4. PREPARATION AND CHARACTERIZATION OF PRAZIQUANTEL-ß-CYCLODEXTRIN NANOSPONGES

4.1. Objective

Praziquantel exhibits poor oral bioavailability due to its low aqueous solubility, extensive hepatic first-pass metabolism and the short plasma half-life (0.8-1.5 hours). This necessitates frequent administration of high oral doses, to overcome first pass metabolism and thereby achieve sufficient drug concentrations at larval tissues. In view of above a nano-particulate drug delivery system, i.e. cyclodextrin based nanosponges (NS), using different ratios of cross linker (Dimethyl carbonate) was formulated. The objective of this chapter was to study the capacity of the nanosponges for incorporating PZQ within their structure along with enhancement in solubility and release characteristics.

4.2. Materials and methods

4.2.1. Materials

Praziquantel (PZQ) was a kind gift from Wockhardt Research Centre, Aurangabad, India. β -Cyclodextrin (β -CD) was provided by ISP (Hongkong Ltd), Hyderabad (India). Dimethyl carbonate was purchased from Lobachemie, Pvt. Ltd. (Mumbai, India). Milli Q water (Millipore) was used throughout the studies. All other chemicals and reagents were of analytical grade while solvents used for HPLC analysis were of HPLC grade unless otherwise specified.

4.2.2. Methods

4.2.2.1. HPLC analytical method for quantification of Praziquantel

Praziquantel was quantified by a validated HPLC method as described by Hanpitakpong et al., 2004. HPLC assay was carried out on a Waters 2998 system (Waters, USA) consisting of binary pumps (Waters 515) equipped with a 20-µl loop, rheodyne sample injector, PDA detector (Waters 2998) and Empower Node-2054 software. The column Spherisorb ODS2 C18 (250mm x 4.6 mm i.d.; 5µm particle size) from Waters (Miliford, MA, USA) was maintained at room temperature (20 ± 2°C). UV spectra from 190 - 400 nm were recorded online for peak identification. The wavelength (λ_{max}) was set at 217 nm. The mobile phase was acetonitrile and water (70:30, %v/v) and flow rate was kept at 1.0 ml/min at ambient temperature. Aliquots of 20 µL clear supernatant samples were injected into the HPLC system.

4.2.2.2. Synthesis of β -cyclodextrin nanosponges

β-cyclodextrin (MW 1,135 g/mol) nanosponges were synthesized by following the procedure reported elsewere (Trotta et al., 2003). Briefly, anhydrous β- CD (2g) was dissolved in anhydrous dimethylformamide (DMF, 30 ml), then triethylamine (1 ml) was added. Dimethyl carbonate (14 ml) was added to the above solution as cross linker and allowed to react in reflux condenser at 90◦C for 4 hrs. The reaction was carried out using a crosslinker excess, at three different molar ratios, e.g. 1:2, 1:4, 1:8 (β CD: cross-linker). The crude nanosponges were then purified by

washings with distilled water (25 ml) and ethanol (25 ml). The mass was then dried overnight at 60°C. Finally, residual by-products or unreacted reagents were completely removed by Soxhlet extraction with ethanol.

4.2.2.3. Preparation of PZQ-loaded nanosponges (PZQ-NS)

The PZQ-loaded nanosponges were prepared by the freeze drying method. Briefly, nanosponges and PZQ as powders were mixed at two different weight ratios of 1.5 and 1:10 (PZQ to NS by weight) and then resultant mixture was suspended in 50 ml of distilled water. The suspension was sonicated for 10 min and was kept for 24 h under-stirring in the dark. After 24 h, the resultant suspension was centrifuged at 2000 rpm for 10 min to separate the uncomplexed drug as a residue below the colloidal supernatant. The filtrate was then lyophilized for 48 h (Lypholizer, Decibel, India) to obtain the PZQ-loaded nanosponges as a powder. The dried powder was sieved through 60# and stored in a desiccator at room temperature until further use.

4.2.2.4. Solubilization efficiency of PZQ-NS

Solubilization efficiency was determined by suspending the excess quantity of PZQ in distilled water as well as with fixed quantities of NS and β -cyclodextrin in mili Q water separately. The glass vials were placed on a mechanical shaker (Remi, India) at ambient temperature for 24 h. Obtained suspension was centrifuged (Remi C-24 BL Centrifuge) at 10,000 rpm for 10 min and filtrate analyzed for PZQ concentration by HPLC method described earlier (Hanpitakpong et al.,2004). Briefly, PZQ

analysis was performed using a waters 2998 system (Waters, USA) consisting of binary pumps (Waters 515) equipped with a 20-µl loop, rheodyne sample injector, PDA detector (Waters 2998)and Empower Node-2054 software. The column Spherisorb ODS2 C18(250mm x 4.6 mm i.d.; 5µm particle size) from Waters(Miliford, MA, USA) was maintained at room temperature(20 ± 2°C). The wavelength was set at 217 nm. The mobile phase was acetonitrile and water (70:30, v/v) and flow rate was kept at 1.0 ml/min.

4.2.2.5. Characterisation of PZQ-loaded Nanosponges

4.2.2.5.1. Particle size and Zeta Potential (ZP) analysis

The mean Particle size, Polydispersity Index (PDI) and Zeta Potential of the formulations were determined by using Particle Size analyzer, Delsa Nano C (Beckman Coulter Counter, USA) based on dynamic light scattering technique. The PZQ-NS sample was diluted in distilled water prior to measurement.

4.2.2.5.2. Drug Entrapment efficiency

Weighed amount of PZQ loaded NS were dissolved in 10 ml with chloroform/methanol mixture (1:1) and vortexed to extract drug from NS. The obtained solution was filtered through 0.45 µm PVDF membrane filter (Whatman). Total drug content (TDC) was determined by HPLC.

The drug-loaded NS (500µl) was transferred to the upper chamber of Nanosep® centrifuge tubes fitted with an omega ultrafilter (molecular weight cutoff (MWCO) 30KD, Pall Life Sciences, India).The Nanosep® was

centrifuged (Cooling Centrifuge BL 24; Remi, India) at 5000 rpm for 10 mins at -4ºC**.** The NS along with encapsulated drug remained in the upper chamber and aqueous phase moved into the sample recovery chamber through filter membrane. The amount of un-entrapped PZQ present in the aqueous phase was estimated by UV spectrophotometer at 262 nm. Entrapment efficiency of SLN formulation was calculated using below formulae

$$\text{Percentage of Entrapment Efficiency (EE \%)} = [(A_t - A_{un})/ A_{t]} \text{ X } 100$$

Where, A_t = Total amount of PZQ in NS; A_{un} = Unentrapped PZQ in NS

4.2.2.5.3. Morphological evaluation

4.2.2.5.3.1. Scanning Electron Microscopy (SEM)

The surface morphologies of NS and PZQ loaded NS were examined using a Scanning Electron Microscope (SEM; Quanta W, FEI Company). The coated pellets were mounted onto stubs using Silver glue as adhesive which is obtained by combining Silver paste and n-butyl acetate. Silver adhesive is used to maintain conductivity. The samples were examined at 15 kV accelerating voltage and in low vacuum.

4.2.2.5.3.2. Transmission Electron Microscopy (TEM)

The suspension of NS and PZQ loaded NS were observed using a TECNAI 20 G^2 (FEI Company) to elucidate the particle shape. The sample was prepared by a formvar resin grid method. Briefly, a 0.5 % w/v suspension of NS was sprayed on a formvar resin coated TEM grid and air dried for 10

min before observation. The negative films (Kodak SO163) were digitized off line using Kodak mega plus CCD camera. Contrast enhancement and particle measurement were performed using the NIH image software.

4.2.2.5.4. Fourier transformed infra-red (FT-IR) spectroscopy

FTIR spectra of β CD, plain NS, PZQ, and PZQ loaded NS were obtained by the conventional potassium bromide (KBr) disk/pellet method using FTIR(SCHIMADZU, Model 8400S, Japan). The sample was grounded gently with anhydrous KBr and compressed to form pellet. The scanning range was between 4000 cm^{-1} to 400 cm^{-1}.

4.2.2.5.5. Differential Scanning Calorimetry (DSC)

The thermal behaviors of the samples (Pure PZQ, plain NS and PZQ-NS) were determined by using DSC (Perkin Elmer) equipped with an intracooler and a refrigerated cooling system. Each sample placed in an aluminum pan was hermetically sealed with an aluminum cover. All measurements were performed over 20-200^{0}C at a heating rate of 10^0/ min. nitrogen was purged at 50 ml/ min through cooling unit.

4.2.2.5.6. X-Ray Powder Diffraction (XRPD)

The physical states of the samples (Pure PZQ, plain NS and PZQ-NS) were characterized using X-Ray Diffractometer (X'Pert PRO, PANalytical) with the sequential collection between 10^0 and 80^0 at 2θ. 2θ at a step size of 0.045° and step time of 0.5 s.

4.2.2.6. In vitro release studies

The drug release study was performed in 0.1NHCl and pH 6.8 Phosphate buffer using the dialysis bag diffusion method. The dialysis membranes (cellophane membrane, molecular weight cut off between 12 -14 kDa, Hi-Media, India) were kept overnight in the dissolution medium to ensure thorough wetting of the membrane. Weighed amount of PZQ loaded nanosponges equivalent to 2mg of Drug were suspended in 2 ml of dissolution medium and suspension was placed inside the dialysis bag with the two ends tied and fixed by clamps. This procedure is carried out with both types of drug loaded nanosponges (1:5, 1:10%w/w) and plain PZQ. The bag was inserted into a beaker containing the respective dissolution medium (37^0C and stirring at 100 rpm) to maintain sink condition. 2 ml of aliquots were withdrawn at predetermined time intervals and replaced by an equal volume of fresh dissolution medium. The samples were filtered through 0.2 micron syringe filters and the drug content was determined. All the operations were carried out in triplicate. The amount of released drug was determined by HPLC All the operations were carried out in triplicate.

4.2.2.7. In vivo studies

4.2.2.7. 1. HPLC bio-analytical method for determination of PZQ

Samples were analyzed by an HPLC method previously reported with little modification (Hanpitakpong *et al*. 2004). Briefly, 200 ng internal standard working solution (diazepam 20 µg/ml) was added to 200 µl plasma. The samples were mixed in a vortex for 4–5 s, then 200 µl mixture

of methyl alcohol and acetonitrile (1: 1, v/v) was added. The mixture was vortexed for 3.0 min to allow complete mixing, followed by centrifugation at 15,000 rpm (Cooling Centrifuge, Remi, India) for 30 min. The 20 µl of supernatant were injected onto the HPLC column. The HPLC system (Waters, USA) consisted of a two solvent delivery pumps (Waters 515) and a PDA detector (Waters 2998). The wavelength was set at 217 nm. The separation was carried out on a reversed phase column Spherisorb ODS2 C18, 250 x 4.6 mm, 5µ column C_{18} (Waters, USA).The mobile phase consisted of acetonitrile and distilled water (70: 30, v/v), running through the column at a flow rate of 1.0 ml/min. The chromatographic analysis was operated at room temperature. The data was processed by means of Ezchrome Elite software (Waters, USA).

4.2.2.7. 2. Calibration curves

Stock solution of PZQ was prepared by dissolving 10mg of drug in 100 ml methanol and stored at 4°C in a glass volumetric flask. The stock solution was appropriately diluted to obtain 0.025, 1, 2, 4, 6, 8, 12 ng/ml concentration of drug. Calibration curves were prepared by linear regression analysis of ten blank plasma samples (0.2 ml each) added with varying concentrations of PZQ covering the range of 0.025–8.0 ng / ml and assayed in triplicate to evaluate linearity.

4.2.2.7.3. Animal Study protocol

In vivo studies were carried out according to the guidelines of the Council for the Purpose of Control and Supervision of Experiments on Animals

(CPCSEA), and were approved by the animal ethical committee of the Institute of Medical Sciences, Banaras Hindu University, Varanasi , India. Healthy adult rats (250 ± 20 g, either sex) of Wistar stock were quarantined in the animal house and handled according to institutional guidelines under controlled environmental conditions of temperature at 30 ± 2◦C and 60 ± 5% RH. A 12-h dark/light cycle was maintained throughout the study. Rats had free access to food (pellet diet supplied) and distilled water *ad libitum*. All animals were starved overnight prior use and divided into two groups comprising six animals in each group (n = 6).

4.2.2.7.4. Pharmacokinetic Study

The in vivo performance of PZQ-NS was evaluated via oral administration of the PZQ formulations at a dose of 50 mg/kg. All animals of group I (control group) were given an oral dose of PZQ suspension (0.5% w/v Methyl Cellulose suspension of pure drug); group II (treated group) was administered orally with an equivalent dose of PZQ-NS. The formulations were administered orally with the aid of a syringe and infant feeding tube to bypass trachea. Blood samples (0.3 - 0.5ml) were drawn by retro-orbital venous plexus puncture with the aid of glass capillary tubes in heparinized Eppendorf tubes at 0.08, 0.17, 0.33, 0.5, 1, 2, 4, 6, and 8 hrs post oral dose. Each blood sample was centrifuged at 12,000 rpm for 10 min; the plasma obtained was stored at −20 ◦C until analysis by HPLC.

4.2.2.7.5. Pharmacokinetic Analysis

Pharmacokinetic analysis were performed on each individual set of data, using the pharmacokinetic software Winnonlin® 5.3 software(Pharsight, California) using a non-compartmental method.

4.3. Results and discussion

4.3.1. HPLC Analytical method

Figure 4.2 shows a representative chromatogram of PZQ obtained from the HPLC analysis. The retention time of 4.64±0.01 min allows rapid determination of PZQ.

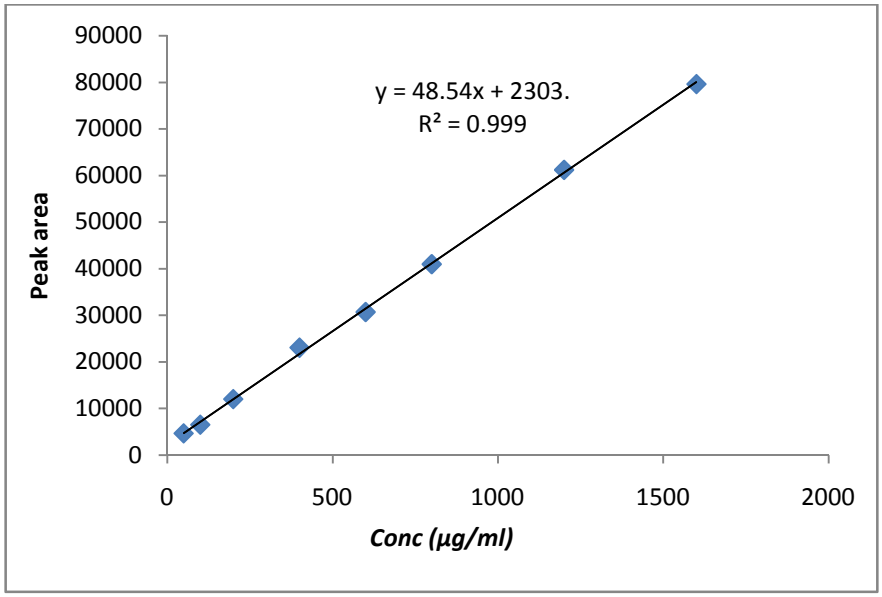


Figure 4.1: Standard calibration curve for PZQ

The regression equation obtained was; y = 48.54x + 2303. The calibration curve of PZQ was linear over the concentration range of 0.25µg/ml to 1500 µg/ml and the mean correlation coefficient (R^2) was 0.999 (Figure 4.1).

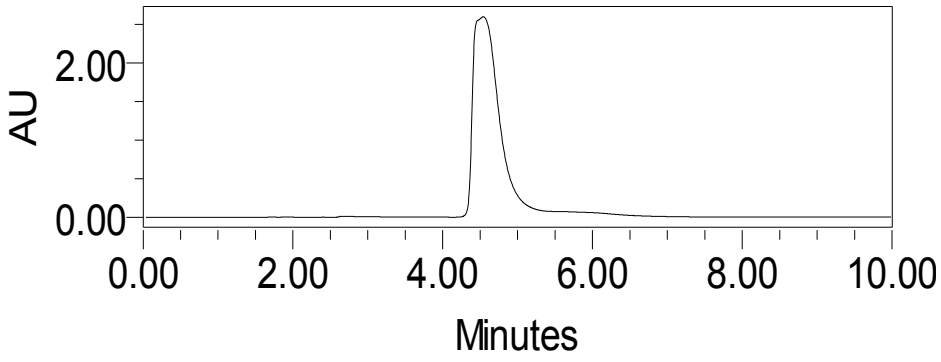


Figure 4.2: HPLC analytical chromatogram of praziquantel

Table 4.1: Validation parameters of HPLC analytical method

Validation parameter	**Result**
Standard regression Equation	y = 48.54x + 2303
Regression coefficient(R^2)	R^2=0.999
Accuracy(%recovery ± SD)	96.74 ± 4.28%.
Precision intra- day and inter- day (%CV)	both below 5% of the actual value
Linearity	0.25–1500 µg/ml
LOD	0.075 µg/ml
LOQ	0.05 µg/ml

4.3.2. Particle Size analysis

NS has been synthesized by various researchers mainly by modification of the earlier developed method (Trota *et al.,*2003). The variation carried out by different researchers was only the uses of various carbonate cross linkers in different ratios. The CD cross-linker ratio varied during their preparation resulted in improved drug loading and a tailored release profile. In the present study, the reaction was carried out using β- CD: cross-linker ratio at three different molar ratios, 1:2, 1:4, 1:8 (β - CD: cross-linker).

Among the β - CD: cross-linker, at three different molar ratios, 1:2, 1:4, 1:8 (β - CD: cross-linker), NS (1:4) showed the lowest size. It can be observed that as the ratio changes from 1:4 to 1:2, there is decrement in the particle size of the nanosponges (Table 4.2). This is possibly due to the increment of cross-linker in NS 1:4, which in turn increases the cross-linking between β -CD units. It is also observed that there is again an increment in the particle size of NS (1:8) which may be attributed to the saturation of the crosslinking between β -CD units.

Table 4.2: Effect of □□CD: cross linker ratio on the Particle Size, PI and ZP of different batches of nanosponges

S. No.	NS formulation	Size (nm)	PI	ZP (mV)
1	Blank NS (1:2)	287.6 ± 6.2	0.095± 0.03	-25.90 ± 2.1
2	Blank NS (1:4)	196.2 ± 4.7	0.111±0.04	-23.55 ± 1.7
2	Blank NS (1:8)	256.9 ± 5.4	0.198± 0.04	-24.70 ± 2.9

Mean ± SD; n = 3

Zeta potential of nanosponges was determined as a measure of surface charge. The Zeta potential for different ratio (the β - CD: cross-linker) were obtained around -24mV (Table 4.2), which means that the particles have little tendency to aggregate.

4.3.3. Solubilisation Efficiency

Drug loaded nanosponge complexes were prepared at the weight ratio of 1:5 (Drug: NS by weight) and saturation solubility studies were performed to obtain the solubilisation efficiency of the prepared β -CD nanosponges. It is evident from the table 4.3 that the solubility was maximum in case of NS (1:4) followed by NS (1:2) and NS (1:8). The enhancement factors were also calculated for the same (Table 4.3). There

were 52.8 fold increments in the solubilisation of drug in the case of NS (1:4), while in the case of NS (1:2) and NS (1:8), the solubilisation enhancement factor was only 35.3 and 27.5, respectively. The total solubility effect exerted by NS may be because of entrapment in the matrix as well as the formation of inclusion complex with drug in case of NS (1:4).

Table 4.3: Saturation solubility studies of formulations with their enhancement factors

Type	Solubility (mg/ml)	Enhancement Factor
Plain Drug PZQ	0.41 ± 0.005	-
NS (1:2) (1:5 w/w)	14.47 ± 0.58	35.3
NS (1:4) (1:5 w/w)	21.64 ± 0.69	52.8
NS (1:8) (1:5 w/w)	11.27 ± 0.78	27.5

Mean ± SD; n = 3

In case of NS (1:2) solubilization decreases due to the lesser cross-linking between β -CD and cross-linker as the cross-linker ratio decreases, so there lower degree of entrapment in the matrix. But NS (1:2) showed more solubility than NS (1:8) because there was a possibility of formation of inclusion complexes in case of NS (1:2) as it was having more ratio of β -CD than NS (1:8). The β -CD formed the inclusion complexes with the Praziquantel (Becket et al., 1999).On the basis of particle size analysis and solubilisation efficiency of different type of nanosponges, NS (1:4) was selected for further studies.

4.3.4. Encapsulation Efficiency (EE %)

The drug at two different weight ratios 1:5 and 1:10 (Drug: NS by weight) was loaded in NS (1:4) and the entrapment efficiency NS was calculated. From the Table 4.4, it can be observed that in the case of 1:10 w/w drug loading, the particle size was found to be 526.7 ± 18.4 nm while in case of 1:5 w/w drug loading, it was 417.3 ± 13.1nm. However, 1:5 drug loaded NS shown encapsulation efficiency of 26% while encapsulation efficiency was 31 % in 1:10 drug loaded NS

Table 4.4: Particle size analysis and EE of different batches of Drug loaded nanosponges

Batches	Size (nm)	PI	ZP	EE
NS (1:4) (1:5 w/w)	417.3 ± 13.1	0.122	-21.55 ± 1.3	26%
NS (1:4) (1:10 w/w)	526.7 ± 18.4	0.098	-24.90 ± 2.5	31%

Mean ± SD; n = 3

This may be due to increase in the solubility of PZQ as the NS concentration increases in the system up to 1:5 weight ratios (drug, NS). Above this concentration, there is no significant enhancement in solubility of PZQ might be because of saturation solubility of PZQ in NS.

4.3.5. FT-IR studies

The FTIR spectra of plain β-CD, plain β CD nanosponge, pure PZQ and PZQ-NS have been shown in Figure 4.3 (a-d). Nanosponge formation was confirmed by FTIR analysis. The blank nanosponge spectrum showed the presence of the carbonate bond as evidenced by a peak at around 1700 to1750 cm^{-1} corresponding to the stretching of carbonyl which was not present in the parent β-cyclodextrin spectrum (Figure 4.3a,4.3b). In addition, the other characteristics peak of NS were found at 2928 cm^{-1} due to the C– H stretching vibration, 1400 cm^{-1} due to C–H bending vibration and 1028 cm^{-1} due to C–O stretching vibration of primary alcohol. The pure PZQ sample (Figure 4.3c) showed characteristic peaks at 2930 and 2852 cm^{-1}, due to the C–H and $C–H_2$ stretching vibration, and at 1649–1627 cm−1, due to the amide stretching vibrations carbonyl –C=O stretching (1630 cm^{-1}), –CH, –CH2, –CH3 stretching (2900-3000 cm^{-1}), –C–N stretching (1000-1350 cm^{-1}). Moreover, the peaks in the region 3300–3700 cm^{-1} and in the fingerprint region below1500 cm^{-1} confirms the racemic form of the drug (Liu et al., 2004). The absorption bands in 1200 – 800 cm^{-1} suggest C-H stretching vibrations and bands at 1250 - 1020 cm^{-1} shows C-N stretching vibrations. The medium absorption band at 997 cm^{-1} show out of plane C-H bending vibrations. FTIR spectra of PZQ-NS(Figure 4.3d) showed that there were interactions between NS and PZQ that were evident from broadenings and disappearance of PZQ peaks. All the sharp peaks belonging to the NS were present while only few characteristics peak of PZQ are visible in FTIR spectra of PZQ-NS. The

FTIR spectra of PZQ-NS showed major changes in the fingerprint region i.e. 800 to 1400 cm^{-1}. There is intense peak due to O-H stretching typical to the carbohydrates in the spectrum of NS was shifted to 3273 cm^{-1}in the complex indicating there is interaction of O-H groups of NS with Praziquantel. However, the main characteristic peaks of PZQ were broadened or shifted in the PZQ -NS due of complexation of PZQ with NS, suggested definite interactions between drug and NS. The thermal analysis of drug- loaded NS confirmed the drug complexation.

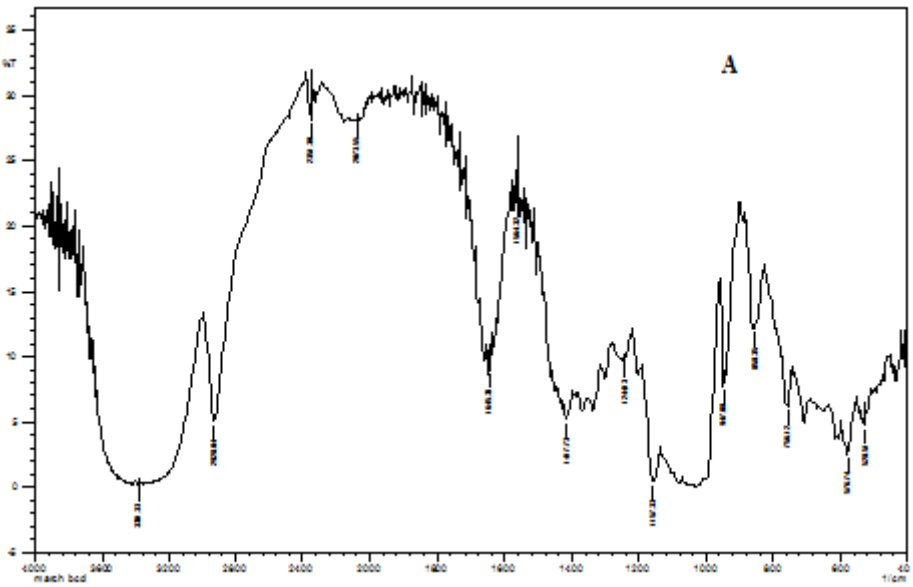


Figure 4.3(a): FT-IR of pure β-CD

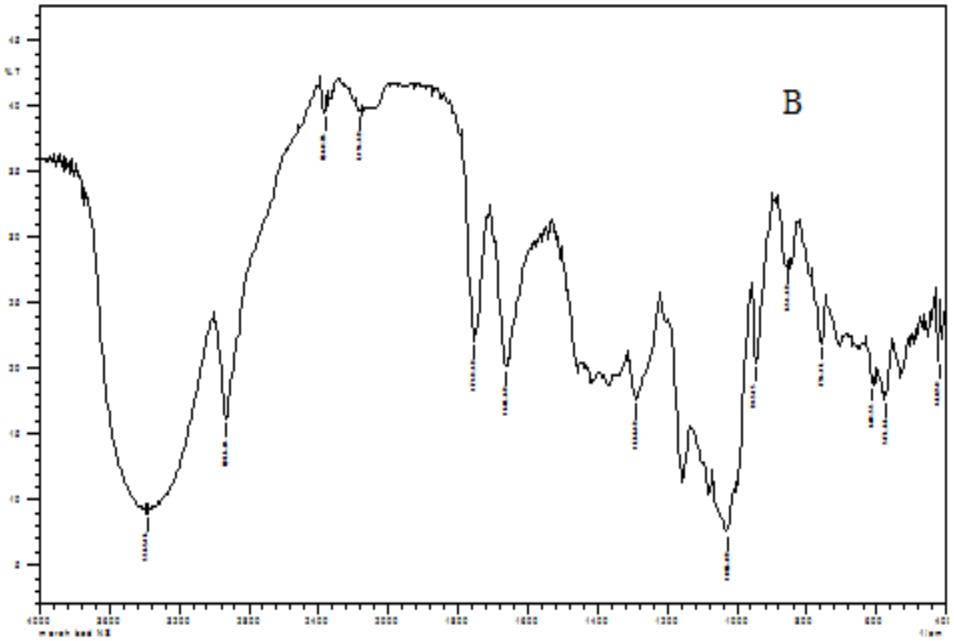


Figure 4.3(b): FT-IR of β-CD Nanosponges

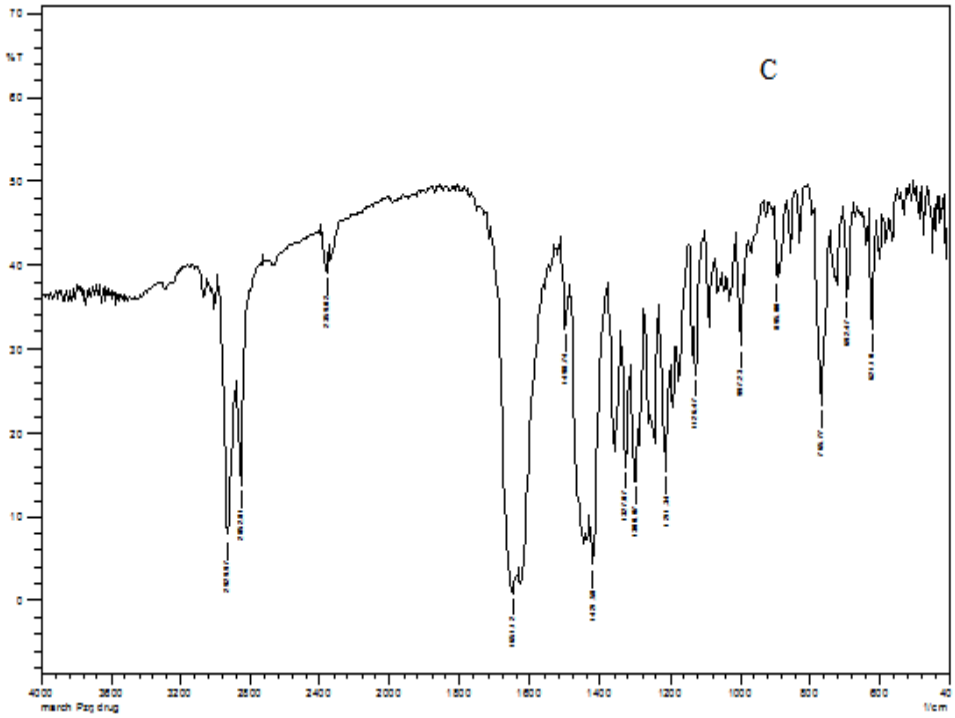


Figure 4.3(c): FT-IR of pure PZQ

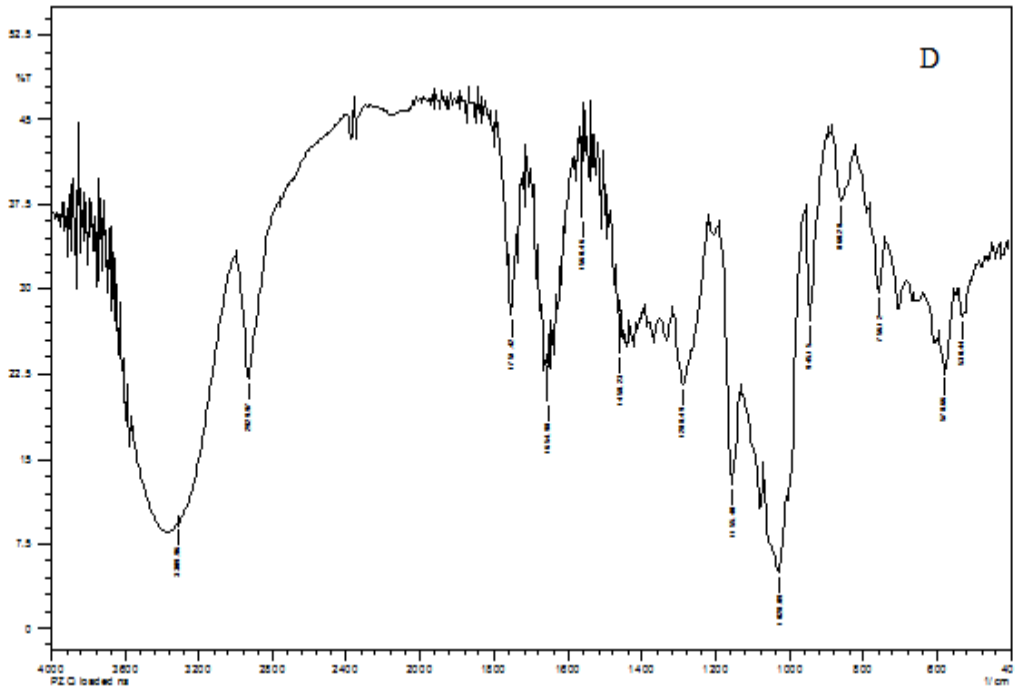


Figure 4.3(d): FT-IR of PZQ-NS

4.3.6. DSC studies

DSC thermograms of pure pzq, plain β CD-NS and PZQ-NS have been shown in Figure 4.4. The physical state of drug in the polymeric matrix influences drug release. The pure PZQ shows a sharp endothermic peak that corresponds to melting at 143 °C, indicating its crystalline nature. The disappearance of this characteristic peak in the PZQ-NS formulation indicates that PZQ was well complexed with NSs. This might also be because of inclusion as well as non-inclusion phenomenon of NS with PZQ.

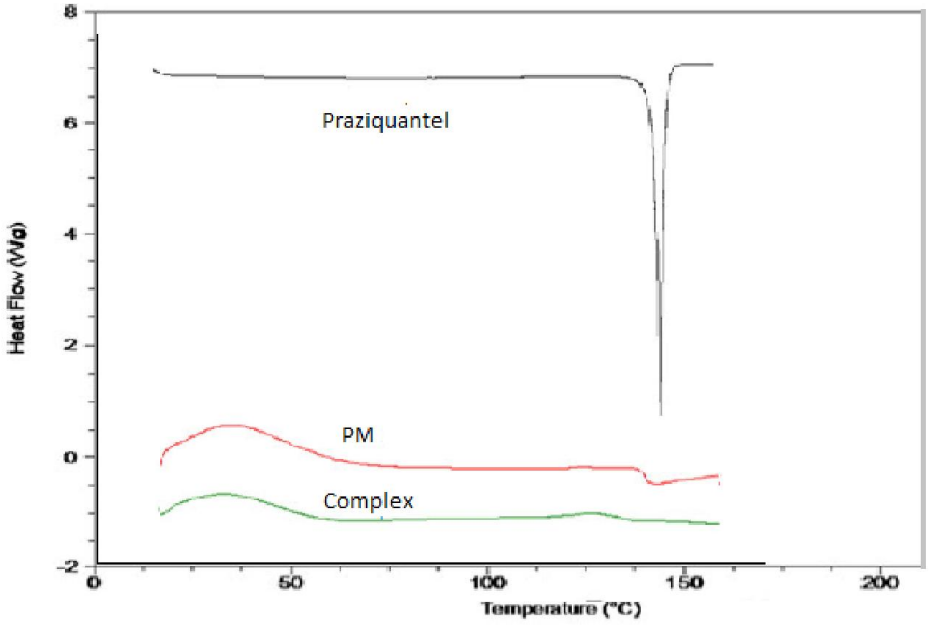


Figure 4.4: DSC thermograms of PZQ, Physical Mixture and Drug NS complex

4.3.7. XRD studies

XRD studies were carried out to consolidate the DSC data indicating the reduction of the crystallinity of PZQ in nanosponges. XRD patterns of pure PZQ, β-CD, blank nanosponges and PZQ-NS were shown in Figure. 4.5(a-d). Powder X-Ray diffraction patterns, displayed in Figure 4.5(a), indicate that β-CD itself is crystalline in nature and have characteristic peaks in its diffractograms, The characteristics peaks of PZQ demonstrated the high crystalline structure in Figure 4.5(c). The comparison of PZQ with their PZQ -NS showed that there was a significant change in the intensities of powder, 2θ values (Figure 4.5d). XRD studies revealed the formation of a new state that is poorly crystalline in nature. It can also be seen from the reduced number of peaks and reduced peak areas indicating that PZQ

encapsulated in NS and is in the disordered crystalline phase or amorphous or in the solid-state solubilized form in the polymeric matrix (Swaminathan *et al*., 2012). When the drug is in amorphous or in disordered crystalline phase, the drug molecules can easily diffuse through the polymeric matrix, leading to a controlled release of the encapsulated drug (Darandale *et al.*, 2012).

Different packing of PZQ in the crystal structure of NS complexes might affect physical and pharmaceutical properties of PZQ as drug loading, drug stability and drug release. The observed differences could be related to the presence of channels or cavities running through the NS crystal structure and working as sites for PZQ molecules besides cyclodextrin cavities. When the structure of the NS collapses, the beehive-like structure of the complex fails, and the PZQ molecules lose their preferential crystallographic sites (Shanker et al., 2012).

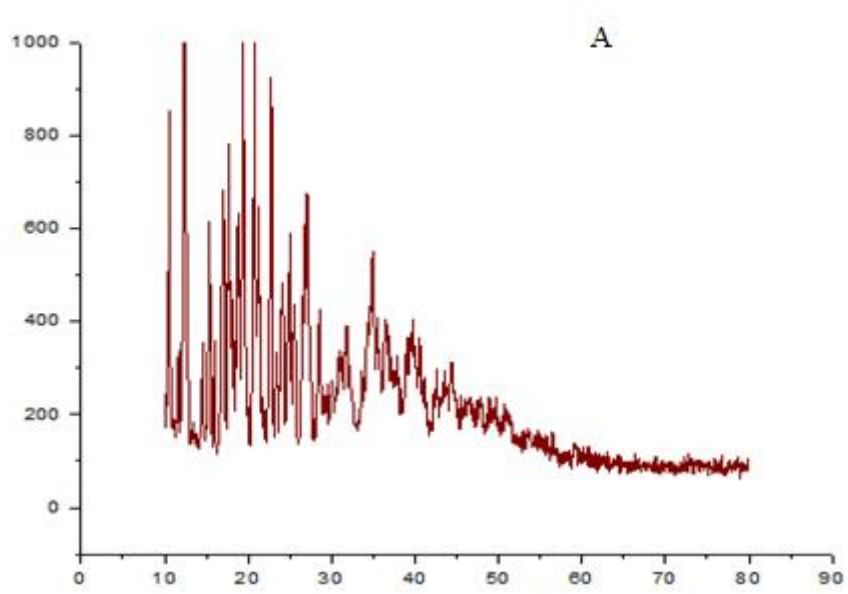


Figure 4.5(a): XRD spectra of β- CD

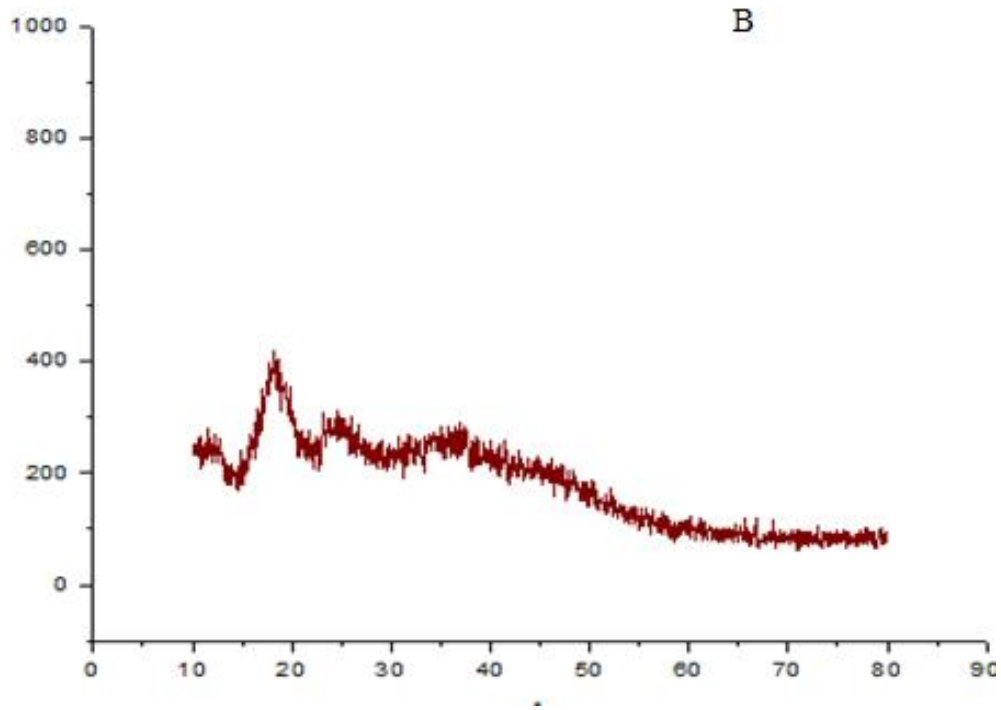


Figure 4.5(b): XRD spectra of β- CD Nanosponges

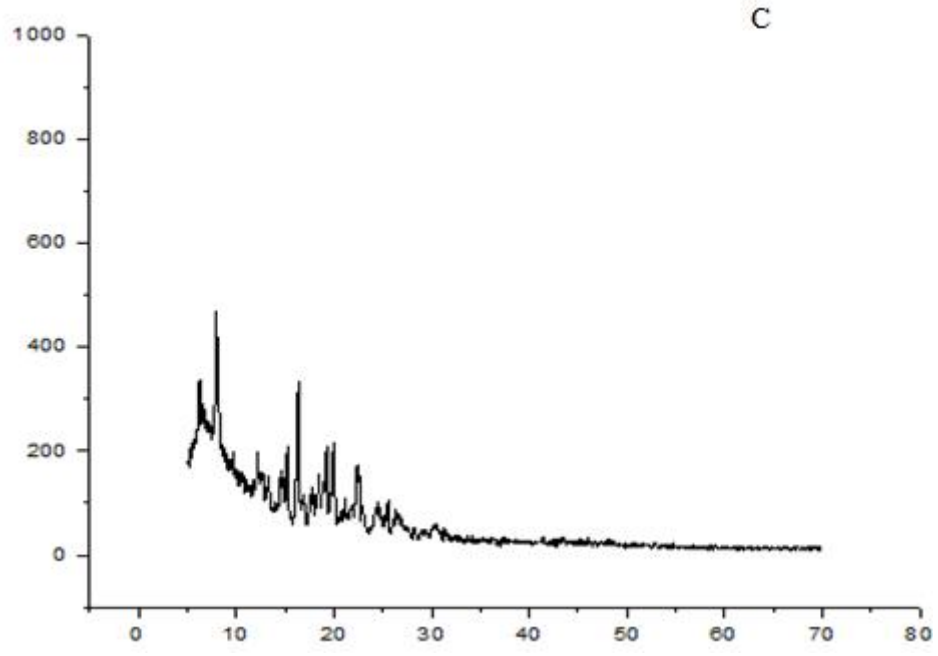


Figure 4.5(c): XRD spectra of PZQ

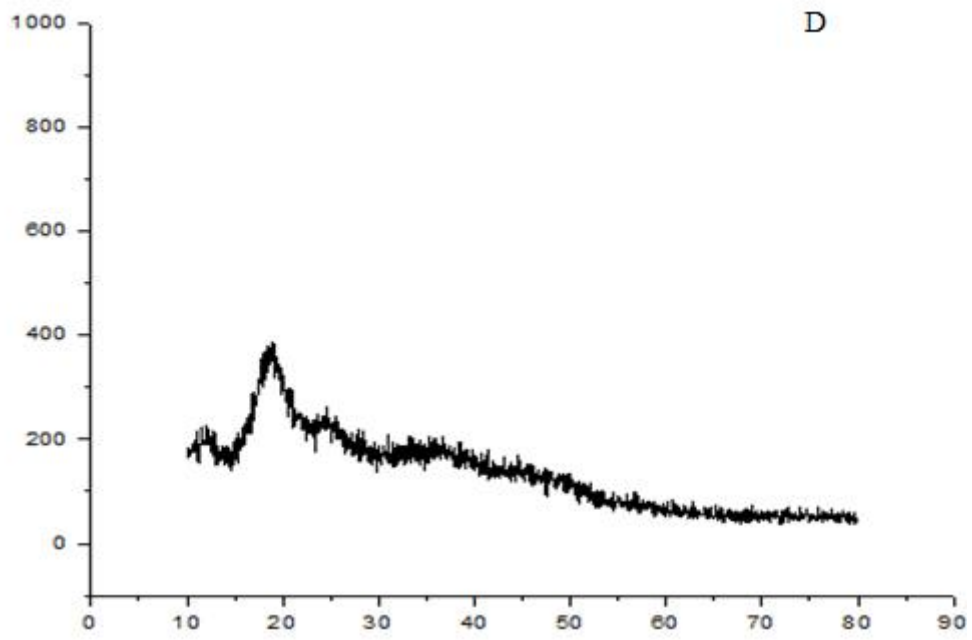
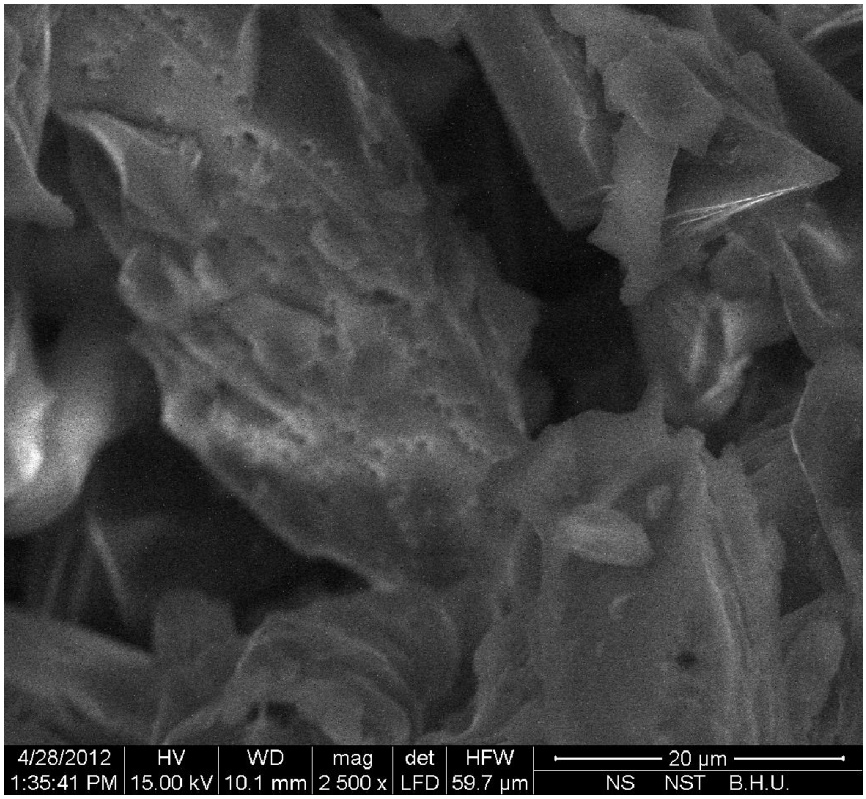


Figure 4.5(d): XRD spectra of PZQ loaded NS

4.3.8. Morphological evaluation

TEM measurement also revealed a particle size around 400 nm for NS complex. The results obtained by dynamic light scattering were in agreement with the TEM studies and showed sizes between 400 to 600 nm, for drug loaded NS. TEM analysis of the nanosponge evidenced a quite spherical morphology (Figure 4.7). SEM studies showed that the nanosponges are having a cluster forming capacity when suspended in water and have a highly porous crystalline structure (Figure 4.6).



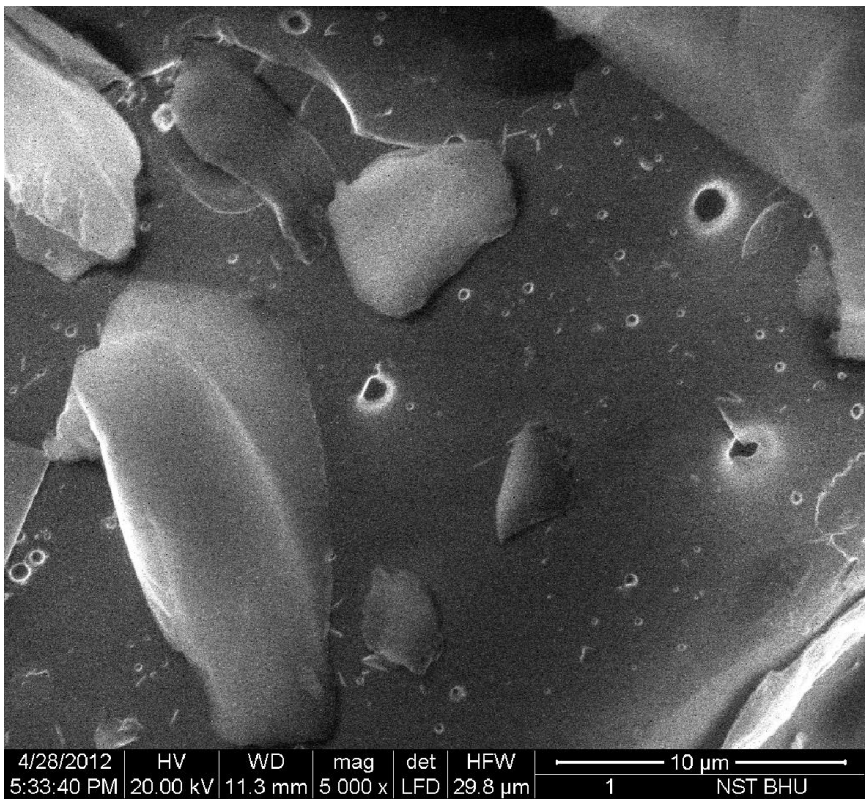
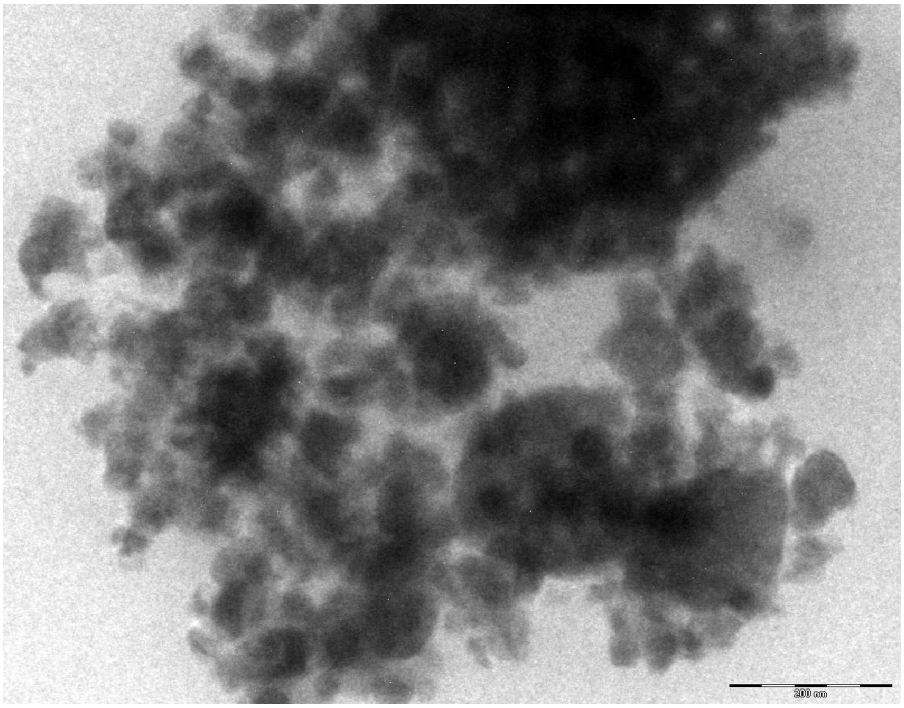


Figure 4.6: SEM of (a) NS; (b) PZQ loaded NS

(a)



(b)

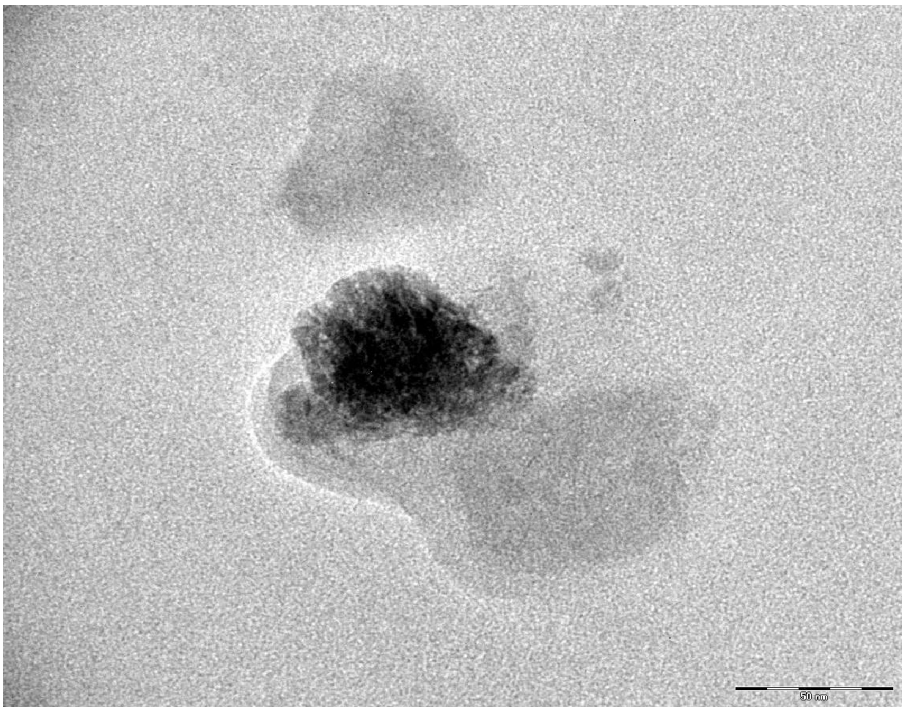


Figure 4.7: TEM of (a) NS; (b) PZQ loaded NS

4.3.9. In vitro release studies

The in vitro release profile of pure PZQ and PZQ-NS in 0.1N HCl and phosphate buffer (pH6.8) has been shown in Figure 4.8.It is evident from

the release profiles that as compared to plain PZQ, nanosponge complexes showed faster release in 0.1NHCl(Figure 4.8a).

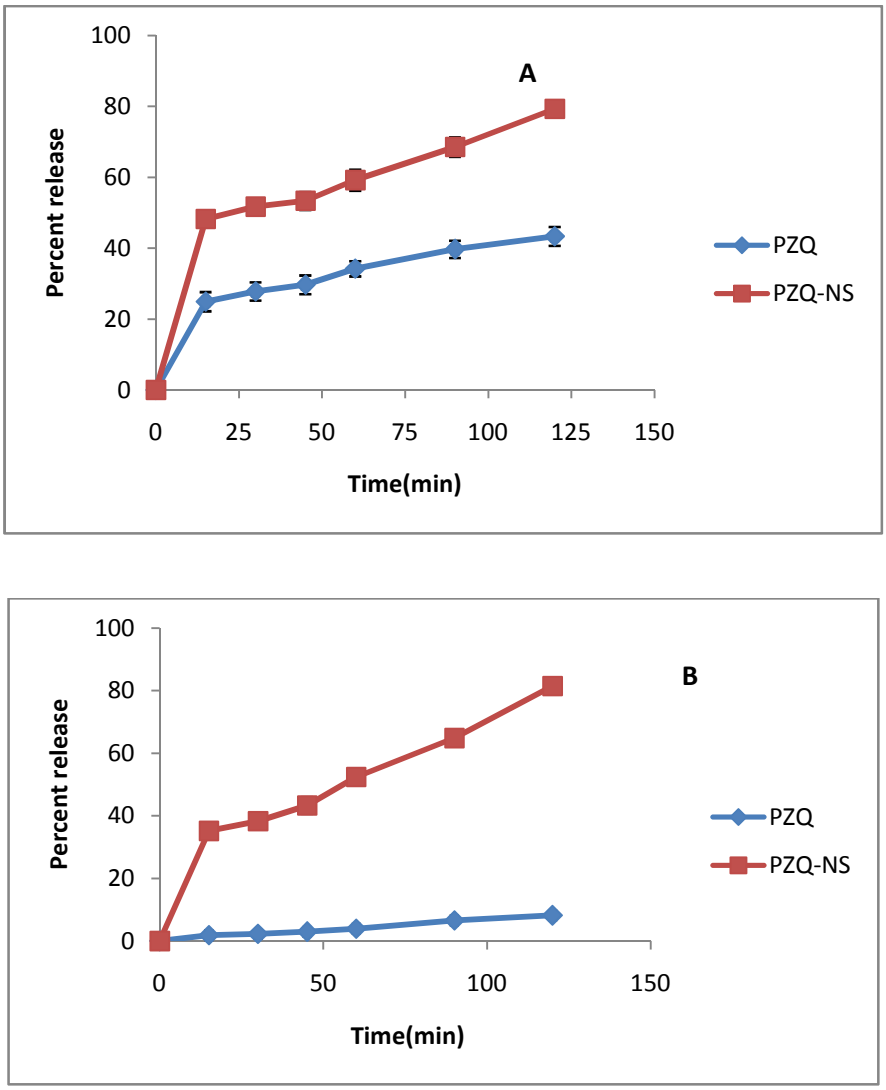


Figure.4.8: Dissolution profiles of PZQ and PZQ-NS in (a) 0.1 N HCl and (b) phosphate buffer pH 6.8

The initial burst effect seen in PZQ NS was probably due to dissolution of PZQ which is not present in the formulations as inclusion complex, but is adsorbed or encapsulated as non-inclusion complex on the NS complexes surfaces. After the initial effect, a linear and controlled release profile of the drug was observed. Pure PZQ release profiles were not depended on

the physiological medium, suggesting that PZQ show pH independent solubility Over a period of 2 h, 85% drug was released in 0.1 N HCl in case of PZQ-NS complexes (Figure 4.8 a). The release study in phosphate buffer (pH 6.8) indicated that almost 83% drug was released during the period of 2 hours (Figure 4.8 b). It can be inferred from this that crystalline complexes of guest molecule (PZQ) are formed within the intricate network of nanochannels present in NS.

4.3.10. Bioanalytical method

Figure 4.9 shows the representative chromatogram of plasma sample spiked with 6400 ng/ml. The run time was short requiring only 10 min (retention time for PZQ was 4.64 ± 0.1 min) to complete the entire run for each sample. Short retention time is generally considered suitable for quick plasma sample analysis.

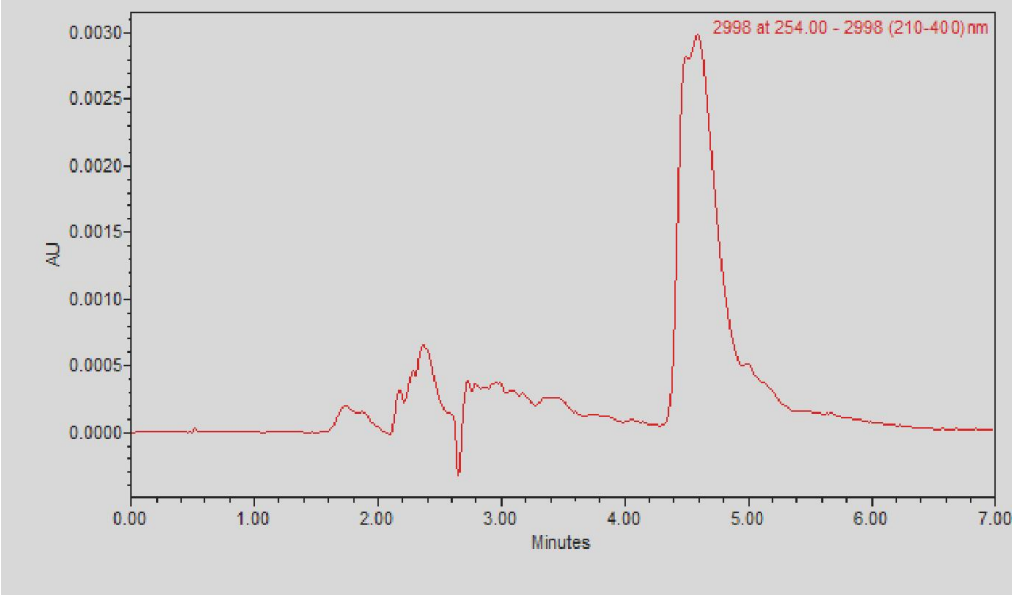


Figure 4.9: HPLC chromatogram for praziquantel

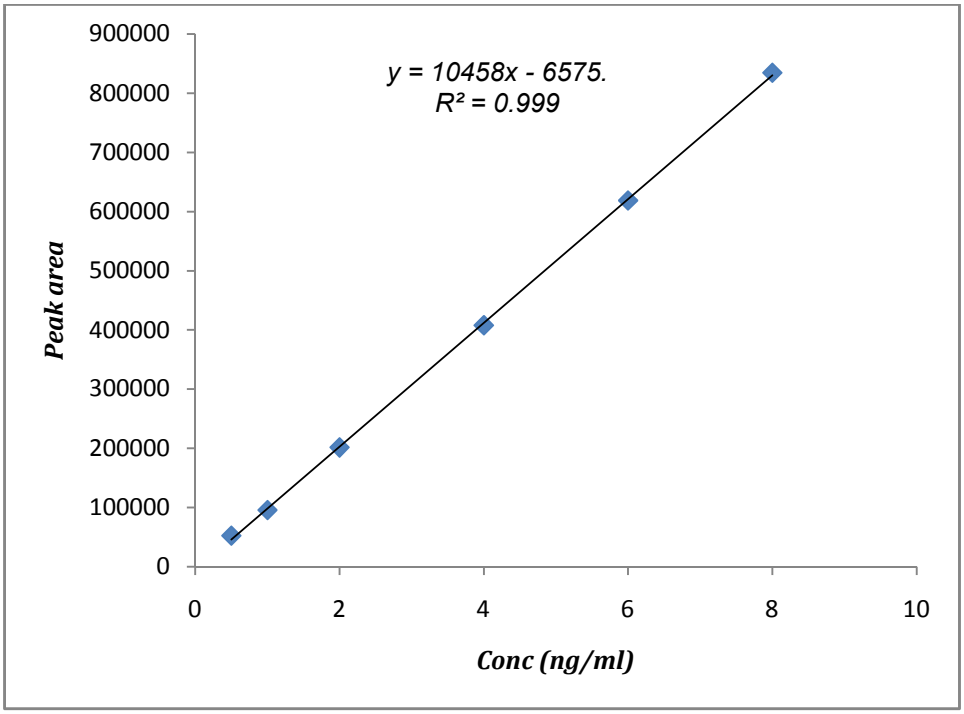


Figure 4.10: Calibration curve for praziquantel

Linear calibration curves for PZQ in the range of 0.025 – 8 ng/ml were constructed from a serial of blank plasma spiked with six different concentrations. The regression equation obtained was; y = 10458x - 6575. The plasma PZQ concentration was found to be linear over the range of 0.025ng/ml to 8ng/ml. The mean correlation coefficient (R^2) was 0.999 (Figure 4.10).

The inter-day and intra-day precision (CV %) were both below 10% of the actual value. The mean recovery of PZQ from plasma was 94.58 ± 6.31%. The limit of detection (LOD) was 0.0075 ng/ml and the limit of quantification (LOQ) was 0.025 ng/ml. (Table 4.5)

Table 4.5: Validation parameters for HPLC bioanalytical method

Validation parameter	Result
Standard regression Equation	y = 10458x - 6575
Regression coefficient(R^2)	R^2=0.999
Accuracy (%recovery ± SD)	94.58 ± 6.31%.
Precision intra-day and inter-day (%CV)	both below 10% of the actual value
Linearity	0.025–8 ng/ml
LOD	0.0075 ng/ml
LOQ	0.025 ng/ml

4.3.11. In vivo studies

The mean plasma concentration time curve for PZQ suspension and PZQ NS is showed in Figure 4.11. It can be evident from Table 4.6 that the C_{max} was enhanced from 2.28 µg/ml for plain PZQ to 3.61 µg/ml for PZQ-NS. Moreover, The MRT value was significantly increased from 2.67 hours to 7.13 hours for PZQ suspension and PZQ NS, respectively. A 3.26 fold increase in AUC was observed in case the PZQ-NS complex (Table 4.6).

The relatively higher $AUC_{0-\infty}$ displayed by the NS complex corroborates in vitro findings that NS have the ability to form crystalline complexes with PZQ. The nanocavities within the NS structure facilitate entrapment of the drug molecule as well as permit a controlled release under in vivo conditions.

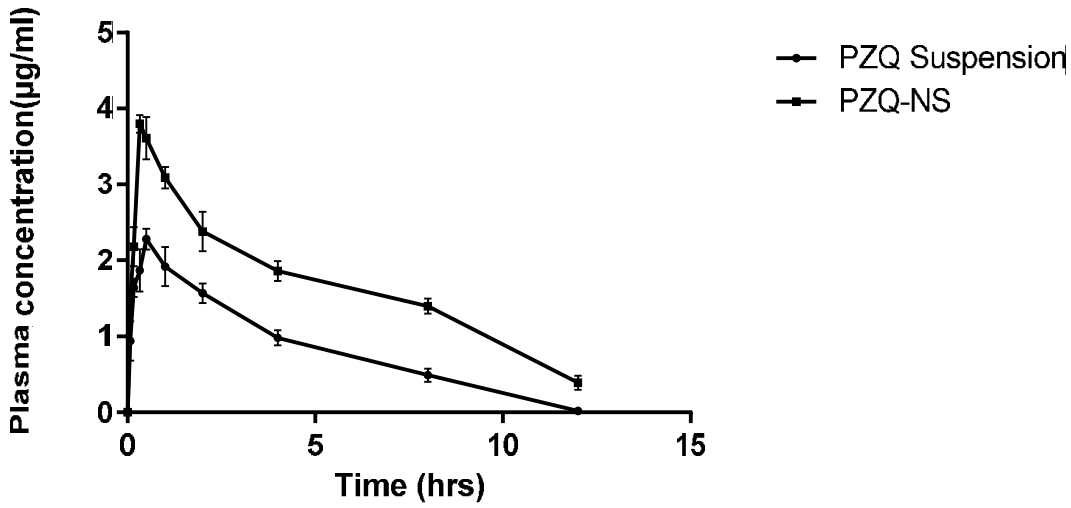


Figure.4.11: Plasma concentration time profile of PZQ suspension and PZQ-NS in rats

The pharmacokinetic parameters showed improved bioavailability of PZQ with NS as compared to pure drug (Table 4.6).

Table 4.6: Pharmacokinetic parameters for praziquantel in rats after oral administration of PZQ-NS and PZQ suspension(0.5% w/v Methyl Cellulose suspension of pure drug)at an equivalent praziquantel dose of 50 mg/kg (values are reported as mean ±s.d., n=6)

Pharmacokinetic parameters	**PZQ Suspension**	**PZQ-NS**
$AUC_{0-\infty}$(µg h/ml)	3.86±0.51	12.61±1.57
Cmax(µg/ml)	2.28 ±0.24	3.61±0.29
Tmax(h)	0.5±0.08	0.5±0.08
MRT(h)	2.67±0.82	7.13±0.68