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Formulation and in-vivo evaluation of nanosponges based tramadol HCL C/R tablets using design of experiment

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***Abstract*---**The current study was aimed to develop a controlled release formulation of tramadol utilizing the cyclodextrin based nanosponges. Box-Behnken design was employed to study the effect of each independent variable on dependent variables. Five types of nanosponges from β-cyclodextrin (NS1-NS5) were purposely designed. The prepared nanosponges by freeze drying method were characterized and formulated into tablets and evaluated for in-vitro and in-vivo studies. The particle sizes of tramadol-nanosponges are in between 34.38 to 134.26 nm, encapsulation efficiency of 41.13-86.72% and drug release% at 6h of 52.34-81.12%. More than 90 % of drug was found to be released from nanosponge formulations. The FTIR, DSC and XRPD studies confirmed the interaction of Tramadol with nanosponges. The nanosponges C_{max} of 915.24±1.67 ng/ml was significantly higher ($p<0.05$) than the marketed product's C_{max} of 411.5±1.25 ng/ml. Both the nanosponges formulation and the commercial product had T_{max} values of 1.5±0.06 and 2.0±0.05 hours, respectively. When compared to the marketed product (342.6±0.65 ng h/ml), the AUC_{0-t} of the nanosponges formulation (643.2±0.56 ng h/ml) was substantially greater ($p<0.05$) indicating better systemic drug absorption from nanosponges formulation. Cyclodextrin based nanosponges showed enhanced solubility with increased bioavailability of poorly soluble Tramadol for controlled drug delivery.

***Keywords*---**Tramadol, Opioid analgesic, Nanosponges, Experimental design, bioavailability.

Introduction

Tramadol is a centrally acting synthetic opioid analgesic and SNRI (serotonin/norepinephrine reuptake-inhibitor) that is structurally related to codeine and morphine. It is readily soluble in water and ethanol and has a pKa of 9.41. The n-octanol/water, log partition coefficient (logP) is 1.35 at pH 7. Tramadol is available in several commercial products in immediate-release and extended-release formulations (Mongin., 2007). Several different technologies are used to achieve the prolonged release of the drug (Zehr., 2010). Most of these formulations are available in doses of 100, 200, 300 and 400 mg. In general, controlled release delivery attempts to; sustain drug action at a predetermined rate by maintaining a relatively constant, effective drug level in the body with a minimization of undesirable side effects (Joshi et al., 2011).

The use of cyclodextrin based nanosponges represents another emerging technological approach to increase drug solubility and stability. Cyclodextrin-based nanosponges showed superior complexing ability than natural cyclodextrins towards many molecules (Bhowmik et al., 2018). Over the years, nanosponges have been extensively explored for solubilization, chemical stabilization, enhancement of permeability, ocular delivery, potentiating of cytotoxicity, modulation of drug release, reduction of toxicity, protein delivery and others (Subramanian et al., 2012). Nanosponges have proven capable of keeping up with the advances in nanomedicine, responding positively to the need for targeted treatments, aimed at improving the efficacy and reducing the adverse effects of the drugs. Cyclodextrin based nanosponges have been extensively investigated for the effective and targeted delivery of several anticancer drugs such as camptothecin, resveratrol, paclitaxel, tamoxifen, curcumin, dexamethasone etc., to enhance bioavailability and therapeutic effects of these drugs (Pawar et al., 2019).

In the present study, we intended to develop controlled release formulation of tramadol using cyclodextrin nanosponges as novel nanocarriers (Kaur et al., 2019). Cyclodextrin based nanosponges were prepared in our laboratory using β-Cyclodextrin and diphenyl carbonate as cross-linking agent.

Materials and Methods

Materials

Tramadol was obtained as a gift sample from MSN laboratories Pvt. Ltd, β-Cyclodextrin was obtained from Gangwal Chemicals Pvt. Ltd. (Mumbai, India)., Diphenyl carbonate purchased from Euclid Pharmaceuticals Limited, Mumbai, Dimethyl sulfoxide and Ethanol was
purchased from Qualigens, Thermo Fisher Scientific India Ltd, Mumbai.

Preparation of β-Cyclodextrin Nanosponges (NS)

Cyclodextrin based nanosponges were prepared in our laboratory using diphenyl carbonate for the crosslinking as reported elsewhere (Swaminathan et al., 2007). Five types (NS1-NS5) of nanosponges were prepared using different molar ratios of

reactants. The molar ratios and concentrations of both the reactants were used as shown in table 1.

Characterization of β-cyclodextrin nanosponges

Characterization of the prepared β-cyclodextrin nanosponges for Particle size, polydispersity index and zeta potential were analysed using a Mastersizer 2000 (Malvern Instruments Ltd, Worcestershire, UK). (Bindiya et al., 2010)

Fabrication of tramadol -loaded β-Cyclodextrin Nanosponges

Tramadol loaded nanosponges were prepared by lyophilisation technique as reported elsewhere (Singireddy et al., 2019; Nagarjun rangaraj et al., 2019).

Design of experiments

Based on Box-Behnken design model provided by Stat-Ease Design Expert® software V8.0.1, 17 model experiments were randomly arranged. (Table 2 and 3) (Anandam et al.,2014)

Data analysis

The obtained results were subject to statistical analysis. (Shivakumar et al.,2007).

Optimization

The nano formulation was prepared in triplicate under optimal conditions to verify the validity optimization technique.

Physico-chemical characterization of tramadol NS

Particle size, polydispersity index and zeta potential were determined as per the procedure adopted for β-Cyclodextrin nanosponges. The formulations analysed for FTIR, DSC, PXRD as per the procedure adopted in reference (Nait bachir et al.,2017).

Characterisation of prepared tramadol nanosponges

The “percent drug pay load” and “percent drug encapsulation efficiency” were calculated using the following equation 1 and 2:

$$\%\ \text{Drug pay load} = \frac{\text{Weight of drug encapsulated in NS formulation}}{\text{Weight of the NS formulation taken for analysis}} \times 100 \qquad (1)$$

$$\%\ \text{Drug encapsulation efficiency} = \frac{\text{Weight of drug encapsulated in NSformulation}}{\text{Initial weight of the drug fed for loading}} \times 100 \qquad (2)$$

Preparation of Tramadol loaded nanosponges tablets

An accurately weighed quantities of tramadol loaded nanosponges corresponding to 100 mg tramadol and the calculated Avicel pH-102, which was added to attain 300 mg tablet, were mixed for 10 min using mortar and pestle after which the magnesium stearate (6 mg) was added and blended for another 2 min. The final mixtures were compressed using a single punch tablet machine with 8 mm, round, flat-faced single punch.

Evaluation of tablet formulation
Uniformity of weight, Hardness test, Friability test, Drug content, In-vitro disintegration test (Moin et al.,2020)

In-vitro release study of Tramadol

In vitro release of drug from tramadol loaded tablets and marketed tramadol tablet (Tramazac 100 mg) was performed using the type II USP dissolution apparatus (Gupta et al.,2019). The dissolution medium was 900 ml 0.1 N HCl for first 2 h then replaced with phosphate buffer pH 6.8 at a speed of 50 rpm and a temperature of 37±0.5 °C. The samples were withdrawn at 0, 0.25, 0.5, 0.75, 1, 1.5, 2, 3, 4, 6, 8, 12, and 24 h. Equal amount of the fresh dissolution medium, retained at the same temperature, was immediately replaced. The samples were suitably diluted and analysed using UC- spectrophotometer at 271.32 nm. The dissolution experiments were conducted in triplicate.

Short Term Stability Studies
Stability studies of the optimized formulation was carried out according to ICH guidelines and were examined for appearance, hardness, disintegration time, dissolution, and drug content.

In vivo pharmacokinetic analysis
Animals

Eighteen healthy male Wistar rats were (Weighing 180-220 g) selected for this study, all the animals were healthy during the period of the experiment. All efforts were made to maintain the animals under controlled environmental conditions (Temperature 25^0C, Relative Humidity 45% and 12 h alternate light and dark cycle) with 100 % fresh air exchange in animal rooms, uninterrupted power and water supply. Rats were fed with standard diet and *water ad libitum.* The protocol of animal study was approved by the institutional animal ethics committee (**IAEC NO: 1447/PO/Re/S/11/CPCSEA-45/A).**

Study Design

Rats were divided in to three groups at random (Group A, Group B and Group C). Each group containing six rats. The treatments as given below were administered to the rats. The rats were fasted for 24 hours prior to the experiments. After 4 hours of dosing, foods were reoffered. First group A, was administered with marketed Tramadol marketed product crushed and made suspension with 0.5% methocel and second group B, was administered Prepared Tramadol optimised nanosponges diluted in 0.5% methocel by oral route at a dose of 1.5625 mg and group C was considered as control. 500 µL blood samples were collected at regular time intervals from the femoral artery at times 0, 0.50, 1, 1.50, 2, 2.50, 3, 4, 6, 8, 12, 16, 20, 24h post dose and transferred into Eppendrof tubes containing heparin in order to prevent blood clotting. Plasma was separated by centrifugation of the blood at 4000 rpm in cooling centrifuge for 5min to 10 minutes and stored frozen at −20°C until analysis (Omar et al.,2020; Gan et al.,2002).

Results and Discussion

Five types of nanosponges were prepared using different molar ratios of reactants (Singireddy et al.,2016). The percent practical yield, Particle size, polydispersity index and zeta potential were measured and are as presented in table 4.

Mean particle size

Particle size of the nano formulation ranges from 34.38 – 134.26 nm. The mathematical model of particle size was found to be significant with model F-value 906.13. The model terms A, B, C, AB, A^2, B^2 and C^2 were found to be significant with p value less than 0.0500. (Figure 1). (Figure 2a and 2b).

Encapsulation efficiency

The encapsulation efficiency of nanosponges was found to be in the range of 41.13 % to 86.72 %. The mathematical model of encapsulation efficiency was found to significant with model F-value 1416.61. The model terms A, B, C, AC, BC, A^2 and C^2 were found to be significant with p value less than 0.0500. (Figure 3) (Figure 4a and 4b). (Figure 5a and 5b).

Percent drug release at 6h

Percent drug release from the nano formulation ranges from 52.34-81.12 %.
The mathematical model of percent drug release at 6h (Y3) was found to be significant with model F-value 896.93. The model term A was found to be significant with p value less than 0.0500. (Figure 6).

Optimization
Derringer's desirability function (D) was used to optimize the selected variables which influences the response parameters. Table 5.

Morphology and sizes of the tramadol loaded nanosponges

The particle size analysis of tramadol loaded nanosponges was around 40- 50 nm with low polydispersity index (table 6). The percent drug loading and encapsulation efficiency of prepared tramadol nanosponges were determined and are presented in table 6. FTIR spectra of free drug tramadol had characteristic peaks at 3421.83, 3308.03, 2929.97, 2860.53, 2602.06, 2513.33, 2482.47, 1606.76, 1579.75, 1481.38, 1288.49, 1244.13, 1161.19, 981.8, 970.23, 866.07, 774.34, 621.1 and 462.93 cm^{-1}. Plain nanosponge showed a characteristic peak of carbonate bond at around 1740–1750 cm^{-1} which confirms the formation of cyclodextrin- based nanosponges. Other characteristics peaks of nanosponges were found at 2918 cm^{-1} due to the C– H stretching vibration, 1418 cm^{-1} due to C–H bending vibration and 1026 cm^{-1} due to C–O stretching vibration of primary alcohol. The FTIR spectra of physical mixtures indicated all the peaks of drug along with some additional peaks of polymers. The Comparison of FTIR spectra of tramadol and tramadol complex showed that there is a major change in the fingerprint region i.e., 900 to 1,400 cm^{-1} as shown in figure 8. The main characteristic peaks of tramadol were disappeared in the formulations suggesting

definite interactions between tramadol and nanosponges (Palanati Mamatha et al.,2021).

The DSC thermogram of free drug shows a sharp melting point at approximately 181.75 °C indicating the crystalline nature of the drug. The DSC thermogram of plain nanosponges (NS2) showed exothermic peaks at around 350 °C. Tramadol nanosponge complex also exhibited a broad exothermic peak at around at 350 °C. The complete disappearance of tramadol endothermic peak was observed for the formulation. This can be considered as indicative of drug amorphization and/or inclusion complex formation (figure 9).

The x-ray diffractograms of plain tramadol exhibited sharp intense peaks at 2θ values of 10.40, 13.00, 15.35, 16.71, 18.49, 20.89, 24.43 and 30.80 confirming the drug's crystal form as shown in figure 10. The absence of such crystalline peaks of tramadol in nanosponge complex clearly indicates that the drug is encapsulated in nanosponges.

Preparation of Tramadol loaded nanosponges tablets

The prepared tablets were evaluated for different quality control parameters.
The mean weight ranged from 299.34 mg ± 2.32 to 301.78 mg ± 1.32.
The mean thickness ranges from 4.89 mm ± 0.76 to 5.23 mm ± 0.64.
The mean hardness ranges from 5.28 kg/cm^2 ± 0.42 to 5.48 kg/cm^2 ± 0.52.
The mean friability values range from 0.53 % ± 0.18 to 0.82 % ± 0.29 and the average percentage drug content ranges from 98.76 % ± 1.22 to 99.54 ± 1.42. The prepared tablets completely disintegrated within 5 min.

In vitro release study

Maximum amount of the drug was released within 2 h from the marketed tablet of tramadol as shown in figure 11. A biphasic release pattern of tramadol from the prepared nanosponges tablets was observed. The initial burst release was ranged from 14.68 % of drug within 1 h, followed by sustained release of the drug for 12 h. The percent of tramadol released from nanosponges tablets after 12 h was 86.58 %.

Short Term Stability Studies
Stability study's results indicated that the there was no significant change in the visual appearance, hardness, disintegration time, dissolution and drug content as shown in table 7.

In-Vivo Studies
Pharmacokinetic parameters comparison for tramadol marketed product and tramadol optimised nanosponges formulation

Figure 12 shows the plasma concentration–time curve in Wister rats after a single oral dose of Tramadol nanosponges formulation as compared to Tramadol marketed product.
C_{max} of the nanosponges 915.24±1.67 ng/ml was significant ($p<0.05$) as compared to the marketed product 411.5±1.25 ng/ml. T_{max} of both nanosponges formulation

and marketed product was 1.5±0.06 and 2.0±0.05h, respectively. $AUC_{0-\infty}$ infinity for nanosponges formulation was higher (972.8±2.48 ng. h/ml) than the marketed product 484.3±1.58 µg h/ml. Statistically, AUC_{0-t} of the nanosponges formulation (643.2±0.56 ng h/ml) was significantly higher ($p<0.05$) as compared to marketed product (342.6±0.65 ng h/ml). (Table 8)

Conclusion

The freeze-drying process was used in this investigation to prepare tramadol-loaded nanosponges. Because of the reduced drug particle size, the creation of a high-energy amorphous state, and intermolecular hydrogen bonding, the dissolution of the tramadol nanosponges was much higher than that of the pure drug. The drug loaded in the nanosponge structure can be retained and released slowly over time. FTIR, DSC and XRD studies confirmed the formation of inclusion complex of tramadol with nanosponges. In-vivo studies revealed enhanced in bioavailability of tramadol from nanosponges when compared to marketed product. Ultimately, this study found that cyclodextrin nanosponges can be a viable technique for regulated drug administration and improving the physicochemical properties, solubility, dissolution, and bioavailability of the opioid analgesic tramadol.

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Tables and Figures

Table 1
Molar ratios and concentrations of β- cyclodextrin and diphenyl carbonate

S.NO	Type of NS	Molar ratio (β-CD: DPC)	Concentration of β-cyclodextrin (gm)	Concentration of diphenyl carbonate (gm)
1	NS1	1:2	4.548	1.712
2	NS2	1:4	4.548	3.424
3	NS3	1:6	4.548	5.136
4	NS4	1:8	4.548	6.848
5	NS5	1:10	4.548	8.560

Table 2
BBD with list of dependent and independent variables with their respective levels and goals

	Independent variables		Levels		
	Variable	Units	Low	Intermediate	High
A	Molar ratio of polymer to cross linker		0.2	0.5	0.8
B	Stirring speed	Rpm	2000	3500	5000
C	Stirring time	Min	360	450	540
	Dependent variables			Goal	
Y1	Mean particle size	Nm		Minimize	
Y2	Encapsulation efficiecny	%		Maximize	
Y3	Percent drug release at 6h	%		Minimize	

Table 3
Observed responses of trial experiments as per BBD

Expt	Molar ratio of polymer to cross linker	Stirring speed (rpm)	Stirring time (min)	Mean particle size (nm)	Encapsulation efficiecny (%)	Percent drug release at 6h (%)
1	0.5	2000	540	104.56	83.74	69.16
2	0.8	3500	540	49.34	86.72	54.34
3	0.8	2000	450	94.46	82.67	53.12
4	0.5	2000	360	128.74	70.56	68.76
5	0.5	3500	450	58.34	76.78	68.12
6	0.5	3500	450	59.16	77.22	67.89
7	0.5	5000	540	34.38	81.22	68.96
8	0.2	5000	450	49.12	46.68	80.86
9	0.5	5000	360	58.34	80.32	69.22
10	0.8	3500	360	78.12	86.56	54.45
11	0.2	3500	540	73.12	55.88	79.12

12	0.5	3500	450	61.62	75.34	69.76
13	0.8	5000	450	41.46	85.12	52.34
14	0.5	3500	450	60.78	76.18	68.92
15	0.2	2000	450	134.26	43.12	80.34
16	0.2	3500	360	101.78	41.13	81.12
17	0.5	3500	450	61.26	75.82	69.28

Table 4
The percent practical yield, Particle size, polydispersity index and zeta potential of different nanosponges

S.NO	Type of NS	Molar ratio (β-CD: DPC)	Practical yield (%)	Mean particle size (nm)	Polydispersity index	Zeta potential
1	NS1	1:2	76.34±2.76	112.56±9.52	0.256±0.005	-23.56±2.12
2	NS2	1:4	81.72±1.98	108.34±6.88	0.312±0.005	-26.56±1.13
3	NS3	1:6	84.58±3.12	116.58±10.42	0.268±0.005	-27.58±3.24
4	NS4	1:8	89.16±2.44	121.42±8.26	0.422±0.005	-24.72±1.74
5	NS5	1:10	91.66±1.89	98.48±5.48	0.272±0.005	-23.98±1.46

(All determinations were performed in triplicate and values were expressed as mean±S.D., n=3)

Table 5
Optimum conditions attained by applying restrictions on response parameters

Independent variables	Optimized values	Predicted values			Actual values			
		Mean particle size (Y_1) Nm	Encapsulation efficiency (Y_2) %	Percent drug release at 6h (Y_3)	Batch	Mean particle size (Y_1) nm	Encapsulation efficiency (Y_2) %	Percent drug release at 6h (Y_3)
Molar ratio of polymer to cross linker	0.73	38.03	87.04	57.84	F1	41.6±10.52	86.82±1.34	56.98±2.12
Stirring speed	4377				F2	47.23±4.56	85.92±1.22	57.24±3.12
Stirring time	540 min				F3	49.02±3.56	86.68±2.12	57.86±1.92

Table 6
Particle Size, polydispersity index and zeta potential of plain nanosponges and drug loaded nanosponge formulation

Sample	Mean particle size ± SD (nm)	Polydispersity Index	Zeta potential (mV)	Drug pay load	Encapsulation efficiency
Plain NS	113.14±5.6	0.32±0.005	-21.76±1.2	-	-
F1	41.6±10.52	0.46±0.005	-20.6±2.1	47.89	86.82±1.34
F2	47.23±4.56	0.11±0.005	-22.3±1.6	48.34	85.92±1.22
F3	49.02±3.56	0.31±0.005	-23.7±1.1	47.12	86.68±2.12

(All determinations were performed in triplicate and values were expressed as mean ± S.D., n=3)

Table 7
Evaluation parameters of tramadol tablets

Formulation	Weight (mg)	Thickness (mm)	Hardness (kg/cm^2)	Friability (%)	Drug content (%)
T1	299.34	4.89	5.28	0.53	98.76
T2	301.78	5.15	5.48	0.66	99.54
T3	300.54	5.23	5.36	0.82	99.17

Table 8
Results of stability studies of the tramadol tablets (T2)

Condition	Days	Appearance	Hardness	Disintegration time (min)	Percent dissolution at 6 h	Drug content
25 ± 2 °C, 60% ± 5 % RH	0	White	5.23±0.36	2.15±0.22 min	58.12±1.78	99.17±0.18
	90	White	5.38±0.42	2.56±0.31 min	59.33±2.28	98.96±0.39
	180	White	5.42±0.24	2.36±0.52 min	57.76±2.52	98.72±0.44
30 ± 2 °C, 65% ± 5	0	White	5.23±0.36	2.15±0.22 min	58.12±1.78	99.17±0.18
	90	White	5.42±0.12	2.32±0.31 min	57.78±2.12	98.52±0.22
	80	White	5.48±0.48	2.10±0.26 min	59.14±1.44	99.04±0.34
40 ± 2 °C, 75% ± 5	0	White	5.23±0.36	2.15±0.22 min	58.12±1.78	99.17±0.18
	90	White	5.33±0.18	2.42±0.16 min	57.34±2.04	99.12±0.28
	180	White	5.46±0.28	2.35±0.12 min	57.62±0.98	98.92±0.20

Table 9
Mean pharmacokinetic parameters of tramadol marketed product and tramadol optimized nanosponges formulation

Pharmacokinetic parameters	Tramadol Marketed product	Tramadol optimised nanosponges
C_{max} (ng/ml)	411.5±1.25	915.24±1.67
AUC_{0-t} (ng. h/ml)	342.6±0.65	643.2±0.56
AUC_{0-inf} (ng. h/ml)	484.3±1.58	972.8±2.48
T_{max} (h)	2.0±0.05	1.5±0.06
$t_{1/2}$ (h)	11.2±0.83	9.82±0.53

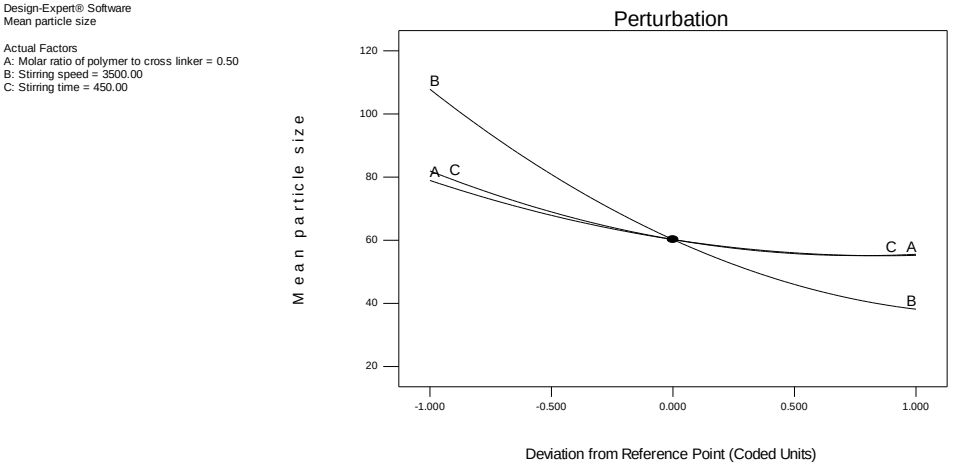
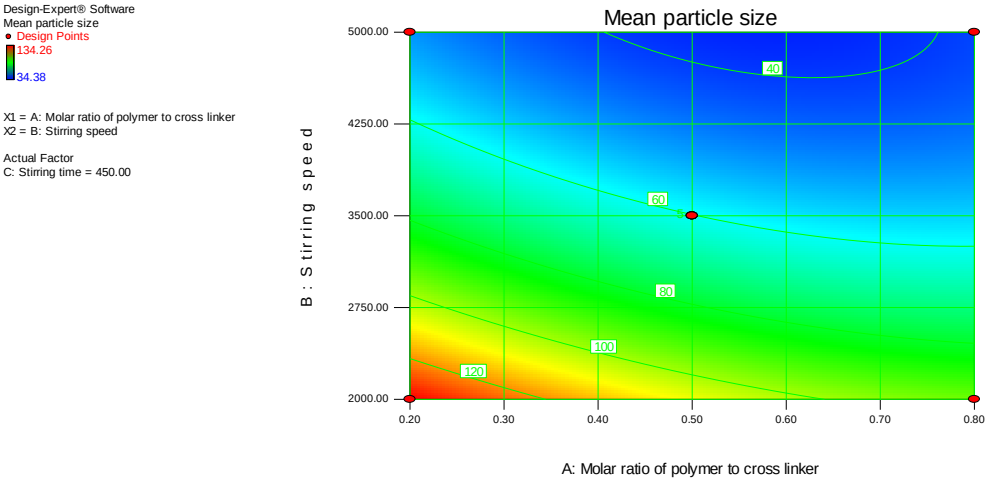


Figure 1. Two-dimensional Perturbation plot- Effect of A, B and C on mean particle size



(a)

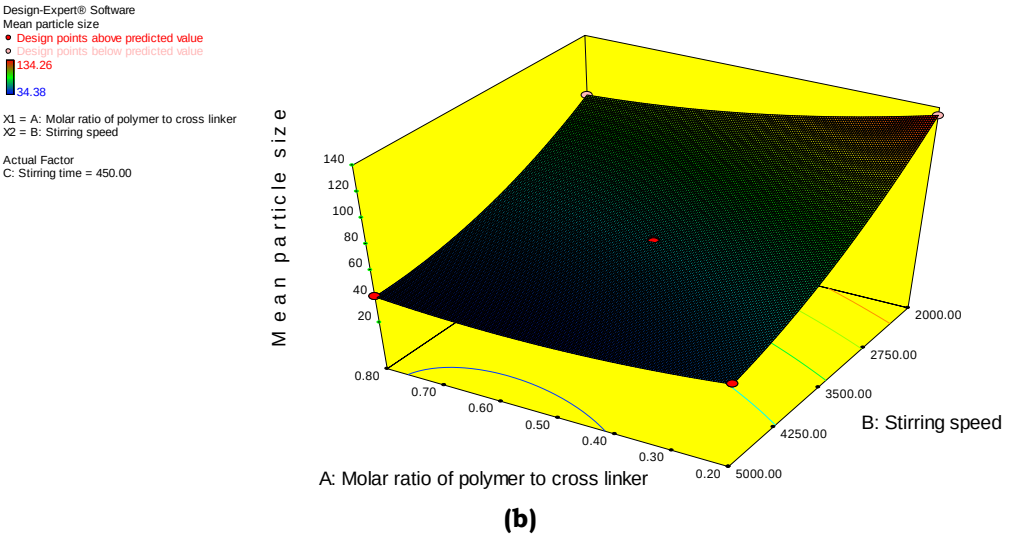


Figure 2. (a). 3D- Contour plot showing the interactive effect of A and B (b). 3D-response surface plot showing the interactive effect of A and B on mean particle size at constant level of C respectively

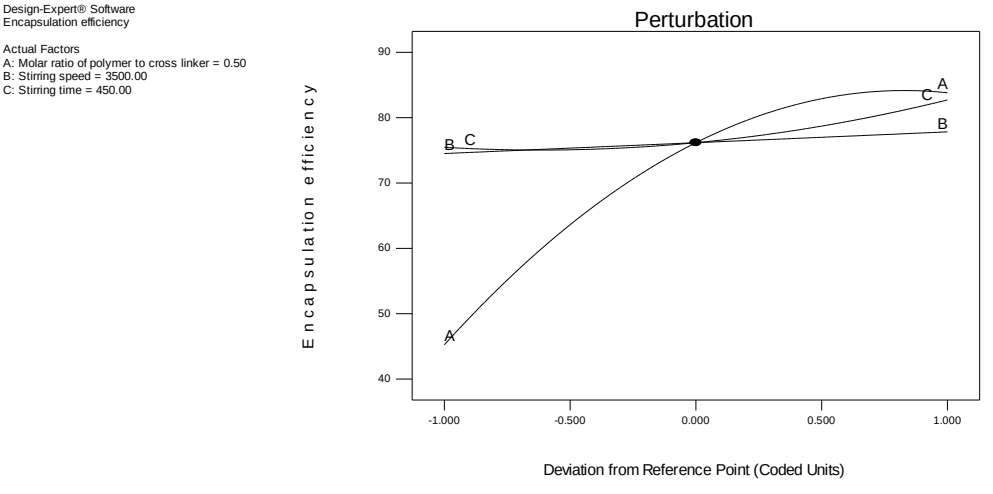
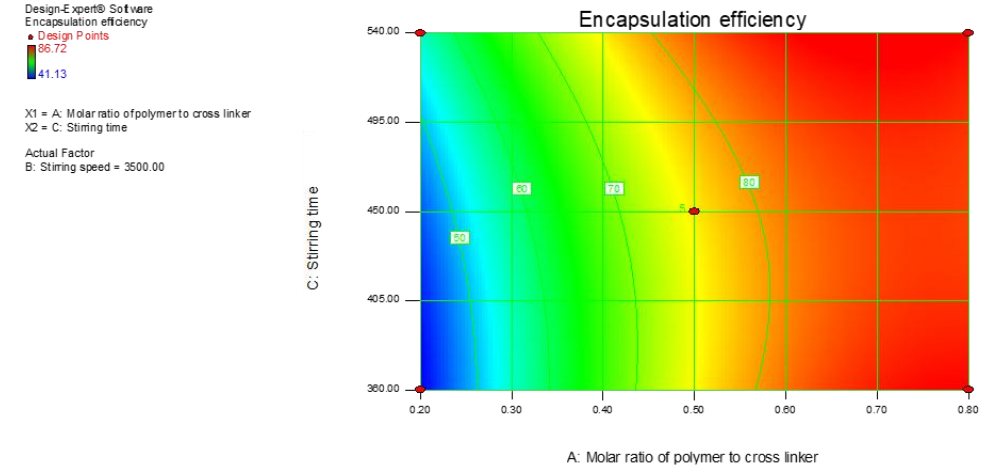
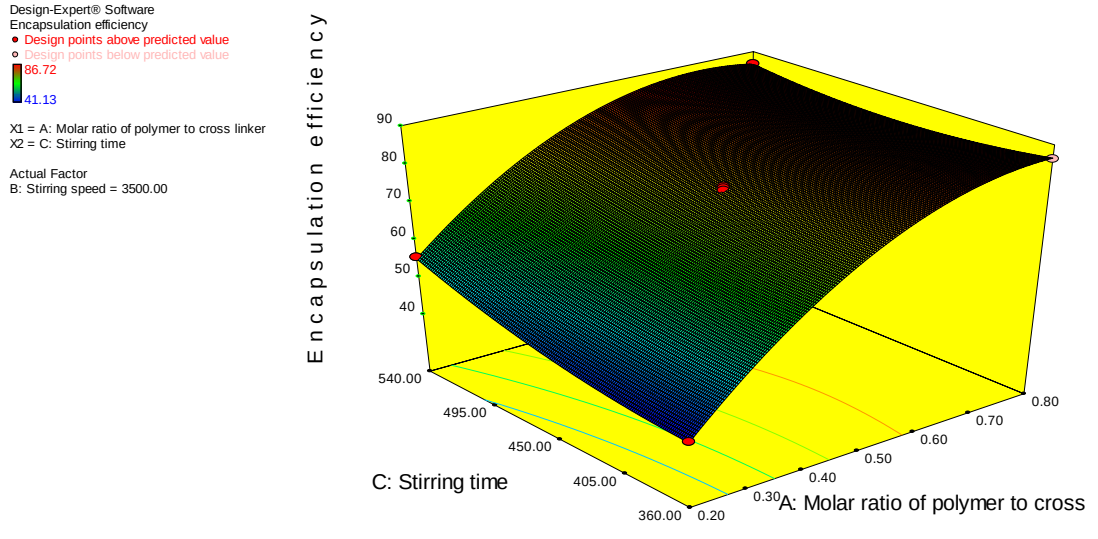


Figure 3. Two-dimensional Perturbation plot- Effect of A, B and C on encapsulation efficiency



(a)



(b)

Figure 4. (a). 3D- Contour plot showing the interactive effect of A and C (b). 3D-response surface plot showing the interactive effect of A and C on encapsulation efficiency at constant level of B respectively.

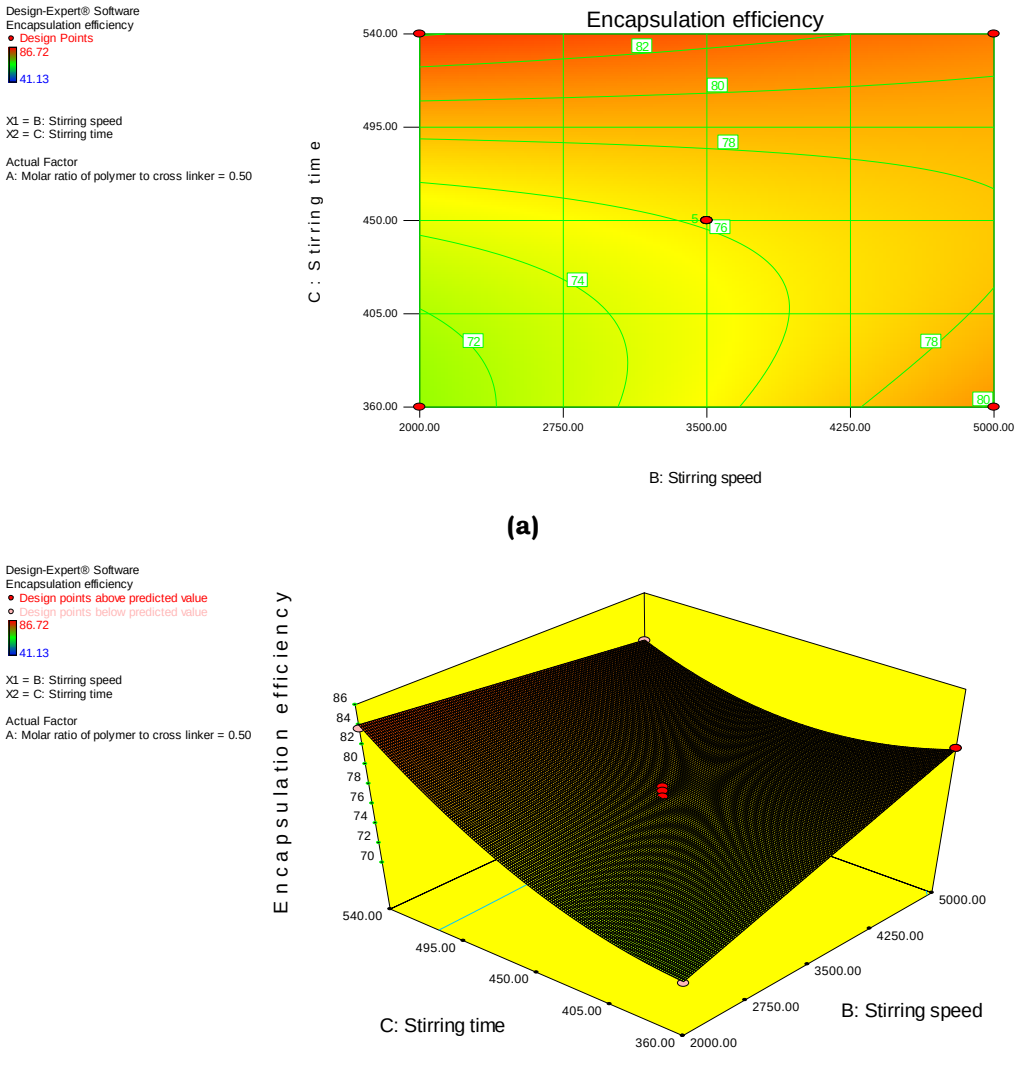


Figure 5. (a). 3D- Contour plot showing the interactive effect of B and C (b). 3D-response surface plot showing the interactive effect of B and C on encapsulation efficiency at constant level of A respectively.

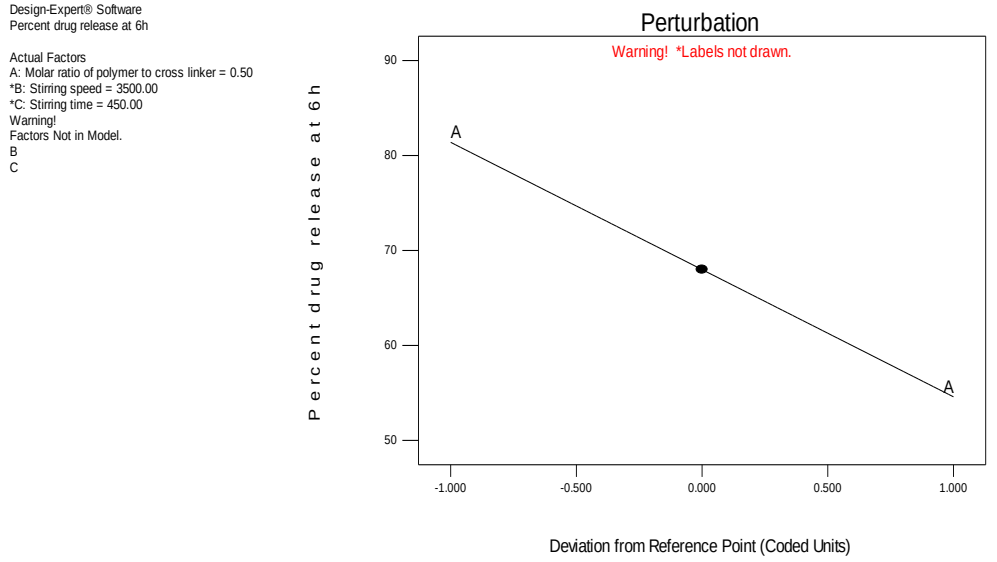


Figure 6. Two-dimensional Perturbation plot- Effect of A on percent drug release at 6h

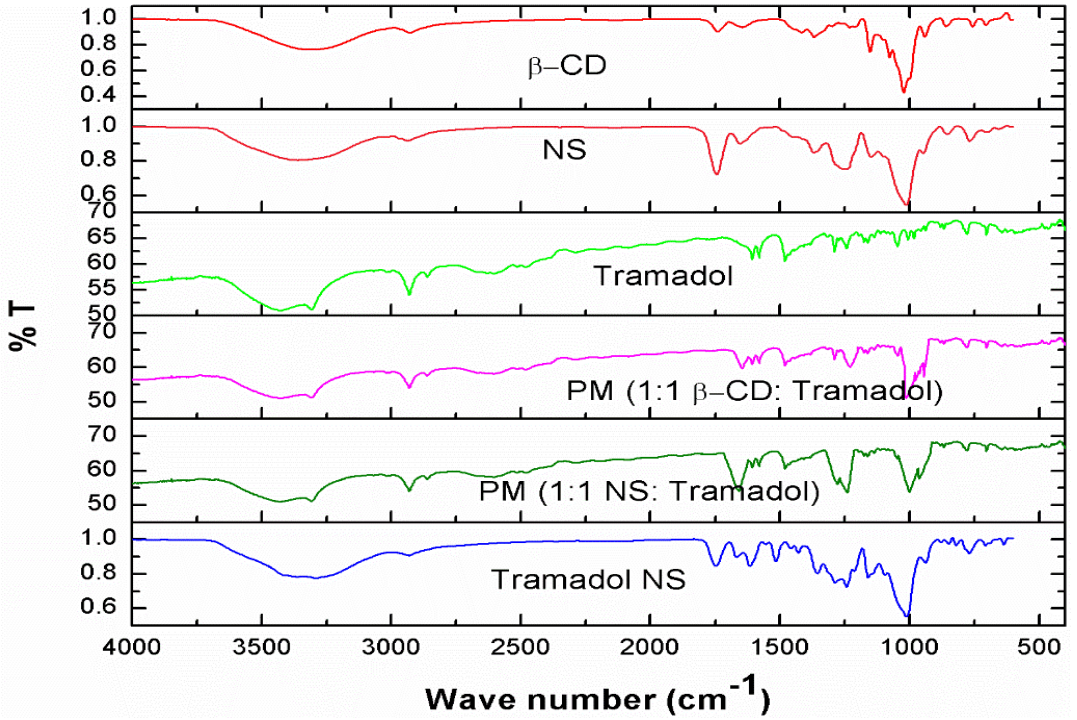


Figure 8. FTIR spectra of β-Cyclodextrin, plain nanosponges, Tramadol, Physical mixture and Tramadol loaded nanosponges

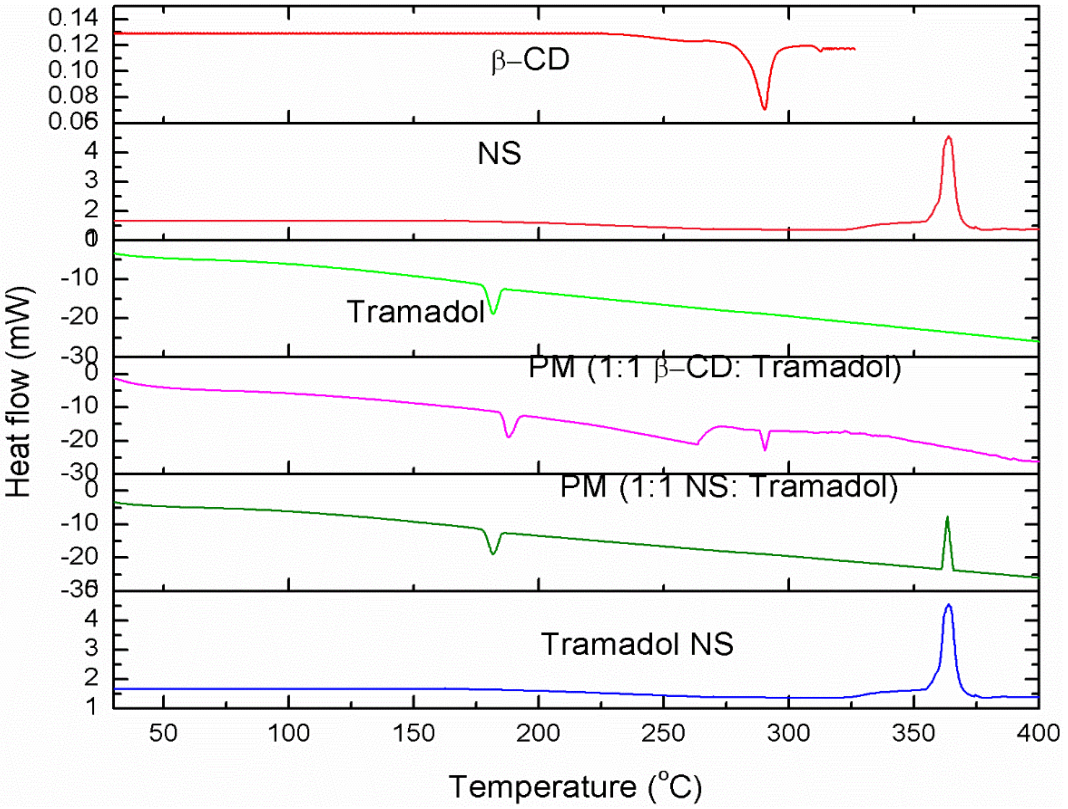


Figure 9. DSC thermograms of β-Cyclodextrin, plain nanosponges, Tramadol, Physical mixture and Tramadol loaded nanosponges

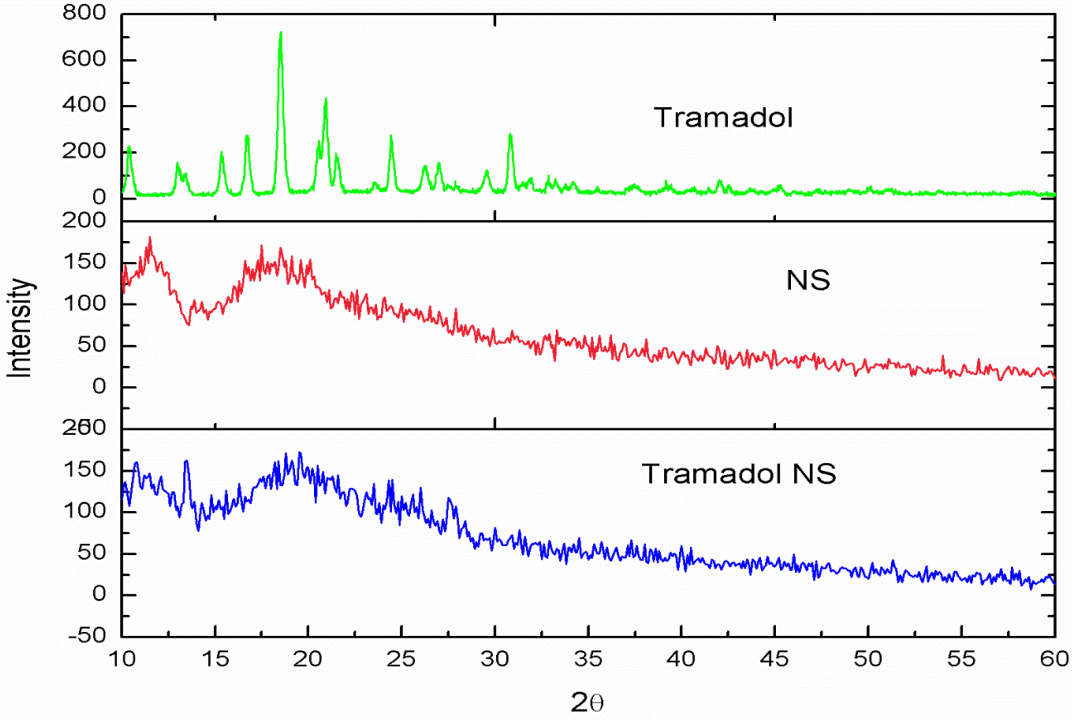
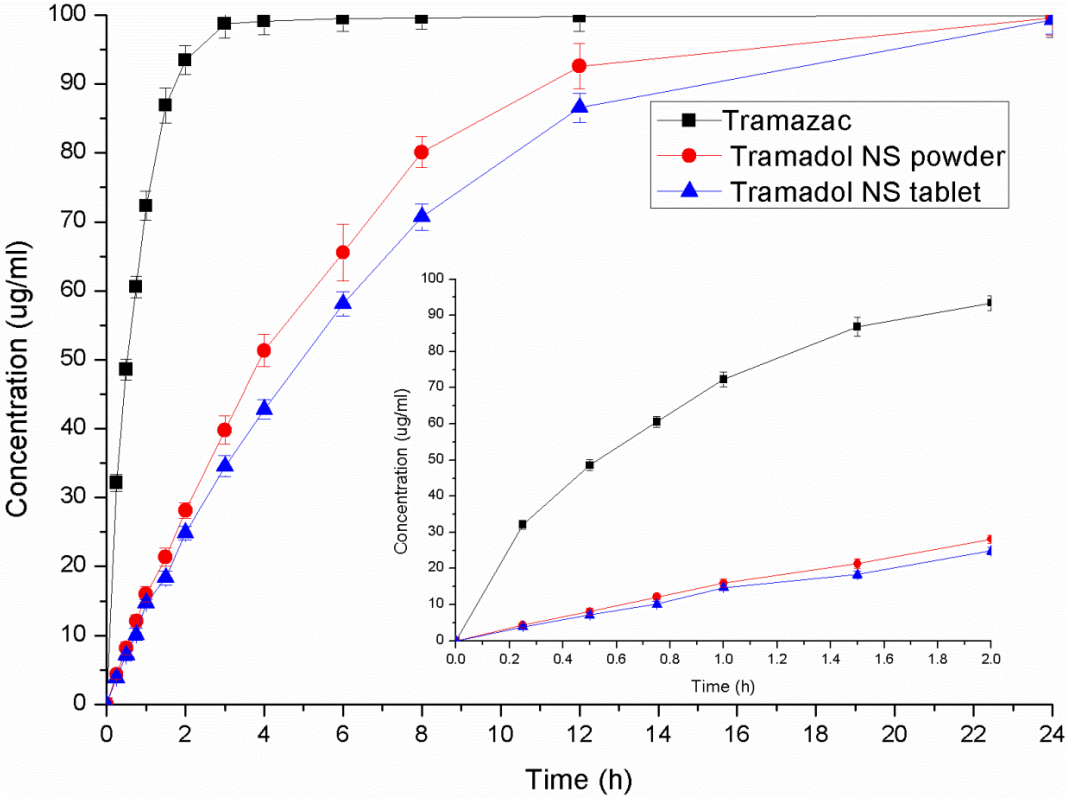
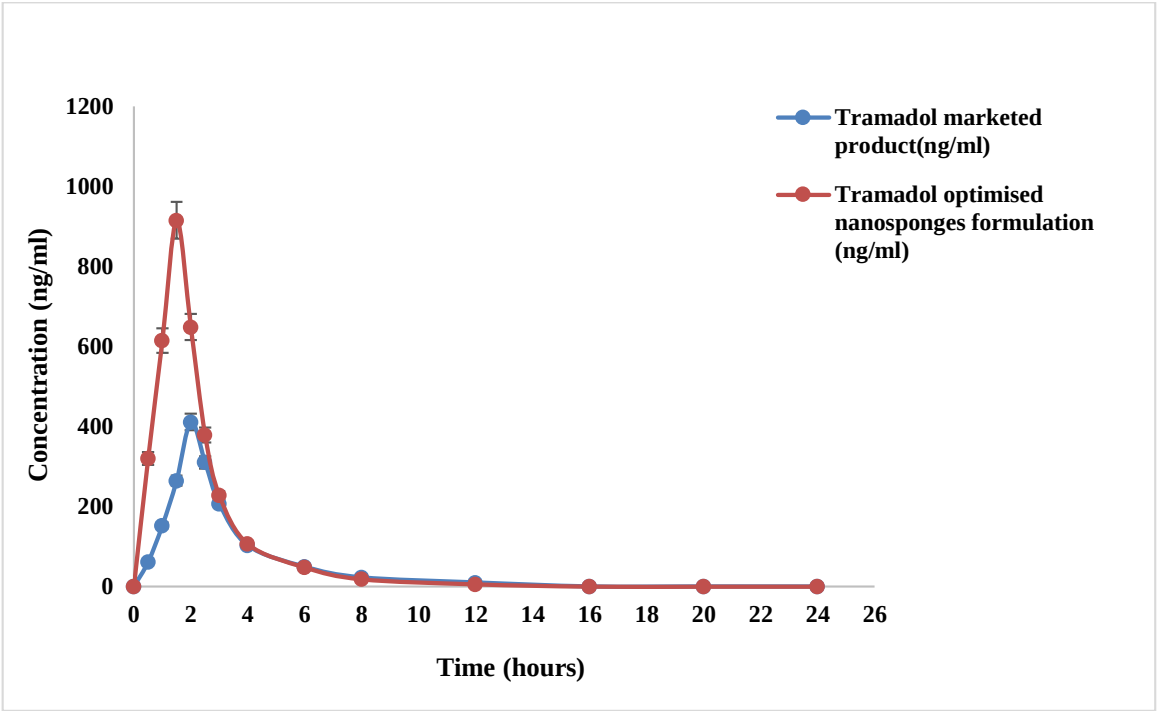


Figure 10. XRPD pattern of Tramadol, plain nanosponges (NS2) and tramadol loaded nanosponge complexes (IBNS)



(All determinations were expressed as mean±S.D. n=3)

Figure 11. In vitro release of Tramadol nanosponges tablets and marketed tablets



Above parameters are communicated as Average ± Standard Deviation; (n=6)
Figure 12: Mean plasma concentration-time profiles for tramadol marketed product and tramadol optimized nanosponges formulation in rats (n=6)