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Formulation & In Vivo Evaluation Of Ibrutinib Loaded Cyclodextrin Nanosponges

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ABSTRACT

The aim of this study was complexing the poorly water-soluble drug ibrutinib with β-cyclodextrin (β-CD) based nanosponges (NS), for improving dissolution oral bioavailability. Blank NS were fabricated by reacting β-CD with the cross-linker carbonyldiimidazole at different molar ratios (1:2, 1:4, and 1:8). The effect of formulation parameters on practical yield and particle size were evaluated by L9 Taguchi orthogonal array design. The NS of highest solubilization extent for drug were complexed with ibrutinib. Drug loaded NS (IBNS) were characterized for various physicochemical properties. The drug loading capacity was 48%. More than 90% of drug was released from IBNS2 over 24h while that of free drug suspension was only 21%. The DSC, FT-IR, and PXRD studies confirmed the complexation of ibrutinib with NS and amorphous state of the drug in the complex. In vivo studies conducted using optimized formulation IBNS2, mean time to attain peak drug concentration (T_{max}) was 1.00±0.04 and 1.5 ±0.05 h for the optimized and pure drug, respectively, while mean maximum drug concentration (C_{max}) was 131.62±0.27 ng/mL was significant ($p<0.05$) as compared to the ibrutinib pure drug 36.94±0.62 ng/mL. Hence, dissolution and bioavailability of the ibrutinib nanosponge formulation were significantly enhanced compared to plain ibrutinib.

Keywords: Ibrutinib, Bruton's tyrosine kinase, β –Cyclodextrin, nanosponges, Taguchi method, Pharmacokinetic parameters.

INTRODUCTION

β -Cyclodextrin-based nanosponges play an important role in new arrays of agriculture, floriculture, cosmetics, medicine, high molecular weight proteins, novel flame retardants, gas carriers and water filters. In recent years, the field of advance nanostructured systems witnesses a rapid development due to miniaturization, dose-reduction, sustained and controlled release of actives and long-term stability of material. β-CDNSs are colloidal and cross-linked Nano carrier comprising of solid mesh-like structure with nano-cavities for encapsulation of complex lipophilic and hydrophilic chemical substances (Swaminathan et al., 2010). Thus, nanosponges are effective carrier for the delivery of actives and developed as a commercial drug delivery system in pharmaceutical industries after certified clinical studies. In the near future, β-CDNS-based product will capture the market due to its diverse applications in anti-cancer, antiviral, antiplatelet, antihypertensive therapy etc. (Kishore et al., 2012).

Ibrutinib is a selective and covalent inhibitor of the enzyme Bruton's tyrosine kinase (BTK), (Amin and

Ranjana et al., 2014) it is used for treatment of B-cell malignancies (Haura and Rix et al., 2014). It has been reported to exhibit pH-dependent solubility as it is slightly soluble at pH 1.2 while practically insoluble at pH 3 to 8, which lead to low bioavailability and impede its in vivo antitumor effect after oral administration (Allahyari et al., 2019)

Taguchi developed a method for designing experiments to investigate how different parameters affect the mean and variance of a process performance characteristic that defines how well the process is functioning. The experimental design proposed by Taguchi involves using orthogonal arrays to organize the parameters affecting the process and the levels at which they should be varies. The Taguchi method is best used when there are an intermediate number of variables (3 to 50), few interactions between variables, and when only a few variables contribute significantly (Taguchi, 1962). The current research deals with formulation and in vivo evaluation of ibrutinib loaded β-Cyclodextrin nanosponges for prolonged drug release and enhanced bioavailability.

MATERIALS AND METHODS

Materials

B-Cyclodextrin (Complexol-B) was obtained as a gift sample from Gangwal Chemicals Pvt. Ltd. (Mumbai, India). Carbonyldiimidazole was purchased from Sigma Aldrich (Milan, Italy). Ibrutinib was obtained as a gift sample from MSN laboratories Pvt. Ltd. Hyderabad, India. All other chemicals and reagents used in the study were of analytical grade. Milli Q water (Millipore) was used throughout the studies.

Preparation of β-cyclodextrin nanosponges

Cyclodextrin based nanosponges were prepared using carbonyl diimidazole for the cross linking as reported elsewhere (Swaminatha et al., 2013). Three types of nanosponges (NS1-NS3) were prepared using different molar ratios of reactants (table1) by dissolving anhydrous β-Cyclodextrin in dimethyl sulphoxide followed by addition of carbonyl diimidazole and refluxed in an oil bath.

Optimization of reaction conditions

The optimization of nanosponges was carried out by Taguchi orthogonal array design (Kacker et al., 1991). Stat-Ease Design Expert® software V8.0.1was used for the optimization wherein the reaction temperature (A), reaction time (B), stirring speed (C), and volume of solvent (D) were varied so as to identify their optimum conditions for the synthesis of nanosponges with reduced particle size, maximum practical yield. (Table 2). The statistical analysis of the results using analysis of variance was performed to determine the factors which had a paramount influence on particle size and practical yield.

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)

Preparation of Ibrutinib-loaded β-cyclodextrin nanosponges (IBNS)

Ibrutinib loaded nanosponges (IBNS1, IBNS2 and IBNS3) were prepared by lyophilization technique as reported elsewhere (Nagarjun Rangaraj et al., 2019) by suspending 200 mg of nanosponges in 100 ml of Milli Q water followed by addition of 140 mg of drug. The colloidal supernatant was separated and freeze dried using a lyophilizer (LARK INDIA) at a temperature of -20° C and pressure of 13.33 mbar.

Physico-chemical characterization of IBNS

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, TEM as per the procedure adopted in reference (Nait bachir et al., 2017).

Determination of Ibrutinib loading in IBNS

Weighed quantity of IBNS were dissolved in methanol and then analysed on a UV spectrophotometer (Labindia UV-3000+ UV–Vis Spectrophotometer) at 260 nm (Qiu et al 2018). The percent drug loading was calculated using the following formula

$$\%\ Drug\ loading = \frac{Weight\ of\ drug\ loaded\ in\ NS\ formulation}{Initial\ weight\ of\ the\ drug\ fed\ for\ loading} \times 100$$

In-vitro release of IBNS

In vitro dissolution study of NS formulations and free ibrutinib was performed using multi compartment (n=6) rotating cells with a dialysis membrane (Sartorius cut off 12,000 Da). The membrane was activated as per the procedure provided by the manufacturer. Formulation equivalent to 5 mg of ibrutinib was filled in the dialysis bag and placed in donor phase consists of 100 ml simulated gastric fluid (pH 6.4). The receptor phase also contains the same medium. The receptor phase was added with 0.5% w/v sodium lauryl sulphate (1 ml) to maintain proper sink conditions. The receptor phase was completely withdrawn at specified intervals and evaluated (Labindia UV-3000+ UV–Vis Spectrophotometer) at 260 nm (Shakeel et al., 2015).

Pharmacokinetic studies of Ibrutinib

Animal preparation

Healthy Wistar rats were (Weighing 150-180 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 study was approved by institutional animal ethics committee with reference no: 1447/PO/Re/S/11/CPCSEA-25/A.

Study design

Rats were divided in to three groups (A, B & C) at random. 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 pure ibrutinib (as such) made suspension with 0.5% methocel and second group (B) was administered

Prepared ibrutinib optimised nanosponges diluted in 0.5% methocel by oral route at a dose of 1.093 mg. Third group (C) was kept as control (Satyen Torne et al., 2013).

Blood sampling

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, 5, 6, 8, 12, 16, 20, 24h post dose and transferred into Eppendr of tubes containing heparin in order to prevent blood clotting. Plasma was separated by centrifugation of the blood at 5000 rpm in cooling centrifuge for 5min to 10 minutes and stored frozen at −20°C until analysis. (Parasuraman et al., 2010)

Determination of Ibrutinib in Rat plasma by HPLC method

Determination of Ibrutinib and internal standard Dasatinib was carried out by using the chromatographic separation was achieved on Separation was achieved on the phenomenex C18 (250 x 4.60), 5 µ particle size column in isocratic mode at room temperature. The sample was introduced through an injector valve with a 20 µl, sample loop. Potassium dihydrogen orthophosphate (0.01M), Methanol and Acetonitrile in ratio of (30:30:40), were used as mobile phase with flow rate of 0.9 ml/min. UV detection was performed at 260 nm. The retention time of Ibrutinib and Dasatinib (Internal Standard) were found to be 5.88 min and 4.1 min respectively. (Reddy et al., 2018)

RESULTS AND DISCUSSION

Through preliminary screening reaction temperature, reaction time, stirring speed and volume of solvent were identified as the most significant process variables for the synthesis of nanosponges. In this study, a four -variable, three -level values matrix was constructed with the target output parameter being the practical yield and particle size. The Taguchi array, which led to an optimized combination through 9 experiments (Table 3) (Figure 1).

Analysis using S/N ratio

In the Taguchi method, S/N ratio is a measure of quality characteristics and deviation from the desired value. The max–min values of reaction temperature and reaction time are the highest value. Therefore, it can be understood that reaction temperature and reaction time are the significant factors influence the practical yield. (Table 4)

Optimization and confirmation experiments

To verify the data, three batches of nanosponges with varied molar concentrations (1:2, 1:4 and 1:8) were prepared according to the predicted levels of A, B, C and D. The predicted and observed values are shown in table 5. Obtained Y1 and Y2 values were in a close agreement with the predicted values. The good agreement between the predicted and experimental results verified the validity of the model and the existence of an optimal point for the synthesis of nanosponges.

Characterization of prepared nanosponges

The particle size analysis was around 139–161 nm with low polydispersity index and a sufficiently high zeta potential values between -12 to -24 mV. (Table 6)

Evaluation of drug loaded nanosponges

Among the three types of nanosponges, the loading efficiency was found to be higher in IBNS2 (1:4 β-Cyclodextrin: Carbonyldiimidazole) as much as 48% w/w. Considering high drug loading capacity IBNS2 was used for further studies (table 7).

In-vitro release of ibrutinib from nanosponge formulations

From in-vitro release studies, it was found that the nanosponge loaded formulations showed a significant improvement in the rate of release as compared with the pure drug. The drug release from the nanosponge formulations is highly significant as compared to free drug suspension ($P < 0.05$). More than 90 % of drug was found to be released from nanosponge formulations as compared to only around 21% from free drug suspension after 24 h of study. (Figure 2)

Characterization of plain (NS2) and drug loaded nanosponge complexes (IBNS2)

Figure 3 shows a comparison of FTIR spectra of Ibrutinib, NS2 and IBNS2 complex. 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 Comparison of FTIR spectra of ibrutinib and ibrutinib complex (IBNS2) showed that there is a major change in the fingerprint region i.e. 900 to 1,400 cm^{-1} as The main characteristic peaks of ibrutinib were broadened or shifted in the formulations suggesting definite interactions between ibrutinib and nanosponges.

The DSC thermogram of free drug shows a sharp melting point at approximately 159 °C indicating the crystalline nature of the drug. The DSC thermogram of plain nanosponges (NS2) showed exothermic peaks at around 350 °C. Ibrutinib nanosponge complex (IBNS2) also exhibited a broad exothermic peak at around at 350 °C. The complete disappearance of ibrutinib endothermic peak was observed for the formulation. (Figure 4)

The x-ray diffractograms of plain ibrutinib exhibited sharp intense peaks at 2θ values of 5.6°, 13.5°, 16.5°, 19.2°, 21.2° and 21.7° confirming the drug's crystal form as shown in figure 5. However, there were no characteristics peak of pure ibrutinib were observed in NS complexes. The absence of such crystalline peaks of ibrutinib in nanosponge complex clearly indicates that the drug is encapsulated in nanosponges.

Morphology and sizes of the ibrutinib loaded nanosponges (IBNS2)

The particle size analysis of plain nanosponges and ibrutinib loaded nanosponges revealed that the average particle size measured by laser light scattering method is around 138-171 nm with low polydispersity index (Table 8). The particle size distribution and zeta potential values are shown in figure 6.

Transmission electron microscopy (TEM) studies showed that the regular spherical shape and size of plain nanosponges that are unaffected even after drug encapsulation. The particle size of the complexes as observed under TEM was consistent with the data obtained through dynamic light scattering. (Figure 6)

Pharmacokinetic studies

Figure 7 shows the plasma concentration–time curve in Wistar rats after a single oral dose of Ibrutinib nanosponges formulation as compared to Ibrutinib pure suspension. At all the indicated time points, the Ibrutinib plasma concentrations in rats treated with nanosponges formulation was significantly

higher than those treated with pure drug. Pharmacokinetic parameters of Ibrutinib after oral administration of the two formulations in Wistar rats are shown in table 9.

C_{max} of the nanosponges 131.62±0.27 ng/ml was significant ($p<0.05$) as compared to the pure drug suspension formulation 36.94±0.62ng/ml. T_{max} of both nanosponges formulation and pure drug suspension was 1.00±0.04 h and 1.50±0.05h, respectively. AUC is an important parameter in evaluating bioavailability of drug from dosage form, as it represents the total integrated area under the blood concentration time profile and represents the total amount of drug reaching the systemic circulation after oral administration. $AUC_{0-\infty}$ infinity for nanosponges formulation was higher (3162.95±1.27 ng. h/ml) than the pure drug suspension formulation 975.42±0.06 ng.h/ml. Statistically, AUC_{0-t} of the nanosponges formulation was significantly higher ($p<0.05$) as compared to pure drug suspension formulation. Higher amount of drug concentration in blood indicated better systemic absorption of Ibrutinib from nanosponges formulation as compared to the pure drug suspension formulation**.**

CONCLUSION

The freeze-drying process was used in this investigation to prepare ibrutinib-loaded nanosponges. The creation of an ibrutinib inclusion complex with nanosponges was confirmed by FTIR, DSC, and XRD. Because of the reduced drug particle size, the creation of a high-energy amorphous state, and intermolecular hydrogen bonding, the dissolution of the ibrutinib nanosponges was much higher than that of the pure drug. Depending on the sort of nanosponges used, the release kinetics can be slowed or sped up. Pharmacokinetic parameters of ibrutinib after oral administration of the two formulations in Wister rats showed that Cmax of the nanosponges formulation was increased 4fold compared to the pure drug, and AUC0-t of the nanosponges formulation was increased 5fold compared to the pure drug, indicating a higher amount of drug concentration in blood, indicating better systemic absorption of ibrutinib from nanosponges formulation. Overall, this study showed that cyclodextrin nanosponges can be used to improve the physicochemical characteristics, oral bioavailability, and therapeutic efficiency of the anticancer medication ibrutinib.

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TABLES

Table 1: Molar ratios and concentrations of cyclodextrins and the cross-linker

S.NO	Type of NS	Molar ratio	Concentration of β-cyclodextrin (gms)	Concentration of carbonyldiimidazole (gms)
1	NS1	1:2 (β-CD: CDI)	2.274	2.484
2	NS2	1:4 (β-CD: CDI)	2.274	4.968
3	NS3	1:8 (β-CD: CDI)	2.274	9.936

Independent variables	Levels

Variable	Name	Units	-1	0	+1
A	Reaction temperature	°C	80	100	120
B	Reaction time	Min	360	420	480
C	Stirring speed	Rpm	1000	2000	3000
D	Volume of solvent	Ml	100	150	200
Dependent variable			**Goal**		
Y1	Practical yield	%	Maximize		
Y2	Particle size	Nm	Minimize		

Table 2. Variables, factors and their levels used in L_9 Taguchi orthogonal array design

Table 3 :Taguchi orthogonal array design with observed responses

Run	Reaction temperature (°C)	Reaction time (min)	Stirring speed (rpm)	Volume of solvent (ml)	Practical yield (%)	Particle size (nm)
1	80	480	3000	200	68.1	189
2	120	360	3000	150	83.5	169
3	100	480	1000	150	96.9	352
4	100	420	3000	100	94.6	142
5	80	360	1000	100	63.3	316
6	120	480	2000	100	88.2	213
7	100	360	2000	200	91.2	272
8	80	420	2000	150	66.2	242
9	120	420	1000	200	86.4	390

Table 4: S/N ratio table for practical yield and particle size

Run	Control factor	Practical yield (%)				Particle size (nm)			
		Mean S/N ratio (dB)				Mean S/N ratio (dB)			
		Level 1	Level 2	Level 3	Max-Min	Level 1	Level 2	Level 3	Max-Min
1	Reaction temperature	-36.37	-39.47	-38.69	3.1	-47.73	-47.55	-47.64	0.17
2	Reaction time	-37.88	-38.22	-38.43	0.54	-47.74	-47.51	-47.67	0.22

3	Stirring speed	-38.16	-38.17	-38.2	0.04	-50.91	-47.64	-44.37	6.53
4	Volume of solvent	-38.15	-38.19	-38.19	0.046	-46.53	-47.72	-48.68	2.14

Table 5: Results of the confirmation experiment by the constraints applies on Y1 and Y2

Independent variable	**Nominal values**	**Predicted values**		**Observed values**		
		Practical yield (Y1)	Particle size (Y2)	Batch	Practical yield (Y1)	Particle size (Y2)
Reaction temperature (A)	100	96.58	146.44	**NS1**	95.34±0.56	161± 2.4
Reaction time (B)	480			**NS2**	94.75±0.34	138± 3.2
Stirring speed (C)	3000			**NS3**	96.12 ± 0.73	155 ± 4.6
Volume of solvent (D)	100					

Table 6: Particle size analysis of cyclodextrin nanosponge

Sample	**Mean hydrodynamic diameter ± SD (nm)**	**Polydispersity Index**	**Zeta potential (mV)**
NS1	161±2.4	0.45±0.005	-24.32±1.8
NS2	139±3.2	0.18±0.005	-12.18±1.3
NS3	155±4.5	0.645±0.005	-16.34±1.1

Table 7: Percent drug loading in nanosponges

S.NO	**Name of the formulation**	**Drug loading (%)**
1	IBNS1	23 ± 0.92
2	IBNS2	48 ± 0.63

3	IBNS3	29± 0.88

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

Table 8: Particle Size, polydispersity index and zeta potential of plain nanosponges and drug loaded nanosponge formulation

Sample	Mean hydrodynamic diameter ± SD (nm)	Polydispersity Index	Zeta potential (mV)
NS2	138.2 ± 3.2	0.18 ± 0.005	-12.18 ± 1.2
IBNS2	171.4 ± 2.3	0.21 ± 0.005	-21.6 ± 2.1

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

Table 9: Mean pharmacokinetic parameters of ibrutinib pure drug and ibrutinib optimized nanosponges formulation in rats

Pharmacokinetic parameters	Ibrutinib Pure drug	Ibrutinib– nanosponges Optimized Formulation
C_{max} (ng/ml)	36.94±0.62	131.62±0.27
AUC_{0-t} (ng. h/ml)	361.96±0.32	1543.29±0.49
AUC_{0-inf} (ng. h/ml)	975.42±0.06	3162.95±1.27
T_{max} (h)	1.50±0.05	1.00±0.04
$t_{1/2}$ (h)	4.56±0.06	2.95±0.05

FIGURES

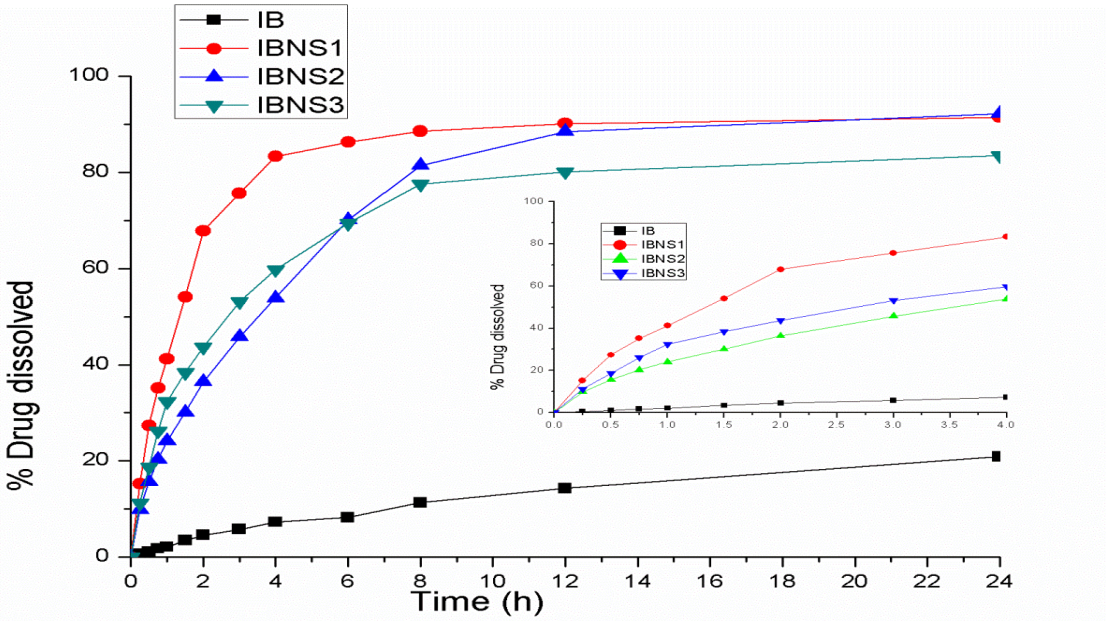
Design Summary

Study Type	Factorial	**Runs**	9
Design Type	Taguchi OA	**Blocks**	No Blocks
Center Points	0		
Design Model	Main effects	**Build Time (ms)**	94.02

Factor	Name	Units	Type	Subtype	Low Actu	High Actual		
A	Reaction temperature	°C	Categoric	Nominal	80	120	**Levels:**	3
B	Reaction time	mn	Categoric	Nominal	360	480	**Levels:**	3
C	Stirring speed	rpm	Categoric	Nominal	1000	3000	**Levels:**	3
D	Volume of solvent	ml	Categoric	Nominal	100	200	**Levels:**	3

Response	Name	Units	Obs	Analysis	Minimum	Maximum	Mean	Std. Dev.	Ratio	Trans	Model
Y1	Practical yield	%	9	Factorial	63.3	96.9	82.0444	12.8354	1.53081	None	RMain effects
Y2	Particle size	nm	9	Factorial	142	390	253.889	85.2107	2.74648	None	RMain effects

Figure 1: Summary of the selected Taguchi OA design



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

Figure 2: Dissolution profile of pure ibrutinib and ibrutinib loaded nanosponge formulations

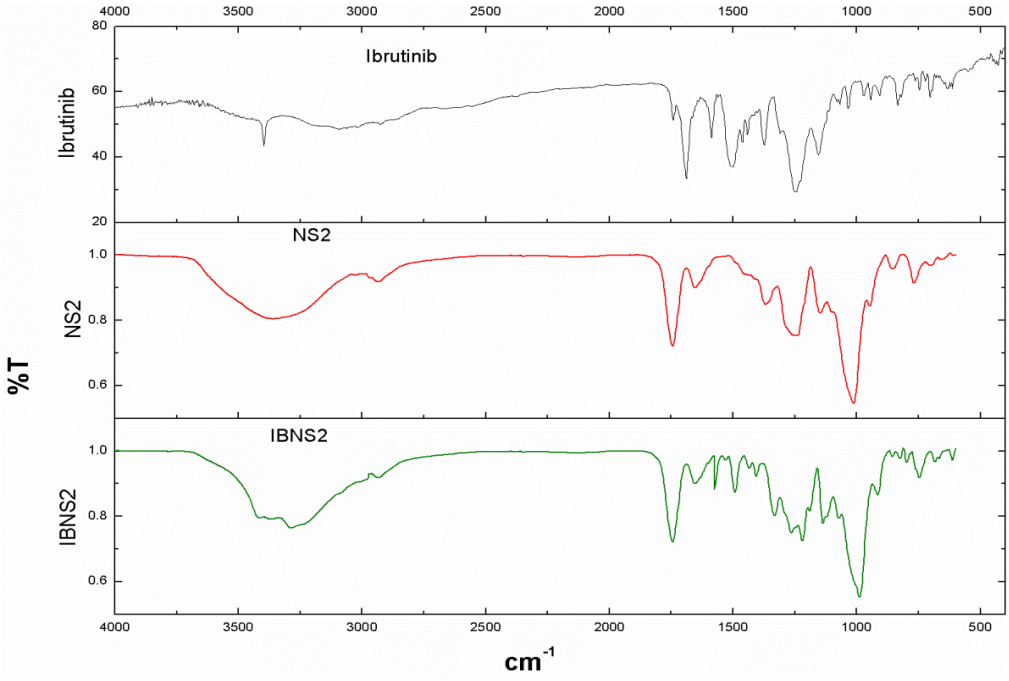


Figure 3: FTIR spectra of free ibrutinib, plain nanosponges (NS2) and ibrutinib loaded nanosponge complexes (IBNS2)

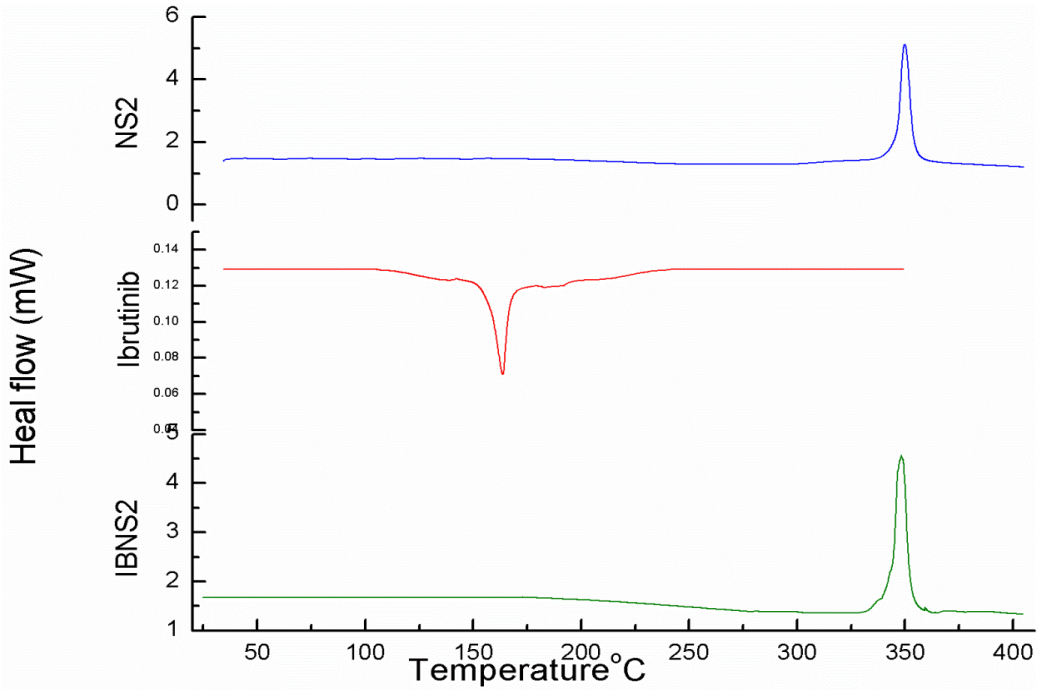


Figure 4: DSC thermograms of plain nanosponges (NS2), free ibrutinib and ibrutinib loaded nanosponge complexes (IBNS2)

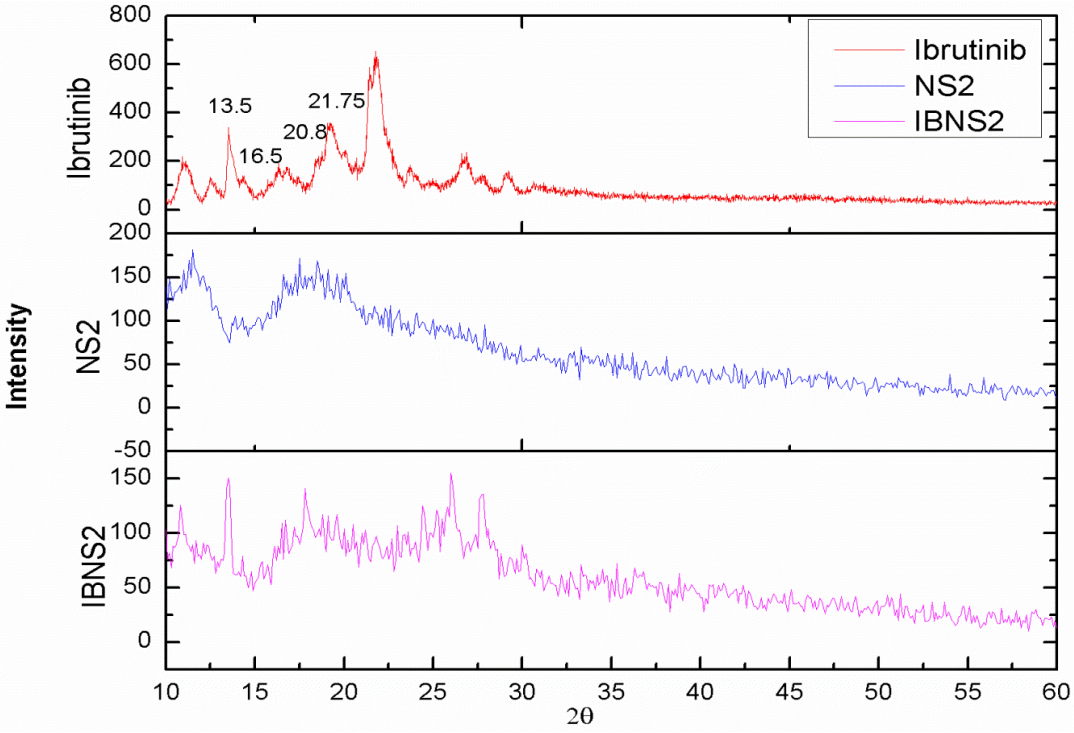


Figure 5: XRPD pattern of Ibrutinib, plain nanosponges (NS2) and ibrutinib loaded nanosponge complexes (IBNS2)

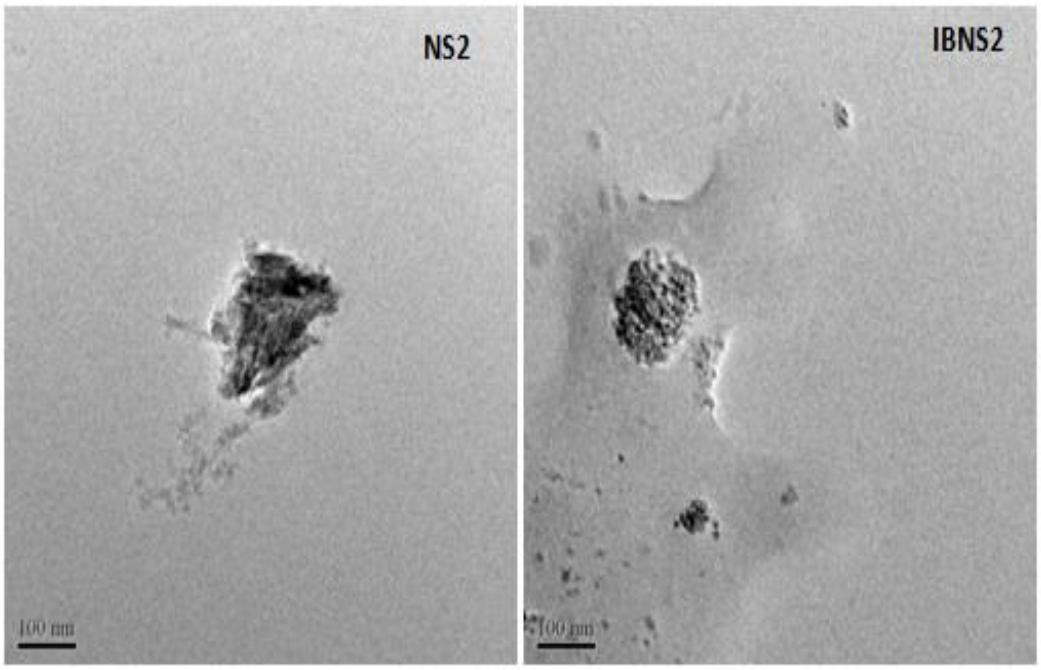


Figure 6: A. TEM image of plain nanosponges (NS2) B. Ibrutinib loaded nanosponge complexes

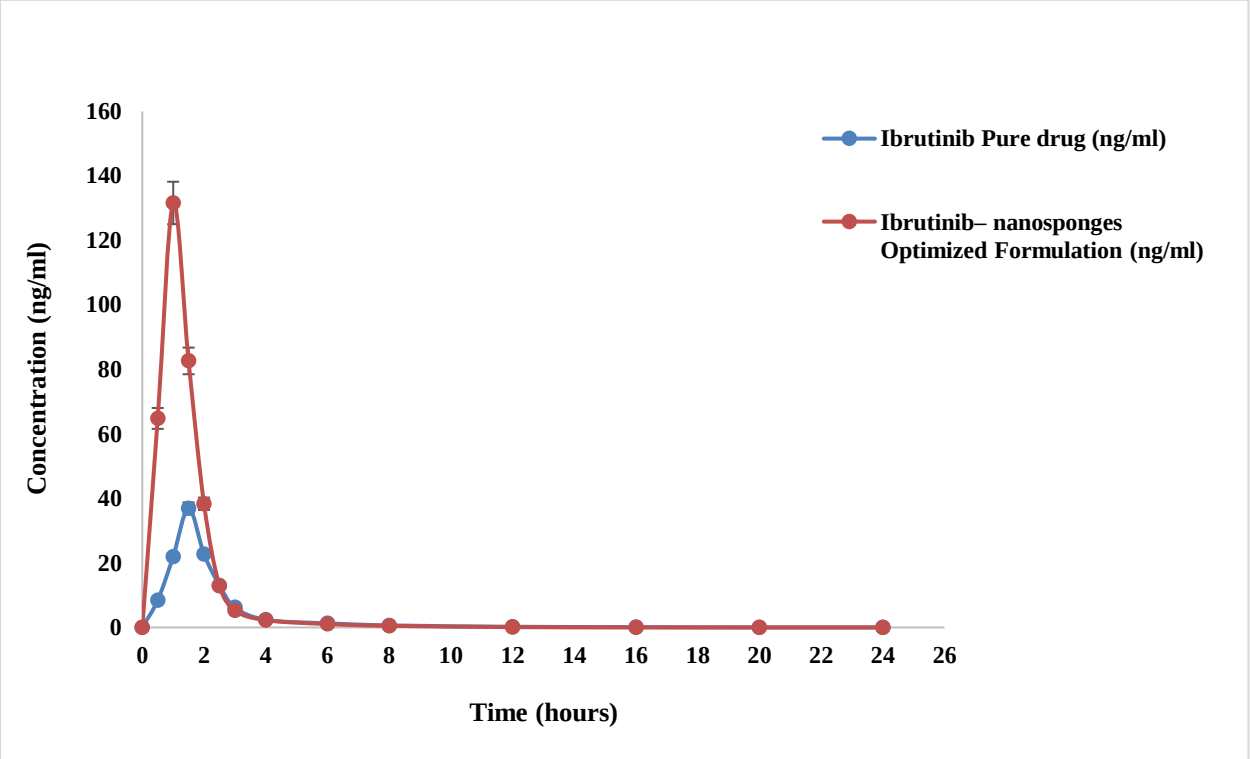


Figure 7: Mean plasma concentration-time profiles for ibrutinib pure drug and ibrutinib optimized nanosponges formulation in rats (n=6)