Research Article

Formulation and evaluation of nanosponge gel containing ketoconazole

Umesh T. Jadhao, Rathod P. Sayali, Dhembre N. Gunesh, Sable D. Shital, Lokhande S. Sneha

Department of Pharmaceutics, SDMVM's SVP College of Pharmacy Hatta, Basmath, Hingoli, Maharashtra, India

Correspondence:
Umesh T. Jadhao, Department of Pharmaceutics, SDMVM's SVP College of Pharmacy Hatta, Basmath, Hingoli, Maharashtra, India.
E-mail: umj81@rediffmail.com

How to cite this article:
Jadhao UT, Sayali RP, Gunesh DN, Shital SD, Sneha LS. Formulation and evaluation of nanosponge gel containing ketoconazole. Innov Pharm Pharmacother 2021;9(1):15-24.

Source of Support: Nil.
Conflicts of Interest: None declared.

ABSTRACT

Aim: The aim of present work is to successfully formulate, evaluate and optimize nanosponges of ketoconazole drug for its efficient delivery through gel base. **Methodology:** Nanosponges were prepared using hyper cross-linked β-cyclodextrin method using different concentration of cross-linker. Diphenyl carbonate was used as the cross-linking polymer. Nanosponge formulations were prepared using β-CD:cross-linker ratios of 1:15–1:3. **Results:** The prepared nanosponges were evaluated for percentage yield, incorporation efficiency, particle size, drug polymer compatibility, scanning electron microscopy (SEM), and *in vitro* drug release. SEM studies confirmed their porous structure with number of nanochannels. The Fourier transform infrared spectra showed stable character of ketoconazole in mixture of polymers. Differential Scanning Calorimetry study revealed that drug was involved in complexation with nanosponges. The average particle size of nanoparticles was found to be 78.81 ± 0.20 nm–336.02 ± 0.124 nm. The drug release from nanosponges was found to extend up to 8 h 82–92%. The nanosponges were formulated into gel using Carbopol 940 Batches G1 to G4 and were prepared by incorporating nanosponges equivalent to 6% w/w of ketoconazole in different polymer concentrations, respectively, and evaluated for percent drug content, viscosity study, spreadability study, and *in vitro* diffusion studies. Drug diffusion from the nanosponge loaded gel formulations was show sustained rate. **Conclusion:** A sustained release topical drug delivery of ketoconazole developed as a nanosponge loaded gel offers solubilizing matrix for the drug, served as a local depot for sustained drug release, and provided a rate limiting matrix barrier for modulation of drug release.

Keywords: Ketoconazole, nanosponges, drug diffusion, β-cyclodextrin

Introduction

The nanosponges are tiny mesh-like structures in which a large variety of substances can be encapsulated.[1,2] They have a proven spherical colloidal nature, reported to have a very high solubilization capacity for poorly soluble drugs by their inclusion and non-inclusion behavior.[3] Nanosponges have recently been developed and proposed for drug delivery. Nanosponges can solubilize poorly water soluble drug and provide prolonged release as well as improving bioavailability of drugs.[4] Nanosponges are able to load both hydrophilic and hydrophobic drug molecules because of their inner hydrophobic cavities and external hydrophilic branching, thereby offering unparalleled flexibility.[5] Nanosponges are more like a three dimensional network or scaffold. The backbone is a long length of polyester which is mixed in solution with small molecules called cross-linkers that act like tiny grappling hooks to fasten different parts of the polymer together.[6] Nanosponges show a remarkable advantage in comparison with the common nanoparticles. Indeed, they can be easily regenerated by different treatments, such as washing with eco-compatible solvents, stripping with moderately inert hot gases, mild heating or changing pH or ionic strength. For all these characteristics, nanosponges have been already employed in different applied fields, such as cosmetic and pharmaceutical sectors.[7,8]

Ketoconazole is antifungal drug often used in the treatment of fungal infection of skin such as athletes foot, jock itch, ringworm, candidiasis, and seborrhea. It has pH-dependent solubility and permeability. The drug has a half-life of 1–2 h. Because of its short biological half-life the drug has to be administered frequently. Furthermore, oral ketoconazole causes irritation in gastric mucosal membrane and possess a bitter taste and after taste. Therefore, present work aims at designing novel nanosponges as carriers for topical delivery of ketoconazole which

Access this article online

Website: www.innpharmacotherapy.com

e-ISSN: 2321-323X
p-ISSN: 2395-0781

minimizes its gastrointestinal side effects and provides consistent drug levels at application site for longer period of time.

Materials and Method

Material

Ketoconazole was obtained as kind gift sample from Zim Pharmaceuticals, Ltd. Nagpur India. B-cyclodextrin and Carbopol 940 was purchased from Research-lab, Mumbai, India. All other materials used of analytical grades.

Method

Formulation of nanosponge[9,10]

Nanosponges were prepared using hyper cross-linked β-cyclodextrin method using different concentration of cross-linker. In this method, anhydrous Dimethyl sulfoxide was placed in round bottom flask and anhydrous β-cyclodextrin was added to achieve complete dissolution. Then diphenylcarbonate was added and the solution was allowed to react for 4 h at 100°C. Once the condensation polymerization was completed, the transparent block of hyper-cross-linked β-cyclodextrin was roughly ground and excess of deionized water were added to remove Dimethyl sulfoxide. Finally, residual byproduct or unreacted reagent was completely removed by Soxhlet extraction with ethanol, the white powder thus obtained was dried overnight in an oven at 60°C and subsequently ground in a mortar. The fine powder obtained was dispersed in water. The colloidal part that remained suspended in water was recovered and lyophilized

Evaluation of ketoconazole loaded nanosponge

Drug loading (DL) determination[11]

An accurately weighed amount of ketoconazole loaded nanosponge (100 mg) was dissolved in 100 ml pH 7.4 phosphate buffers. The nanosponge was soaked for 24 h with stirring. The solution was filtered through Whatman filter paper and analyzed by ultraviolet (UV) Spectrophotometer at 292 nm. DL was calculated according to following equation-

$$\text{Drug loading} = \frac{\text{Actual drug content nanosponges}}{\text{Total amount of nanosponges}} \times 100$$

Entrapment efficiency (EE) determination[12]

The weighed sample of drug loaded nanosponges (30 mg) was dissolved in 100 ml, 7.4 phosphate buffer under magnetic stirrer for 4 h at 30°C. The sample was filtered and sample was read out at 292 nm against blank using spectrophotometer. Moreover, EE was determined using the following formula-

$$\%\ \text{EE} = \frac{\text{Actual drug content nanoponges}}{\text{Theorotical drug content}} \times 100$$

Production yield[12]

Production yield of nanosponges was determined by calculating accurately the initial weight of raw materials and last weight of microsponges obtained. It was determined using following equation.

$$\text{Production yield} = \frac{\text{Practical mass of nanosponges}}{\text{Theorotical mass(drug+polymer)}} \times 100$$

Determination of particle size[13]

The particle size of nanosponge of different batches was measured by dynamic light scattering using a 90 plus particle sizer (Brookhaven Instruments Corporation), with MAS Option particle sizing software was used at the fixed angel of 90°. Particle sizes of each batch and of nanosponge with drug F1 to F4 and without drug batches NS1 to NS4 were determined 3 times and mean values were taken

Surface morphology[14]

The surface morphology of the optimized formulation was examined by scanning electron microscopy (SEM). Nanosponges were spread on a doubled sided adhesive plate, one side of which was stuck to glass slide. Excess nanosponges were removed and the slide was kept on sample holder and SEM was taken using an electron microscope. The SEMs were taken as shown as shown in Figure.

Differential scanning calorimetry (DSC)[15]

A DSC was used for thermal analysis of drug and physical mixture. Drug and its physical mixture were weighted directly in the pierced DSC aluminum pan (Aluminum Standard 40 µl) and scanned at the temperature range of 25–400°C and at heating rate of 10°C/min. in nitrogen atmospheres at flow rate of 20 ml/min, thermogram obtained were observed for any interaction.

Fourier transform infrared (FTIR) spectroscopy

It is important to check any kind of interaction between drug and polymer. It was done using FTIR Spectroscopy. IR has been the method of choice to probe the nature and extent of interaction in polymer blends. The premise of using an IR to study in the polymer blend is that the mixture of two components at molecular level will cause changes in oscillating dipoles of the molecules. This manifest itself as changes in frequency and bandwidth of interacting group, in the spectrum if the drug and polymer interact then functional group in FTIR spectra will show band shifts and broadening compared to the spectra of pure drug. Ketoconazole was mixed thoroughly with potassium bromide as shown in Figures 1-4. This physical mixture was converted in a circular disc. This disc was then placed in the scanning slot of FTIR and scanned between 4000 cm^{-1} and 400 cm^{-1} to obtain the FTIR of ketoconazole.

In vitro drug release study[16,17]

In vitro release study of ketoconazole nanosponge was carried out using USP type 1 apparatus. Nanosponge's equivalent to 500 mg of Ketoconazole were weighed accurately and placed in basket. The dissolution medium used was 900 ml of 7.4 phosphate-buffered maintained at 37 ± 1° and stirred at 150 rpm. 5 ml of the dissolution medium was sampled at certain intervals; fresh dissolution medium was simultaneously replaced in the apparatus to keep the volume constant. The withdrawn samples were filtered and filtrate was assayed spectrophotometrically at 292 nm.

Composition of ketoconazole loaded nanosponge gel[18-20]

Accurately weighed quantity of Carbopol 940 was dissolved in water using stirrer. In another beaker, nanosponges containing ketoconazole (equivalent to 6% w/w) drug dissolved in dimethyl sulfoxide and added to Carbopol solution under continuous stirring, followed by addition of polyethylene glycol 400. The Carbopol solution was neutralized by slowly adding triethanolamine with constant stirring until gel is formed. pH of final gel was determined.

Evaluation of nanosponges loaded gel

Drug content[21,22]

1.0 g of each gel formulations were taken in 100 ml volumetric flask containing 20 ml of phosphate buffer pH 7.4 and stirred for 30 min and allowed to stand for 24 h in case of nanosponge loaded gel formulations. The volume was made up to 100 ml with phosphate buffer. Proper dilutions were made and the formulation was subjected to the spectrophotometric analysis. The content of drug was estimated spectrophotometrically by using standard curve plotted at λ_{max} 292 nm.

pH study[23]

pH of the various gel formulations were determined using digital pH meter. The measurement of pH of each gel was done in triplicate and average values were calculated.

Viscosity study[24]

Viscosity of prepared gel was measured by Brookfield viscometer. The gels were rotated at the speed of 10 rotations/min with spindle no. 3.

Spreadability[25]

Spreadability of formulations was determined by an apparatus suggested by Multimer *et al*. which was fabricated in laboratory and used for study. The apparatus consists of a wooden block with a fixed glass slide and movable glass slide with one end tied to weight pan rolled on the pulley, which was in horizontal level with fixed slide.

Rheological behavior[26]

The rheology of prepared formulations was studied using Brookfield viscometer (CAP-2000). The sample was placed on temperature

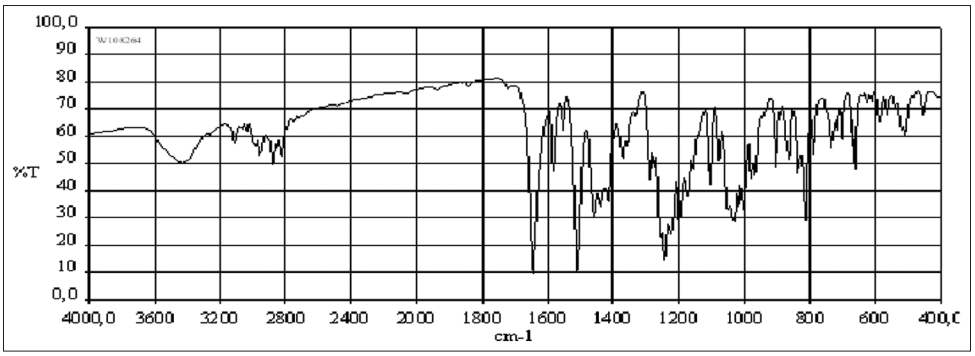

Figure 1: Fourier transform infrared spectral analysis of ketoconazole

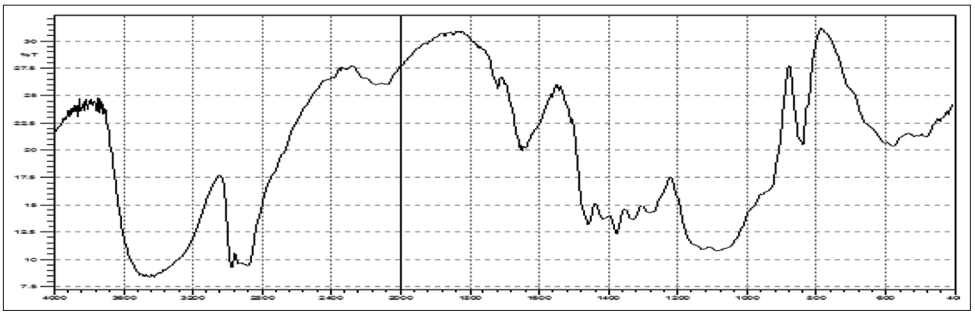

Figure 2: Fourier transform infrared spectrum of diphenyl carbonate

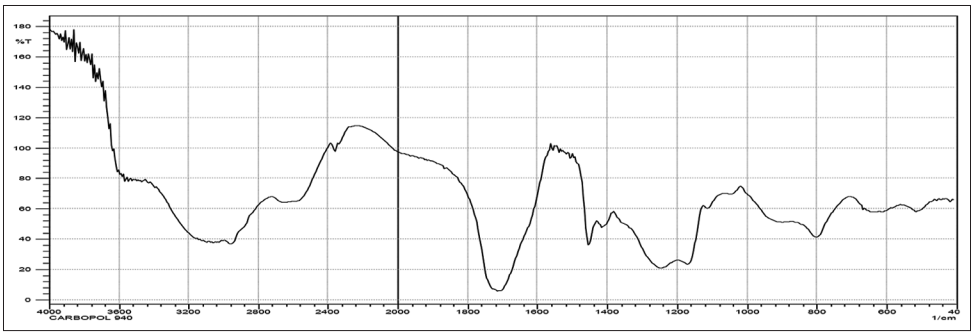

Figure 3: Fourier transform infrared spectrum of Carbopol

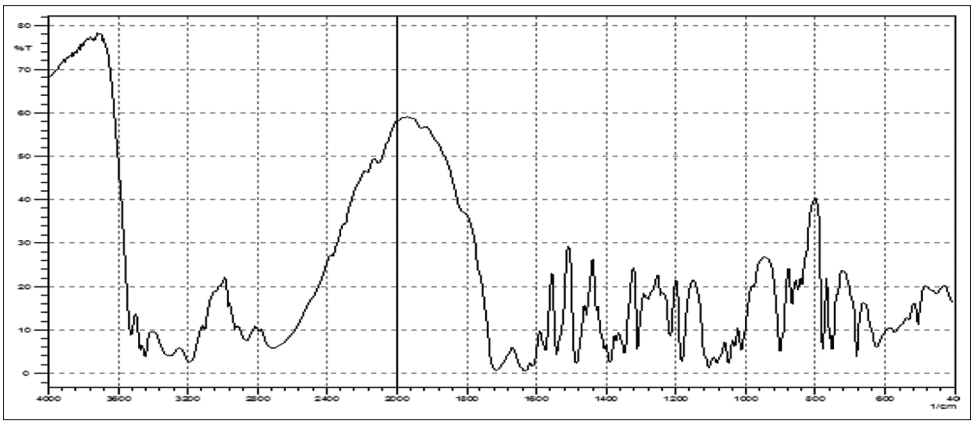

Figure 4: Fourier transform infrared spectrum of physical mixture of ketoconazole

sensitive plate. Temperature was kept at 37 ± 1°C. Cone no. 3 was held on the plate and speed of 10, 20, 30, 40, 50, 60, 70, and 80 rpm were selected. Different viscosities at respective spindle speeds were obtained for an ascending and descending curve.

In vitro diffusion studies[19,27]

The release of ketoconazole from optimized nanosponge gel was determined by membrane diffusion technique using Franz diffusion cell. The nanosponge gel equivalent to 5% w/w of ketoconazole was taken in donor compartment. The donor and receptor compartments were separated by synthetic cellophane membrane. The synthetic cellophane membrane was mounted between donor and receptor compartment of cell. The receptor medium was filled with phosphate buffer pH 7.4. The assembly was stirred at 200 rpm and receptor compartment was replenished with equal volume of phosphate buffer. Aliquots each of 1 ml was withdrawn periodically at an interval of 1, 2, 3, 4, 5, 6, 7, and 8 h and replaced by an equal volume of receptor medium. The aliquots were suitably diluted with receptor medium and analyzed by UV-visible spectrophotometer.

Stability study[28]

The optimized formulation of ketoconazole loaded nanosponge gel was packed in aluminum collapsible tubes and subjected to stability studies at 40 ± 2°C/75 ± 5% RH for a period of 3 months. Formulations were evaluated at periodic intervals for pH, viscosity, and drug content and drug release profiles.

RESULTS

FTIR study

FTIR study of ketoconazole displayed characteristic peaks of C=O stretching vibration of carbonyl group, C-O stretching of aliphatic ether group, and C-O stretching of cyclic ether at 1646.12 cm^{-1}, 1106.27 cm^{-1}, and 1245.12 cm^{-1}, respectively. Physical mixture of drug and polymer was characterized by FTIR spectral analysis for any physical as well as chemical alteration of drug characteristic. From results, it was concluded that there was no interference in the functional group as the principal peaks of ketoconazole were found to be unaltered in the drug polymer physical mixture.

Table 1: Composition of various drug-loaded nanosponge batches

Ingredients	F1	F2	F3	F4
Ketoconazole	33.33	50	100	166.66
B-cyclodextrin	100	100	100	100
Diphenyl carbonate	200	400	600	800
DMSO	100	100	100	100
Ethanol	q.s	q.s	q.s	q.s

Table 2: Composition of ketoconazole loaded nanosponge gel

Ingredients	G1	G2	G3	G4
Nanosponge (F3)	425	425	425	425
Carbopol 940 (%)	0.25	0.50	0.75	1.0
Methanol	2	2	2	2
Polyethylene glycol	1	1	1	1
Triethanolamine	q.s	q.s	q.s	q.s
Distilled water	10	10	10	10

DL and EE

It was found that the DL of the batches F1to F4 ranged from 39.2 ± 0.22 to 87.3 ± 0.45% w/w. From Figure 5, it was found that EE of batches ranged from 22.37 ± 0.25 to 82.71 ± 0.71% w/w. It was observed that as drug:polymer ratio was increased, DL and EE of ketoconazole loaded nanosponges also increased. Figure 6 indicates that loading efficacy of ketoconazole loaded nanosponge of batch F4 showed high loading compared with other batches may be because of high degree of cross-linking ratio.

At all the ratios of drug:polymer employed, the mean amount of drug entrapped in the prepared nanosponges was lower than the theoretical value, since the DL did not reach 100%.

Production yield

The production yield of batches from F1 to F3 ranged from 13.63 ± 0.41% to 52.82 ± 0.54% [Tables 1 and 2 and Figures 7 and 8]. Increase in the drug:polymer ratio increased the production yield.

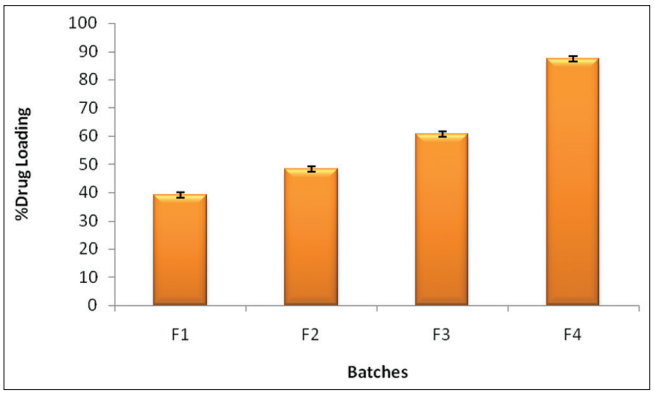

Figure 5: Percent drug loading of batches F1 to F4

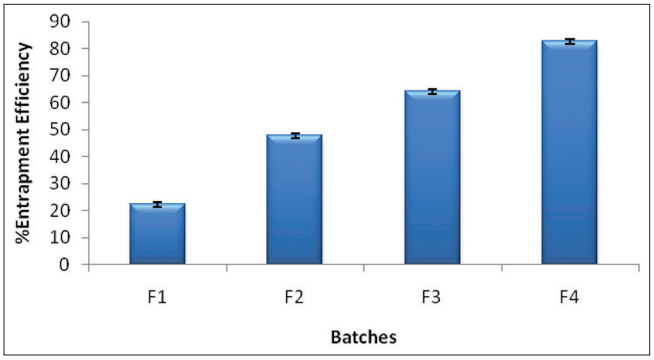

Figure 6: Percent entrapment efficiency of batches F1to F4 * (n = 3)

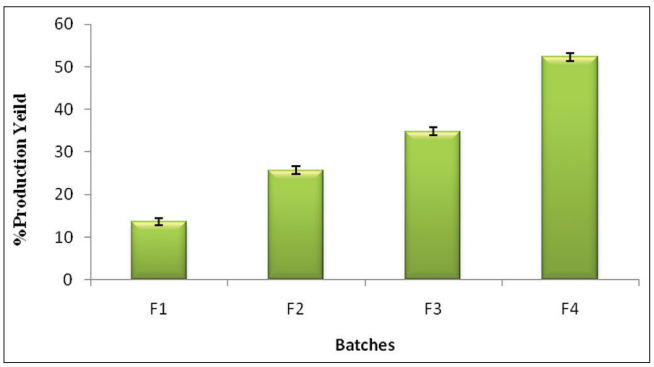

Figure 7: Percent production yield of batches F1 to F4

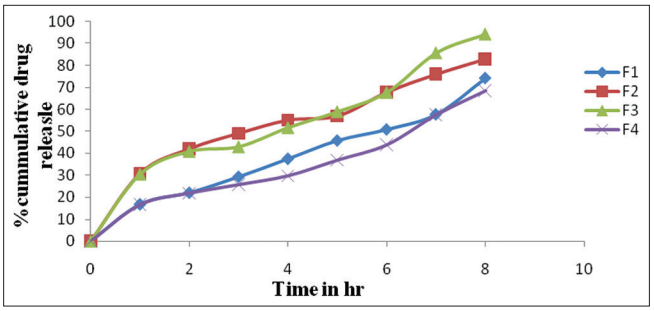

Figure 8: *In vitro* release of batches F1 to F4

The reason for increased in production yield may due to saturation of reactive carbonyl functional group of cross-linker at higher concentration ratio.

Table 3: Particle size of batches from NS1 to NS 4 and F1to F4

Sr. no.	Batches	Diameter (μm)*	PDI *
1	NS1	78.82±0.00	1±0.01
2	NS2	78.82±0.01	0.31±0.020
3	NS3	265.2±0.231	0.706±0.540
4	NS4	336.2±0.124	0.824±0.110
5	F1	78.82±0.214	0.312±0.521
6	F2	183.1±0.127	0.782±0.251
7	F3	204.9±0.154	0.632±0.321
8	F4	265.2±0.71	0.706±0.123

Particle size analysis

The average particle size of plain nanosponge batches NS1 to NS4 ranged from 78.81 ± 0.20 nm to 336.02 ± 0.124 nm [Table 3 and Figures 9-11]. It was found that on increasing the drug:polymer ratio, the mean particle size was increased. This could probably due to the fact that at high drug:a polymer ratio, the amount of cross-linker available per nanosponge was more. Probably at high drug:polymer ratios, the amount of cross-linker available per nanosponge to encapsulate the drug becomes more, thus reducing the thickness of the polymer wall and hence nano size sponges were obtained.

Surface morphology

Surface morphology of nanosponges was studied by SEM Figure 12. The SEM photographs showed that the nanosponges were spherical, uniform, and contained pores.

DSC

DSC curve of plain ketoconazole and the drug loaded nanosponges mixture showed in Figures 13-16. The DSC spectra of ketoconazole showed sharp endothermic peak at 152.25 C. Corresponding to its melting point. The disappearance of the drug endothermic peak was observed for optimized formulation F3 obtained by freeze drying. This phenomenon might be a proof of interaction between the components of the formulation. This may be considered as indicative of drug amorphization and/or inclusion complex formation

Determination of *in vitro* drug release study

The *in vitro* release study was carried out on all the batches. It was observed that the drug release increased with increase in drug:polymer ratio. The percent of ketoconazole release from nanosponge formulation after 8 h 82 to 92%. This may be due to the fact that the cross-linker concentration was varies for each formulation and also the concentration of drug molecules was increased which resulted in reduced thickness of polymer coat surrounding nanoparticle. From the release profiles, it was found that nanosponges from all the batches showed a biphasic release with initial burst effect. The burst effect could be due to two reasons: First due to the drug near or on the surface of nanosponges and second due to porous nature of nanosponges, the pores provided the channel for release of drug.

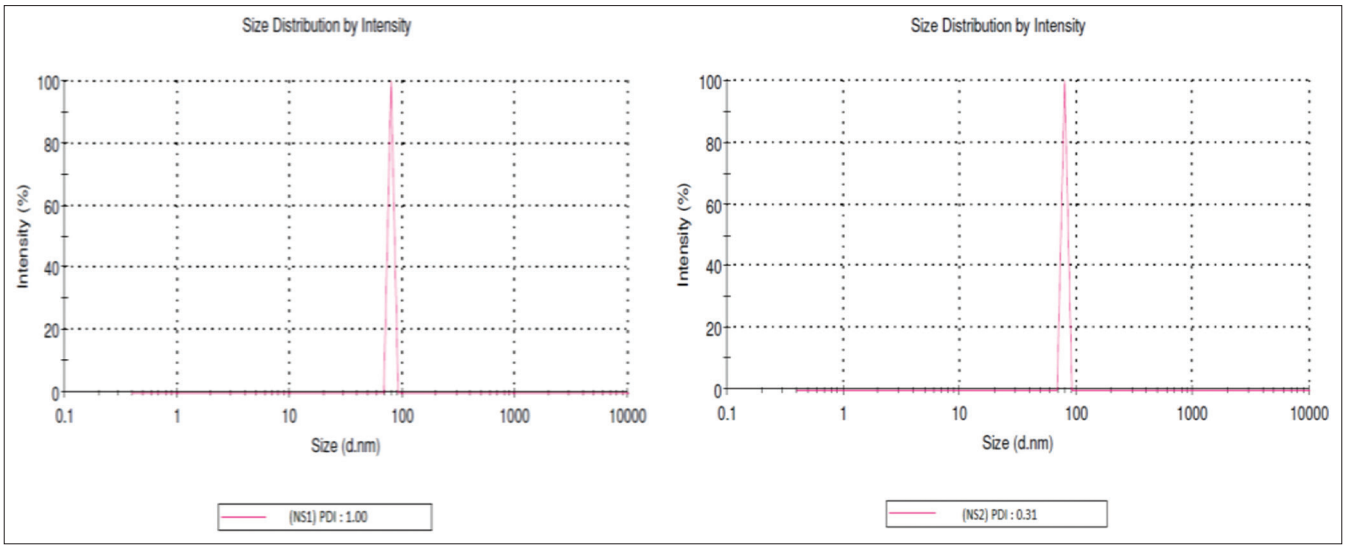

Figure 9: Particle size of nanosponge without drug

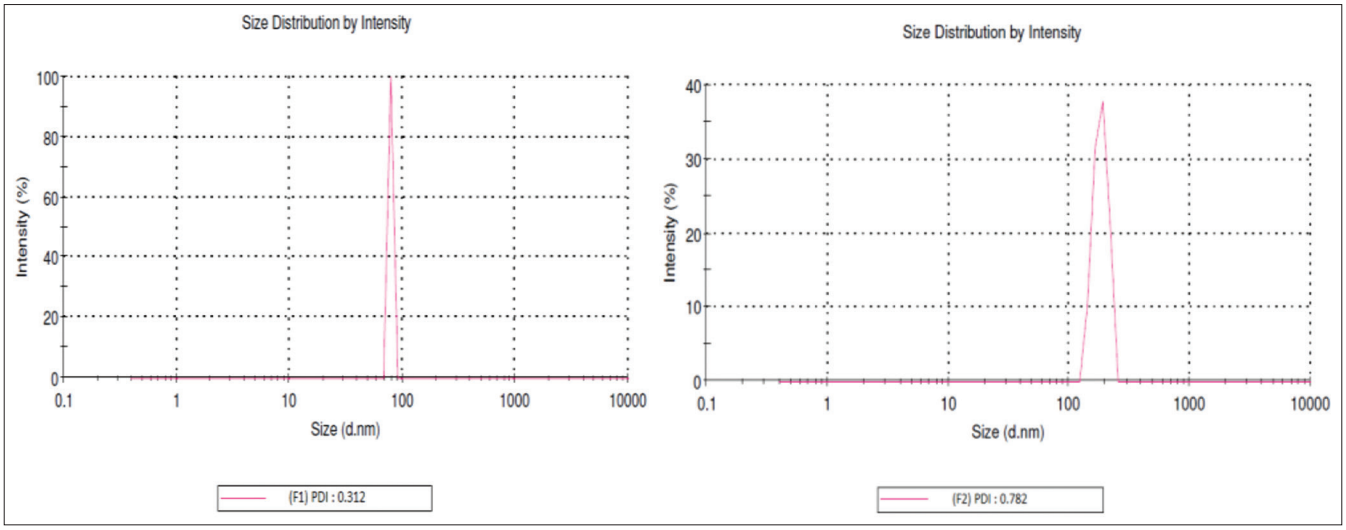

Figure 10: Particle size of ketoconazole loaded nanosponge of batch F1 and F2

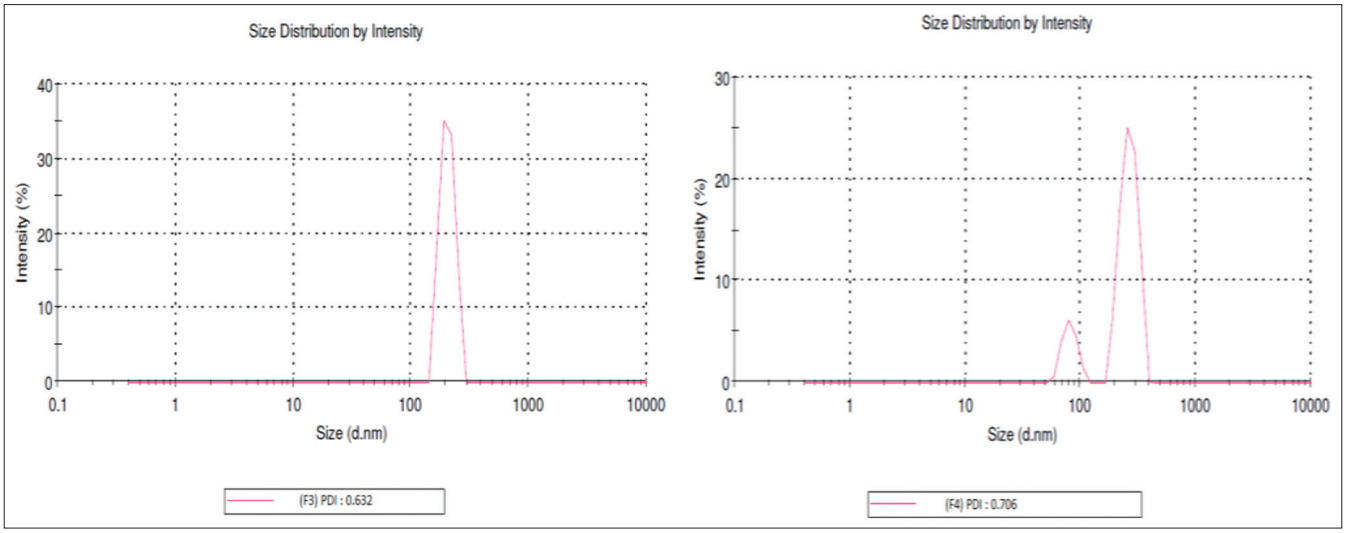

Figure 11: Particle size of ketoconazole nanosponge of batch F3 and F4

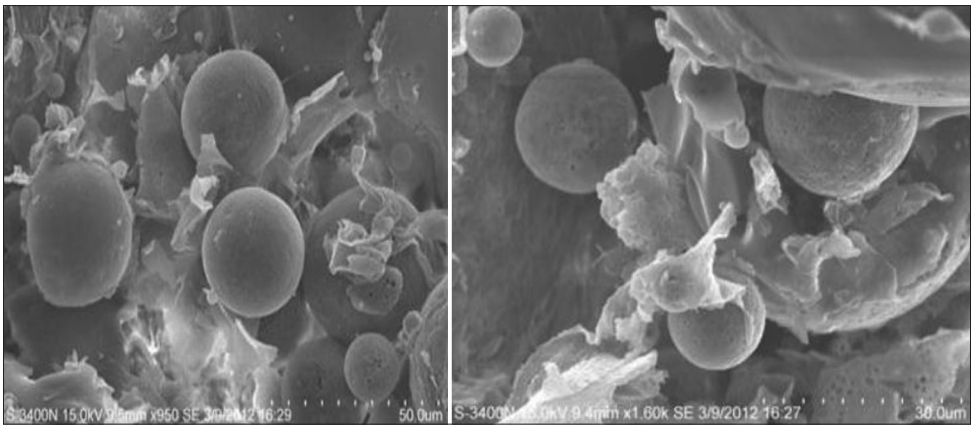

Figure 12: Scanning electron microscopy image of nanosponge without drug nanosponge and batch F3

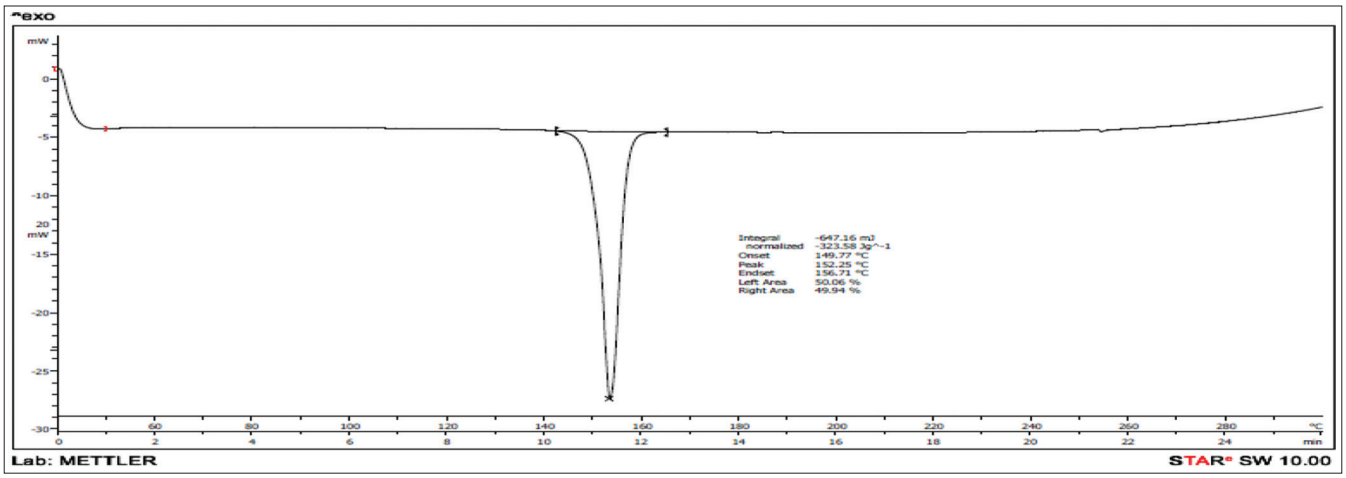

Figure 13: Differential scanning calorimetry curve of ketoconazole

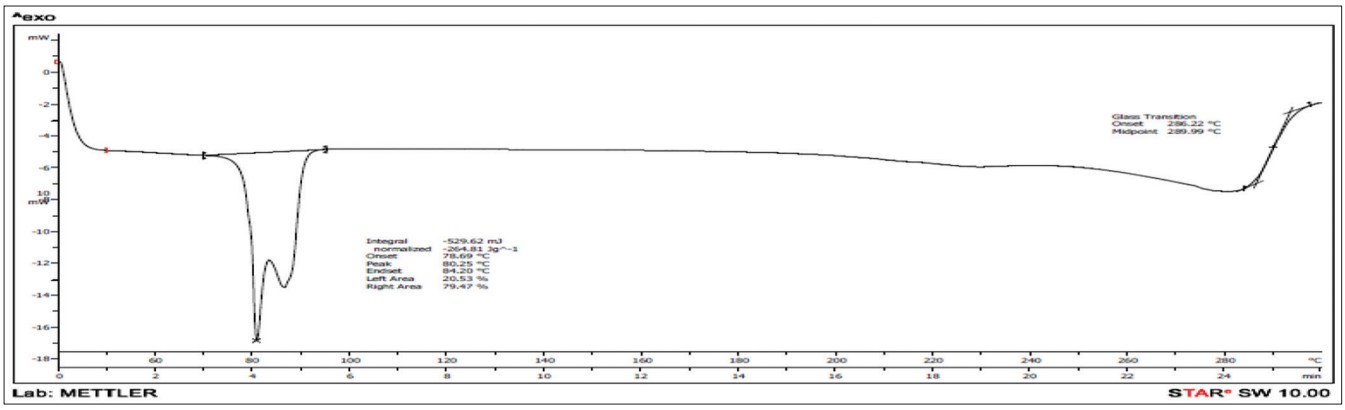

Figure 14: Differential scanning calorimetry curve of ketoconazole loaded nanosponge batch F1

Evaluation of nanosponges loaded gel

The content of drug was estimated spectrophotometrically using standard curve plotted at λ_{max} 292 nm drug content was found 90.55 ± 0.10–92.58 ± 0.14, respectively. All the formulations are with low standard deviation indicating that the drug distribution was uniform. The drug content of ketoconazole loaded gel of batch G4 was found to be higher than that of others batches. pH was found 7.26 ± 0.20–7.53 ± 0.14. That suits the skin pH indicating skin compatibility. Viscosity was found in range of 7543 ± 1.11–8351 ± 2.16 cp. Furthermore, the viscosity increases with increase in pH. As pH increases and carboxylic acid moieties of the polymer are neutralized, therefore viscosity increases and spreadability were found to be 11.87 ± 0.03–11.91 ± 0.05 g cm/s, respectively, as shown in Table 4.

Rheological behavior

Rheograms were obtained by plotting rate of shear on Y-axis versus calculated value of shear stress on X-axis. Rheogram of all formulations is shown, respectively, in Table 5. The viscosity of all

Table 4: Drug content of nanosponge gel batches from G1 to G4

Sr. no.	Batches	Drug content	pH*	viscosity*(cp)	Spreadability (g cm/s)
1	G1	90.55±0.10	7.26±0.20	7543±1.11	11.87±0.03
2	G2	90.72±0.17	7.36±0.35	7806±2.18	11.84±0.04
3	G3	91.81±0.13	7.42±0.32	8087±1.59	11.59±0.01
4	G4	92.58±0.14	7.53±0.14	8351±2.16	11.91±0.05

formulation follows a pseudo plastic behavior. The material flow as soon as a shear stress is applied the slope of the curve gradually decreases with increasing rate of shear. The viscosity was derived from the slop which is found to decrees as the shear rate is increase. Rheological characteristics of all gel formulations were studied and from rheogram it was proved that all gel formulations exhibited shear thinning system (thixotropy) which indicated the quality of developed formulation.

Table 5: Rheological studies of nanosponge gel batches G1 to G4

Sr. no		RPM	G1		G2		G3		G4	
			Shear rate (s^{-1})	Shear stress ($dyne/cm^2$)	Shear rate (s^{-1})	Shear stress ($dyne/cm^2$)	Shear rate (s^{-1})	Shear stress ($dyne/cm^2$)	Shear rate (s^{-1})	Shear stress ($dyne/cm^2$)
1	Ascending	10	133	1,084,700	133	1,211,566	133	1,177,151	133	1,049,769
2		20	267	1,238,454	267	1,473,698	267	1,926,405	267	1,447,407
3		30	400	1,413,600	400	1,752,400	400	28,260,000	400	1,710,400
4		40	533	1,612,858	533	2,039,416	533	3,527,269	533	1,934,619
5		50	667	1,987,542	667	2,444,905	667	4,077,621	667	2,184,875
6		60	800	2,154,782	800	2,734,400	800	4,396,000	800	2,516,000
8		70	933	2,354,872	933	3,071,010	933	4,628,613	933	2,809,946
9		80	1067	2,483,380	1067	3,202,538	1067	4,534,150	1067	3,278,247
10	Descending	70	933	2,465,287	933	3,178,000	933	4,223,200	933	3,069,658
11		60	800	2,315,200	800	2,988,800	800	3,920,800	800	2,710,400
12		50	667	2,154,114	667	2,705,352	667	3,524,845	667	2,438,752
13		40	533	1,890,079	533	2,294,769	533	2,876,068	533	2,203,093
14		30	400	1,663,200	400	2,005,600	400	2,265,200	400	1,961,600
15		20	267	1,452,879	267	1,806,556	267	1,670,352	267	1,692,672
16		10	133	1,239,688	133	1,563,287	133	887,908	133	1,477,768

Table 6: Evaluation of optimized batch G1 at different time intervals after storage under 40 ± 2°C/75 ± 5% RH

Temperature/parameters evaluated		0 month	1 month	2 months	3 months
40±2°C/75±5% RH	Ph	7.32±0.32	7.475±0.34	7.29±0.31	7.36±0.35
	Viscosity* (centipoise)	7547±1.59	7646±1.56	7539±1.51	7638±1.53
	Spreadability*	11.38±0.11	11.36±0.01	11.35±0.10	11.43±0.12
	Drug content (%)*	93.51±0.30	92.599±0.15	92.64±0.01	93.26±0.19
	Cumulative % drug release*	89.21±0.20	89.98±0.03	89.05±0.04	89.04±0.08

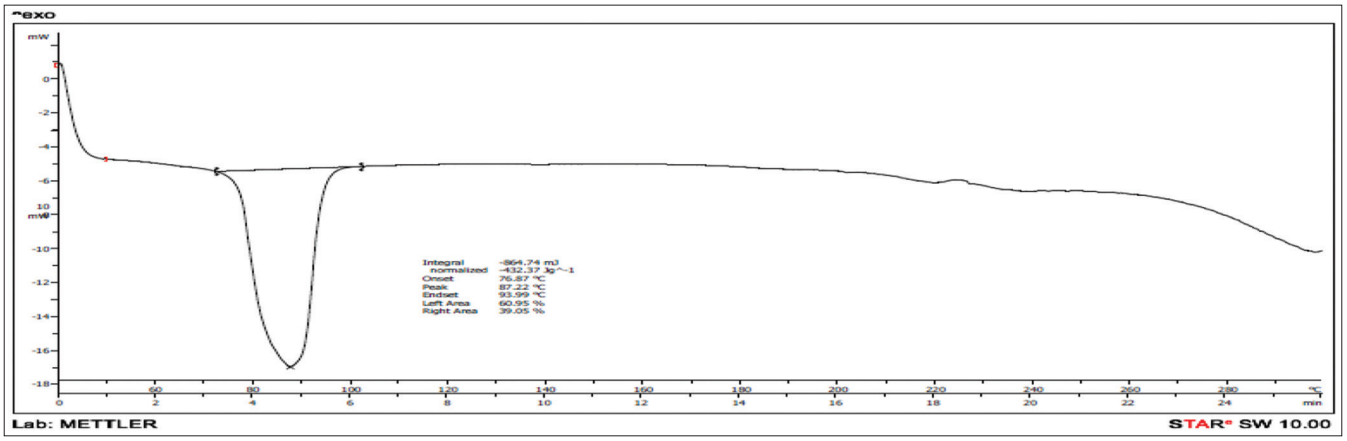

Figure 15: Differential scanning calorimetry curve of ketoconazole loaded nanosponge batchF2

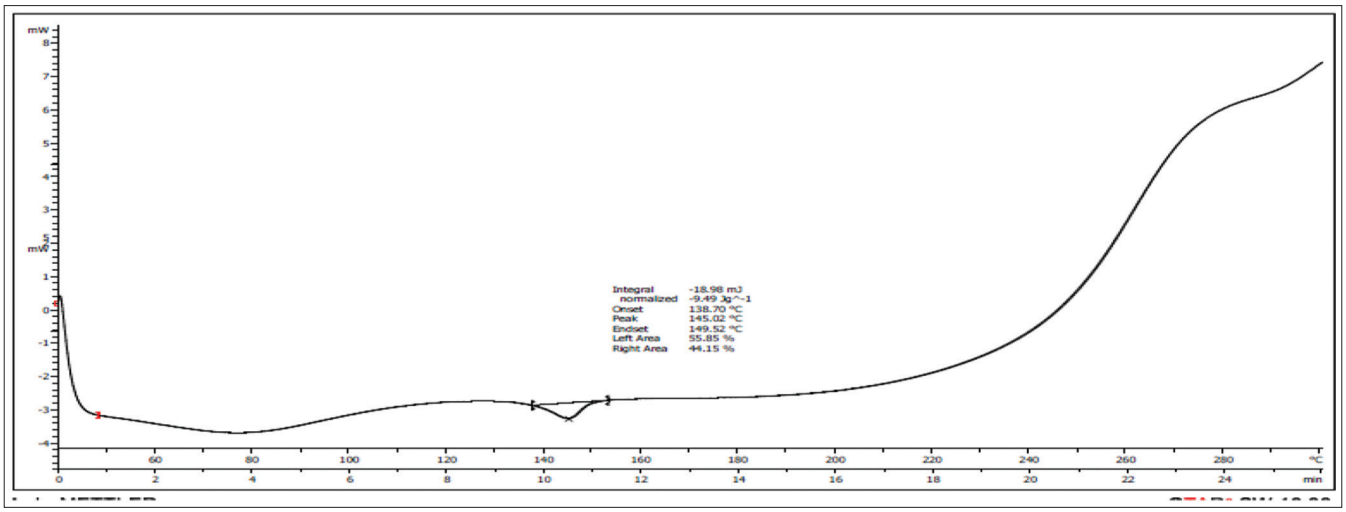

Figure 16: Differential scanning calorimetry curve of ketoconazole loaded nanosponge batch F3

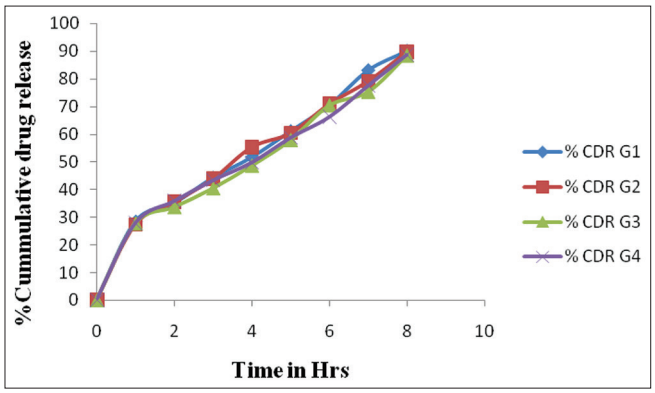

Figure 17: *In vitro* release of batches G1 to G4

In vitro diffusion studies

The *in vitro* diffusion study was taken using Franz diffusion cell which shows cumulative % drug release of ketoconazole gel formulation was G1-90.02 ± 0.30, G2-89.78 ± 1.27, G3-88.32 ± 0.52, and G4-88.72 ± 0.26%, respectively. Among the nanosponge loaded gel formulations highest release was for batch G1 (90.02 ± 0.03%) which may be due to lower polymer concentration (0.25%) used for formulation of gel.

The optimized batch G1 was kept for stability study. The batches showed good stability with no change in drug content as shown in table No.6, pH, viscosity, Spreadability and in vitro release after stability study of 3 months as in Figure 17.

Conclusion

Ketoconazole nanosponges were successfully prepared by hyper cross-linked β-cyclodextrin method. Nanosponge formulation F3 showed good physical parameter study and was used for formulating a gel in the Carbopol base. At the end of 8thh drug release from the gel was found to be in increasing order G1> G2> G3> G4. It can also be used in various dosage forms such as tablet, capsule, emulsion, and suspension.

References

1. Trotta F, Zanetti M, Cavalli R. Cyclodextrin-based nanosponges as drug carriers. Beilstein J Org Chem 2012;8:2091-9.
2. Subramanian S, Singireddy A, Krishnamoorthy K, Rajappan M. Nanosponges: A novel class of drug delivery system-review. J Pharm Pharm Sci 2012;15:103-11.
3. Swaminathan S, Vavia PR, Trotta F, Cavalli R, Tumbiolo S, Bertinetti L. Structural evidence of differential forms of nanosponges of beta-cyclodextrin and its effect on solubilization of a model drug. J Incl Phenom Macrocycl Chem 2012;76:201-11.
4. Patel EK, Oswal RJ. Nanosponge and microsponges: A novel drug delivery system. Int J Res Pharm Chem 2012;2:237-44.
5. Swaminathan S, Darandale S, Vavia PR. Nanosponge-aided drug delivery: A closer look. Pharm Formul Qual 2012;14:12-5.
6. Shinde G, Rajesh KS, Bhatt D, Bangale G, Umalkar D, Virag G. Current status of colloidal system (nano range). Int J Drug Formul Res 2011;2:39-54.
7. Liang L, Liu DP, Liang CC. Optimizing the delivery systems of chimeric RNA, DNA oligonucleotides beyond general oligonucleotide transfer. Eur J Biochem 2002;269:5753-8.
8. Trotta F, Cavalli R, Tumiatti W, Zerbinati O, Rogero C, Vallero R. Ultrasound Assisted Synthesis of Cyclodextrin-Based Nanosponges. European Patent No. 1786841B1, 2007. Vol. 428; 2012. p. 152-63.
9. Ansari KA, Vavia PR, Trotta F, Cavalli R. Cyclodextrin-based nanosponges for delivery of resveratrol: *In vitro* characterisation, stability, cytotoxicity and permeation study. AAPS Pharm Sci Tech 2011;12:279-86.
10. Torne S, Darandale S, Vavia P, Cyclodextrin-based nanosponges: Effective nanocarrier for tamoxifen delivery. Pharm Dev Technol 2013;18:619-25.
11. Rajeswari C, Alka A, Javed A, Khar RK. Cyclodextrins in drug delivery: An update review. AAPS Pharm Sci Tech 2005;6:E329-57.
12. Chandramouli Y, Firoj S, Rajalakshmi R, Vikram AB, Yasmeen R, Chakravarthi RN. Preparation and evaluation of microsponge loaded controlled release topical gelof acyclovir sodium. Int J Biopharm 2012;3:96-102.
13. Deshpande A, Patel PE. Preparation and evaluation of cyclodextrin based atorvastatin nanosponges. Am J Pharm Tech Res 2014;4:2249-3387.
14. Ramnik S, Nitin B, Jyotsana M, Horemat SN. Characterization of cyclodextrin inclusion complexes-a review. J Pharm Sci Tech 2010;2:171-83.
15. Swaminathan S, Vavia PR, Trotta F, Torne S. Formulation of betacyclodextrin based nanosponges of itraconazole. J Pharm Phenom macrocycl Chem 2007;57:89-94.
16. Rao M, Bajaj A, Khole A, Munjapar G, Trotta F. *In vitro* and *in vivo* evaluation of β-cyclodextrin-based nanosponge of telmisartan. J Incl Phenom Macrocycl

Chem 2012;77:135-45.

17. Swaminathan S, Vavia PR, Trotta F, Torne S, Formulation of beta cyclodextrin based nanosponges of itraconazole. J Pharm Phenm Macrocycl Chem 2007;57:89-94.
18. Nevine SA, Menshawe S. New topical fluconazole loaded microsponge hydrogel: Preparation and characterization. Int J Pharm Sci 2012;4:460-8.
19. Patel RP, Patel H, Baria AH. Formulation and evaluation of carbopol gel containing liposome of Ketoconazole. Int J Drug Deliv Technol 2009;1:42-5.
20. Ning M, Guo Y, Pan H, Chen X, Gu Z. Preparation, in vitro and in vivo evaluation of liposomal/niosomal gel delivery systems for clotrimazole. Drug Dev Ind Pharm 2005;31:375-83.
21. Panigrahi L, Ghosal SK, Pattnaik S, Mahasana L, Barik BB. Formulation and evaluation of solid dispersion incorporated gel of ketoconazole. Indian J Pharm Sci 2006;68:205-11.
22. Sreenivasa MR, Mutalik S, Veerabhadra GR. Preparation and evaluation of monoxidil gel for topical application in alopecia. Indian J Pharm Sci 2006;68:432-6.
23. Maiti S, Kaity S, Ray S, Biswanath SA. Developement and evaluation of xanthan gum facilitated ethyl cellulose microsponges for controlled percutaneous delivery of diclofenac sodium. Acta Pharm 2011;61:257-70.
24. Mehta M, Panchal A, Shah VH, Upadhyay U. Formulation and *in vitro* evaluation of controlled release microsponge gel for topical delivery of clotrimazole. Int J Adv Pharm 2012;2:93-101.
25. Kumar JR, Murlidharan S, Ramasamy S. Microsponge enriched gel. A novel strategy for ophthalmic drug delivery system containing ketotifen. J Pharm Sci Res 2013;5:97-102.
26. Nokhodichi A, Jelvehgari M, Siahi MR, Mozafari MR. Factors affecting the morphology of benzoyl peroxide microsponges. Micron 2007;38:834-40.
27. Gupta GD, Goud RS. Release rate of tenoxicam from acrypol gels. Indiana Pharm 2005;4:69-75.
28. Lee JH, Park TG, Mills OH. Development of oral drug delivery system using floating microspheres. J Microencapsul 1999;16:715-29.