45.32±3.4	46.18
17	10	1	1.25	1	10	314.8±12.4	342.6	49.32±3.5	52.79
7	11	1.5	1.25	1	15	380.6±15.6	418.8	43.21±1.2	43.58
9	12	1	1.25	2	5	479.2±11.3	437.5	40.32±5.2	46.83
6	13	1.5	1.25	3	5	523.8±14.5	493.3	37.21±4.6	30.72
16	14	1.5	2	3	10	328.9±16.7	361.7	41.65±3.2	51.08
10	15	2	1.25	2	5	477.6±18.5	488.0	45.77±1.1	49.43
27	16	1.5	1.25	2	10	422.7±19.4	387.3	55.66±4.5	58.23
12	17	2	1.25	2	15	419.6±12.2	442.6	51.78±3.6	50.51
20	18	2	1.25	3	10	438.9±14.8	422.2	46.21±2.1	43.59
21	19	1.5	0.5	2	5	412.5±15.6	461.2	44.32±5.3	45.74
13	20	1.5	0.5	1	10	499.5±13.5	447.9	56.21±2.5	52.02
23	21	1.5	0.5	2	15	392.9±17.6	393.5	53.22±3.4	53.21
5	22	1.5	1.25	1	5	503.2±14.6	505.7	58.32±1.9	52.34
24	23	1.5	2	2	15	337.9±11.3	300.4	58.22±6.4	57.65
4	24	2	2	2	10	433.7±15.7	427.1	62.89±2.5	58.03
28	25	1.5	1.25	2	10	387.5±18.9	387.3	61.56±4.3	58.23
14	26	1.5	2	1	10	369.4±11.3	346.9	52.87±6.3	56.74
19	27	1	1.25	3	10	278.3±12.5	283.8	64.32±5.4	62.74
11	28	1	1.25	2	15	277.4±10.9	248.2	63.12±2.4	64.70
25	29	1.5	1.25	2	10	381.3±12.8	387.3	59.32±5.1	58.23

(Mean ± S.D., $n = 3$)

LMTMC-CBZ NPs- Cyclobenzaprine HCl loaded low molecular weight trimethyl chitosan NPs

*Ratio of trimethyl chitosan: sodium alginate: drug: sodium deoxycholate solution was taken 7.9:0.7:0.7:0.7

Table: 5.24. Matrix of Box Behnken Design, Particle size response and Entrapment Efficiency of each experimental run for MMTMC-TZ NPs

Std	Run	Concentration of TMC (mg/ml) Factor :A	Concentration of SA (mg/ml) Factor :B	Concentration of drug(mg/ml) Factor :C	Concentration of SDC(mg/ml) Factor: D	Particle size (nm)	Predicted particle size (nm)	Entrapment Efficiency (% EE)	Predicted Entrapment Efficiency (% EE)
***3**	**1**	**1**	**2**	**2**	**10**	**226.6±18.1**	**234.3**	**62.27±1.8**	**59.27**
8	2	1.5	1.25	3	15	388.4±12.5	383.8	57.49±2.7	59.98
1	3	1	0.5	2	10	300.2±20.9	304.7	63.56±2.9	65.43
26	4	1.5	1.25	2	10	402.1±19.8	393.7	62.89±3.5	61.04
2	5	2	0.5	2	10	450.5±21.7	439.1	54.66±4.1	54.76
18	6	2	1.25	1	10	477.8±16.7	490.4	59.32±5.5	63.55
15	7	1.5	0.5	3	10	380.5±18.6	371.5	53.89±3.8	54.60
29	8	1.5	1.25	2	10	383.2±14.6	393.7	57.43±4.9	61.04
22	9	1.5	2	2	5	356.2±15.7	362.1	48.54±4.7	48.80
17	10	1	1.25	1	10	280.4±12.4	269.8	51.98±3.9	53.80
7	11	1.5	1.25	1	15	433.2±11.7	427.3	46.32±4.7	47.43
9	12	1	1.25	2	5	350.8±19.6	342.4	43.87±4.3	47.78
6	13	1.5	1.25	3	5	395.8±16.9	398.0	39.43±2.8	35.42
16	14	1.5	2	3	10	333.5±14.5	330.5	45.21±3.8	52.47
10	15	2	1.25	2	5	468.9±15.8	453.3	49.43±5.3	52.69
27	16	1.5	1.25	2	10	394.2±19.2	393.7	58.90±4.4	61.04
12	17	2	1.25	2	15	493.7±13.7	497.6	53.21±5.3	52.39
20	18	2	1.25	3	10	376.8±11.8	395.7	48.43±2.8	46.40
21	19	1.5	0.5	2	5	440.7±12.9	455.8	46.32±3.4	48.26
13	20	1.5	0.5	1	10	410.8±20.7	409.3	59.54±3.8	55.36
23	21	1.5	0.5	2	15	387.6±12.7	389.9	57.33±4.6	56.85
5	22	1.5	1.25	1	5	433.8±22.8	434.8	60.21±3.8	54.82
24	23	1.5	2	2	15	413.2±12.8	406.3	59.54±2.7	57.39
4	24	2	2	2	10	440.3±10.7	432.1	66.78±4.9	62.01
28	25	1.5	1.25	2	10	394.7±9.7	393.7	63.22±3.7	61.04
14	26	1.5	2	1	10	368.5±14.7	373.0	56.20±3.8	58.58
19	27	1	1.25	3	10	288.4±11.8	284.1	68.54±2.8	64.09
11	28	1	1.25	2	15	265.4±12.6	276.5	65.43±3.8	65.25
25	29	1.5	1.25	2	10	394.5±11.1	393.7	62.78±3.2	61.04

(Mean ± S.D., *n* = 3)

MMTMC-TZ NPs- Tizanidine HCl loaded medium molecular weight trimethyl chitosan NPs

*Ratio of trimethyl chitosan: sodium alginate: drug: sodium deoxycholate solution was taken 7.9:0.7:0.7:0.7

Table: 5.25. Matrix of Box Behnken Design, Particle size response and Entrapment Efficiency of each experimental run for MMTMC-CBZ NPs

Std	Run	Concentration of TMC (mg/ml) Factor :A	Concentration of SA (mg/ml) Factor :B	Concentration of drug(mg/ml) Factor :C	Concentration of SDC(mg/ml) Factor: D	Particle size (nm)	Predicted particle size (nm)	Entrapment Efficiency (% EE)	Predicted Entrapment Efficiency (% EE)
***3**	**1**	**1**	**2**	**2**	**10**	**274.5±21.3**	**271.5**	**72.88±2.1**	**69.15**
8	2	1.5	1.25	3	15	389.5±12.5	385.7	60.12±3.2	62.42
1	3	1	0.5	2	10	308.5±13.5	307.2	64.65±4.3	65.21
26	4	1.5	1.25	2	10	415.1±14.6	400.1	61.34±2.9	61.32
2	5	2	0.5	2	10	465.1±13.8	458.4	53.54±5.1	55.18
18	6	2	1.25	1	10	489.4±18.9	501.6	60.56±3.8	63.39
15	7	1.5	0.5	3	10	402.1±21.5	383.1	49.56±4.2	50.01
29	8	1.5	1.25	2	10	388.7±14.5	400.1	59.21±5.7	61.32
22	9	1.5	2	2	5	377.9±17.5	387.0	51.33±3.9	52.46
17	10	1	1.25	1	10	288.4±16.8	284.7	58.90±4.1	61.10
7	11	1.5	1.25	1	15	451.1±21.2	437.6	48.34±4.9	48.19
9	12	1	1.25	2	5	360.7±13.5	352.9	50.65±5.1	54.67
6	13	1.5	1.25	3	5	404.1±17.9	408.0	39.98±3.8	38.03
16	14	1.5	2	3	10	349.5±14.7	341.8	61.89±2.9	64.36
10	15	2	1.25	2	5	488.1±15.9	469.9	48.99±1.8	51.30
27	16	1.5	1.25	2	10	397.4±11.5	400.1	60.32±1.5	61.32
12	17	2	1.25	2	15	499.3±13.2	499.2	55.32±3.7	52.51
20	18	2	1.25	3	10	380.1±12.9	401.3	50.11±1.9	48.77
21	19	1.5	0.5	2	5	444.1±19.8	462.8	52.56±2.4	51.41
13	20	1.5	0.5	1	10	412.3±17.6	412.1	63.97±2.9	62.71
23	21	1.5	0.5	2	15	390.2±18.3	398.6	53.23±3.7	52.96
5	22	1.5	1.25	1	5	450.2±20.2	444.4	64.67±5.2	60.27
24	23	1.5	2	2	15	423.4±18.9	422.2	61.22±6.1	63.23
4	24	2	2	2	10	450.3±17.6	441.9	65.21±7.9	62.56
28	25	1.5	1.25	2	10	401.2±15.9	400.1	63.78±9.8	61.32
14	26	1.5	2	1	10	390.3±16.9	401.3	58.93±8.5	59.68
19	27	1	1.25	3	10	291.2±13.5	296.6	69.66±4.9	67.69
11	28	1	1.25	2	15	284.3±11.8	294.5	66.88±6.2	65.77
25	29	1.5	1.25	2	10	397.9±14.7	400.1	61.99±5.3	61.32

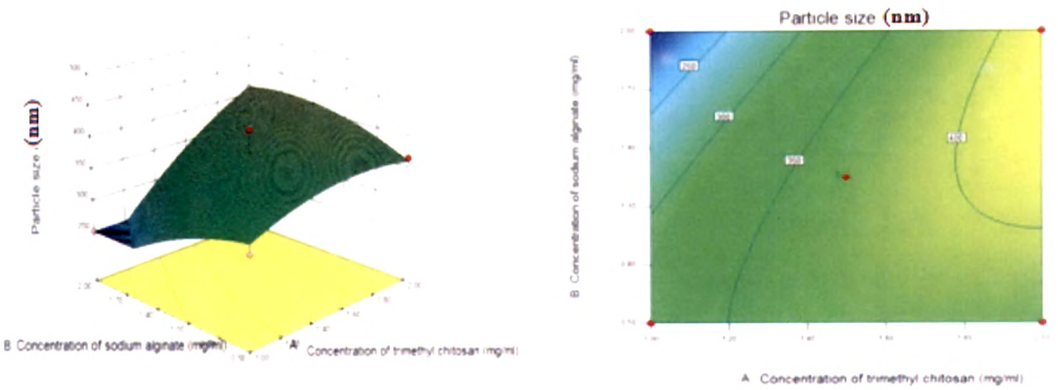
(Mean ± S.D., $n = 3$)

MMTMC-CBZ NPs- Cyclobenzaprine HCl loaded medium molecular weight trimethyl chitosan NPs

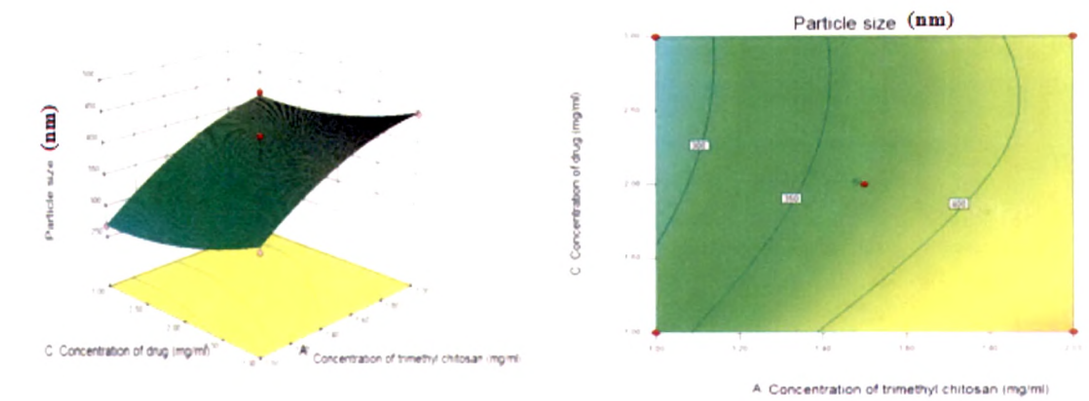
*Ratio of trimethyl chitosan: sodium alginate: drug: sodium deoxycholate solution was taken 7.9:0.7:0.7:0.7

In order to optimize trimethyl chitosan: sodium alginate: drug: sodium deoxycholate solution ratio in each formulations, nanoparticles were formulated using trimethyl chitosan: sodium alginate: drug: sodium deoxycholate solution with different ratio 7.9:0.7:0.7:0.7, 7:1:1:1, 4:2:2:2, 5.5:1.5:1.5:1.5 and the Factor A, B, C and D concentration was kept constant (Wang et al., 2008).

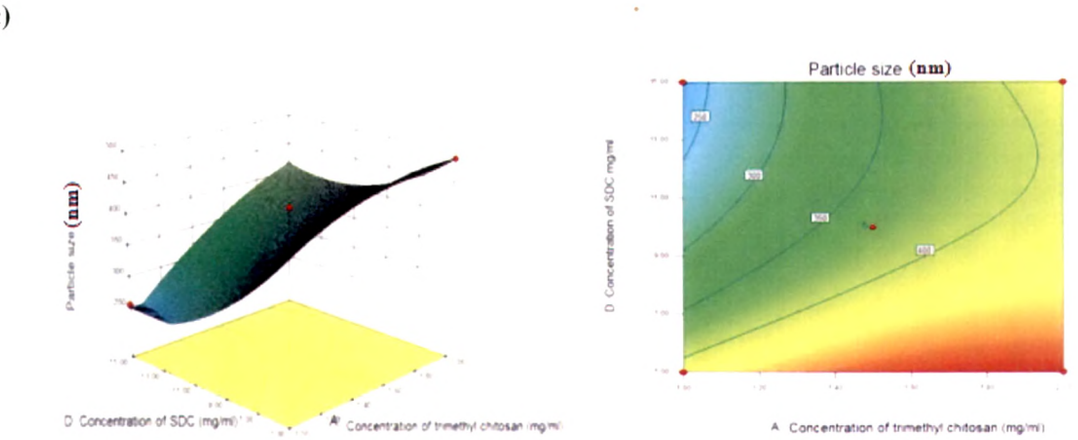
(a)


Particle size (nm)
B: Concentration of sodium alginate (mg/ml)
A: Concentration of trimethyl chitosan (mg/ml)
Particle size (nm)
B: Concentration of sodium alginate (mg/ml)
A: Concentration of trimethyl chitosan (mg/ml)

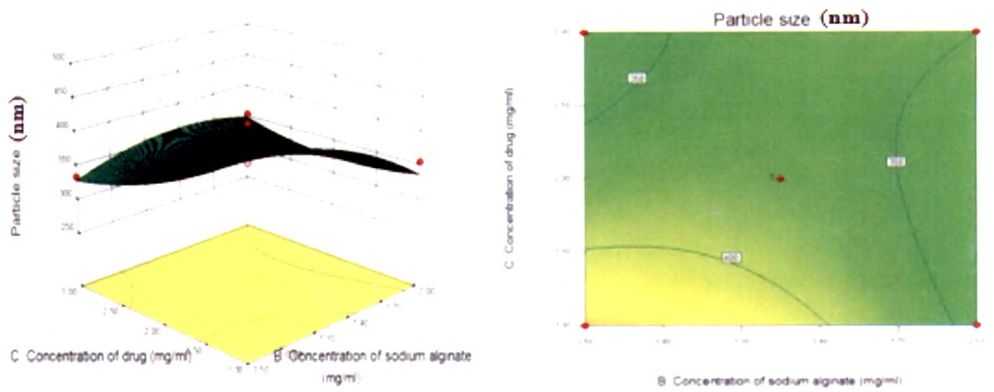
(b)


Particle size (nm)
C: Concentration of drug (mg/ml)
A: Concentration of trimethyl chitosan (mg/ml)
Particle size (nm)
C: Concentration of drug (mg/ml)
A: Concentration of trimethyl chitosan (mg/ml)

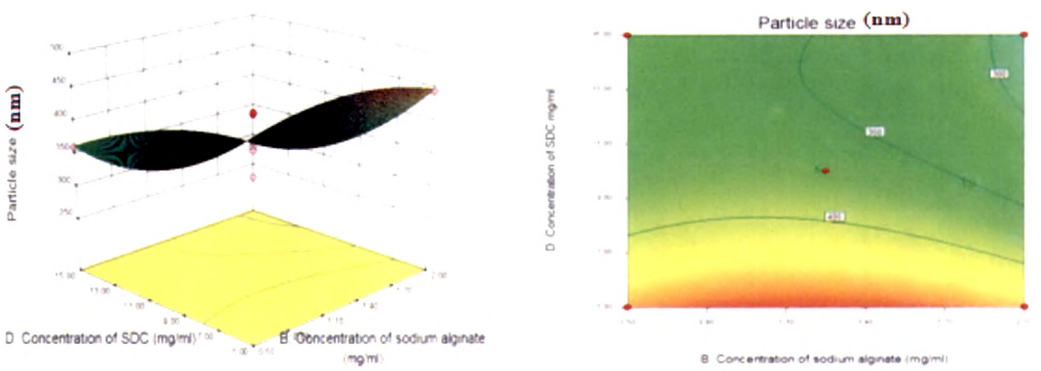
(c)


Particle size (nm)
D: Concentration of SDC (mg/ml)
A: Concentration of trimethyl chitosan (mg/ml)
Particle size (nm)
D: Concentration of SDC (mg/ml)
A: Concentration of trimethyl chitosan (mg/ml)

(d)



(e)



(f)

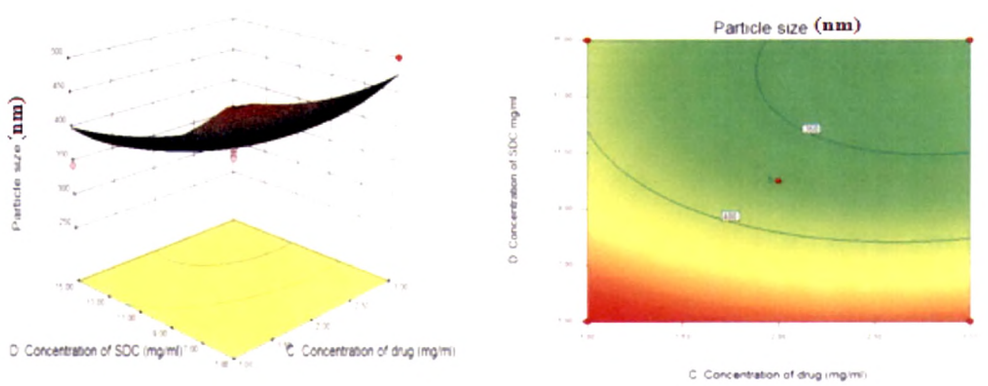
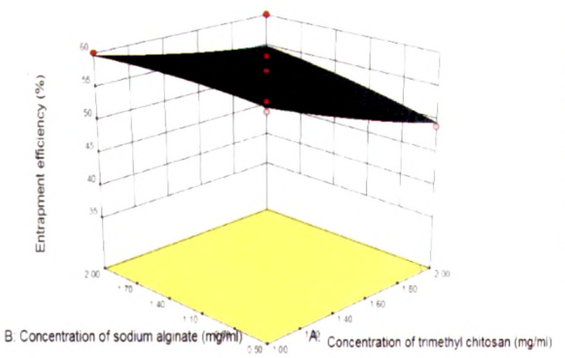
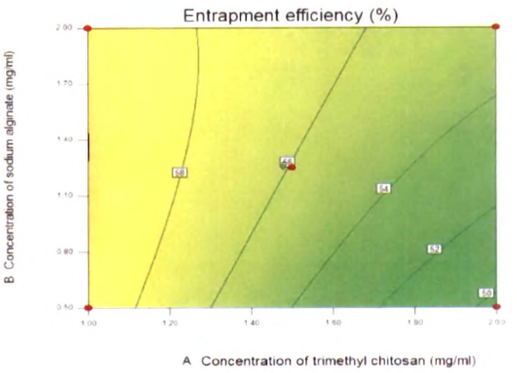


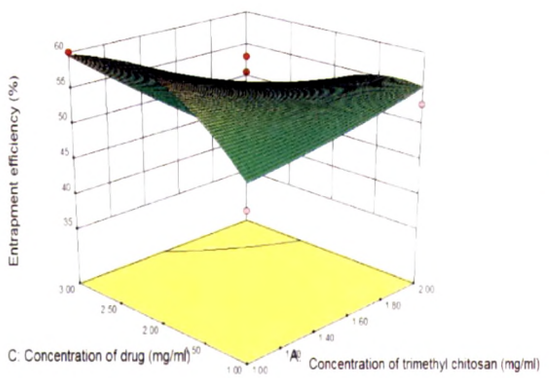
Figure: 5.17. Response surface and contour plots of a) the effects of Concentration of trimethyl chitosan (A) and Concentration of sodium alginate (B) b) effects of Concentration of trimethyl (A) and Concentration of drug (C) c) Concentration of trimethyl chitosan (A) and concentration of sodium deoxycholate (D) d) Concentration of sodium alginate (B) and Concentration of Drug (C) e) Concentration of sodium alginate (B) and concentration of sodium deoxycholate (D) f) Concentration of drug (C) and concentration of sodium deoxycholate (D) on the particle size of the LMTMC-TZ NPs

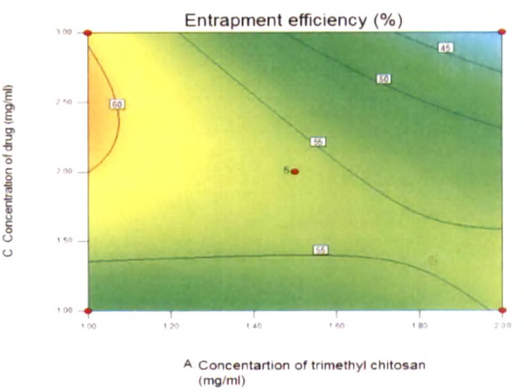
(a)


Entrapment efficiency (%)
B: Concentration of sodium alginate (mg/ml)
A: Concentration of trimethyl chitosan (mg/ml)

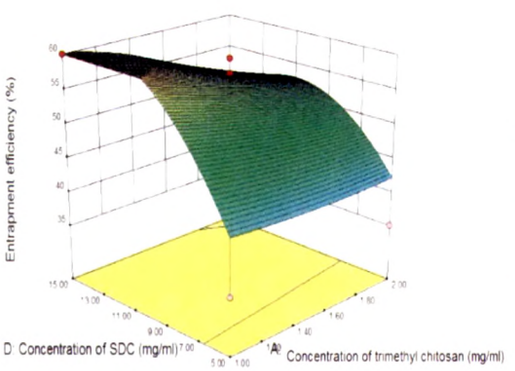

Entrapment efficiency (%)
B: Concentration of sodium alginate (mg/ml)
A: Concentration of trimethyl chitosan (mg/ml)

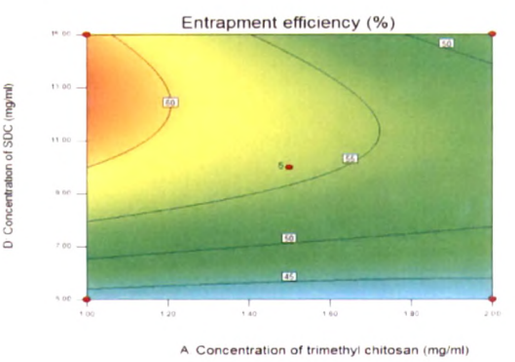
(b)


Entrapment efficiency (%)
C: Concentration of drug (mg/ml)
A: Concentration of trimethyl chitosan (mg/ml)

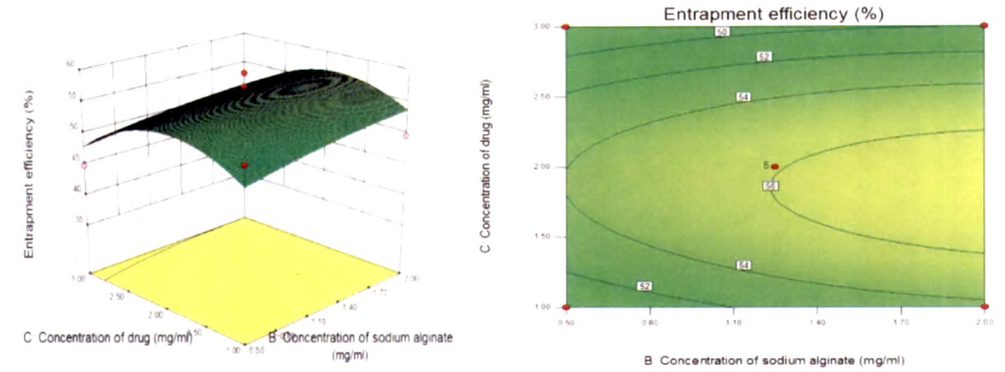

Entrapment efficiency (%)
C: Concentration of drug (mg/ml)
A: Concentartion of trimethyl chitosan (mg/ml)

(c)

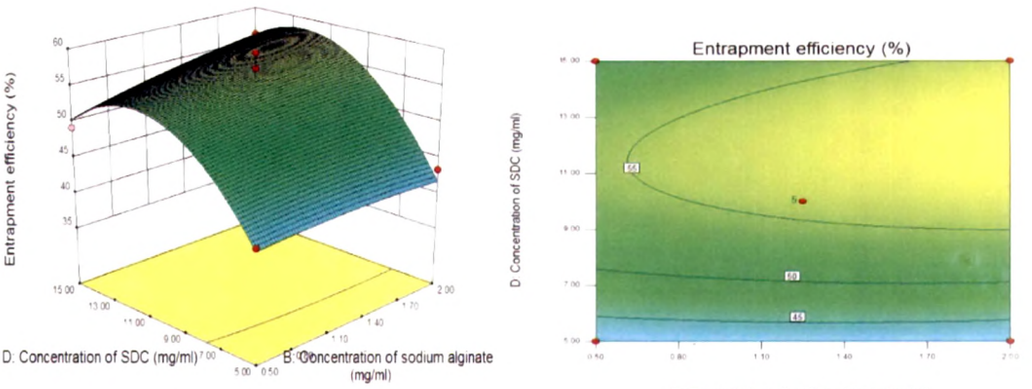

Entrapment efficiency (%)
D: Concentration of SDC (mg/ml)
A: Concentration of trimethyl chitosan (mg/ml)


Entrapment efficiency (%)
D: Concentration of SDC (mg/ml)
A: Concentration of trimethyl chitosan (mg/ml)

(d)



(e)



(f)

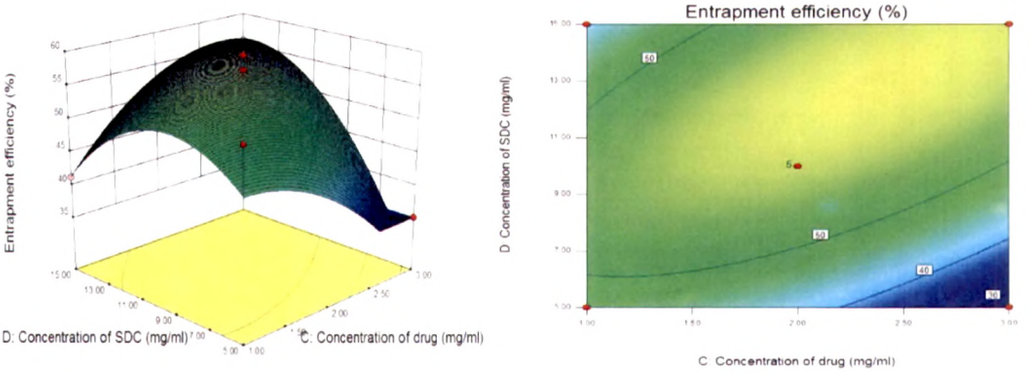
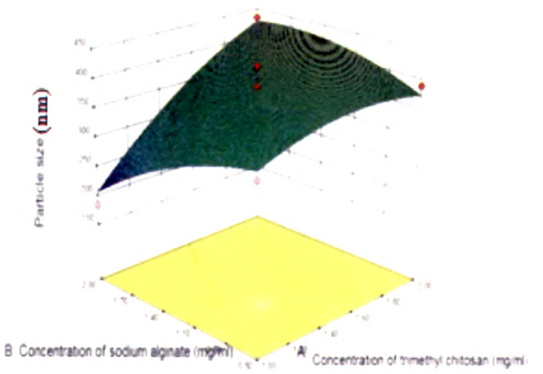
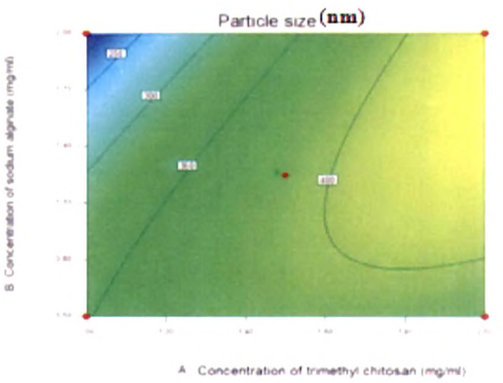


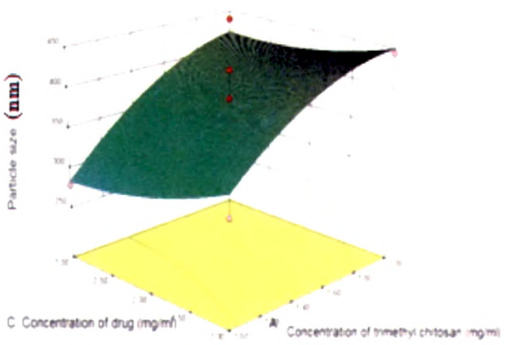
Figure: 5.18. Response surface and contour plots of a) the effects of Concentration of trimethyl chitosan (A) and Concentration of sodium alginate (B) b) effects of Concentration of trimethyl (A) and Concentration of drug (C) c) Concentration of trimethyl chitosan (A) and concentration of sodium deoxycholate (D) d) Concentration of sodium alginate (B) and Concentration of Drug (C) e) Concentration of sodium alginate (B) and concentration of sodium deoxycholate (D) f) Concentration of drug (C) and concentration of sodium deoxycholate (D) on the entrapment efficiency of the LMTMC-TZ NPs

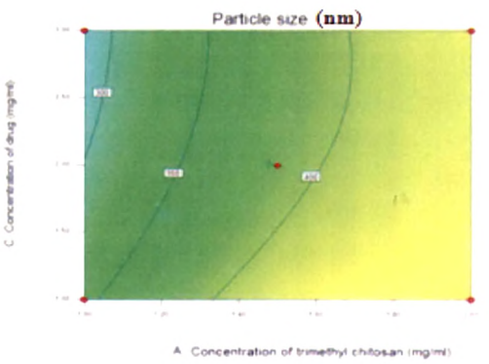
(a)


Particle size (nm)
B: Concentration of sodium alginate (mg/ml)
A: Concentration of trimethyl chitosan (mg/ml)

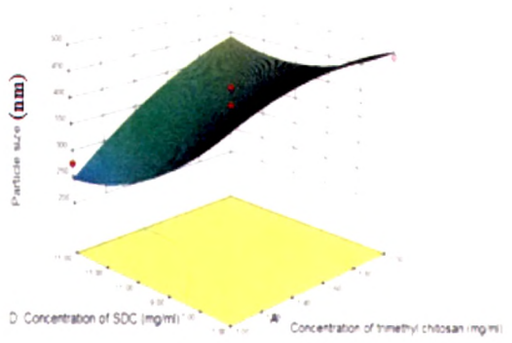

Particle size (nm)
B: Concentration of sodium alginate (mg/ml)
A: Concentration of trimethyl chitosan (mg/ml)

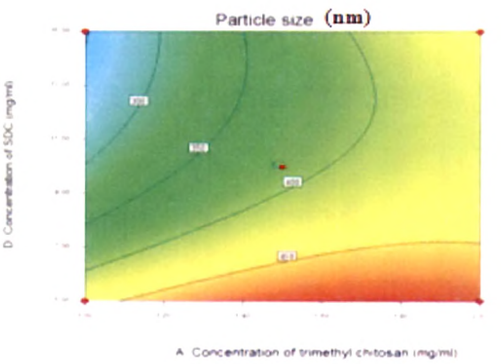
(b)


Particle size (nm)
C: Concentration of drug (mg/ml)
A: Concentration of trimethyl chitosan (mg/ml)

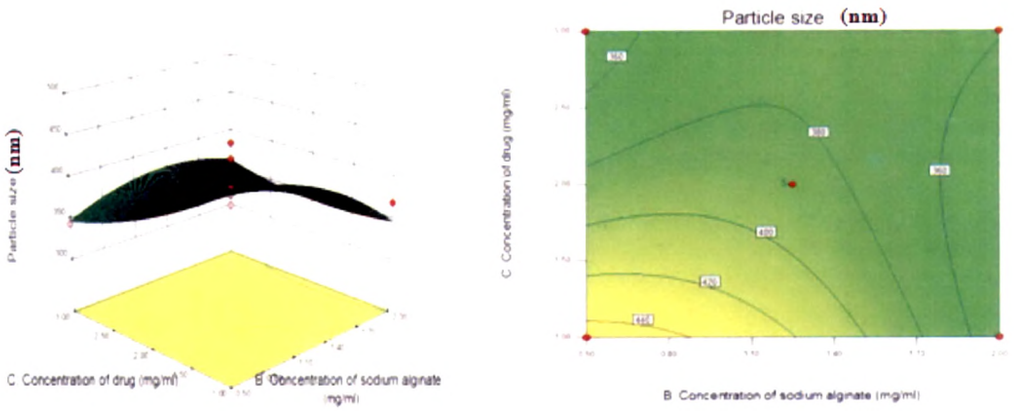

Particle size (nm)
C: Concentration of drug (mg/ml)
A: Concentration of trimethyl chitosan (mg/ml)

(c)

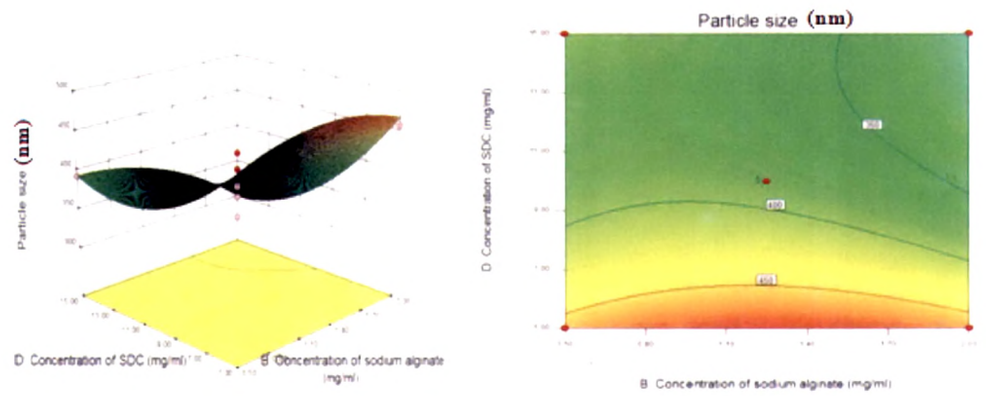

Particle size (nm)
D: Concentration of SDC (mg/ml)
A: Concentration of trimethyl chitosan (mg/ml)


Particle size (nm)
D: Concentration of SDC (mg/ml)
A: Concentration of trimethyl chitosan (mg/ml)

(d)



(e)



(f)

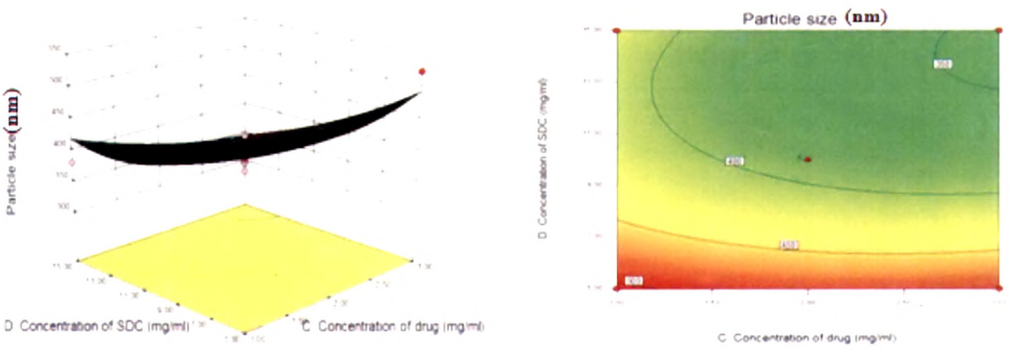
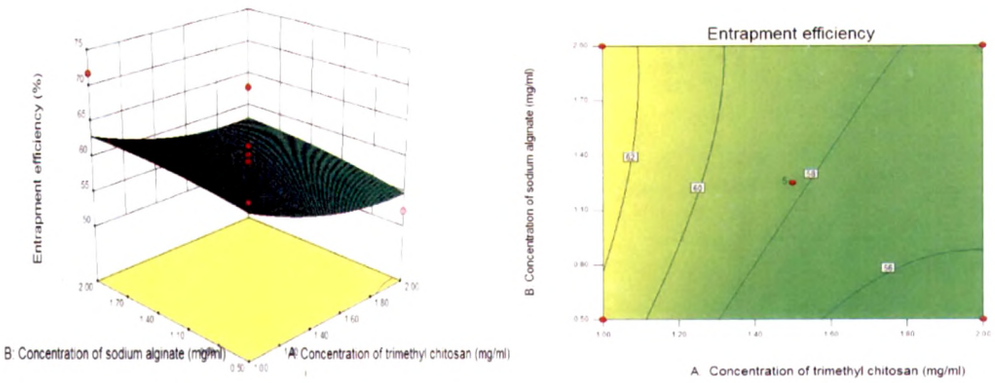
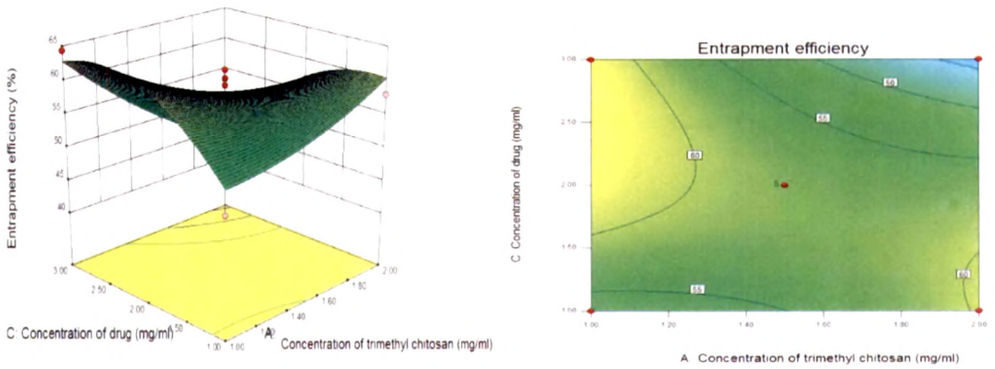


Figure: 5.19. Response surface and contour plots of a) the effects of Concentration of trimethyl chitosan (A) and Concentration of sodium alginate (B) b) effects of Concentration of trimethyl (A) and Concentration of drug (C) c) Concentration of trimethyl chitosan (A) and concentration of sodium deoxycholate (D) d) Concentration of sodium alginate (B) and Concentration of Drug (C) e) Concentration of sodium alginate (B) and concentration of sodium deoxycholate (D) f) Concentration of drug (C) and concentration of sodium deoxycholate (D) on the particle size of the LMTMC-CBZ NPs

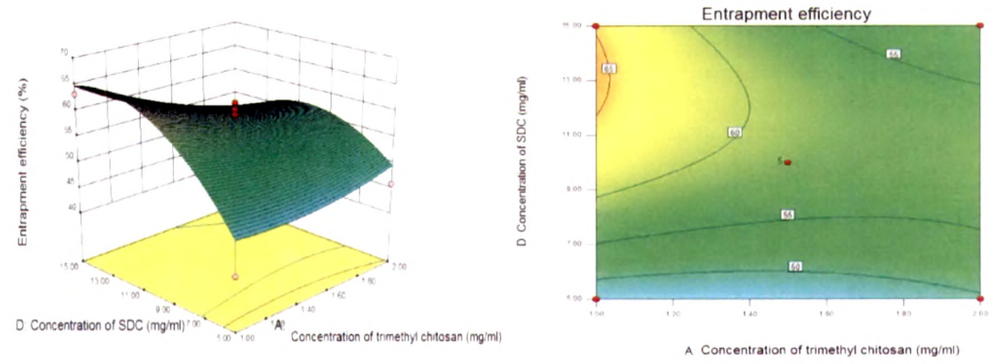
(a)


Entrapment efficiency (%)
B: Concentration of sodium alginate (mg/ml)
A: Concentration of trimethyl chitosan (mg/ml)
Entrapment efficiency
B: Concentration of sodium alginate (mg/ml)
A: Concentration of trimethyl chitosan (mg/ml)

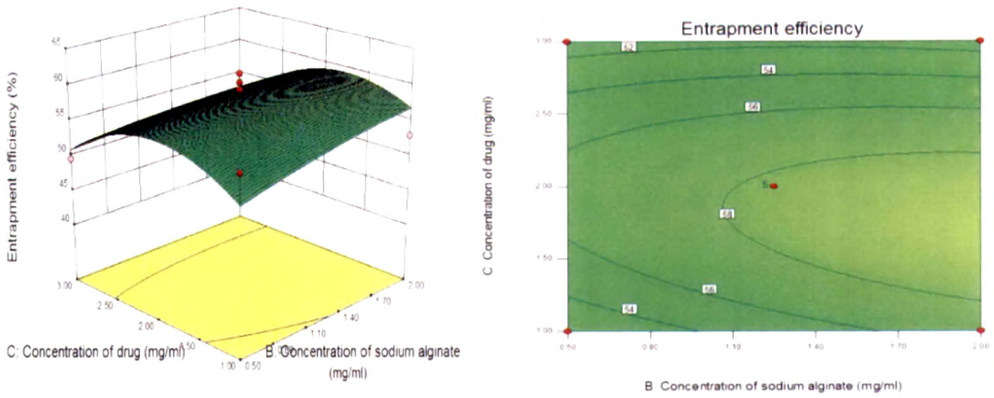
(b)


Entrapment efficiency (%)
C: Concentration of drug (mg/ml)
A: Concentration of trimethyl chitosan (mg/ml)
Entrapment efficiency
C: Concentration of drug (mg/ml)
A: Concentration of trimethyl chitosan (mg/ml)

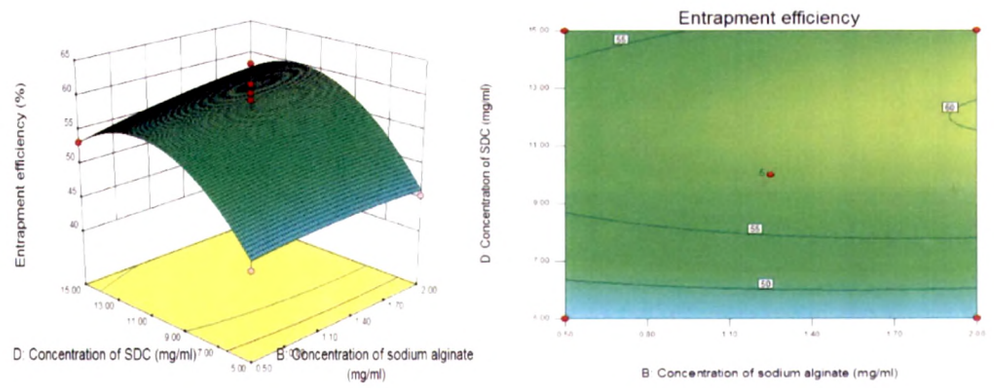
(c)


Entrapment efficiency (%)
D: Concentration of SDC (mg/ml)
A: Concentration of trimethyl chitosan (mg/ml)
Entrapment efficiency
D: Concentration of SDC (mg/ml)
A: Concentration of trimethyl chitosan (mg/ml)

(d)



(e)



(f)

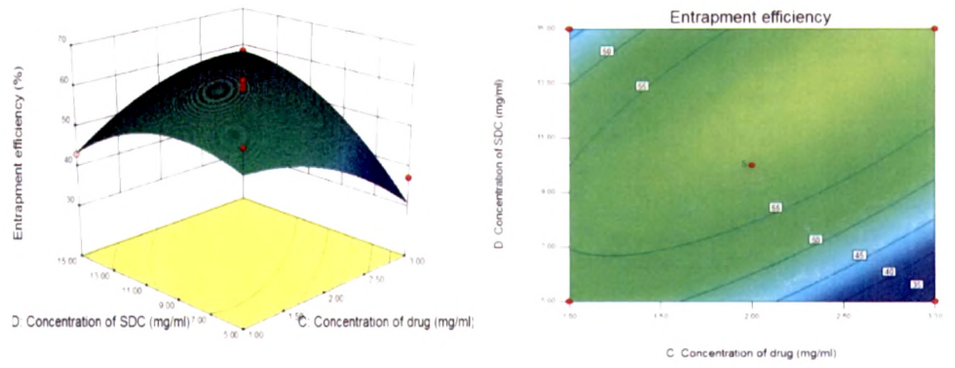
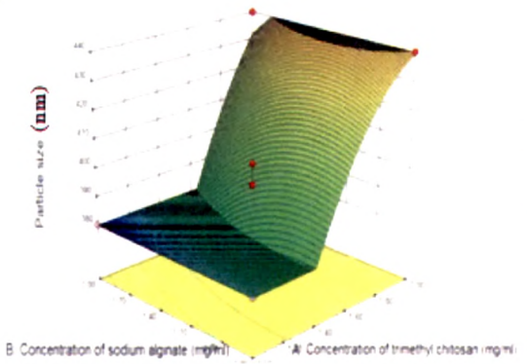
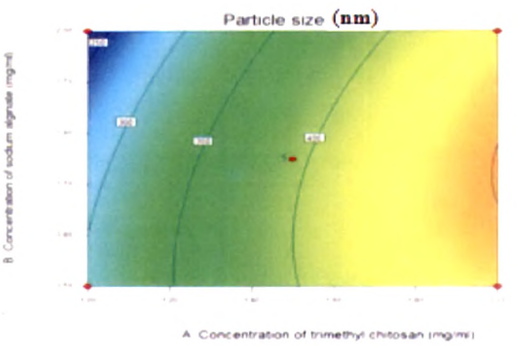


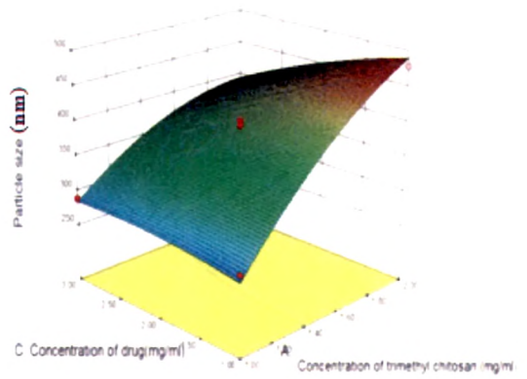
Figure: 5.20. Response surface and contour plots of a) the effects of Concentration of trimethyl chitosan (A) and Concentration of sodium alginate (B) b) effects of Concentration of trimethyl (A) and Concentration of drug (C) c) Concentration of trimethyl chitosan (A) and concentration of sodium deoxycholate (D) d) Concentration of sodium alginate (B) and Concentration of Drug (C) e) Concentration of sodium alginate (B) and concentration of sodium deoxycholate (D) f) Concentration of drug (C) and concentration of sodium deoxycholate (D) on the entrapment efficiency of the LMTMC-CBZ NPs

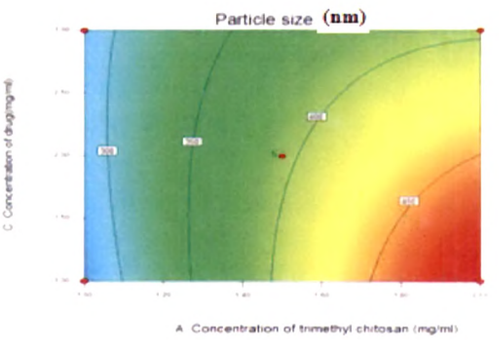
(a)


Particle size (nm)
B: Concentration of sodium alginate (mg/ml)
A: Concentration of trimethyl chitosan (mg/ml)

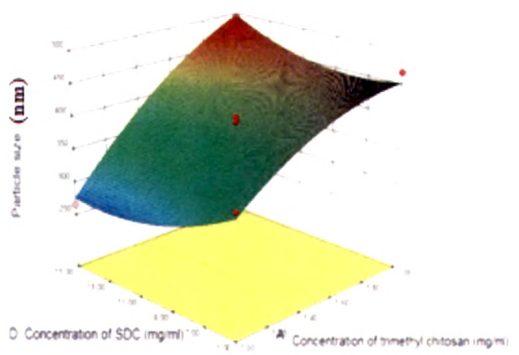

Particle size (nm)
B: Concentration of sodium alginate (mg/ml)
A: Concentration of trimethyl chitosan (mg/ml)

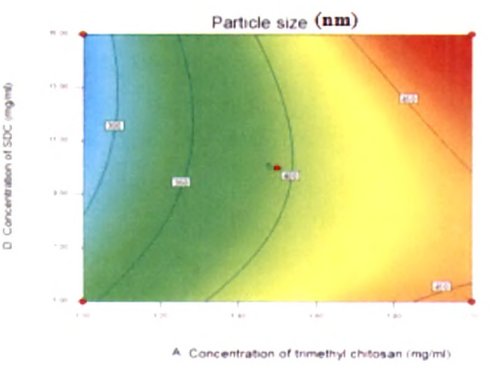
(b)


Particle size (nm)
C: Concentration of drug (mg/ml)
A: Concentration of trimethyl chitosan (mg/ml)

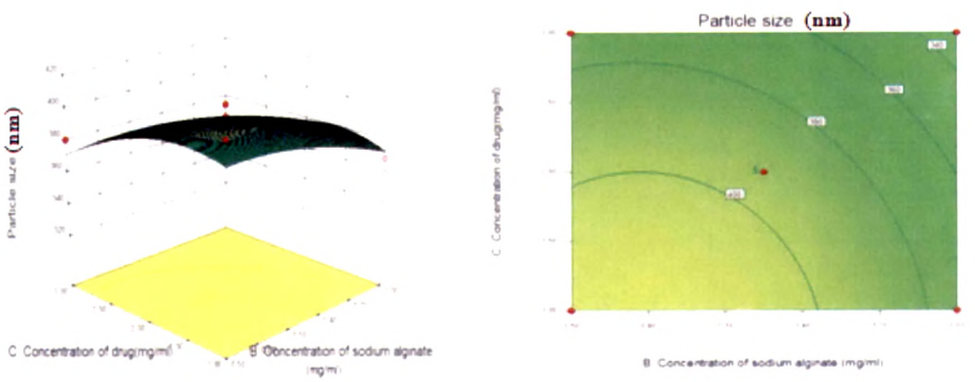

Particle size (nm)
C: Concentration of drug (mg/ml)
A: Concentration of trimethyl chitosan (mg/ml)

(c)

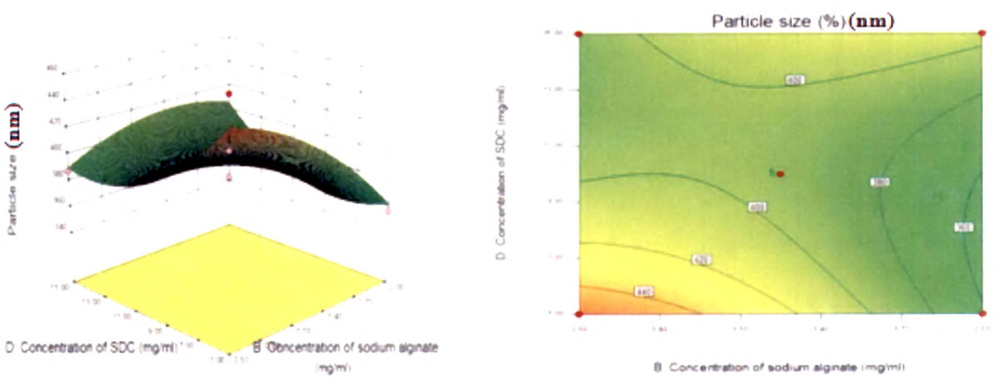

Particle size (nm)
D: Concentration of SDC (mg/ml)
A: Concentration of trimethyl chitosan (mg/ml)


Particle size (nm)
D: Concentration of SDC (mg/ml)
A: Concentration of trimethyl chitosan (mg/ml)

(d)



(e)



(f)

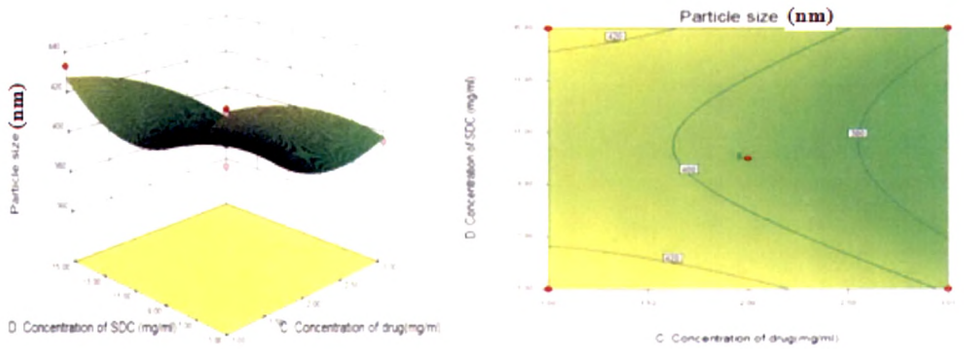
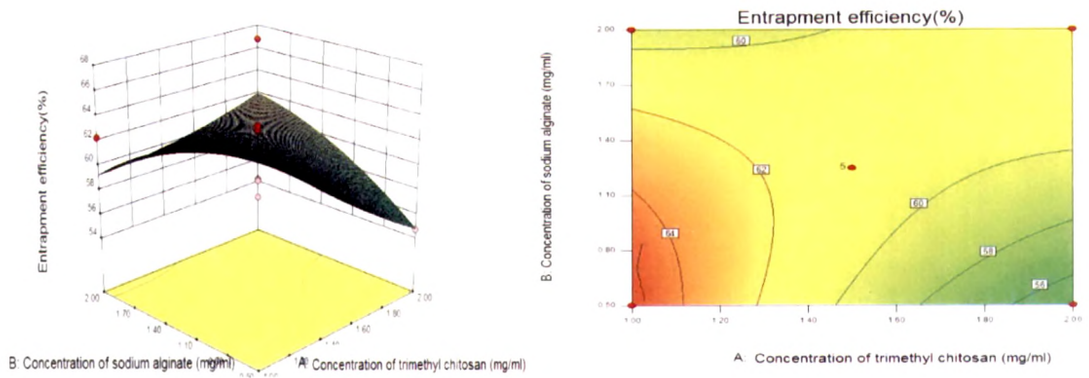
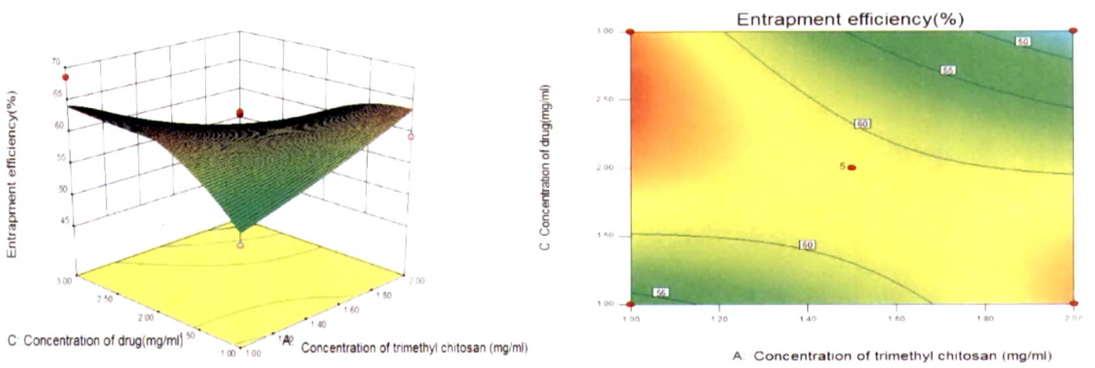


Figure: 5.21. Response surface and contour plots of a) the effects of Concentration of trimethyl chitosan (A) and Concentration of sodium alginate (B) b) effects of Concentration of trimethyl (A) and Concentration of drug (C) c) Concentration of trimethyl chitosan (A) and concentration of sodium deoxycholate (D) d) Concentration of sodium alginate (B) and Concentration of Drug (C) e) Concentration of sodium alginate (B) and concentration of sodium deoxycholate (D) f) Concentration of drug (C) and concentration of sodium deoxycholate (D) on the particle size of the MMTMC-TZ NPs

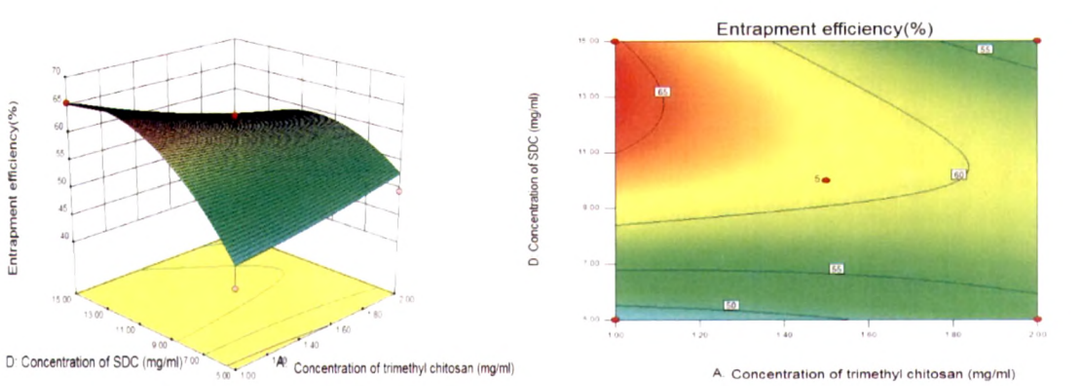
(a)


Entrapment efficiency(%)
Entrapment efficiency(%)
B: Concentration of sodium alginate (mg/ml)
A: Concentration of trimethyl chitosan (mg/ml)
Entrapment efficiency(%)
B: Concentration of sodium alginate (mg/ml)
A: Concentration of trimethyl chitosan (mg/ml)

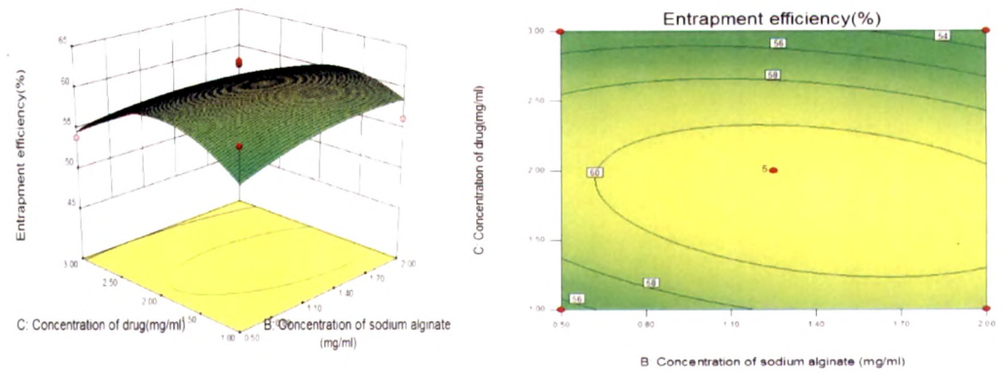
(b)


Entrapment efficiency(%)
Entrapment efficiency(%)
C: Concentration of drug(mg/ml)
A: Concentration of trimethyl chitosan (mg/ml)
Entrapment efficiency(%)
C: Concentration of drug(mg/ml)
A: Concentration of trimethyl chitosan (mg/ml)

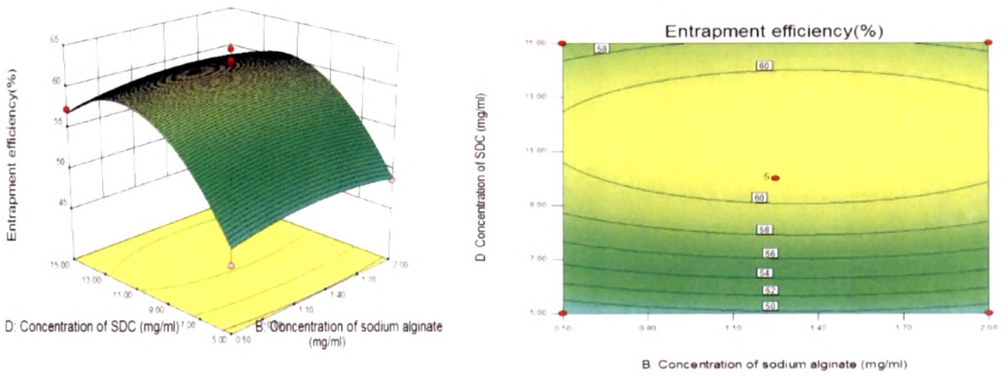
(c)


Entrapment efficiency(%)
Entrapment efficiency(%)
D: Concentration of SDC (mg/ml)
A: Concentration of trimethyl chitosan (mg/ml)
Entrapment efficiency(%)
D: Concentration of SDC (mg/ml)
A: Concentration of trimethyl chitosan (mg/ml)

(d)



(e)



(f)

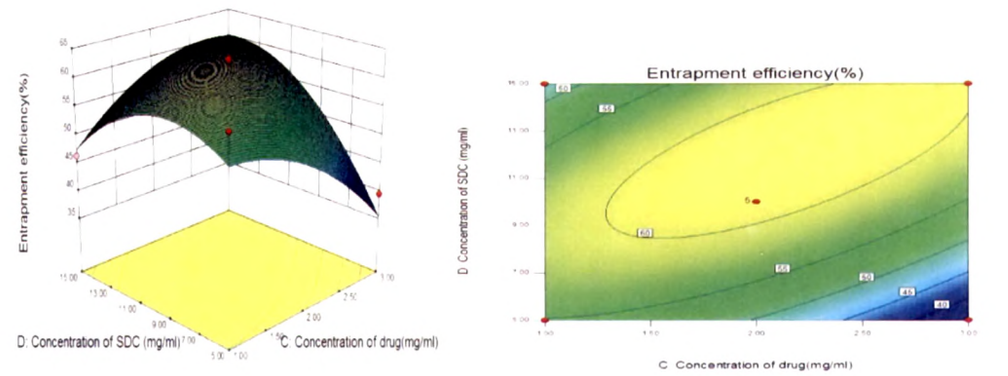
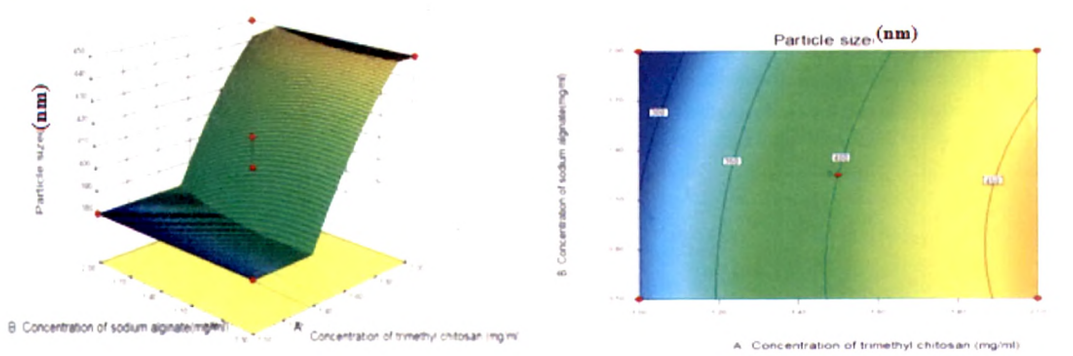
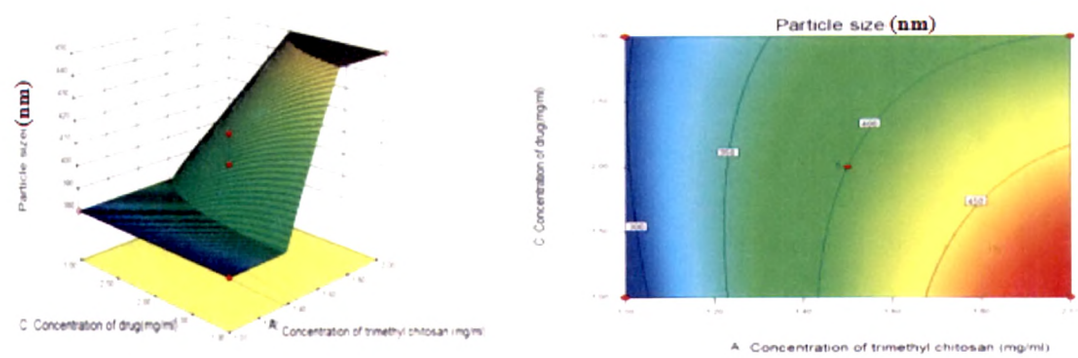


Figure: 5.22. Response surface and contour plots of a) the effects of Concentration of trimethyl chitosan (A) and Concentration of sodium alginate (B) b) effects of Concentration of trimethyl (A) and Concentration of drug (C) c) Concentration of trimethyl chitosan (A) and concentration of sodium deoxycholate (D) d) Concentration of sodium alginate (B) and Concentration of Drug (C) e) Concentration of sodium alginate (B) and concentration of sodium deoxycholate (D) f) Concentration of drug (C) and concentration of sodium deoxycholate (D) on the entrapment efficiency of the MMTMC-TZ NPs

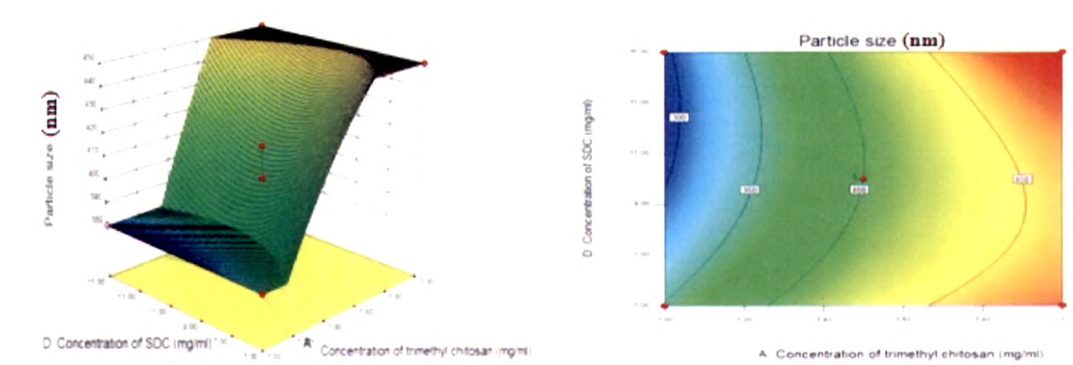
(a)


Particle size (nm)
B Concentration of sodium alginate(mg/ml)
A Concentration of trimethyl chitosan (mg/ml)
Particle size (nm)
B Concentration of sodium alginate(mg/ml)
A Concentration of trimethyl chitosan (mg/ml)

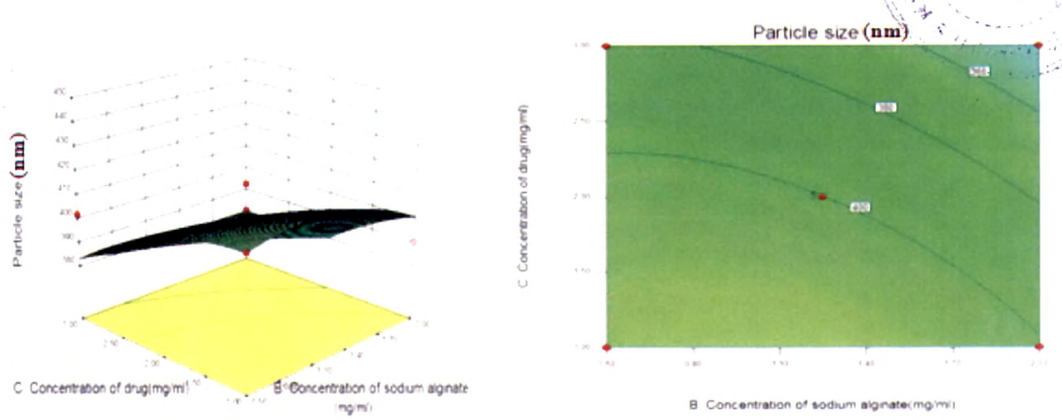
(b)


Particle size (nm)
C Concentration of drug(mg/ml)
A Concentration of trimethyl chitosan (mg/ml)
Particle size (nm)
C Concentration of drug(mg/ml)
A Concentration of trimethyl chitosan (mg/ml)

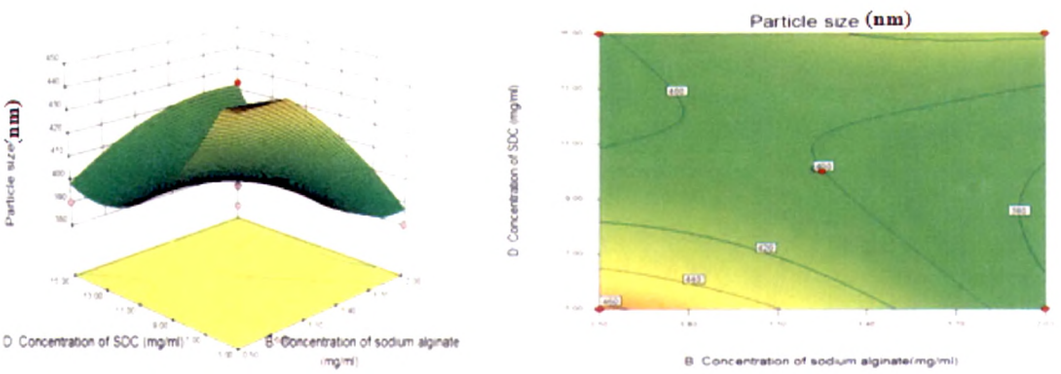
(c)


Particle size (nm)
D Concentration of SDC (mg/ml)
A Concentration of trimethyl chitosan (mg/ml)
Particle size (nm)
D Concentration of SDC (mg/ml)
A Concentration of trimethyl chitosan (mg/ml)

(d)



(e)



(f)

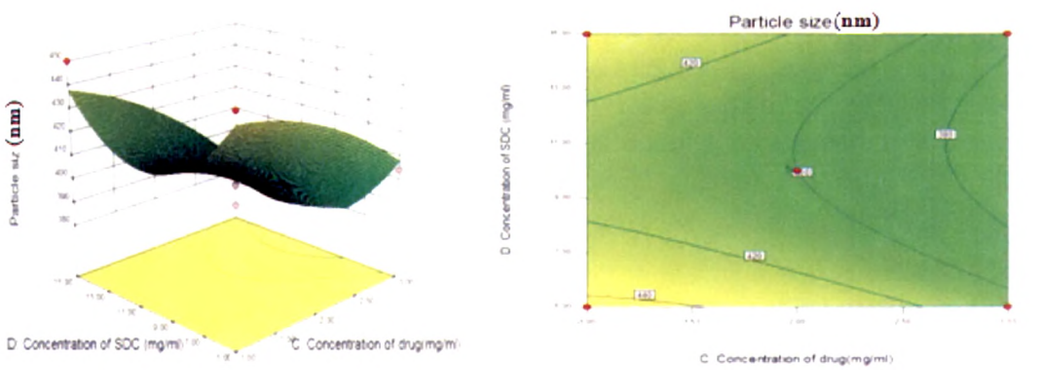
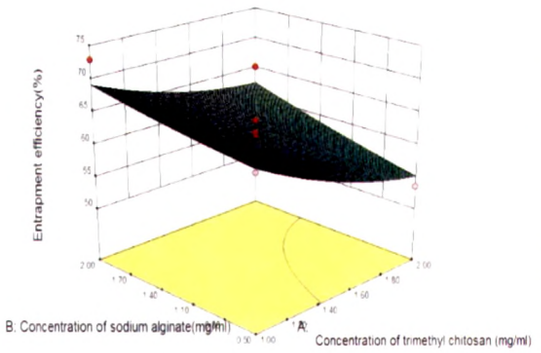
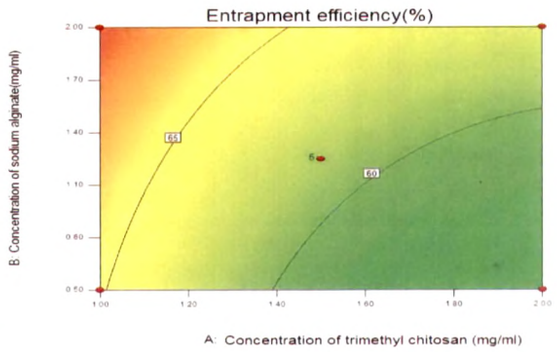


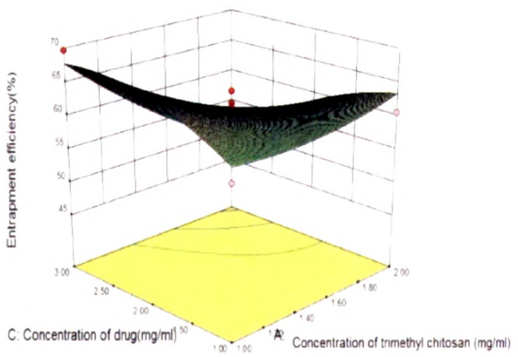
Figure: 5.23. Response surface and contour plots of a) the effects of Concentration of trimethyl chitosan (A) and Concentration of sodium alginate (B) b) effects of Concentration of trimethyl (A) and Concentration of drug (C) c) Concentration of trimethyl chitosan (A) and concentration of sodium deoxycholate (D) d) Concentration of sodium alginate (B) and Concentration of Drug (C) e) Concentration of sodium alginate (B) and concentration of sodium deoxycholate (D) f) Concentration of drug (C) and concentration of sodium deoxycholate (D) on the particle size of the MMTMC-CBZ NPs

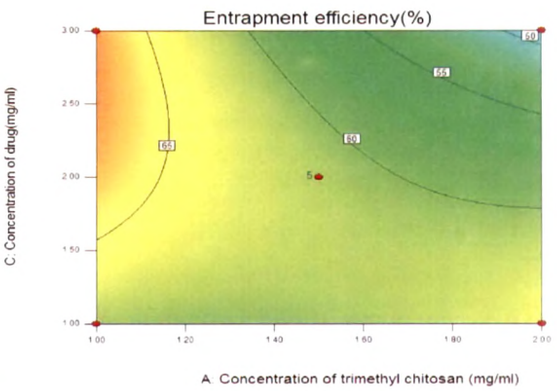
(a)


Entrapment efficiency(%)
B: Concentration of sodium alginate(mg/ml)
A: Concentration of trimethyl chitosan (mg/ml)

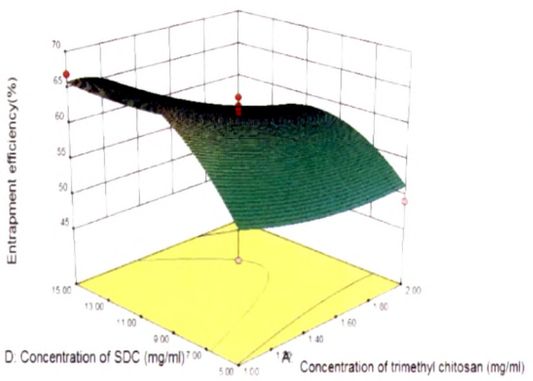

Entrapment efficiency(%)
B: Concentration of sodium alginate(mg/ml)
A: Concentration of trimethyl chitosan (mg/ml)

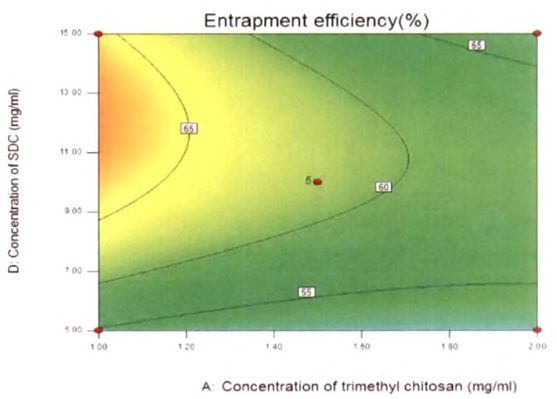
(b)


Entrapment efficiency(%)
C: Concentration of drug(mg/ml)
A: Concentration of trimethyl chitosan (mg/ml)

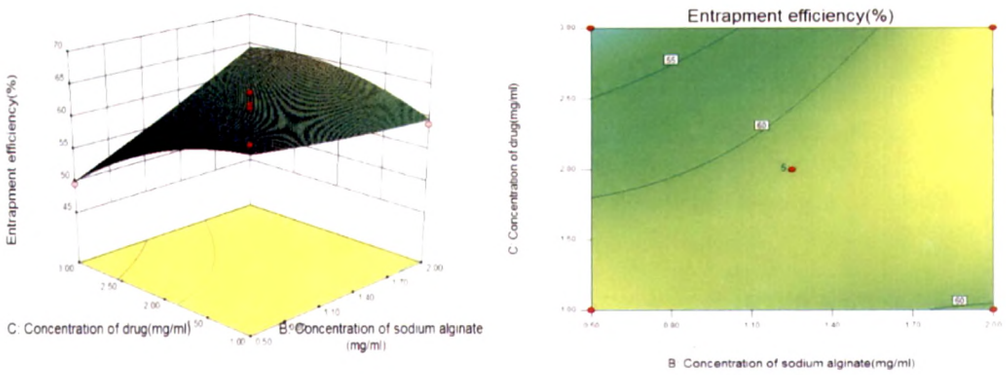

Entrapment efficiency(%)
C: Concentration of drug(mg/ml)
A: Concentration of trimethyl chitosan (mg/ml)

(c)

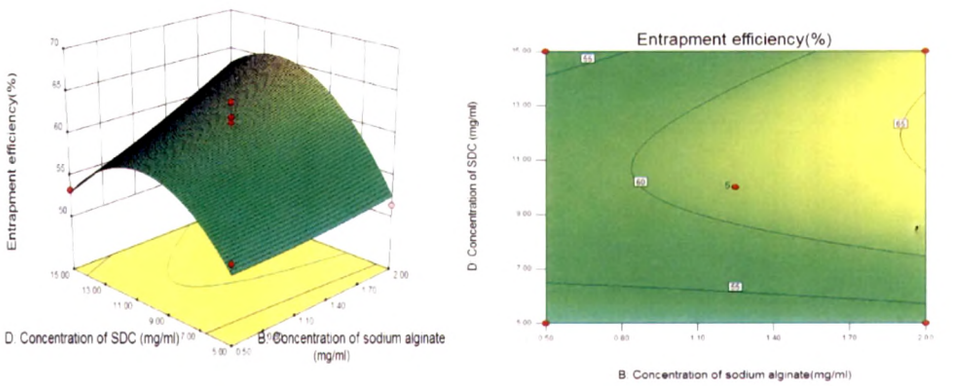

Entrapment efficiency(%)
D: Concentration of SDC (mg/ml)
A: Concentration of trimethyl chitosan (mg/ml)


Entrapment efficiency(%)
D: Concentration of SDC (mg/ml)
A: Concentration of trimethyl chitosan (mg/ml)

(d)



(e)



(f)

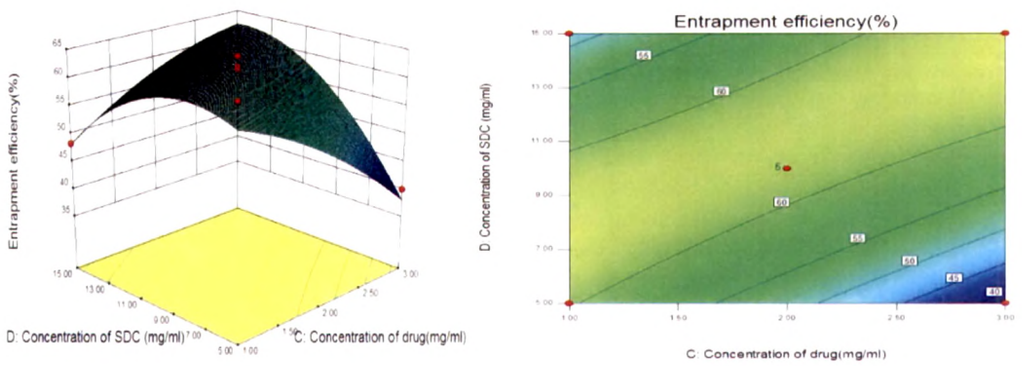


Figure: 5.24. Response surface and contour plots of a) the effects of Concentration of trimethyl chitosan (A) and Concentration of sodium alginate (B) b) effects of Concentration of trimethyl (A) and Concentration of drug (C) c) Concentration of trimethyl chitosan (A) and concentration of sodium deoxycholate (D) d) Concentration of sodium alginate (B) and Concentration of Drug (C) e) Concentration of sodium alginate (B) and concentration of sodium deoxycholate (D) f) Concentration of drug (C) and concentration of sodium deoxycholate (D) on the entrapment of the MMTMC-CBZ NPs

Data processing:

Trimethyl chitosan nanoparticles were prepared by Ionotropic gelation with slight modification using Trimethyl chitosan/sodium alginate/drug/sodium deoxycholate. Formulation parameters, such as concentration of trimethyl chitosan, concentration of sodium alginate, concentration of drug and concentration of sodium deoxycholate found to have significant influence on preparation of nanoparticles. Hence, formulation parameters were optimized and kept unaltered in subsequent experiments. In order to optimize formulation parameters, a Box Behnken Design (BBD) of RSM was used. Twenty nine batches of nanoparticles for each were prepared by Ionotropic gelation method using a 4-factor, 3-level BBD varying four independent variables Concentration of trimethyl chitosan (mg/ml) (A), Concentration of sodium alginate (mg/ml) (B), concentration of drug (mg/ml) (C) and concentration of sodium deoxycholate (D) of the formulation according to Table 5.21. The influence of these variables on observed response (Y, particle size and entrapment efficiency) is recorded in Table 5.22 to Table 5.25 for each formulation. Each batch of nanoparticles was prepared three times and was evaluated for particle size and entrapment efficiency.

Polynomial equation for each formulation:

LMTMC-TZ NPs- The maximum response as particle size was 513.89nm and minimum response was 168nm. The mathematical relationship for the same in terms of a polynomial equation relating the response Y and independent variables was: (Y1) =+371.17+64.16 * A-21.17 * B-25.56 * C-63.77 * D+47.17 * A * B+6.50 *A * C+28.59 * A * D+31.53 * B * C-16.77 * B * D-9.33 * C * D-23.03 * A^2-23.53 * B^2+17.65 * C^2+40.25 * D^2 and (Y2) =+55.93-3.61* A+1.41 * B-1.45 * C+5.73* D+1.25* A * B-5.98 *A * C-3.17 * A * D-0.36 * B * C+1.38 * B * D+8.75* C * D+0.46 * A^2-0.54 * B^2-4.99 * C^2-7.97 * D^2 for particle size and entrapment efficiency respectively. ..... Equation [5.20] & [5.21]

LMTMC-CBZ NPs- The maximum response as particle size was 523.89nm and minimum response was 184.6nm. The mathematical relationship for the same in terms of a polynomial equation relating the response Y and independent variables was :(Y1) =+387.40+61.23 * A-21.71 * B-21.41 * C-58.68 * D+48.38 * A * B+7.97 * A * C+35.95 * A * D+28.81 * B * C-24.83*B*D-15.20*C*D-24.86*A^2-23.31*B^2+11.94 * C^2+41.57 * $D^2$and (Y2) =+58.24-2.90* A+1.22 * B-1.69 * C+4.74 * D+0.42* A *

B-6.67 * A * C-4.20 * A * D-1.14* B * C+1.00 * B * D+9.12 * C * D+1.62* A^2-0.56 * B^2-4.99 * C^2-6.98 * D^2 for particle size and entrapment efficiency respectively.

.....Equation [5.22] & [5.23]

MMTMC-TZ NPs- The maximum response as particle size was 493.78nm and minimum response was 226.6nm. The mathematical relationship for the same in terms of a polynomial equation relating the response Y and independent variables was :(Y1) =+393.78+83.03 * A-19.35 * B-20.09* C-5.40 * D+15.85* A * B-27.25* A *C+27.55 * A * D-1.17 * B * C+27.53*B*D-1.69*C*D-26.13*A^2-15.05*B^2-7.62*C^2+24.86 * D^2 and (Y2) =+61.04-1.99* A+0.27 * B-1.71 * C+4.29 * D+3.35* A * B-6.86 * A * C-4.45 * A * D-1.34 * B * C-2.500E-003 * B * D+7.99 * C * D+0.52 * A^2-1.19 * B^2-4.60 * C^2-7.03 * D^2 for particle size and entrapment efficiency respectively. ..... Equation [5.24] & [5.25]

MMTMC-CBZ NPs- The maximum response as particle size was 499.34nm and minimum response was 274.5nm. The mathematical relationship for the same in terms of a polynomial equation relating the response Y and independent variables was: (Y1) =+400.11+80.39 * A-13.04 * B-22.10 * C-7.27 * D+4.82 * A * B-28.03 * A * C+21.92*A*D-7.64*B*C+24.86*B*D-3.88*C*D-21.91*A^2-8.38*B^2-7.11*C^2+25.97* D^2 and (Y2) =+61.33-4.16* A+2.83* B-2.00* C+3.08* D+0.86 * A * B-5.30 * A * C-2.47* A * D+4.34* B * C+2.31* B * D+9.12* C * D+1.37* A^2+0.33* B^2-2.46 * C^2-6.63 * D^2 for particle size and entrapment efficiency respectively.

..... Equation [5.26] & [5.27]

Equation expresses the quantitative effect of the individual formulation components (A, B, C and D) and combination thereof on the response (Y) in terms of interaction coefficients. The values of the coefficients A to D are related to the effect of these variables on the response (Y). Coefficients with more than one factor term and those with higher order terms represent interaction terms and quadratic relationships respectively. A positive and negative signs suggest a positive and negative effect on response respectively. The theoretical (predicted) values and the observed values were in reasonably good agreement as seen from Table 5.22 to Table 5.25 for each formulation. The significance of the ratio of mean square variation due to regression and residual error was tested using analysis of variance (ANOVA). It was observed that the best-fitted models were quadratic. The values of R^2, adjusted R^2, predicted R^2, Degree of freedom (Df), Sum of squares(SS) and Mean square(MS) are given in

Table 5.26 to Table 5.29 for each formulation, along with the regression equation generated for each response. The results of ANOVA in Table 5.26 to Table 5.29 for the dependent variables demonstrate that the model was significant for both the response variables.

Table: 5.26. Analysis of Variance for BBD Model of LMTMC-TZ NPs

Parameters		Df	SS	MS	R^2	AdjR^2	PredR^2	F	Lack of fit
Particle size	Model	14	1.532E+005	10942.7	0.8849	0.769	0.3924	0.0002	0.1433
	Residual	14	19919.07	1422.79	-	-	-	-	-
Entrapment efficiency	Model	14	1650.66	117.90	0.7464	0.4928	-0.3427	0.0262	0.1364
	Residual	14	560.83	40.06	-	-	-	-	-

*Df- Degree of freedom; SS- Sum of squares; MS-Mean square; F- Significance AdjR^2 -Adjusted R^2; PredR^2- Predicted R^2

Table: 5.27. Analysis of Variance for BBD Model of LMTMC-CBZ NPs

Parameters		Df	SS	MS	R^2	AdjR^2	PredR^2	F	Lack of fit
Particle size	Model	14	1.432E+005	10227.5	0.8898	0.7795	0.4120	0.0002	0.1170
	Residual	14	17738.78	1267.06	-	-	-	-	-
Entrapment efficiency	Model	14	1510.54	107.90	0.7748	0.5497	-0.2140	0.0137	0.0911
	Residual	14	438.96	31.35	-	-	-	-	-

*Df- Degree of freedom; SS- Sum of squares; MS-Mean square; F- Significance AdjR^2 -Adjusted R^2; PredR^2- Predicted R^2

Table: 5.28. Analysis of Variance for BBD Model of MMTMC-TZ NPs

Parameters		Df	SS	MS	R^2	AdjR^2	PredR^2	F	Lack of fit
Particle size	Model	14	1.146E+005	8186.6	0.9825	0.9650	0.9056	0.0001	0.0943
	Residual	14	2044.32	146.02	-	-	-	-	-
Entrapment efficiency	Model	14	1311.15	93.65	0.8274	0.6549	0.0823	0.0030	0.1249
	Residual	14	273.45	19.53	-	-	-	-	-

*Df- Degree of freedom; SS- Sum of squares; MS-Mean square; F- Significance AdjR^2 -Adjusted R^2; PredR^2- Predicted R^2

Table: 5.29. Analysis of Variance for BBD Model of MMTMC-CBZ NPs

Parameters		Df	SS	MS	R^2	AdjR^2	PredR^2	F	Lack of fit
Particle size	Model	14	1.041E+005	7434.4	0.9727	0.9454	0.8572	0.0001	0.1674
	Residual	13	2920.53	208.61	-	-	-	-	-
Entrapment efficiency	Model	14	1398.15	99.87	0.9158	0.8316	0.5478	0.0001	0.1007
	Residual	13	128.56	9.18	-	-	-	-	-

*Df- Degree of freedom; SS- Sum of squares; MS-Mean square; F- Significance
AdjR^2 -Adjusted R^2; PredR^2- Predicted R^2

The ANOVA indicated a significant ($P < 0.05$) effect of factors on response. Lack of fit was not significant and regression was strongly significant for all formulations. The Adjusted R-square and Predicted R-square should be within approximately 0.20 of each other to be in reasonable agreement. The "Pred R-Square" is in reasonable agreement with the "Adj R-Square" of all the formulations, which is very good for chosen factorial model. So it was concluded that the second-order model adequately approximated the true surface.

5.3. Lyophilization and optimization of cryoprotectant concentration

The nanoparticles dispersions have thermodynamic instability upon storage and lead to the formation of aggregates. Freeze drying/lyophilization are one of the known methods to recover the nanoparticles in the dried form and suitably redisperse it at the time of administration. To the suspension of the nanoparticles different cryoprotectants like sucrose, mannitol and trehalose were added in different concentrations at nanoparticles (NPs): cryoprotectant (CP) ratio of 1:1, 1:2 and 1:3 before freeze-drying. The effect these cryoprotectants on the redispersibility of the freeze-dried formulations and the size of the nanoparticles after freeze-drying was investigated and recorded in Table 5.30 to Table 5.35.

Table: 5.30: Effect of different cryoprotectants on the particle size and redispersion of NPs (LMC-TZ NPs and MMC-TZ NPs)

Type of cryoprotectant	NPs: CP	Particle size (nm)				S_f/S_i		Redispersion	
		Before lyophilization S_i		After lyophilisation S_f		A		B	
		A	B	A	B				
Initial	1:0	473.5 ± 13.5	598.7 ±18	NA	NA	NA	NA	NA	NA
Sucrose	1: 1	--	--	--	--	--	--	PR	PR
Sucrose	1:2	--	--	1721.4 ± 52.5	1989.9 ± 62.5	4.3	3.3	PR	PR
Sucrose	1:3	--	--	1251.4 ± 45.5	1654.7 ± 55.5	3.1	2.7	PR	PR
Mannitol	1: 1	--	--	1045 ± 55.5	1347 ± 45.5	2.6	2.2	DR	DR
Mannitol	1:2	--	--	937.6 ± 47.9	1137.8 ± 57.7	2.3	1.9	DR	DR
Mannitol	1:3	--	--	772.6 ±34.8	976.4 ± 44.8	1.9	1.6	DR	DR
Trehalose	1: 1	--	--	696.5 ± 39.8	886.6 ± 40.5	1.7	1.4	ER	ER
Trehalose	1:2	--	--	528.9 ± 33.3	718.4 ± 38.6	1.3	1.1	ER	ER
Trehalose	1:3	--	--	488.4 ± 24.5	628.7 ± 28.8	1.0	1.1	ER	ER

(Mean ± S.D., *n* = 3)

PR- Poor redispersibility
DR- Difficult redispersibility
ER- Easy redispersibility
A= LMC-TZ NPs, B= MMC-TZ NPs
NPs-Nanoparticles
CP-Cryoprotectant

Table: 5.31: Effect of different cryoprotectants on the particle size and redispersion of NPs (LMC-CBZ NPs and MMC-CBZ NPs)

Type of cryoprotectant	NPs: CP	Particle size (nm)				S_f/S_i		Redispersion	
		Before lyophilization S_i		After lyophilisation S_f		C		D	
		C	D	C	D				
Initial	1:0	472.6 ±12.7	628.2 ±13.1	NA	NA	NA	NA	NA	NA
Sucrose	1: 1	--	--	--	--	--	--	PR	PR
Sucrose	1:2	--	--	1825.3 ± 42.6	1999.2 ± 49.4	3.8	3.2	PR	PR
Sucrose	1:3	--	--	1351.4 ± 35.3	1674.5 ± 48.9	2.8	2.6	PR	PR
Mannitol	1: 1	--	--	1143 ± 41.6	1357.1 ± 25.8	2.4	2.1	DR	DR
Mannitol	1:2	--	--	989.4 ± 51.2	1166.7 ± 47.2	2.0	1.8	DR	DR
Mannitol	1:3	--	--	878.9 ±44.3	986.7 ± 35.9	1.8	1.5	DR	DR
Trehalose	1: 1	--	--	666.7 ± 28.7	891.5 ± 33.8	1.4	1.4	ER	ER
Trehalose	1:2	--	--	538.3 ± 23.5	721.6 ± 16.7	1.1	1.1	ER	ER
Trehalose	1:3	--	--	480.9 ± 21.7	648.6 ± 27.1	1.0	1.0	ER	ER

(Mean ± S.D., $n = 3$)

PR- Poor redispersibility
DR- Difficult redispersibility
ER- Easy redispersibility
C= LMC-CBZ NPs, D= MMC-CBZ NPs
NPs-Nanoparticles
CP-Cryoprotectant

Table: 5.32: Effect of different cryoprotectants on the particle size and redispersion of NPs (LMTC-TZ NPs and MMTC-TZ NPs)

Type of cryoprotectant	NPs: CP	Particle size (nm)				S_f/S_i		Redispersion	
		Before lyophilization S_i		After lyophilisation S_f		C		D	
		A	B	A	B				
Initial	1:0	262.5 ±12.4	264.9 ±15	NA	NA	NA	NA	NA	NA
Sucrose	1: 1	--	--	--	--	--	--	PR	PR
Sucrose	1:2	--	--	721.4 ± 22.5	811.6 ± 28.5	2.7	3.0	PR	PR
Sucrose	1:3	--	--	625.4 ± 15.5	678.4 ± 22.5	2.3	2.5	PR	PR
Mannitol	1: 1	--	--	437.6 ± 17.9	527.7 ± 18.9	1.7	1.9	DR	DR
Mannitol	1:2	--	--	472.6 ±14.8	492.8 ±19.8	1.8	1.8	DR	DR
Mannitol	1:3	--	--	396.5 ± 17.8	399.9 ± 12.8	1.5	1.5	DR	DR
Trehalose	1: 1	--	--	346.5 ± 13.7	356.8 ± 13.7	1.3	1.4	ER	ER
Trehalose	1:2	--	--	328.9 ± 13.3	344.7 ± 16.3	1.3	1.3	ER	ER
Trehalose	1:3	--	--	296.4 ± 14.9	292.4 ± 13.4	1.1	1.1	ER	ER

(Mean ± S.D., *n* = 3)

PR- Poor redispersibility
DR- Difficult redispersibility
ER- Easy redispersibility
A= LMTC-TZ NPs, B= MMTC-TZ NPs
NPs-Nanoparticles
CP-Cryoprotectant

Table: 5.33: Effect of different cryoprotectants on the particle size and redispersion of NPs (LMTC-CBZ NPs and MMTC-CBZ NPs)

Type of cryoprotectant	NPs: CP	Particle size (nm)				S_f/S_i		Redispersion	
		Before lyophilization S_i		After lyophilisation S_f		C		D	
		C	D	C	D				
Initial	1:0	272.1 ±11.5	282.9 ±15.6	NA	NA	NA	NA	NA	NA
Sucrose	1: 1	--	--	--	--	--	--	PR	PR
Sucrose	1:2	--	--	849.4 ± 21.2	899.1 ± 25.3	3.1	3.1	PR	PR
Sucrose	1:3	--	--	728.6 ± 13.9	747.2 ± 22.5	2.6	2.6	PR	PR
Mannitol	1: 1	--	--	517.7 ± 20.3	621.3 ± 19.1	1.9	2.1	DR	DR
Mannitol	1:2	--	--	479.3 ±11.7	499.5 ±18.7	1.7	1.7	DR	DR
Mannitol	1:3	--	--	399.7 ± 14.2	403.8 ± 10.4	1.4	1.4	DR	DR
Trehalose	1: 1	--	--	366.7 ± 10.8	376.9 ± 9.7	1.3	1.3	ER	ER
Trehalose	1:2	--	--	339.8 ± 12.1	348.6 ± 10.3	1.2	1.2	ER	ER
Trehalose	1:3	--	--	289.8 ± 15.9	294.8 ± 11.4	1.0	1.0	ER	ER

(Mean ± S.D., $n = 3$)

PR- Poor redispersibility
DR- Difficult redispersibility
ER- Easy redispersibility
C= LMTC-CBZ NPs, D= MMTC-CBZ NPs
NPs-Nanoparticles
CP-Cryoprotectant

Table: 5.34: Effect of different cryoprotectants on the particle size and redispersion of NPs (LMTMC-TZ NPs and MMTMC-TZ NPs)

Type of cryoprotectant	NPs: CP	Particle size (nm)				S_f/S_i		Redispersion	
		Before lyophilization S_i		After lyophilisation S_f		C		D	
		A	B	A	B				
Initial	1:0	168±1 0.2	226.6 ±18.1	NA	NA	NA	NA	NA	NA
Sucrose	1: 1	--	--	--	--	--	--	PR	PR
Sucrose	1:2	--	--	529.6 ± 20.3	561.9 ± 23.8	3.1	2.4	PR	PR
Sucrose	1:3	--	--	499.6 ± 11.7	518.4 ± 19.2	2.9	2.2	PR	PR
Mannitol	1: 1	--	--	427.8 ± 16.8	487.3 ± 13.7	2.5	2.1	DR	DR
Mannitol	1:2	--	--	397.4 ±11.9	422.7 ±15.4	2.3	1.8	DR	DR
Mannitol	1:3	--	--	316.4 ± 12.2	388.5 ± 12.8	1.8	1.7	DR	DR
Trehalose	1: 1	--	--	278.8 ± 19.4	345.7 ± 18.5	1.6	1.5	ER	ER
Trehalose	1:2	--	--	228.6 ± 9.9	299.6 ± 14.3	1.3	1.3	ER	ER
Trehalose	1:3	--	--	184.5 ± 18.1	246.7 ± 11.7	1.0	1.0	ER	ER

(Mean ± S.D., n = 3)

PR- Poor redispersibility
DR- Difficult redispersibility
ER- Easy redispersibility
A= LMTMC-TZ NPs, B= MMTMC-TZ NPs
NPs-Nanoparticles
CP-Cryoprotectant

Table: 5.35: Effect of different cryoprotectants on the particle size and redispersion of NPs (LMTMC-CBZ NPs and MMTMC-CBZ NPs)

Type of cryoprotectant	NPs: CP	Particle size (nm)				S_f/S_i		Redispersion	
		Before lyophilization S_i		After lyophilisation S_f		C		D	
		C	D	C	D				
Initial	1:0	184.6 ±13.9	274.5 ±21.3	NA	NA	NA	NA	NA	NA
Sucrose	1: 1	--	--	--	--	--	--	PR	PR
Sucrose	1:2	--	--	721.7 ± 29.5	809.3 ± 31.2	3.9	2.9	PR	PR
Sucrose	1:3	--	--	633.5 ± 30.8	717.9 ± 28.4	3.4	2.6	PR	PR
Mannitol	1: 1	--	--	527.3 ± 25.1	602.8 ± 26.8	2.8	2.1	DR	DR
Mannitol	1:2	--	--	467.2 ±21.6	519.9 ±17.3	2.5	1.8	DR	DR
Mannitol	1:3	--	--	397.7 ± 19.1	467.3 ± 13.9	2.1	1.7	DR	DR
Trehalose	1: 1	--	--	316.8 ± 12.1	402.1 ± 18.3	1.7	1.4	ER	ER
Trehalose	1:2	--	--	259.2 ± 11.4	338.9 ± 12.9	1.4	1.2	ER	ER
Trehalose	1:3	--	--	202.3 ± 10.5	291.6 ± 15.6	1.0	1.0	ER	ER

(Mean ± S.D., $n = 3$)

PR- Poor redispersibility
DR- Difficult redispersibility
ER- Easy redispersibility
C= LMTMC-CBZ NPs, D= MMTMC-CBZ NPs
NPs-Nanoparticles
CP-Cryoprotectant

5.4. Preparation of Rhodamine B loaded chitosan, thiolated chitosan and trimethyl chitosan nanoparticles

Nanoparticles containing fluorescent dye Rhodamine B were formulated using same ionotropic gelation method. The dye acts as a fluorescent probe for NPs and offers a sensitive method to qualitative determine their penetration into the mucus layer. Briefly, Rhodamine B loaded chitosan NPs prepared by dissolving chitosan (1 mg/ml) in 1% acetic acid aqueous solution. After stirring overnight Rhodamine B (2.5 mg/ml)

was added in chitosan solution. Sodium alginate (0.5mg/ml) solution was added drop wise to above chitosan solution with ratio of chitosan to sodium alginate solution 2:1 under moderate stirring led to the immediate formation of NPs. Following stirring for 20 minutes, the particle suspension was centrifuged at 4°C at 18,000 rpm for 30 minutes. The particles were washed and subjected to characterize particle size and zeta potential. The supernatant was analyzed for entrapment efficiency using U.V spectroscopy.

Rhodamine B loaded thiolated chitosan NPs were prepared by ionic gelation of polymer using sodium alginate with slight modification in above method. Thiolated chitosan NPs prepared by dissolving thiolated chitosan (2 mg/ml) in distilled water. Rhodamine B solution was incubated with sodium deoxycholate (10mg/ml) for 30 seconds. The resulting complex was added to thiolated chitosan solution. The addition of sodium alginate solution (0.5 mg/ml) with stirring to the above mixture with (ratio of thiolated chitosan: sodium deoxycholate: sodium alginate: Rhodamine B was taken 7:1:1:1) moderate stirring led to the immediate formation of NPs. The particle suspension was centrifuged at 4°C at 18,000 rpm for 30 minutes. The particles were washed and subjected to characterize zeta potential, and particle size. The supernatant was analyzed for entrapment efficiency using U.V spectroscopy.

Rhodamine B loaded trimethyl chitosan NPs were prepared by same method as thiolated chitosan NPs. Trimethyl chitosan NPs prepared by dissolving trimethyl chitosan (1 mg/ml) in distilled water. Rhodamine B solution was incubated with sodium deoxycholate (10mg/ml) for 30 seconds. The resulting complex was added to trimethyl chitosan solution. The addition of sodium alginate solution (2 mg/ml) with stirring to the above mixture with (ratio of trimethyl chitosan: sodium deoxycholate: sodium alginate: Rhodamine B was taken 7.9:0.7:0.7:0.7) moderate stirring led to the immediate formation of NPs. The particle suspension was centrifuged at 4°C at 18,000 rpm for 30 minutes. The particles were washed and subjected to characterize zeta potential and particle size. The supernatant was analyzed for entrapment efficiency using U.V spectroscopy.

Results of characterization of Rhodamine B loaded chitosan thiolated chitosan and trimethyl chitosan nanoparticles are tabulated and discussed in chapter 6.

5.5. Results and discussion

5.5.1. Preparation of Tizanidine HCl (TZ) and Cyclobenzaprine HCl (CBZ) loaded chitosan (C), thiolated chitosan (TC) and trimethyl chitosan (TMC) nanoparticles

Ionotropic gelation method was easy and reproducible for preparation of drug loaded chitosan thiolated chitosan and trimethyl chitosan NPs. There are at least four methods available for nanoparticles preparation namely: ionotropic gelation, microemulsion, emulsification solvent diffusion and polyelectrolyte complex. The most widely developed methods are ionotropic gelation and self assemble polyelectrolytes. These methods offer many advantages such as simple and mild preparation method without the use of organic solvent or high shear force.

5.5.2 Optimization of Formulation Parameters for chitosan Nanoparticles

The formulation parameters *viz.* Concentration of chitosan, Concentration of sodium alginate and concentration of Drug were optimized for maximum %EE and minimum particle size. The relationship between the dependent and independent variables was further elucidated using contour and response surface plots.

LMC-TZ NPs

The effect of concentration of chitosan (A) and concentration of sodium alginate (B) and their interaction on response Y at a fixed level of drug concentration (C) are given in response surface and contour plot shown in Figures 5.1 (a) and Figure 5.2 (a) for particles size and % EE respectively. As shown in figures, at mean level concentration of chitosan (A) 1.5 mg/ml and lower level concentration of drug (C) 1 mg/ml, particle size increases from489.9 to 1119.7nm and %EE increases from 16.99 to 17.50 % (w/w), when the concentration of sodium alginate (B) increases from 0.5 to 1.5 mg/ml. Similarly, at higher level concentration chitosan (A) 2mg/ml, particle size and %EE increases from 768.5 to 1105.4nm and 24.19 to 25.41% (w/w), respectively when concentration of sodium alginate (B) increases from 0.5 to 1.5mg/ml.

The effect of concentration of chitosan (A) and concentration of drug(C) and their interaction on response Y at a fixed level of concentration of sodium alginate (B) are given in response surface and contour plot shown in Figures 5.1 (b) and Figure 5.2 (b) for particles size and % EE respectively. As shown in figures, at mean level concentration of sodium alginate (B) 1mg/ml and at the lower level concentration of drug (C) 1mg/ml, particle size decreases from 726.9 to 536.2nm when the

concentration of chitosan (A) increases from 1 to 2 mg/ml with non significant change in drug entrapment efficiency.

The effect of concentration of sodium alginate (B) and concentration of drug(C) and their interaction on response Y at a fixed level of concentration of chitosan (A) are given in response surface and contour plot shown in Figures 5.1 (c) and Figure 5.2 (c) for particles size and % EE respectively. As shown in figures, at mean level concentration of chitosan (A) 1.5 mg/ml and at the higher level concentration of sodium alginate (B) 1.5 mg/ml, particle size increases from 1119.7 to 1299.8nm and %EE increases from 17.50 to 25.0%(w/w), when the concentration of drug (C) increases from 1 to 4 mg/ml.

LMC-CBZ NPs

The effect of concentration of chitosan (A) and concentration of sodium alginate (B) and their interaction on response Y at a fixed level of drug concentration (C) are given in response surface and contour plot shown in Figures 5.3 (a) and Figure 5.4 (a) for particles size and % EE respectively. As shown in figures, at higher level concentration of chitosan (A) 2 mg/ml and mean level concentration of drug (C) 2.5 mg/ml, particle size increases from 708.9 to 1023.4nm and %EE increases from 24.20 to 25.10 % (w/w), when the concentration of sodium alginate (B) increases from 0.5 to 1.5 mg/ml.

The effect of concentration of chitosan (A) and concentration of drug(C) and their interaction on response Y at a fixed level of concentration of sodium alginate (B) are given in response surface and contour plot shown in Figures 5.3 (b) and Figure 5.4 (b) for particles size and % EE respectively. As shown in figures, at mean level concentration of sodium alginate (B) 1mg/ml and at the lower level concentration of drug (C). 1mg/ml, particle size decreases from 666.5to 589.7nm and %EE decreases from16.39 to 15.43 % (w/w) when the concentration of chitosan (A) increases from 1 to 2 mg/ml.

The effect of concentration of sodium alginate (B) and concentration of drug(C) and their interaction on response Y at a fixed level of concentration of chitosan (A) are given in response surface and contour plot shown in Figures 5.3 (c) and Figure 5.4 (c) for particles size and % EE respectively. As shown in figures, at mean level concentration of chitosan (A) 1.5 mg/ml and at the higher level concentration of sodium alginate (B) 1.5 mg/ml, particle size increases from 1110.3 to 1189.0±23nm

and %EE increases from 17.44 to 24.43%(w/w), when the concentration of drug (C) increases from 1 to 4 mg/ml.

MMC-TZ NPs

The effect of concentration of chitosan (A) and concentration of sodium alginate (B) and their interaction on response Y at a fixed level of drug concentration (C) are given in response surface and contour plot shown in Figures 5.5 (a) and Figure 5.6 (a) for particles size and % EE respectively. As shown in figures, at higher level concentration of chitosan (A) 2 mg/ml and mean level concentration of drug (C) 2.5 mg/ml, particle size increases from 895.7 to 1145nm and %EE increases from 18.65 to 24.34 % (w/w), when the concentration of sodium alginate (B) increases from 0.5 to 1.5 mg/ml.

The effect of concentration of chitosan (A) and concentration of drug(C) and their interaction on response Y at a fixed level of concentration of sodium alginate (B) are given in response surface and contour plot shown in Figures 5.5 (b) and Figure 5.6 (b) for particles size and % EE respectively. As shown in figures, at mean level concentration of sodium alginate (B) 1mg/ml and at the lower level concentration of drug (C) 1 mg/ml, particle size decreases from 856.7to 769.7nm and %EE decreases from 19.12 to 16.90 % (w/w), when the concentration of chitosan (A) increases from 1 to 2 mg/ml.

The effect of concentration of sodium alginate (B) and concentration of drug(C) and their interaction on response Y at a fixed level of concentration of chitosan (A) are given in response surface and contour plot shown in Figures 5.5 (c) and Figure 5.6 (c) for particles size and % EE respectively. As shown in figures, at mean level concentration of chitosan (A) 1.5 mg/ml and at the higher level concentration of sodium alginate (B) 1.5 mg/ml, particle size increases from 1123.6 to 1309.4nm and %EE increases from 18.65to 24.38% (w/w), when the concentration of drug (C) increases from 1 to 4 mg/ml.

MMC-CBZ NPs

The effect of concentration of chitosan (A) and concentration of sodium alginate (B) and their interaction on response Y at a fixed level of drug concentration (C) are given in response surface and contour plot shown in Figures 5.7 (a) and Figure 5.8 (a) for particles size and % EE respectively. As shown in figures, at higher level concentration of chitosan (A) 2 mg/ml and mean level concentration of drug (C) 2.5

mg/ml, particle size increases from 923.9 to 1287.8nm and %EE increases from 23.56to 24.89 % (w/w), when the concentration of sodium alginate (B) increases from 0.5 to 1.5 mg/ml.

The effect of concentration of chitosan (A) and concentration of drug(C) and their interaction on response Y at a fixed level of concentration of sodium alginate (B) are given in response surface and contour plot shown in Figures 5.7 (b) and Figure 5.8 (b) for particles size and % EE respectively. As shown in figures, at mean level concentration of sodium alginate (B) 1mg/ml and at the lower level concentration of drug (C) 1 mg/ml, particle size decreases from 799.8 to 705.9nm and %EE decreases from18.99 to 16.56 % (w/w), when the concentration of chitosan (A) increases from 1 to 2 mg/ml.

The effect of concentration of sodium alginate (B) and concentration of drug(C) and their interaction on response Y at a fixed level of concentration of chitosan (A) are given in response surface and contour plot shown in Figures 5.7 (c) and Figure 5.8 (c) for particles size and % EE respectively. As shown in figures, at mean level concentration of chitosan (A) 1.5 mg/ml and at the higher level concentration of sodium alginate (B) 1.5 mg/ml, particle size increases from 1167.5to 1388nm and %EE increases from 18.78 to 24.66%(w/w), when the concentration of drug (C) increases from 1 to 4 mg/ml.

Overall conclusions based on above results, the effect of sodium alginate concentration and drug concentration was higher than the effect chitosan concentration because chitosan and drug both are cationic in nature and sodium alginate was polyanions so its effect was more prominent toward the cationic drug. Drug entrapment and particle size both was greatly affected by sodium alginate concentration.

Chitosan NPs with different molecular weight were prepared by ionotropic gelation of chitosan with sodium alginate, which involves the mixing of two aqueous solutions at ambient temperature while stirring without using sonication or organic solvents. Various formulations were made with different initial concentrations of chitosan (1, 1.5, 2 mg/ml) and sodium alginate (0.5, 1, 1.5 mg/ml) to establish preparation conditions at which NPs are formed. Since smaller particles generally show a higher uptake by nasal epithelia than larger ones (Desai et al., 1996, Delie et al., 1998, Huang et al., 1998, Brooking et al., 2001).The criteria size, size distribution, colloidal

stability and reproducibility of NPs production were used to select the best formulation parameters to prepare NPs. The optimal chitosan NPs were formed when the chitosan solution (1 mg/ml) to sodium alginate solution (0.5 mg/ml) ratio was 2:1. At very low or high chitosan to sodium alginate ratio either a clear solution or large NPs with a low colloidal stability were obtained. When sodium alginate concentration was 1.5 mg/ml, too high chitosan concentration (2 mg/ml) made encapsulation extremely difficult, and too low chitosan concentration (0.5 mg/ml) made some aggregates with large diameter. The formation of nanoparticles is only possible within some moderate concentrations of chitosan and sodium alginate. As for gelation between sodium alginate solution of 0.5 mg/ml and chitosan solution of 1–2 mg/ml, we usually observed some opalescent suspension of nanoparticles. Increase in chitosan concentration led to decrease of encapsulation efficiency of drug. It has been previously reported that the highly viscous nature of the gelation medium hinders the encapsulation of drug in case of chitosan microspheres (Vandenberg et al., 2001). So it was proposed that relatively lower viscosity of chitosan with lower concentration promotes the encapsulation of drug and gelation between chitosan and sodium alginate (Wu et al., 2005).

With increase in the concentration of drug in optimal formulation (1, 2.5, 4 mg/ml) drug entrapment was increases but above the 2.5 mg/ml further entrapment was decreased (Wang et al., 2008). In general, the size of the nanoparticles increased when drug was loaded on the surface. Encapsulation of medium molecular weight chitosan is more than that of low molecular weight chitosan. This is possibly attributed to their longer chains of medium molecular weight chitosan, which can entrap more amount of drug when gelated with sodium alginate (Wu et al., 2005).

Moreover impact of chitosan solution pH (4 and 5) and sodium alginate pH (8, 9, 10 and 11) was seen on particle size and drug entrapment on optimal formulations. Higher entrapment and lower size of NPs was found at the 5 pH of chitosan solution and 8 pH value of sodium alginate solution. One can argue that in this method when the pH of the polymers is adjusted to 5 the NH_2^- groups of the chitosan are mostly protonated and are better accessible for interaction (Sadeghi et al., 2008). As with increase in the pH of sodium alginate 8 to 11, particle size of NPs and drug entrapment was increase up to pH 10 and decreased at above pH 10. To elucidate the influence of chitosan molecular weight on the particle size, as the molecular weight

increases the particle size was increased and this trend may be explained by the fact that a medium molecular weight chitosan can more interact and associate drug more efficiently than a lower molecular weight chitosan. The factor is out-weighted by the fact that medium molecular weight chitosan is less soluble compared to low molecular weight chitosan and as a result, an increase in particle diameter or even aggregation may be obtained (Wu et al., 2005). Chitosan free amino groups were responsible for the measured positive zeta potential values obtained for all formulations, which might ensure the electrostatic interactions with the anionic groups of the mucus.

5.5.3 Optimization of Formulation Parameters for Thiolated Chitosan (TC) Nanoparticles

The formulation parameters *viz.* concentration of thiolated chitosan, concentration of sodium alginate, concentration of sodium deoxycholate and concentration of drug were optimized for maximum %EE and minimum particle size. The relationship between the dependent and independent variables was further elucidated using contour and response surface plots.

LMTC-TZ NPs

The effect of concentration of thiolated chitosan (A) and concentration of sodium alginate (B) and their interaction on response Y at a fixed level concentration of drug (C) and concentration of sodium deoxycholate (D) are given in response surface and contour plot shown in Figures 5.9 (a) and Figure 5.10 (a) for particles size and % EE respectively.

As shown in figures, at mean level concentration of thiolated chitosan (A) 2 mg/ml, at the higher level concentration of drug (C) 3 mg/ml, and mean level concentration of sodium deoxycholate (D) 10 mg/ml, particle size decreases from 355.5 to 299.6 nm and %EE increases from 51.44 to 60.59 % (w/w), when the concentration of sodium alginate (B) increases from 0.25 to 0.75 mg/ml.

Similarly, at higher level of concentration of thiolated chitosan (A) 3 mg/ml, at the mean level of concentration of drug (C) 2 mg/ml, and mean level concentration of sodium deoxycholate (D) 10 mg/ml, particle size increases from 377.1to 408.7nm and %EE increases from 66.87to 67.43% (w/w), when the concentration of sodium alginate (B) increases from 0.25 to 0.75 mg/ml.

The effect of concentration of thiolated chitosan (A) and concentration of drug(C) and their interaction on response Y at a fixed level concentration of sodium alginate (B)

and concentration of sodium deoxycholate (D) are given in response surface and contour plot shown in Figures 5.9 (b) and Figure 5.10 (b) for particles size and % EE respectively.

As shown in figures, at lower level concentration of sodium alginate (B) 0.25mg/ml, at the mean level concentration of drug (C) 2mg/ml and at the mean level concentration of sodium deoxycholate (D) 10 mg/ml, particle size and % EE increases from 303.6 to 377.1nm and 64.32 to 66.87 % (w/w) respectively, when the concentration of thiolated chitosan (A) increases from 1 to 3 mg/ml.

The effect of concentration of thiolated chitosan (A) and concentration of sodium deoxycholate (D) and their interaction on response Y at a fixed level concentration of sodium alginate (B) and concentration of drug (C) are given in response surface and contour plot shown in Figures 5.9 (c) and Figure 5.10 (c) for particles size and % EE respectively.

As shown in figures, at mean level concentration of thiolated chitosan (A) 2mg/ml, at the mean level of concentration of drug (C) 2mg/ml and at the higher level concentration of sodium alginate (B) 0.75 mg/ml, particle size decreases from 456.7to 299.5nm and % EE increases from 43.98 to 64.90 % (w/w), when the concentration of sodium deoxycholate (D) increases from 5 to 15 mg/ml.

The effect of concentration of sodium alginate (B) and concentration of drug (C) and their interaction on response Y at a fixed level concentration of thiolated chitosan (A) and concentration of sodium deoxycholate (D) are given in response surface and contour plot shown in Figures 5.19 (d) and Figure 5.10 (d) for particles size and % EE respectively.

As shown in figures, at mean level concentration of sodium alginate (B) 0.5mg/ml, at the mean level concentration of sodium deoxycholate (D) 10 mg/ml and at the higher level concentration of thiolated chitosan (A) 3 mg/ml, particle size increase from 354.8 to 378.1nm, when the concentration of drug (C) increases from 1 to 3 mg/ml with non significant change in drug entrapment efficiency.

The effect of concentration of sodium alginate (B) and concentration of sodium deoxycholate (D) and their interaction on response Y at a fixed level concentration of thiolated chitosan (A) and concentration of drug (C) are given in response surface and contour plot shown in Figures 5.9 (e) and Figure 5.10 (e) for particles size and % EE respectively.

As shown in figures, mean level concentration of thiolated chitosan (A) 2 mg/ml, at the mean level concentration of sodium alginate (B) 0.5 mg/ml and at the higher level concentration of drug (C) 3 mg/ml, particle size decreases from 373.5 to 289.6 nm and % EE increases from 44.23 to 63.32 % (w/w), when the concentration of sodium deoxycholate (D) increases from 5 to 15 mg/ml.

The effect of concentration of drug (C) and concentration of sodium deoxycholate (D) and their interaction on response Y at a fixed level concentration of thiolated chitosan (A) and concentration of sodium alginate (B) are given in response surface and contour plot shown in Figures 5.9 (f) and Figure 5.10 (f) for particles size and % EE respectively.

As shown in figures, at lower level of thiolated chitosan (A) 1 mg/ml, at the mean level concentration of sodium alginate (B) 0.5mg/ml and at the mean level concentration of drug (C) 2 mg/ml, particle size decreases from 455.7to 276.0nm and % EE increases from 48.93 to 56.43% (w/w), when the concentration of sodium deoxycholate (D) increases from 5 to 15 mg/ml.

LMTC-CBZ NPs

The effect of concentration of thiolated chitosan (A) and concentration of sodium alginate (B) and their interaction on response Y at a fixed level concentration of drug (C) and concentration of sodium deoxycholate (D) are given in response surface and contour plot shown in Figures 5.11 (a) and Figure 5.12 (a) for particles size and % EE respectively.

As shown in figures, at higher level of concentration of thiolated chitosan (A) 3 mg/ml, at the mean level of concentration of drug (C) 2 mg/ml, and mean level concentration of sodium deoxycholate (D) 10 mg/ml, particle size increases from 345.5to 421.2nm and %EE increases from 64.04 to 69.11% (w/w), when the concentration of sodium alginate (B) increases from 0.25 to 0.75 mg/ml.

The effect of concentration of thiolated chitosan (A) and concentration of drug(C) and their interaction on response Y at a fixed level concentration of sodium alginate (B) and concentration of sodium deoxycholate (D) are given in response surface and contour plot shown in Figures 5.11 (b) and Figure 5.12 (b) for particles size and % EE respectively.

As shown in figures, at higher level concentration of sodium alginate (B) 0.75mg/ml, at the mean level concentration of drug (C) 2mg/ml and at the mean level

concentration of sodium deoxycholate (D) 10 mg/ml, particle size and % EE increases from 350.4 to 421.2nm and 63.87 to 69.11 % (w/w) respectively, when the concentration of thiolated chitosan (A) increases from 1 to 3 mg/ml.

The effect of concentration of thiolated chitosan (A) and concentration of sodium deoxycholate (D) and their interaction on response Y at a fixed level concentration of sodium alginate (B) and concentration of drug (C) are given in response surface and contour plot shown in Figures 5.11 (c) and Figure 5.12 (c) for particles size and % EE respectively.

As shown in figures, at mean level concentration of thiolated chitosan (A) 2mg/ml, at the mean level of concentration of drug (C) 2mg/ml and at the higher level concentration of sodium alginate (B) 0.75 mg/ml, particle size decreases from 466.2 to 318.6 nm and % EE increases from 45.18 to 63.87 % (w/w), when the concentration of sodium deoxycholate (D) increases from 5 to 15 mg/ml.

The effect of concentration of sodium alginate (B) and concentration of drug (C) and their interaction on response Y at a fixed level concentration of thiolated chitosan (A) and concentration of sodium deoxycholate (D) are given in response surface and contour plot shown in Figures 5.11 (d) and Figure 5.12 (d) for particles size and % EE respectively.

As shown in figures, at mean level concentration of sodium alginate (B) 0.5mg/ml, at the mean level concentration of sodium deoxycholate (D) 10 mg/ml and at the higher level concentration of thiolated chitosan (A) 3 mg/ml, particle size increases from 364.0 to 388.1nm, when the concentration of drug (C) increases from 1 to 3 mg/ml with non significant change in drug entrapment efficiency.

The effect of concentration of sodium alginate (B) and concentration of sodium deoxycholate (D) and their interaction on response Y at a fixed level concentration of thiolated chitosan (A) and concentration of drug (C) are given in response surface and contour plot shown in Figures 5.11 (e) and Figure 5.12 (e) for particles size and % EE respectively.

As shown in figures, mean level concentration of thiolated chitosan (A) 2 mg/ml, at the mean level concentration of sodium alginate (B) 0.5 mg/ml and at the higher level concentration of drug (C) 3 mg/ml, particle size decreases from 395.6 to 303.6nm and % EE increases from 54.16 to 63.70% (w/w), when the concentration of sodium deoxycholate (D) increases from 5 to 15 mg/ml.

The effect of concentration of drug (C) and concentration of sodium deoxycholate (D) and their interaction on response Y at a fixed level concentration of thiolated chitosan (A) and concentration of sodium alginate (B) are given in response surface and contour plot shown in Figures 5.11 (f) and Figure 5.12 (f) for particles size and % EE respectively.

As shown in figures, at lower level of thiolated chitosan (A) 1 mg/ml, at the mean level concentration of sodium alginate (B) 0.5mg/ml and at the mean level concentration of drug (C) 2 mg/ml, particle size decreases from 467.7 to 268.5nm and % EE increases from 49.09 to 59.21% (w/w), when the concentration of sodium deoxycholate (D) increases from 5 to 15 mg/ml.

MMTC-TZ NPs

The effect of concentration of thiolated chitosan (A) and concentration of sodium alginate (B) and their interaction on response Y at a fixed level concentration of drug (C) and concentration of sodium deoxycholate (D) are given in response surface and contour plot shown in Figures 5.13 (a) and Figure 5.14 (a) for particles size and % EE respectively.

As shown in figures, at higher level of concentration of thiolated chitosan (A) 3 mg/ml, at the mean level of concentration of drug (C) 2 mg/ml, and mean level concentration of sodium deoxycholate (D) 10 mg/ml, particle size increases from 378.2 to 415.6nm and %EE increases from 68.41 to 69.87% (w/w), when the concentration of sodium alginate (B) increases from 0.25 to 0.75 mg/ml.

The effect of concentration of thiolated chitosan (A) and concentration of drug(C) and their interaction on response Y at a fixed level concentration of sodium alginate (B) and concentration of sodium deoxycholate (D) are given in response surface and contour plot shown in Figures 5.13 (b) and Figure 5.14 (b) for particles size and % EE respectively.

As shown in figures, at higher level concentration of sodium alginate (B) 0.75mg/ml, at the mean level concentration of drug (C) 2mg/ml and at the mean level concentration of sodium deoxycholate (D) 10 mg/ml, particle size and % EE increases from 395.1 to 415.6nm and 63.59 to 69.87 % (w/w) respectively, when the concentration of thiolated chitosan (A) increases from 1 to 3 mg/ml.

The effect of concentration of thiolated chitosan (A) and concentration of sodium deoxycholate (D) and their interaction on response Y at a fixed level concentration of

sodium alginate (B) and concentration of drug (C) are given in response surface and contour plot shown in Figures 5.13 (c) and Figure 5.14 (c) for particles size and % EE respectively.

As shown in figures, at mean level concentration of thiolated chitosan (A) 2mg/ml, at the mean level of concentration of drug (C) 2mg/ml and at the higher level concentration of sodium alginate (B) 0.75 mg/ml, particle size decreases from 464.7to 333.9 nm and % EE increases from 45.77 to 64.76 % (w/w), when the concentration of sodium deoxycholate (D) increases from 5 to 15 mg/ml.

The effect of concentration of sodium alginate (B) and concentration of drug (C) and their interaction on response Y at a fixed level concentration of thiolated chitosan (A) and concentration of sodium deoxycholate (D) are given in response surface and contour plot shown in Figures 5.13 (d) and Figure 5.14 (d) for particles size and % EE respectively.

As shown in figures, at mean level concentration of sodium alginate (B) 0.5mg/ml, at the mean level concentration of sodium deoxycholate (D) 10 mg/ml and at the higher level concentration of thiolated chitosan (A) 3 mg/ml, particle size increase from 361.0 to 381.8nm, when the concentration of drug (C) increases from 1 to 3 mg/ml with non significant change in drug entrapment efficiency.

The effect of concentration of sodium alginate (B) and concentration of sodium deoxycholate (D) and their interaction on response Y at a fixed level concentration of thiolated chitosan (A) and concentration of drug (C) are given in response surface and contour plot shown in Figures 5.13 (e) and Figure 5.14 (e) for particles size and % EE respectively.

As shown in figures, mean level concentration of thiolated chitosan (A) 2 mg/ml, at the mean level concentration of sodium alginate (B) 0.5 mg/ml and at the higher level concentration of drug (C) 3 mg/ml, particle size decreases from 387.1to 293.4nm and % EE increases from 45.65 to 64.91% (w/w), when the concentration of sodium deoxycholate (D) increases from 5 to 15 mg/ml.

The effect of concentration of drug (C) and concentration of sodium deoxycholate (D) and their interaction on response Y at a fixed level concentration of thiolated chitosan (A) and concentration of sodium alginate (B) are given in response surface and contour plot shown in Figures 5.13 (f) and Figure 5.14 (f) for particles size and % EE respectively.

As shown in figures, at lower level of thiolated chitosan (A) 1 mg/ml, at the mean level concentration of sodium alginate (B) 0.5mg/ml and at the mean level concentration of drug (C) 2 mg/ml, particle size decreases from 434.9 to 281.5nm and % EE increases from 49.45 to 63.59% (w/w), when the concentration of sodium deoxycholate (D) increases from 5 to 15 mg/ml.

MMTC-CBZ NPs

The effect of concentration of thiolated chitosan (A) and concentration of sodium alginate (B) and their interaction on response Y at a fixed level concentration of drug (C) and concentration of sodium deoxycholate (D) are given in response surface and contour plot shown in Figures 5.15 (a) and Figure 5.16 (a) for particles size and % EE respectively.

As shown in figures, at higher level of concentration of thiolated chitosan (A) 3 mg/ml, at the mean level of concentration of drug (C) 2 mg/ml, and mean level concentration of sodium deoxy cholate (D) 10 mg/ml, particle size increases from 365.1to 425.8nm and %EE increases from 70.44 to 71.12% (w/w), when the concentration of sodium alginate (B) increases from 0.25 to 0.75 mg/ml.

The effect of concentration of thiolated chitosan (A) and concentration of drug(C) and their interaction on response Y at a fixed level concentration of sodium alginate (B) and concentration of sodium deoxycholate (D) are given in response surface and contour plot shown in Figures 5.15 (b) and Figure 5.16 (b) for particles size and % EE respectively.

As shown in figures, at higher level concentration of sodium alginate (B) 0.75mg/ml, at the mean level concentration of drug (C) 2mg/ml and at the mean level concentration of sodium deoxycholate (D) 10 mg/ml, particle size and % EE increases from 370.9to 425.8nm and 66.12 to 71.12 % (w/w) respectively, when the concentration of thiolated chitosan (A) increases from 1 to 3 mg/ml.

The effect of concentration of thiolated chitosan (A) and concentration of sodium deoxycholate (D) and their interaction on response Y at a fixed level concentration of sodium alginate (B) and concentration of drug (C) are given in response surface and contour plot shown in Figures 5.15 (c) and Figure 5.16 (c) for particles size and % EE respectively.

As shown in figures, at mean level concentration of thiolated chitosan (A) 2mg/ml, at the mean level of concentration of drug (C) 2mg/ml and at the higher level

concentration of sodium alginate (B) 0.75 mg/ml, particle size decreases from 473.5 to 313.4nm and % EE increases from 47.11 to 64.34 % (w/w), when the concentration of sodium deoxycholate (D) increases from 5 to 15 mg/ml.

The effect of concentration of sodium alginate (B) and concentration of drug (C) and their interaction on response Y at a fixed level concentration of thiolated chitosan (A) and concentration of sodium deoxycholate (D) are given in response surface and contour plot shown in Figures 5.15 (d) and Figure 5.16 (d) for particles size and % EE respectively.

As shown in figures, at mean level concentration of sodium alginate (B) 0.5mg/ml, at the mean level concentration of sodium deoxycholate (D) 10 mg/ml and at the higher level concentration of thiolated chitosan (A) 3 mg/ml, particle size increase from 374.5to 397.4nm, when the concentration of drug (C) increases from 1 to 3 mg/ml with non significant change in drug entrapment efficiency.

The effect of concentration of sodium alginate (B) and concentration of sodium deoxycholate (D) and their interaction on response Y at a fixed level concentration of thiolated chitosan (A) and concentration of drug (C) are given in response surface and contour plot shown in Figures 5.15 (e) and Figure 5.16 (e) for particles size and % EE respectively.

As shown in figures, mean level concentration of thiolated chitosan (A) 2 mg/ml, at the mean level concentration of sodium alginate (B) 0.5 mg/ml and at the higher level concentration of drug (C) 3 mg/ml, particle size decreases from 395.9 to 309.2nm and % EE increases from 55.43 to 69.33% (w/w), when the concentration of sodium deoxycholate (D) increases from 5 to 15 mg/ml.

The effect of concentration of drug (C) and concentration of sodium deoxycholate (D) and their interaction on response Y at a fixed level concentration of thiolated chitosan (A) and concentration of sodium alginate (B) are given in response surface and contour plot shown in Figures 5.15 (f) and Figure 5.16 (f) for particles size and % EE respectively.

As shown in figures, at lower level of thiolated chitosan (A) 1 mg/ml, at the mean level concentration of sodium alginate (B) 0.5mg/ml and at the mean level concentration of drug (C) 2 mg/ml, particle size decreases from 477.9 to 276.4 nm and % EE increases from 50.44 to 65.58 % (w/w), when the concentration of sodium deoxycholate (D) increases from 5 to 15 mg/ml.

Over all conclusion based on the above results is that the concentration of sodium deoxycholate and sodium alginate was affect more than the other factors on particle size and %EE. . This result may be due to the strong interactions between anions and TC because TC has a higher positive charge than chitosan (Worawan et al., 2008). Nanoparticles from thiolated chitosan with difference moleculer weight were prepared by ionic gelation of thiolated chitosan using sodium alginate with slight modification in above method. Various formulations were made with different initial concentrations of thiolated chitosan (1, 2 and 3 mg/ml) and sodium alginate solutions (0.25, 0.5 and 0.75 mg/ml) to establish preparation conditions at which NPs are formed. The effect of low or medium molecular weight thiolated chitosan solution to sodium alginate solution ratio was found to be similar as of chitosan NPs (Dong-Won et al., 2006). The impact of pH change on drug entrapment was negligible as thiolation increases positive charge and improves solubility of chitosan at physiological pH. Moreover, tizanidine HCl and cyclobenzaprine HCl were highly cationic hydrophilic drugs that also affect the drug entrapment with highly positively charged polymer. The optimal thiolated chitosan NPs were formed when the thiolated chitosan (2 mg/ml) was dissolved in distilled water. Drug solution (2mg/ml) was incubated with anionic sodium deoxycholate (10 mg/ml) for 30 seconds. The resulting complex was added to thiolated chitosan solution. The addition of sodium alginate solution (0.5 mg/ml) with stirring to the above mixture with ratio of thiolated chitosan: sodium alginate: drug: sodium deoxycholate solution **7:1:1:1** led to the immediate formation of NPs. Further with increases in the concentration of drug (1, 2 and 3 mg/ml) in optimal formulation, drug entrapment was increases up to 2 mg/ml and decreases above the 2 mg/ml concentration. Moreover, with increase in the concentration of anionic surfactant up to 10 mg/ml, the drug entrapment was increased because of the formation of neutral drug-sodium deoxycholate complexes, which adsorbed more on the positively charged thiolated chitosan than the highly cationic drug (Carmen et al., 2009). With increase in the concentration of sodium deoxycholate solution, the size was also decrease and surface area was increased. Hence, more drug-sodium deoxycholate complexes adsorbed on the surface of thiolated chitosan leads to high drug entrapment. The mean hydrodynamic diameter of these types of nanoparticles showed a clear increased with the molecular weight of

thiolated chitosan, which is in agreement with previous works (Chauvierre et al., 2003, Bertholon-Rajot et al., 2005).

5.5.4. Optimization of Formulation Parameters for Trimethyl chitosan (TMC) Nanoparticles

The formulation parameters *viz.* concentration of trimethyl chitosan, concentration of sodium alginate, concentration of sodium deoxy cholate and concentration of Drug were optimized for maximum %EE and minimum particle size. The relationship between the dependent and independent variables was further elucidated using contour and response surface plots.

LMTMC-TZ NPs

The effect of concentration of trimethyl chitosan (A) and concentration of sodium alginate (B) and their interaction on response Y at a fixed level concentration of drug (C) and concentration of sodium deoxycholate (D) are given in response surface and contour plot shown in Figures 5.17 (a) and Figure 5.18 (a) for particles size and % EE respectively.

As shown in figures, at lower level concentration of trimethyl chitosan (A) 1 mg/ml, at the mean level concentration of drug (C) 2 mg/ml, and mean level concentration of sodium deoxycholate (D) 10 mg/ml, particle size decreases from 312.6to 168.0 nm and %EE increases from 60.57to 66.90 % (w/w), when the concentration of sodium alginate (B) increases from 0.5 to 2 mg/ml.

The effect of concentration of trimethyl chitosan (A) and concentration of drug(C) and their interaction on response Y at a fixed level concentration of sodium alginate (B) and concentration of sodium deoxycholate (D) are given in response surface and contour plot shown in Figures 5.17 (b) and Figure 5.18 (b) for particles size and % EE respectively.

As shown in figures, at mean level concentration of sodium alginate (B) 1.25mg/ml, at the lower level concentration of drug (C) 1mg/ml and at the mean level concentration of sodium deoxycholate (D) 10 mg/ml, particle size and % EE increases from 323.8to 445.8nm and 46.98 to 52.78 % (w/w) respectively, when the concentration of trimethyl chitosan (A) increases from 1 to 2 mg/ml.

The effect of concentration of trimethyl chitosan (A) and concentration of sodium deoxycholate (D) and their interaction on response Y at a fixed level concentration of sodium alginate (B) and concentration of drug (C) are given in response surface and

contour plot shown in Figures 5.17 (c) and Figure 5.18 (c) for particles size and % EE respectively.

As shown in figures, at lower level concentration of trimethyl chitosan (A) 1mg/ml, at the mean level of concentration of drug (C) 2mg/ml and at the mean level concentration of sodium alginate (B) 1.25 mg/ml, particle size decreases from 444.8 to 255.3 nm and % EE increases from 34.78 to 61.34 % (w/w), when the concentration of sodium deoxycholate (D) increases from 5 to 15 mg/ml.

The effect of concentration of sodium alginate (B) and concentration of drug (C) and their interaction on response Y at a fixed level concentration of trimethyl chitosan (A) and concentration of sodium deoxycholate (D) are given in response surface and contour plot shown in Figures 5.17 (d) and Figure 5.18 (d) for particles size and % EE respectively.

As shown in figures, at lower level concentration of sodium alginate (B) 0.5mg/ml, at the mean level concentration of sodium deoxycholate (D) 10 mg/ml and at the mean level concentration of trimethyl chitosan (A) 1.5 mg/ml, particle size decreases from 503.7to 334.8 nm and % EE decreases from 53.23 to 44.78% (w/w), when the concentration of drug (C) increases from 1 to 3 mg/ml.

The effect of concentration of sodium alginate (B) and concentration of sodium deoxycholate (D) and their interaction on response Y at a fixed level concentration of trimethyl chitosan (A) and concentration of drug (C) are given in response surface and contour plot shown in Figures 5.17 (e) and Figure 5.18 (e) for particles size and % EE respectively.

As shown in figures, higher level concentration of trimethyl chitosan (A) 2 mg/ml, at the mean level concentration of sodium alginate (B) 1.25 mg/ml and at the mean level concentration of drug (C) 2 mg/ml, particle size decreases from 488.0 to 412.8nm and % EE increases from 34.67to 48.56 % (w/w), when the concentration of sodium deoxycholate (D) increases from 5 to 15 mg/ml.

The effect of concentration of drug (C) and concentration of sodium deoxycholate (D) and their interaction on response Y at a fixed level concentration of trimethyl chitosan (A) and concentration of sodium alginate (B) are given in response surface and contour plot shown in Figures 5.17 (f) and Figure 5.18 (f) for particles size and % EE respectively.

As shown in figures, at mean level of trimethyl chitosan (A) 1.5 mg/ml, at the lower level concentration of sodium alginate (B) 0.5mg/ml and at the mean level concentration of drug (C) 2 mg/ml, particle size decreases from 402.7 to 360.6nm and % EE increases from 42.11 to 49.21% (w/w), when the concentration of sodium deoxycholate (D) increases from 5 to 15 mg/ml.

LMTMC-CBZ NPs

The effect of concentration of trimethyl chitosan (A) and concentration of sodium alginate (B) and their interaction on response Y at a fixed level concentration of drug (C) and concentration of sodium deoxycholate (D) are given in response surface and contour plot shown in Figures 5.19 (a) and Figure 5.20 (a) for particles size and % EE respectively.

As shown in figures, at lower level concentration of trimethyl chitosan (A) 1 mg/ml, at the mean level concentration of drug (C) 2 mg/ml, and mean level concentration of sodium deoxycholate (D) 10 mg/ml, particle size decreases from 333.7 to 184.6 nm and %EE increases from 62.65 to 71.75 % (w/w), when the concentration of sodium alginate (B) increases from 0.5 to 2 mg/ml.

The effect of concentration of trimethyl chitosan (A) and concentration of drug(C) and their interaction on response Y at a fixed level concentration of sodium alginate (B) and concentration of sodium deoxycholate (D) are given in response surface and contour plot shown in Figures 5.19 (b) and Figure 5.20 (b) for particles size and % EE respectively.

As shown in figures, at mean level concentration of sodium alginate (B) 1.25mg/ml, at the lower level concentration of drug (C) 1mg/ml and at the mean level concentration of sodium deoxycholate (D) 10 mg/ml, particle size and % EE increases from 314.8 to 443.5nm and 49.32 to 57.89 % (w/w) respectively, when the concentration of trimethyl chitosan (A) increases from 1 to 2 mg/ml.

The effect of concentration of trimethyl chitosan (A) and concentration of sodium deoxycholate (D) and their interaction on response Y at a fixed level concentration of sodium alginate (B) and concentration of drug (C) are given in response surface and contour plot shown in Figures 5.19 (c) and Figure 5.20 (c) for particles size and % EE respectively.

As shown in figures, at lower level concentration of trimethyl chitosan (A) 1mg/ml, at the mean level of concentration of drug (C) 2mg/ml and at the mean level

concentration of sodium alginate (B) 1.25 mg/ml, particle size decreases from 479.2 to 277.4 nm and % EE increases from 40.32 to 63.12 % (w/w), when the concentration of sodium deoxycholate (D) increases from 5 to 15 mg/ml.

The effect of concentration of sodium alginate (B) and concentration of drug (C) and their interaction on response Y at a fixed level concentration of trimethyl chitosan (A) and concentration of sodium deoxycholate (D) are given in response surface and contour plot shown in Figures 5.19 (d) and Figure 5.20 (d) for particles size and % EE respectively.

As shown in figures, at lower level concentration of sodium alginate (B) 0.5mg/ml, at the mean level concentration of sodium deoxycholate (D) 10 mg/ml and at the mean level concentration of trimethyl chitosan (A) 1.5 mg/ml, particle size decreases from 499.5to 343.7 nm and % EE decreases from 56.21 to 49.54 % (w/w), when the concentration of drug (C) increases from 1 to 3 mg/ml.

The effect of concentration of sodium alginate (B) and concentration of sodium deoxycholate (D) and their interaction on response Y at a fixed level concentration of trimethyl chitosan (A) and concentration of drug (C) are given in response surface and contour plot shown in Figures 5.19 (e) and Figure 5.20 (e) for particles size and % EE respectively.

As shown in figures, higher level concentration of trimethyl chitosan (A) 2 mg/ml, at the mean level concentration of sodium alginate (B) 1.25 mg/ml and at the mean level concentration of drug (C) 2 mg/ml, particle size decreases from 477.6 to 419.6 nm and % EE increases from 45.77 to 51.78 % (w/w), when the concentration of sodium deoxycholate (D) increases from 5 to 15 mg/ml.

The effect of concentration of drug (C) and concentration of sodium deoxycholate (D) and their interaction on response Y at a fixed level concentration of trimethyl chitosan (A) and concentration of sodium alginate (B) are given in response surface and contour plot shown in Figures 5.19 (f) and Figure 5.20 (f) for particles size and % EE respectively.

As shown in figures, at mean level of trimethyl chitosan (A) 1.5 mg/ml, at the lower level concentration of sodium alginate (B) 0.5mg/ml and at the mean level concentration of drug (C) 2 mg/ml, particle size decreases from 412.5 to 392.9 nm and % EE increases from 44.32 to 53.22% (w/w), when the concentration of sodium deoxycholate (D) increases from 5 to 15 mg/ml.

MMTMC-TZ NPs

The effect of concentration of trimethyl chitosan (A) and concentration of sodium alginate (B) and their interaction on response Y at a fixed level concentration of drug (C) and concentration of sodium deoxycholate (D) are given in response surface and contour plot shown in Figures 5.21 (a) and Figure 5.22 (a) for particles size and % EE respectively.

As shown in figures, at higher level concentration of trimethyl chitosan (A) 2 mg/ml, at the mean level concentration of drug (C) 2 mg/ml, and mean level concentration of sodium deoxycholate (D) 10 mg/ml, particle size decreases from 450.5 to 440.3 nm and %EE increases from 54.66 to 66.78 % (w/w), when the concentration of sodium alginate (B) increases from 0.5 to 2 mg/ml.

The effect of concentration of trimethyl chitosan (A) and concentration of drug(C) and their interaction on response Y at a fixed level concentration of sodium alginate (B) and concentration of sodium deoxy cholate (D) are given in response surface and contour plot shown in Figures 5.21 (b) and Figure 5.22 (b) for particles size and % EE respectively.

As shown in figures, at mean level concentration of sodium alginate (B) 1.25mg/ml, at the lower level concentration of drug (C) 1mg/ml and at the mean level concentration of sodium deoxycholate (D) 10 mg/ml, particle size and % EE increases from 280.4 to 477.8 nm and 51.98 to 59.32 % (w/w) respectively, when the concentration of trimethyl chitosan (A) increases from 1 to 2 mg/ml.

The effect of concentration of trimethyl chitosan (A) and concentration of sodium deoxycholate (D) and their interaction on response Y at a fixed level concentration of sodium alginate (B) and concentration of drug (C) are given in response surface and contour plot shown in Figures 5.21 (c) and Figure 5.22 (c) for particles size and % EE respectively.

As shown in figures, at lower level concentration of trimethyl chitosan (A) 1mg/ml, at the mean level of concentration of drug (C) 2mg/ml and at the mean level concentration of sodium alginate (B) 1.25 mg/ml, particle size decreases from 350.8 to 265.4 nm and % EE increases from 43.87 to 65.43 % (w/w), when the concentration of sodium deoxycholate (D) increases from 5 to 15 mg/ml.

The effect of concentration of sodium alginate (B) and concentration of drug (C) and their interaction on response Y at a fixed level concentration of trimethyl chitosan (A)

and concentration of sodium deoxy cholate (D) are given in response surface and contour plot shown in Figures 5.21 (d) and Figure 5.22 (d) for particles size and % EE respectively.

As shown in figures, at lower level concentration of sodium alginate (B) 0.5mg/ml, at the mean level concentration of sodium deoxycholate (D) 10 mg/ml and at the mean level concentration of trimethyl chitosan (A) 1.5 mg/ml, particle size decreases from 410.8 to 380.5 nm and % EE decreases from 59.54 to 53.89 % (w/w), when the concentration of drug (C) increases from 1 to 3 mg/ml.

The effect of concentration of sodium alginate (B) and concentration of sodium deoxycholate (D) and their interaction on response Y at a fixed level concentration of trimethyl chitosan (A) and concentration of drug (C) are given in response surface and contour plot shown in Figures 5.21 (e) and Figure 5.22 (e) for particles size and % EE respectively.

As shown in figures, higher level concentration of trimethyl chitosan (A) 2 mg/ml, at the mean level concentration of sodium alginate (B) 1.25 mg/ml and at the mean level concentration of drug (C) 2 mg/ml, particle size decreases from 493.7 to 468.9nm and % EE increases from 49.43to 53.21 % (w/w), when the concentration of sodium deoxycholate (D) increases from 5 to 15 mg/ml.

The effect of concentration of drug (C) and concentration of sodium deoxycholate (D) and their interaction on response Y at a fixed level concentration of trimethyl chitosan (A) and concentration of sodium alginate (B) are given in response surface and contour plot shown in Figures 5.21 (f) and Figure 5.22 (f) for particles size and % EE respectively.

As shown in figures, at mean level of trimethyl chitosan (A) 1.5 mg/ml, at the lower level concentration of sodium alginate (B) 0.5mg/ml and at the mean level concentration of drug (C) 2 mg/ml, particle size decreases from 440.7 to 387.6 nm and % EE increases from 46.32to 57.33% (w/w), when the concentration of sodium deoxycholate (D) increases from 5 to 15 mg/ml.

MMTMC-CBZ NPs

The effect of concentration of trimethyl chitosan (A) and concentration of sodium alginate (B) and their interaction on response Y at a fixed level concentration of drug (C) and concentration of sodium deoxycholate (D) are given in response surface and

contour plot shown in Figures 5.23 (a) and Figure 5.24 (a) for particles size and % EE respectively.

As shown in figures, at lower level concentration of trimethyl chitosan (A) 1 mg/ml, at the mean level concentration of drug (C) 2 mg/ml, and mean level concentration of sodium deoxycholate (D) 10 mg/ml, particle size decreases from 308.5 to 274.5 nm and %EE increases from 64.65 to 72.88 % (w/w), when the concentration of sodium alginate (B) increases from 0.5 to 2 mg/ml.

The effect of concentration of trimethyl chitosan (A) and concentration of drug(C) and their interaction on response Y at a fixed level concentration of sodium alginate (B) and concentration of sodium deoxycholate (D) are given in response surface and contour plot shown in Figures 5.23 (b) and Figure 5.24 (b) for particles size and % EE respectively.

As shown in figures, at mean level concentration of sodium alginate (B) 1.25mg/ml, at the lower level concentration of drug (C) 1mg/ml and at the mean level concentration of sodium deoxycholate (D) 10 mg/ml, particle size and % EE increases from 288.4 to 489.4 nm and 58.90 to 60.56 % (w/w) respectively, when the concentration of trimethyl chitosan (A) increases from 1 to 2 mg/ml.

The effect of concentration of trimethyl chitosan (A) and concentration of sodium deoxycholate (D) and their interaction on response Y at a fixed level concentration of sodium alginate (B) and concentration of drug (C) are given in response surface and contour plot shown in Figures 5.23 (c) and Figure 5.24 (c) for particles size and % EE respectively.

As shown in figures, at lower level concentration of trimethyl chitosan (A) 1mg/ml, at the mean level of concentration of drug (C) 2mg/ml and at the mean level concentration of sodium alginate (B) 1.25 mg/ml, particle size decreases from 360.7 to 284.3 nm and % EE increases from 50.65to 66.88 % (w/w), when the concentration of sodium deoxycholate (D) increases from 5 to 15 mg/ml.

The effect of concentration of sodium alginate (B) and concentration of drug (C) and their interaction on response Y at a fixed level concentration of trimethyl chitosan (A) and concentration of sodium deoxy cholate (D) are given in response surface and contour plot shown in Figures 5.23 (d) and Figure 5.24 (d) for particles size and % EE respectively.

As shown in figures, at lower level concentration of sodium alginate (B) 0.5mg/ml, at the mean level concentration of sodium deoxycholate (D) 10 mg/ml and at the mean level concentration of trimethyl chitosan (A) 1.5 mg/ml, particle size decreases from 412.3 to 402.1 nm and % EE decreases from 63.97 to 49.56 % (w/w), when the concentration of drug (C) increases from 1 to 3 mg/ml.

The effect of concentration of sodium alginate (B) and concentration of sodium deoxycholate (D) and their interaction on response Y at a fixed level concentration of trimethyl chitosan (A) and concentration of drug (C) are given in response surface and contour plot shown in Figures 5.23 (e) and Figure 5.24 (e) for particles size and % EE respectively.

As shown in figures, higher level concentration of trimethyl chitosan (A) 2 mg/ml, at the mean level concentration of sodium alginate (B) 1.25 mg/ml and at the mean level concentration of drug (C) 2 mg/ml, particle size decreases from 499.3 to 488.1 nm and % EE increases from 48.99 to 55.32 % (w/w), when the concentration of sodium deoxycholate (D) increases from 5 to 15 mg/ml.

The effect of concentration of drug (C) and concentration of sodium deoxycholate (D) and their interaction on response Y at a fixed level concentration of trimethyl chitosan (A) and concentration of sodium alginate (B) are given in response surface and contour plot shown in Figures 5.23 (f) and Figure 5.24 (f) for particles size and % EE respectively.

As shown in figures, at mean level of trimethyl chitosan (A) 1.5 mg/ml, at the lower level concentration of sodium alginate (B) 0.5mg/ml and at the mean level concentration of drug (C) 2 mg/ml, particle size decreases from 444.1 to 390.2 nm and % EE increases from 52.56 to 53.23% (w/w), when the concentration of sodium deoxycholate (D) increases from 5 to 15 mg/ml.

Over all conclusions based on the above results the concentration of sodium deoxycholate and sodium alginate was affect more than the other factors on particle size and %EE. . This result may be due to the strong interactions between anions and TMC because TMC has a higher positive charge than chitosan (Worawan et al., 2008). Trimethyl chitosan NPs with difference moleculer weight were prepared by ionic gelation of TMC using sodium alginate with slight modification same as thiolated chitosan. Various formulations were made with different initial concentrations of chitosan (1, 1.5 and 2 mg/ml) and sodium alginate solutions (0.5,

1.5 and 2 mg/ml) to establish preparation conditions at which NPs are formed. The effect of low or medium molecular weight TMC solution to sodium alginate solution ratio was found to be similar as of chitosan NPs (Dong-Won et al., 2006). The impact of pH change on drug entrapment was negligible as methylation increases positive charge and improves solubility of chitosan at physiological pH. Moreover, tizanidine HCl and cyclobenzaprine HCl were the highly cationic hydrophilic drugs that also affect the drug entrapment with highly positively charged polymer. The optimal TMC NPs were formed when the TMC (1 mg/ml) was dissolved in distilled water. Drug solution (2mg/ml) was incubated with anionic sodium deoxycholate (10 mg/ml) for 30 seconds. The resulting complex was added to TMC solution. The addition of sodium alginate solution (2 mg/ml) with stirring to the above mixture with ratio of TMC: sodium alginate: drug: sodium deoxycholate solution 7.9:0.7:0.7:0.7 led to the immediate formation of NPs. Further with increase in the concentration of drug solution (1, 2 and 3 mg/ml) in optimal formulation, drug entrapment was increased up to 2 mg/ml drug concentration but above the 2 mg/ml further entrapment was decreased. Moreover, with increase in the concentration of anionic surfactant up to 10 mg/ml, the drug entrapment was increased because of the formation of drug-sodium deoxycholate complexes, which adsorbed more on the positively charged TMC than the highly cationic drug (Carmen et al., 2009). With increase in the concentration of sodium deoxycholate solution, the size was decreased and surface area was increased. Hence, more drug-sodium deoxycholate complexes adsorbed on the surface of TMC leads to high drug entrapment. The mean hydrodynamic diameter of these types of nanoparticles showed a clear increased with the molecular weight of TMC, which is in agreement with previous works (Chauvierre et al., 2003, Bertholon-Rajot et al., 2005).

5.6. Lyophilization and optimization of cryoprotectant concentration

The results for lyophilization of nanoparticles are shown in Table 5.30 and Table 5.35. In aqueous suspensions, the chemical and physical stability of nanoparticles has been reported to be poor (Saez et al., 2000 & Konan et al., 2002). Freeze-drying has been the most utilized drying method of nanoparticles suspensions. Because the freeze-drying process is highly stressful for nanoparticles, addition of cryoprotectants becomes essential. For nanoparticles carbohydrates have been perceived to be suitable freeze-drying protectants. There are considerable differences in the cryoprotective

abilities of different carbohydrates. The optimized batch of nanoparticles was lyophilized using sucrose, mannitol and trehalose (at 1:1, 1:2 and 1:3 NPs: cryoprotectant) to select suitable cryoprotectant and its concentration. The redispersibility of the freeze-dried formulations and particle size of the NPs before and after freeze-drying were evaluated and recorded in Table 5.30 to Table 5.35.

When sucrose was used as a cryoprotectant, at all concentrations studied, the redispersion of freeze-dried NPs was difficult due to the formation of flakes or aggregates and also there was substantial increase in particle size after lyophilization. The Sf/Si values were higher than the 1. The increase in the particle size could have been due to the cohesive nature of the sucrose. Further, it was observed that the lyophilized nanoparticles with sucrose had tendency to absorb moisture very quickly.

With mannitol, the nanoparticle formulation showed free flowing ability, however the redispersion was difficult and possible only after vigorous shaking. The Sf/Si values were slightly greater than the 1. With trehalose as cryoprotectant, the lyophilized nanoparticles were redispersed easily and the increase in particle size was not significant as indicated by S_f/S_i values which were almost nearer to 1. The redispersion of the nanoparticles depends on the hydrophilicity of the surface. The easy redispersibility is probably due to the higher solubility of trehalose in water i.e. 0.7 parts in 1 part of water. The cryoprotective effect may be attributed to the ability of trehalose to form a glassy amorphous matrix around the particles, preventing the particles from sticking together during removal of water (Konan et al., 2002). Furthermore, trehalose, a non-reducing disaccharide of glucose, has previously exhibited satisfactory cryoprotective effects for pharmaceutical and biological materials.

5.7. Conclusions

Tizanidine HCl and Cyclobenzaprine loaded chitosan/thiolated chitosan/trimethyl chitosan NPs were successfully prepared by ionotropin gelation method using sodium alginate as a ionic crosslinking agent and sodium deoxycholate as a anionic surfactant for entrapment improvement in thiolated chitosan and trimethyl chitosan NPs preparation. The NPs of chitosan, thiolated chitosan and trimethyl chitosan were found to be in nanometer range with the positive zeta potential that suitable for intranasal administration. The prepared nanoparticles were lyophilized using trehalose successfully with good redespesibility and not significant change in particle size.

Rhodamine B loaded chitosan, thiolated chitosan and trimethyl loaded chitosan NPs were also successfully prepared using ionotropic gelation method with not significant change in results as a drug loaded NPs.

5.8. References

Bertholon-Rajot, I., Labarre, D., Vauthier, C. (2005). Influence of the initiator system, cerium–polysaccharide, on the surface properties of poly(isobutylcyanoacrylate) nanoparticles. Polymer, 46(4), 1407–1415.

Brooking, J., Davis, S.S., Illum, L. (2001). Transport of nanoparticles across the ratnasal mucosa. J. Drug Target., 9 (4), 267– 279.

Carmen, P., Hayat, O. (2009). Biodegradable Nanoparticles Incorporating Highly Hydrophilic Positively Charged Drugs, United States Patent Application 20090117195.

Chauvierre, C., Labarre, D., Couvreur, P., Vauthier, C. (2003). Radical polymerization of alkylcyanoacrylates initiated by the redox system dextran-cerium (IV) under acidic aqueous conditions. Macromolecules, 36(16), 6018–6027.

Delie, F. (1998). Evaluation of nano- and microparticle uptake by the gastrointestinal tract. Adv. Drug Del. Rev., 34 (2,3), 221– 233.

Desai, M. P., Labhasetwar, V., Amidon, G., Levy, R. (1996). Gastrointestinal uptake of biodegradable microparticles: effect of particle size. Pharm. Res., 13 (12), 1838–1845.

Dong-Won, L., Shawna, A. S., Richard, F. L., Shyam, S. M. (2006). Thiolated chitosan nanoparticles enhance anti-inflammatory effects of intranasally delivered theophylline. Respiratory Research, 7 (1), 112.

Huang, Y., Donovan, M.D. (1998). Large molecule and particulate uptake in the nasal cavity: the effect of size on nasal absorption. Adv. Drug Del. Rev., 29 (1-3), 147–155.

Illum, L., Farraj, N.F., Davis, S.S. (1994). Chitosan as a novel nasal delivery system for peptide drugs. Pharm. Res., 11 (8), 1186-1189.

Illum, L., Jabbal-Gill, I., Hinchcliffe, M., Fisher, A.N., Davis, S.S. (2001). Chitosan as a novel nasal delivery system for vaccines. Adv. Drug Delivery Rev., 51 (1-3), 81-96.

Konan, Y.N., Gurny, R., Allémann. E. (2002). Preparation and characterization of sterile and freeze-dried sub-200nm nanoparticles. Int. J. Pharm., 233(1-2), 239–252.

Sadeghi, A.M.M., Dorkoosh, F.A., Avadi, M.R., Saadat, P., Rafiee-Tehrani, M., Junginger, H.E. (2008). Preparation, characterization and antibacterial activities of

chitosan, N-trimethyl chitosan (TMC) and N-diethylmethyl chitosan (DEMC) nanoparticles loaded with insulin using both the ionotropic gelation and polyelectrolyte complexation methods. International Journal of Pharmaceutics, 355, (1-2), 299–306.

Saez, A., Guzmán, M., Molpeceres, J., Aberturas, M.R. (2000). Freeze-drying of polycaprolactone and poly(D,L-lactic-glycolic) nanoparticles induce minor particle size changes affecting the oral pharmacokinetics of loaded drugs. Eur J Pharm Biopharm., 50(3), 379-387.

Tiyaboonchai, W. (2003). Chitosan Nanoparticles: A Promising System for Drug Delivery, Naresuan University Journal, 11(3), 51-66.

Vandenberg, G.W., Drolet, C., Scott, S.L., Noue, J.D.(2001). Factors affecting protein release from alginate-chitosan coacervate microcapsules during production and gastric/intestinal simulation. J. Control. Release, 77(3), 297–307.

Wang, X., Chi, N., Tang, T. (2008). Preparation of estradiol chitosan nanoparticles for improving nasal absorption and brain targeting. European Journal of Pharmaceutics and Biopharmaceutics, 70 (3), 735–740.

Worawan, B., Hans E. J., Neti, W., Assadang, P., Tasana, P. (2008). Preparation and Characterization of Particles from Chitosan with Different Molecular Weights and Their Trimethyl Chitosan Derivatives for Nasal Immunization. Journal of Metals, Materials and Minerals, 18 (2), 59-65.

Wu, Y., Yang, W., Wang, C., Hu, J., Fu, S. (2005). Chitosan nanoparticles as a novel delivery system for ammonium glycyrrhizinate. International Journal of pharmaceutics, 295 (1-2), 235–245.

CHAPTER 5 135
5.1. Introduction 136
5.2. Methods 137
5.2.1. Preparation of Tizanidine HCl (TZ) and Cyclobenzaprine HCl (CBZ) loaded chitosan, thiolated chitosan nanoparticles (TC) and trimethyl chitosan (TMC) nanoparticles 137
5.2.2 Optimization of Formulation Parameters for chitosan Nanoparticles 138
5.2.3. Optimization of Formulation Parameters for Thiolated Chitosan (TC) Nanoparticles 155
5.2.4 Optimization of Formulation Parameters for trimethyl chitosan Nanoparticles 181
5.3. Lyophilization and optimization of cryoprotectant concentration 207
5.4. Preparation of Rhodamine B loaded chitosan, thiolated chitosan and trimethyl chitosan nanoparticles 213
5.5. Results and discussion 215
5.5.1. Preparation of Tizanidine HCl (TZ) and Cyclobenzaprine HCl (CBZ) loaded chitosan (C), thiolated chitosan (TC) and trimethyl chitosan (TMC) nanoparticles 215
5.5.2 Optimization of Formulation Parameters for chitosan Nanoparticles 215
5.5.3 Optimization of Formulation Parameters for Thiolated Chitosan (TC) Nanoparticles 220
5.5.4. Optimization of Formulation Parameters for Trimethyl chitosan (TMC) Nanoparticles 229
5.6. Lyophilization and optimization of cryoprotectant concentration 237
5.7. Conclusions 238
5.8. References 239