Formulation and Evaluation of Ketoconazole Nanosponge gel.

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Abstract

Ketoconazole Nanosponges were prepared by using Hyper cross linked β-cyclodextrin method by using different concentration of cross-linker. Diphenyl carbonate was used as the cross linking polymer. Nanosponge formulations were prepared by using β-CD: cross linker ratios of 1:15, 1:10, 1:5 and 1:3.Thepreparednanosponges were evaluated for percentage yield, incorporation efficiency, particle size, drug polymer compatibility, scanning electron microscopy andin-vitrodrugrelease.SEM studies confirmed their porous structure with number of nano channels. The FTIR spectra showed stable character of Ketoconazole in mixture of polymers and revealed the absence of drug polymer interactions. DSC study revealed that drug was involved in complexation with nanosponges. The average particle size of Ketoconazole nanoparticles was found to be in the range of 78.81± 0.20 nm to336.02 ± 0.124nm.The drug release from nanosponges was found to extended upto 8hr. 82 to 92%.The nano sponges were formulated into gel using Carbopol 940Batches G1 to G4 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, In vitro diffusion studies. Nanosponge gel G1 showed the optimum pH, viscosity, Spread ability and In vitro release. Drug diffusion from the nanosponge loaded gel formulations was show sustained rate. 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, seborrhea. It has pH dependent solubility and permeability. The drug has a half-life of 1 to 2 hours. Because of its short biological half-life the drug has to be administered frequently. Furthermore oral Ketoconazolecauses 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 minimizes its gastro intestinal side effects and provides consistent drug levels at application site for longer period of time.

Material and Method

Material

Ketoconazole was obtained as kind gift sample from Zim Pharmaceuticles, Ltd. Nagpur India. B-cyclodextrin & 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 by using Hyper cross linked β-cyclodextrin method by using different concentration of cross-linker. In this method, anhydrous Dimethyl sulfoxide was placed in round bottom flask and anhydrous β-cyclodextrin were added to achieve complete dissolution. Then diphenyl carbonate were added and the solution was allowed to react for 4 h at 100^0 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 were completely removed by Soxhlet extraction with ethanol, the white powder thus obtained was dried over night in an oven at 60^0 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

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

Evaluation of Ketoconazole loaded Nanosponge.

Drug loading determination[11]

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

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

Entrapment efficiency determination[12]

The weighed sample of drug loaded nanosponges(30mg) were dissolved in 100ml, 7.4 phosphate buffer under magnetic stirrer for 4hrs at 30^0C. The sample were filtered and

sample was read out at 292 nm against blank using spectrophotometer. And entrapment efficiency was determined by 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 by 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 Optionparticle 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 three times and mean values was taken

Surface morphology[14]

The surface morphology of the optimized formulation was examined by scanning electron microscopy. 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 scanning electron micrograph was taken using an electron microscope. The scanning electron micrographs were taken as shown as shown in **Figure**

Differential scanning calorimetry[15]

A differential scanning calorimeter was used for thermal analysis of drug and physical mixture. Drug and its physical mixture were weighted directly in was weighed 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 Spectroscopy

It is important to check any kind of interaction between drug and polymer. It was done using Fourier Transform Infrared 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. This physical mixture was converted in a circular disc. This disc was then placed in the scanning slot of FTIR and scanned between 4000cm^{-1} to 400cm^{-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. nanosponges equivalent to 500 mg of Ketoconazole were weighed accurately and placed in basket .The dissolution medium used was 900ml of 7.4 phosphate buffer maintained at 37 ± 1^0 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 spectrophoto metrically at 292 nm.

Composition of Ketoconazole Loaded Nanoponge 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 (PEG) 400.The Carbopol solution was neutralized by slowly adding triethanol amine with constant stirring until gel is formed. pH of final gel was determined.

Table 2 .Composition Of Ketoconazole Loaded Nanoponge 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

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 hrs in case of nanosponge loaded gel formulations. The volume was made upto 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}292nm.

pH study[23]

pH of the various gel formulations were determined by 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 per minute 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 consist 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 rheologies of prepared formulations were studied using Brookfield viscometer (CAP-2000). The sample was placed on temperature sensitive plate. Temperature was kept at 37 ± 1^0 C. Cone no. 3 was held on the plate and speed of 10,20, 30, 40,50,60,70,80 rpm were selected. Different viscosities at respective spindle speeds were obtained for an ascending and descending curve.

***In vitro* diffusion studies** [27-28]

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 compartment 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 hrs 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 [29]

The optimized formulation of Ketoconazole loaded nanosponge gel was packed in aluminum collapsible tubes and subjected to stability studies at $40^0C\pm 2^0C/75\% \pm 5\%$ RH for a period of 3 months. Formulations were evaluated at periodic intervals for pH, viscosity, and drug content and drug release profiles.

RESULT

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 1646.12cm^{-1}, 1106.27 cm^{-1} ,1245.12cm^{-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.

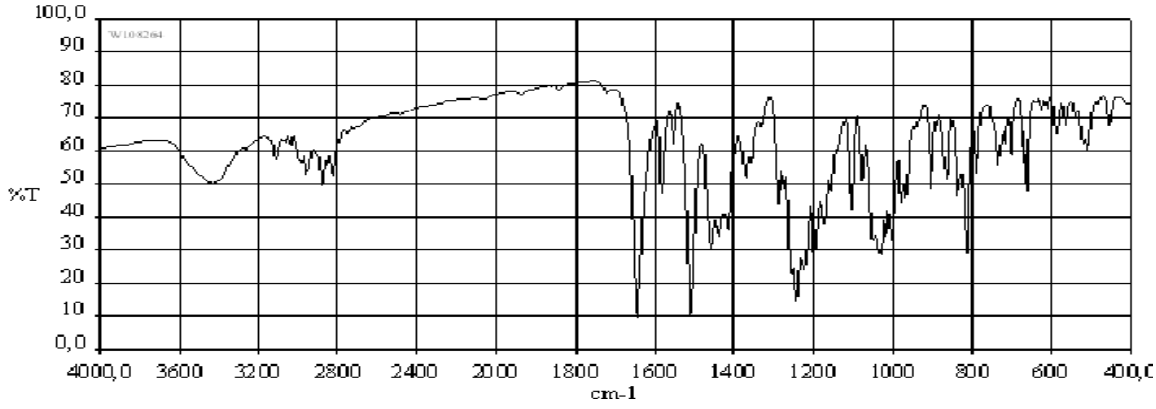


Figure No 1: FTIR Spectral Analysis of Ketoconazole

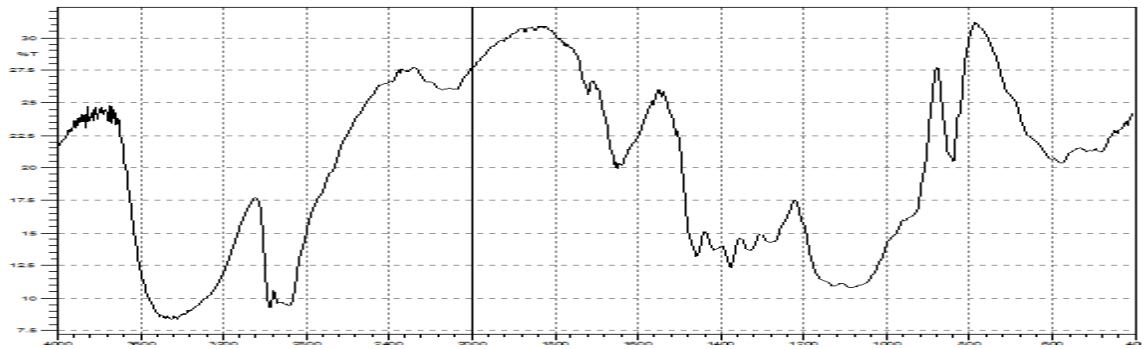


Figure No.2 FTIR Spectrum of Diphenyl Carbonate

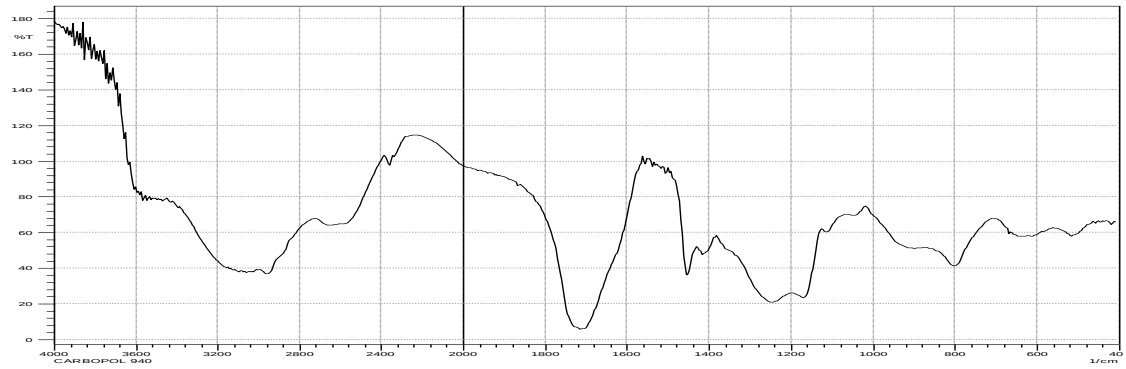


Figure No. 3 FTIR Spectrum of Carbopol.

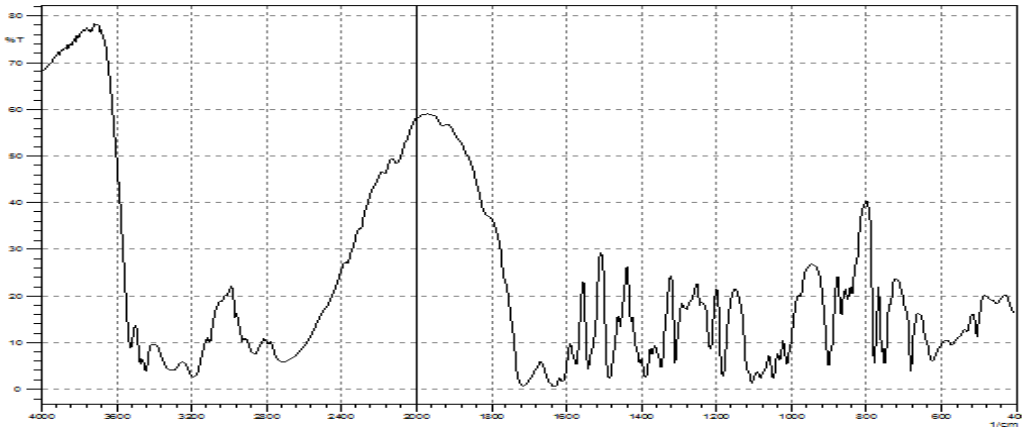


Figure No 4.FTIR Spectrum of Physical mixture of ketoconazole**.**

Drug loading (DL) and Entrapment efficiency (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 the Figure No. 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 indicate 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.

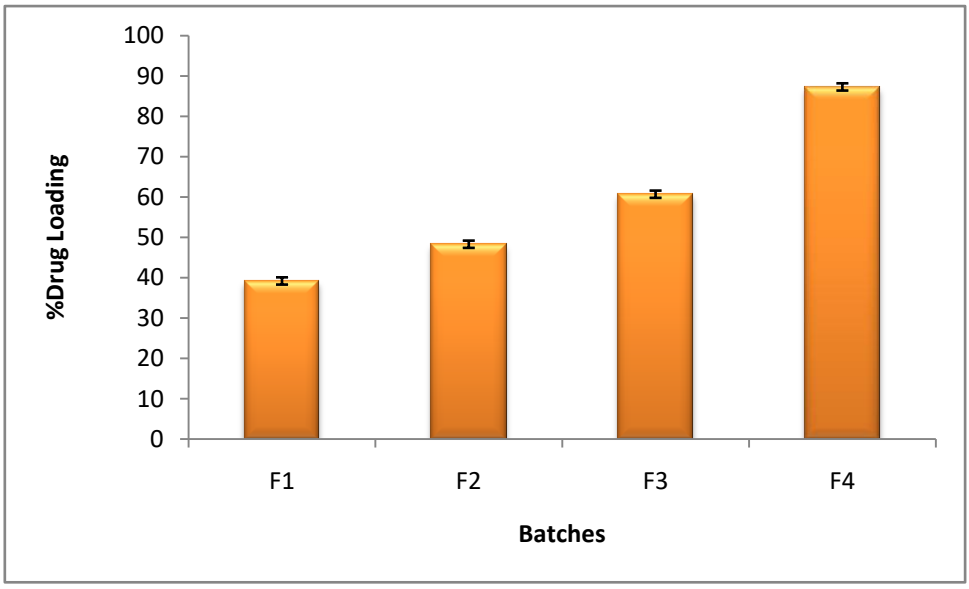
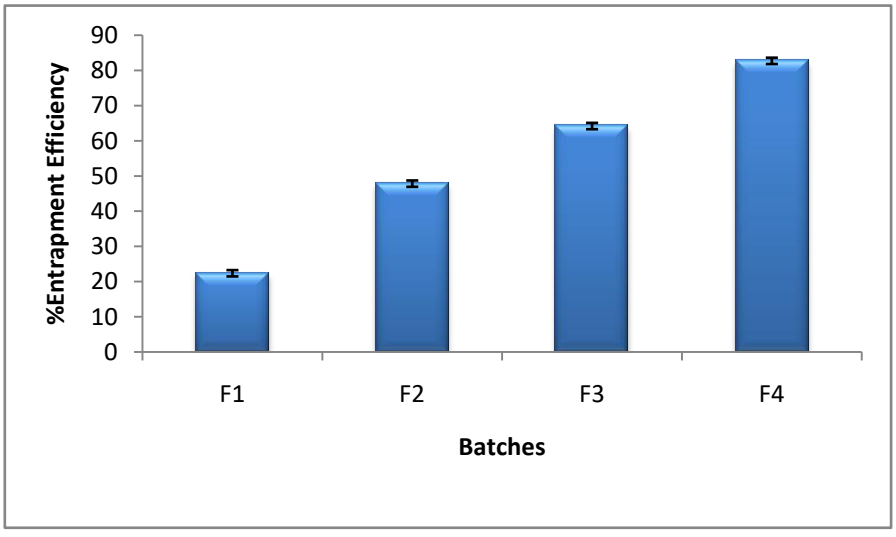


Figure No 5 : Percent drug loading of batches F1 to F4

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 drug loading did not reach 100%.



*** (n =3)**

Figure No 6 : Percent entrapment efficiency of batches F1to F4

Production yield

The production yield of batches from F1 to F3 ranged from 13.63 ± 0.41% to 52.82 ± 0.54% (**Table 8 and Figure 16)**. Increase in the drug: polymer ratio increased the production yield. The reason for increased in production yield may due to saturation of reactive carbonyl functional group of cross linker at higher concentration ratio.

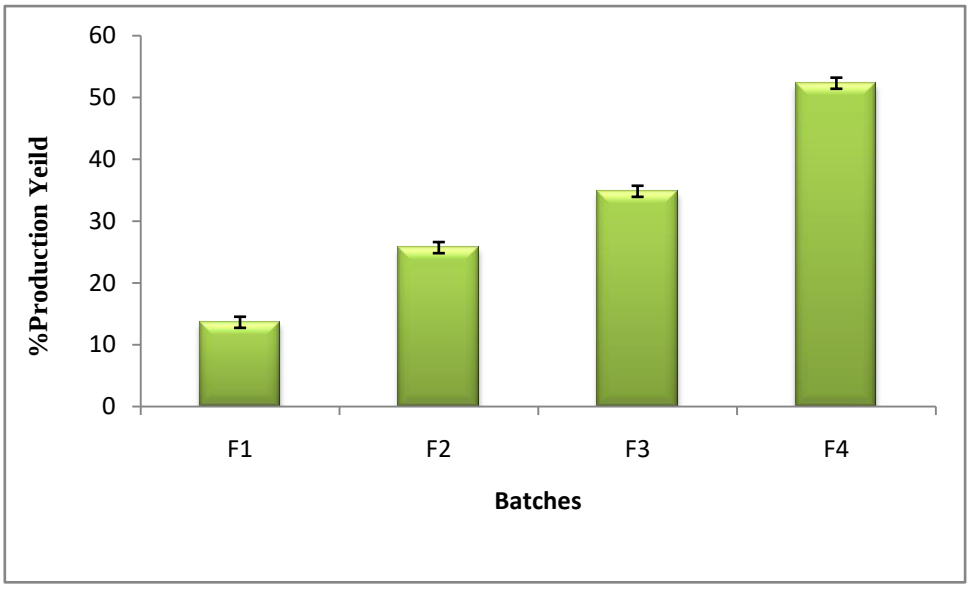


Figure No 7: Percent production yield of batches F1 to F4

Particle size analysis

The average particle size of plain nanosponge batches NS1 to NS4 ranged from 78.81± 0.20 nm to336.02 ± 0.124nm **(**Table 3 and Figure 8,9 and 10**).** 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.

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

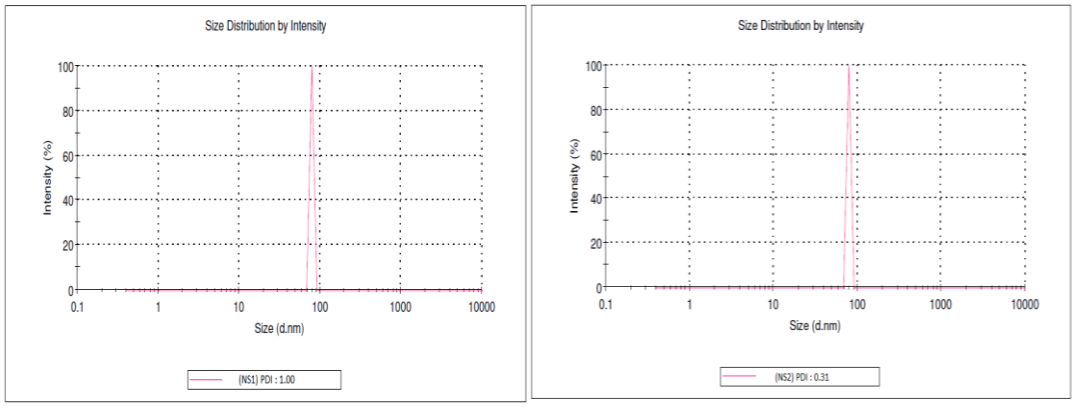


Figure no.8. Particle size of nanosponge without drug

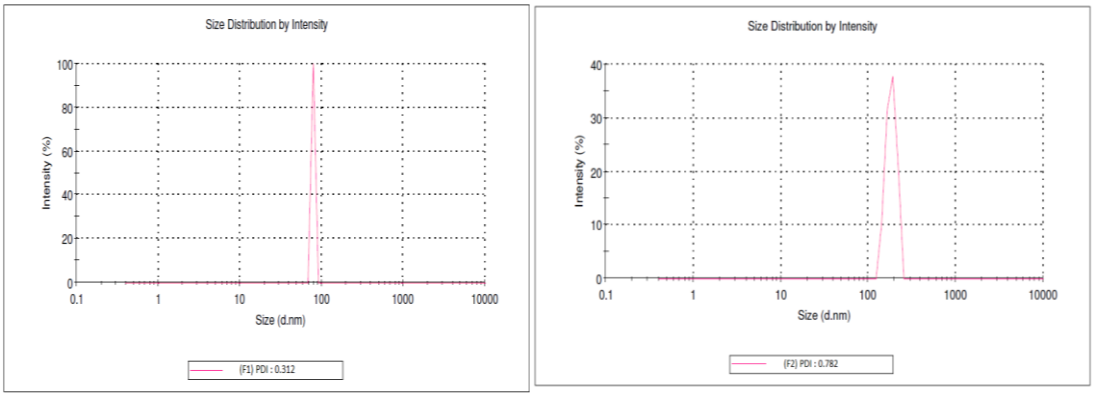


Figure no.9.Particle size of ketoconazole loaded nanosponge of batch F1&F2

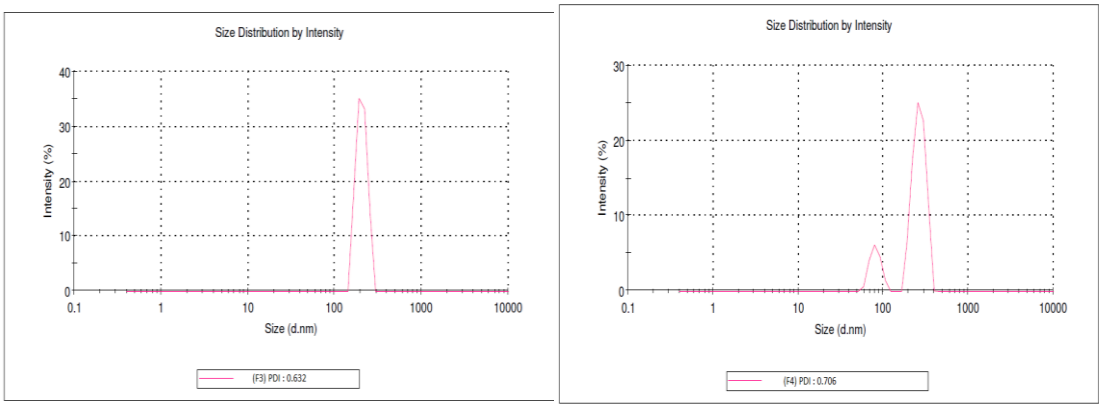


Figure no.10.Particle size of Ketoconazole nanosponge of batch F3 & F4

Surface morphology

Surface morphology of nanosponges was studied by scanning electron microscopy **Figure11** The SEM photographs showed that the nanosponges were spherical, uniform and contained pores.

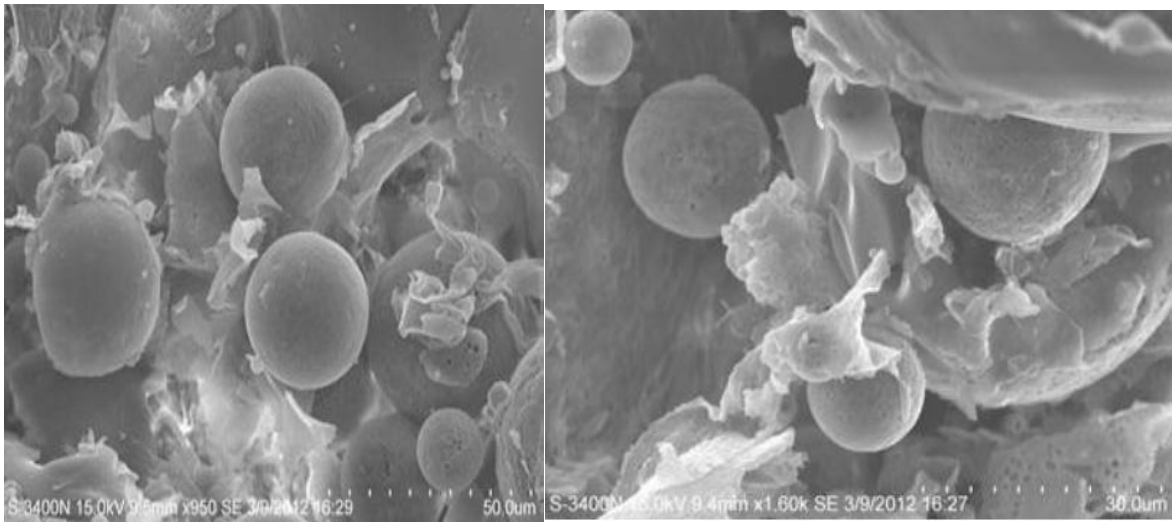


Figure No.11.SEM image of nanosponge without drug nanosponge & batch F3

Differential scanning calorimetry

DSC curve of plain Ketoconazole and the drug loaded nanosponges mixture showed in **Figure19a.** The DSC spectra of Ketoconazole showed sharp endothermic peak at 152.25 C.Corresponding to its melting point. The disapperence 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

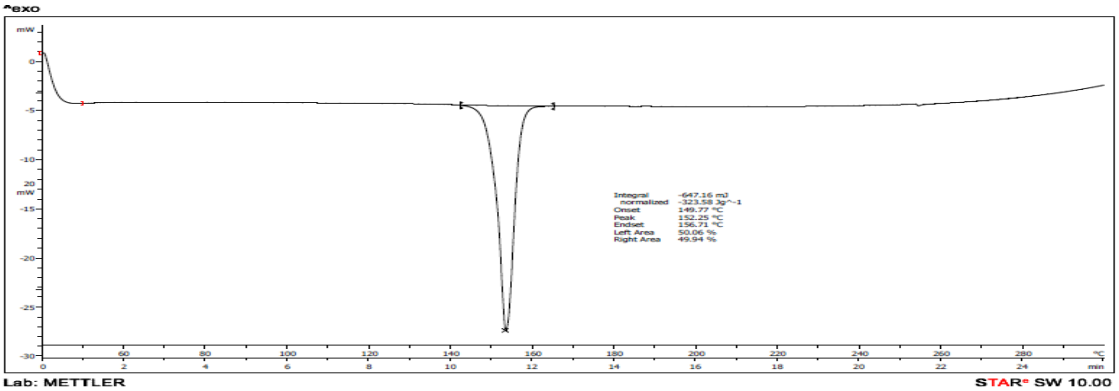


Figure no.12. DSC curve of ketoconazole

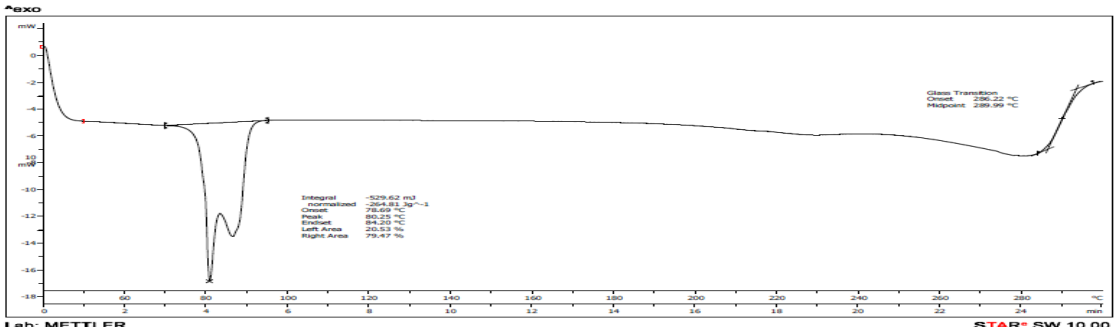


Figure No.13. DSC curve of ketoconazole loaded nanosponge batch F1

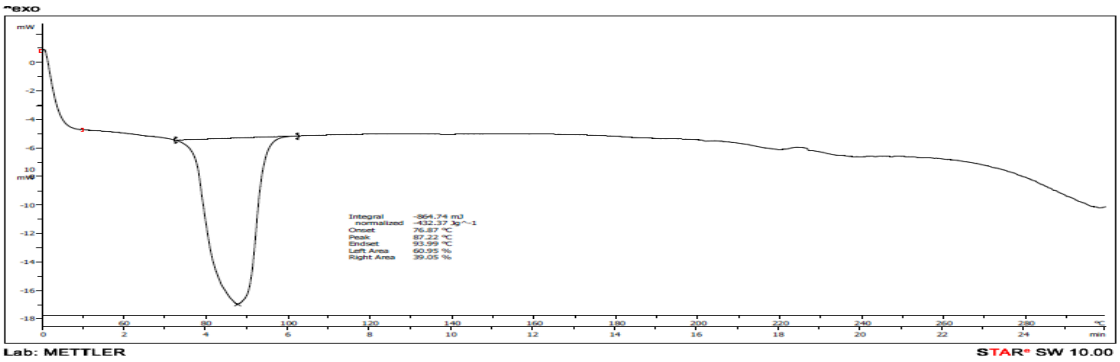


Figure No.14.DSC curve of ketoconazole loaded nanosponge batchF2

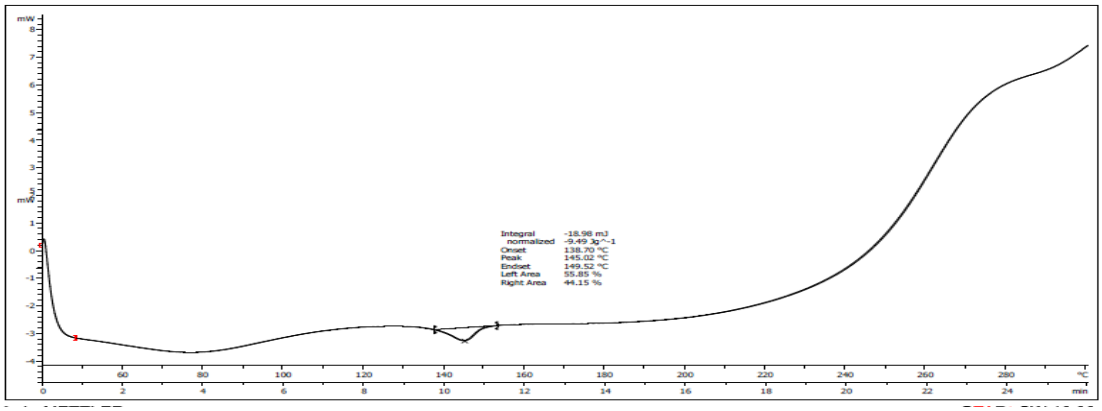


Figure No.15.DSC curve of ketoconazole loaded nanosponge batch F3

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: polymerratio. The percent of ketoconazole release from nanosonge 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.

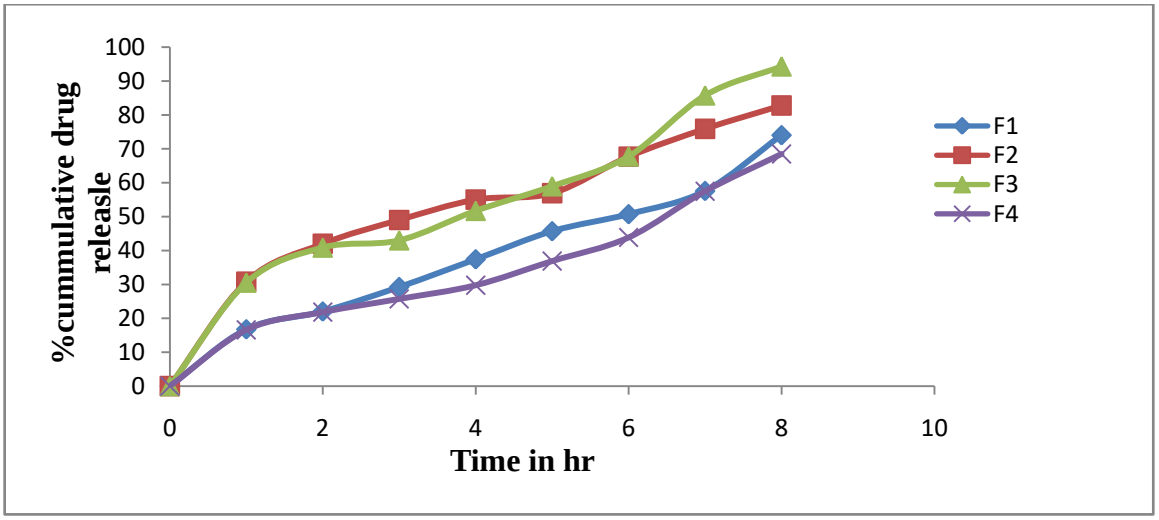


Figure 16: *In vitro* release of batches F1 to F4

Evaluation of Nanosponges loaded Gel

The content of drug was estimated spectrophoto metrically by using standard curve plotted at λ_{max}292nm.drug content was found 90.55 ±0.10 to 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 to 8351±2.16 cp. Also the viscosity increases with increase in pH. As pH increases and carboxylic acid moieties of the polymer are neutralized, therefore viscosity increases. and spreadability was found to be 11.87±0.03 to11.91±0.05g.cm/secrespectively were shown in table no.4.

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

Sr. No	Batches	Drug content	pH*	viscosity*(cp)	Spreadability (g.cm/sec)
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

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 were shown respectively in table no.5**.**The viscosity of all 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 nanoosponge gel batches G1 to G4

Sr. No		RPM	G1		G2		G3		G4	
			Shear rate (sec^{-1})	Shear stress ($dyne/cm^2$)	Shear rate (sec^{-1})	Shear stress ($dyne/cm^2$)	Shear rate (sec^{-1})	Shear stress ($dyne/cm^2$)	Shear rate(sec^{-1})	Shear stress ($dyne/cm^2$)
1	Ascending	10	133	1084700	133	1211566	133	1177151	133	1049769
2		20	267	1238454	267	1473698	267	1926405	267	1447407
3		30	400	1413600	400	1752400	400	28260000	400	1710400
4		40	533	1612858	533	2039416	533	3527269	533	1934619
5		50	667	1987542	667	2444905	667	4077621	667	2184875
6		60	800	2154782	800	2734400	800	4396000	800	2516000

8		70	933	2354872	933	3071010	933	4628613	933	2809946
9		80	1067	2483380	1067	3202538	1067	4534150	1067	3278247
10	**Descending**	70	933	2465287	933	3178000	933	4223200	933	3069658
11		60	800	2315200	800	2988800	800	3920800	800	2710400
12		50	667	2154114	667	2705352	667	3524845	667	2438752
13		40	533	1890079	533	2294769	533	2876068	533	2203093
14		30	400	1663200	400	2005600	400	2265200	400	1961600
15		20	267	1452879	267	1806556	267	1670352	267	1692672
16		10	133	1239688	133	1563287	133	887908	133	1477768

***In vitro* diffusion studies-**

The In-vitro diffusion study was taken by using franz diffusion cell which shows cumulative % drug release of ketoconazole gel formulation wasG1-90.02±0.30,G2-89.78±1.27,G3-88.32±0.52,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.

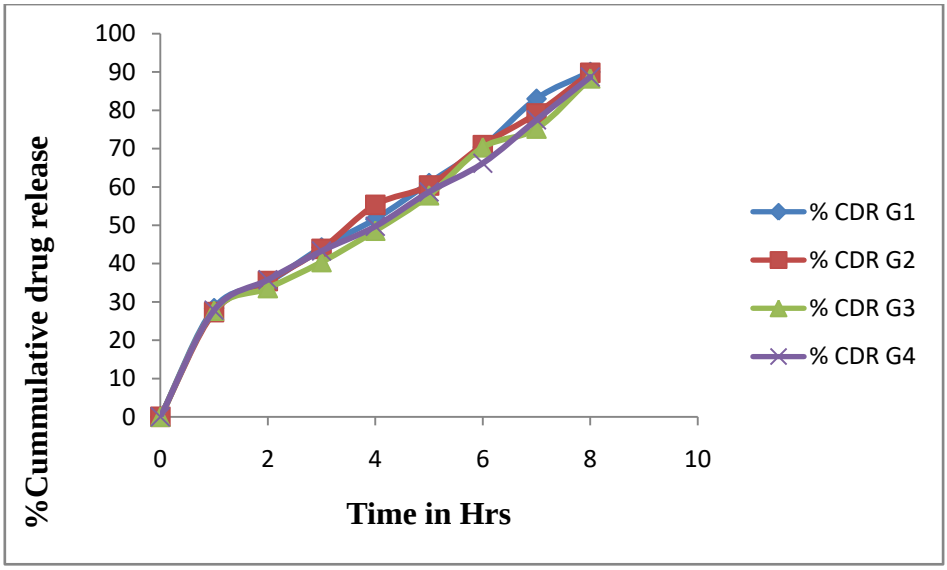


Figure No.17.*In vitro* release of batches G1toG4.

The optimized batch G1was kept for stability study. The batches showed good stability with no change in drug content, pH, viscosity, Spreadability and *in vitro* release after stability study of three months.

Table 6: Evaluation of optimized batch G1 at different time intervals after storage under 40 ± 2^0C/ 75 ± 5% RH

Temperature/parameters Evaluated		**0 month**	**1month**	**2 months**	**3months**
	pH	7.32± 0.32	7.475± 0.34	7.29± 0.31	7.36±0.35

$40^0C±2^0C$/ 75% ± 5% RH	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

Conclusion-

In this study, Ketoconazole nanosponges were successfully prepared by hyper cross linked β-cyclodexrin method. The method was found to be simple and reproducible. Nanosponge formulation F3showed good physical parameter study and was used for formulating a gel in the Carbopol base. At the end of 8^{th} hour drug release from the gel was found to be in increasing order G1 > G2 >G3 >G4.It can also be used in various dosage form like tablet, capsule, emulsion, suspension, etc .,for delivering other BCS classII drugs for getting therapeutic effect at the desired site

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