

**RESEARCH PAPER**ISSN:2394-2371
CODEN (USA):IJPTIL**FORMULATION AND EVALUATION OF NANOSPONGE BASED CONTROLLED RELEASE TOPICAL GEL PREPARATION OF KETOCONAZOLE****Jyoti Pandey*, Amandeep Singh**

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*Corresponding Author: **Jyoti Pandey****ABSTRACT**

Controlled release topical drug delivery system is most prominent system which gaining specific drug delivery. But Controlled and targeted topical drug delivery to specific site of body to prevent overdosing and to attain control release is significant problem which is confronted by the medical researcher. To resolve such problems developed a new creative colloidal carrier called nanosponge. A nanosponge is a type of nanoparticles which are spongy in nature and capable to load both hydrophilic and hydrophobic drug molecules. Such system can solubilize poorly water soluble drug and provide prolong drug release as well as improving drugs bioavailability. In the present study, the nanosponge based ketoconazole topical gel formulation was prepared by using several substances such as, Ethyl cellulose (polymer), Polyvinyl alcohol (surfactant), Dichloromethane (cross-linking agent) and Carbopol-934 (polymer), Triethanolamine (pH neutralizer), Propylene glycol (Permeation enhancer) etc. Formulation batches starting from F1 through F6 the final batch (F6) is considered as the best entrapped (80.80%) nanosponge with greater percentage drug release (95.0%). The SEM analysis concluded that the formulation particles are porous in nature and particle size determination finally concluded that particles are nano in size which is recognized the preparation as a “Nano-sponge”.

Keywords: - Nanosponges, Colloids, Ketoconazole, pH neutralizer, Permeation enhancer.

INTRODUCTION

Nanotechnology is an imperative part of engineering revolution since the industrial age. [1]. Nanotechnology resulted in multifarious

formulations like nanoparticles, nanocapsules, nanospheres, nanosuspensions, nanocrystals, nano-erythosomes etc. Nanotechnology is a technology relates with creation and manipulation [2]. Nanoparticles are newer development which is accessible in several forms like polymeric nanoparticles, solid-lipid nanoparticles, nanoemulsions, **nanosponges**,

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Article Published: April-June 2018

carbon nanotubes, micellar systems, dendrimers etc. [3]. Nanosponges are lucrative to solubilize poorly-water soluble drug and provide prolonged release as well as mended the drugs bioavailability [4]. Such system of

drug delivery is enabling to seize, carrying and selectively assertion a wide variety of elements due to their specialty of 3D-structure. They are applied to mask spiteful flavors and used to change fluid substances into solid [5, 6].

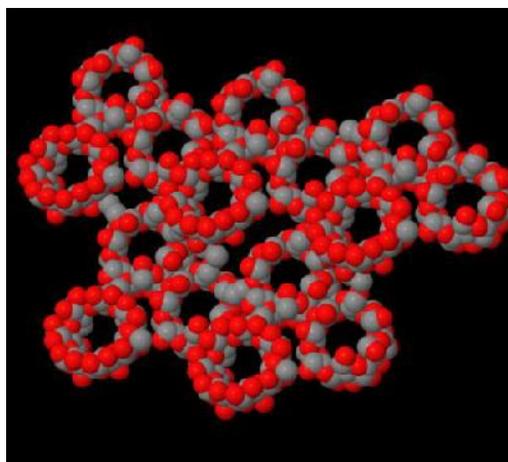
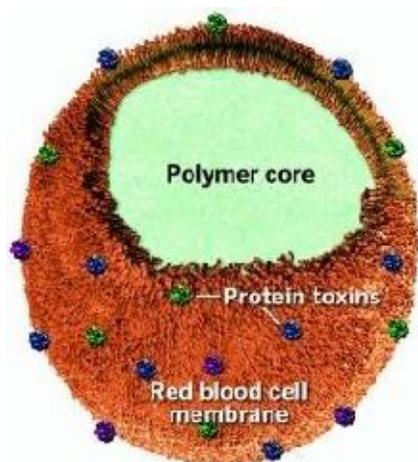


Fig 1.1: Nanosponge formed by Polymer

Formation of Nanosponges:

Nanosponges possess a three-dimensional network or scaffold. These are formed by reacting polyesters (Cyclodextrin) with

Fig1.2: Nanosponges formed by Cyclodextrin

appropriate crosslinking agents, a novel nanostructured material can be obtained, known as nanosponges [7,8].

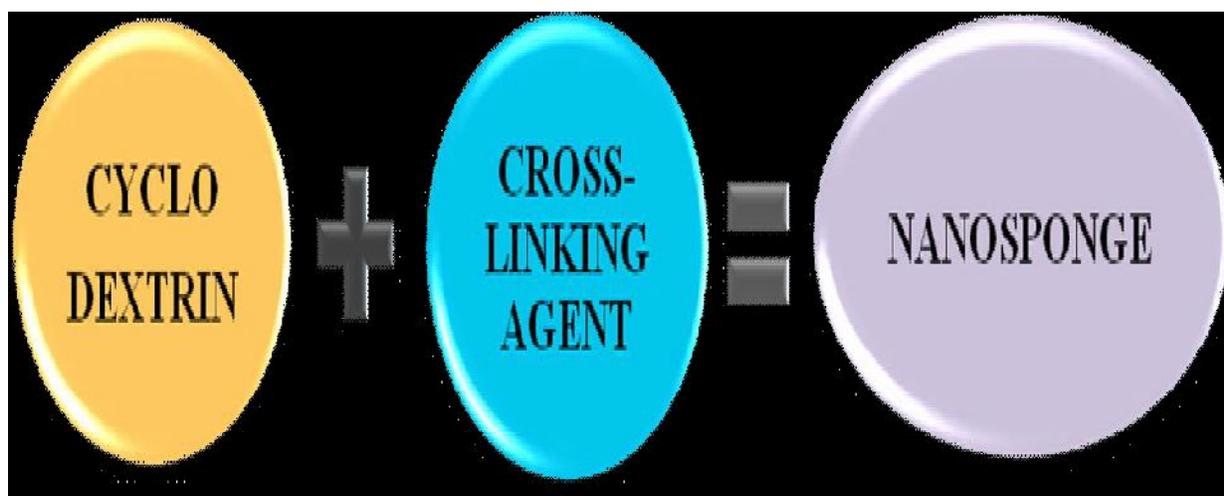


Figure 1.3: Formation of Nanosponges

Types of Nanosponges:

Nanosponges divided into following types which is given below [9]:

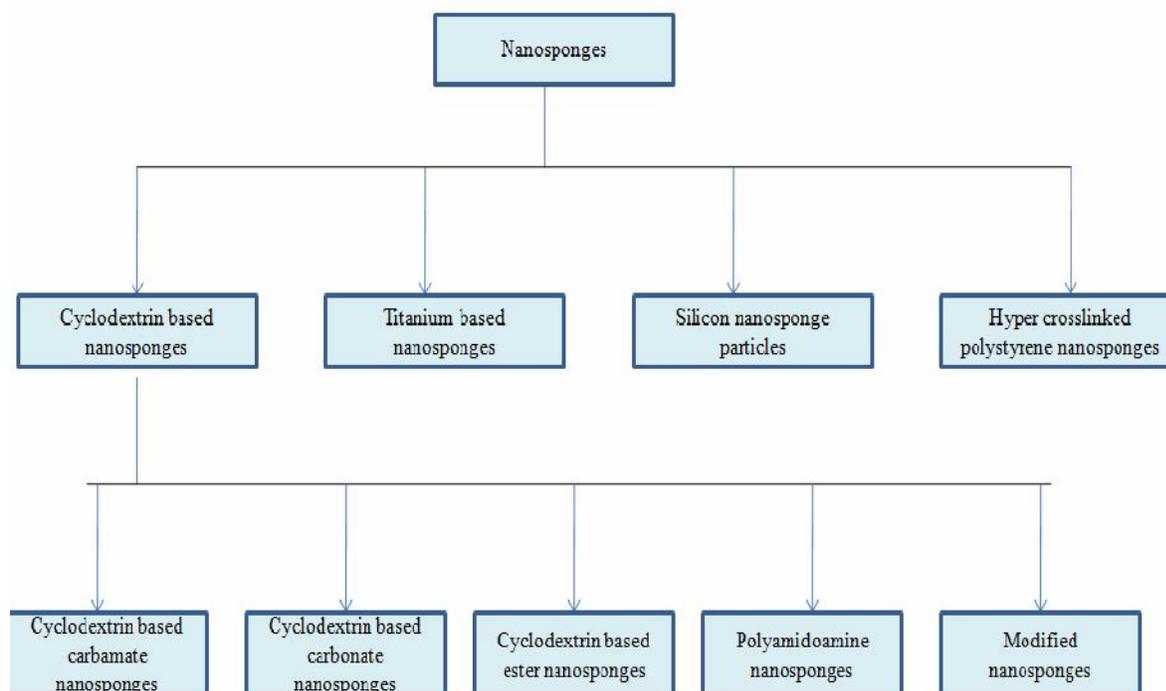


Figure 1.4: Types of Nanosponges

Ketoconazole (Nizoral®) is an imidazole derivative. It comes under the category of class II drugs. It can be absorbed orally, but an acidic gastric environment provides more absorption. It remains useful in the treatment of cutaneous dermatophyte and yeast infections, but it has been fungible by the newer triazolesin the treatment of severe Candida infections and protrudes mycoses. Ketoconazole is usually effective in the treatment of ringworm, jock itch, thrush (oral candidiasis), athlete's foot, seborrheic dermatitis & leishmaniasis are widespread dermatophyte infections on topical surface of

skin can be cure easily with oral ketoconazole drug [10].

EXPERIMENTAL WORK**Materials:**

Ketoconazole (KTZ) was the generous gift from Aarti laborates Ltd, Uttarakhand. Polyvinyl alcohol (PVA), Ethyl cellulose, Methanol (99%) and Triethanolamine was purchased from Merck (P) Ltd., Mumbai. Carbopol 934 was procured from Molychem, Mumbai. All other reagents were available at the highest grade and were obtained from commercial sources.

Methods of Preparation of Nanosponges [16].

1. Melt Method.
2. Solvent diffusion methods:
 - (a) Emulsion Solvent diffusion method.
 - b) Quasi-emulsion solvent diffusion
3. Ultra Sound Assisted Method.
4. Solvent method.

Table 1.1: Preparation of Ketoconazole loaded nanosponge

COMPOSITION	F1	F2	F3	F4	F5	F6
Drug :Polymer (mg)	50:100	50:150	50:200	50:250	50:300	50:350
(KTZ : Ethyl cellulose)						
Poly vinyl alcohol (%w/v)	0.5	0.5	0.5	0.5	0.5	0.5
Dichloromethane (ml)	20	20	20	20	20	20
Purified water (ml)	100	100	100	100	100	100

Drug loading in Nanosponges:

Nanosponges mean particle size below 500-600 nm should be needed to penetrated or better drug delivery. Nanosponges were pendent in water and sonicated to avoid the presence of masses. The obtained suspension is centrifuged to attain the colloidal fraction. Unglued the supernatant and dehydrated by freeze drying. Then the prepared aqueous suspension of nanosponges was distributed to the superfluous amount of drug. Such mixture placed under constant stirring up to a specified period of time for complexation. As a final point the solid crystals of nanosponges are obtained by solvent evaporation or freeze drying [12].

Development of Nanosponge loaded gel:

Initially the polymer Carbopol 934P (100 mg) was moistened in solvent i.e., water (5 ml) for the gel for 2-3 hrs and dispersed by constant stirring at 600 rpm with aid of magnetic stirrer to get smooth dispersion. Then to the above dispersion added Triethanolamine (2% v/v) to neutralize the pH. The previously prepared ameliorate nanosponge suspension and Propylene glycol as permeability enhancers was added to the above aqueous dispersion and make up the volume upto 100 ml with distilled water [16].

EVALUATION OF DRUG LOADED NANOSPONGES:

Physical properties of ketoconazole:

Physical parameters of drug like appearance, melting point, strength and solubility was determined by visual examination.

UV Spectrophotometric Analysis:

UV spectrum measurement was done by using methanol and Phosphate buffer of pH 6.8 solvent solution. Sample in different concentration of suitable solvent was calibrated under UV spectrophotometer and absorbance was recorded. Then plot the calibration curve between the ratio of concentration ($\mu\text{g/ml}$) and absorbance.

FT-IR Analysis of Drug and Drug-Polymer Mixture:

Fourier transformed-infrared spectroscopy (FT-IR) spectra were obtained using Shimadzu IR-prestige 21 FTIR spectrometer. Such analysis significant to estimate the FT-IR spectrum of nanosponges. For this Potassium Bromide (10 mg) was mixed with 2 mg of sample and allow for spectral studies within the range of wave number region of $4000\text{-}5000\text{ cm}^{-1}$, which is significant to determine the conformational changes of optimized drug when compared with pure drug and pure excipients.

Scanning Electron Microscopy (SEM) analysis:

SEM analysis significant for determination of surface characteristics and size of particle. Scanning electron microscope was operated at acceleration voltage of 15 kV. A concentrated aqueous suspension was spread in an equipment cell receiver and dried under

vacuum. The sample was shadowed in a gold layer 20 mm thickened cathodic evaporator attached with monitor which represents the images of sample. The processed images were recorded and individual formulated nanosponges particle diameter were measured to obtain average particle size.

Particle size

Nanosponges particles dispersions was characterized for average particle size (z-average size) using PCS (Photon Correlation Spectroscopy) [12].

Zeta Potential

Zeta potential is measured by an instrument known as zetasizer. It is used to measure the charge on particle surface in nanosponge formulation. Solvent not only deed as mechanical barrier but also concluded formation of surface charges Zeta potential, which can yield repulsive electrical forces. The more negative zeta potential, greater the net charge of particles and more steady the nanosponge preparation. Zeta potential values lower than -30 mv commonly indicate a high degree of physical stability [13].

Polydispersity index

Polydispersity index was resolute, which is the measurement of width of the particle size distribution is given by $d_{0.9}$, $d_{0.1}$ and $d_{0.5}$ are the particle size dogged at 90th, 50th and 10th percentile of particle undersized [14].

The particle size and polydispersity index of nanosponges was measured by Photon Correlation Spectroscopy using a Zetasizer Malvern, Version 7.03. Samples were diluted suitably with the aqueous phase of the formulation to get optimum kilo counts per second (Kcps) of 208.2–602.0 for measurements and the pH of diluted samples ranged from 6.9 to 7.2. The measurements were carried out in disposable sizing cuvette at 25°C, in 75%-100% intensity. The samples were analyzed at IIT, Roorkee and Troikaa Pharmaceuticals Limited.

Evaluation of Gel

Estimation of nanosponge based gel was performed by evaluating the formulation for pH measurement, clarity examination, spreadability, homogeneity, viscosity, skin irritability, drug content %, in-vitro drug diffusion profile. Such mention estimation was measured in triplicate and average values were projected. Such characterization performed by following process.

i) pH measurement :

For pH determination digital pH meter was used. weighed about 2.5 gm of nanosponge based gel and dispersed in 25 ml volume of water.

ii) Clarity examination:

The clarity testing of all prepared formulation was done by visual observation under black

and white background and it was categorized by following symbol; Turbid(+), clear(++), transparent /glassy (+++).

iii) Spreadability:

It is assessed by wooden base block and glass slide apparatus. For detection of spreadability, sample spread in between two slides and was compressed to uniform thickness by employing 1000 gm weight for a time period of 5 minutes. Weighed about 50 gm added to pan. Then quantify the time required to separate the slides from each other. So, spreadability was dignified as a time that was taken to separate upper glass slide moves over the lower plates. Following formula used to measured spreadability of gels:

$$S=ML/T$$

Where,

S= Spreadability,

M= Weight applied upon upper slide,

L= Length moved on the glass slide,

T= Time utilized to separate the slide from each other.

iv) Homogeneity:

This testing performed by visual examination of all nanosponge base gel formulations. For this evaluation process gel dispensed in transparent container and after settling of gel observed the formulation either it contain any

lump, particles, air entrapment or free from them [15].

v) Extrudability:

This evaluation was performed by using Pfizer hardness tester. In an aluminium tube about 15 gm of gel was filled. Then the plunger of hardness tester was attuned for wisely holding the aluminum tube. Then the pressure of 1kg/cm² was smeared for 30 sec. Due to pressure the quantity of gel extrude was noted

and in triplicate same procedure implemented in equidistance sides of tube.

vi) Viscosity determination:

Viscosity measurement performed by using Brookfield viscometer by using sample adapter which have spindle no. SC4-18/13R. In this viscometer nanosponge based gel formulation was subjected to a torque ranging from 10 to 100% and attained the viscosity through 'Rheocal software' [16].

Table 1.2 Estimation parameter to evaluating nanosponge based topical gels

Formulation code	pH data	Clarity testing	Homogeneity	Spreadibility (gm-cm/sec)	% drug content	Extrudability
A1	6.78	**	Good	22.04	88.3	*
A2	6.0	**	Good	17.3	86.04	**
A3	6.2	**	Good	21.07	90.25	*
A4	6.8	**	Good	18.3	90.15	*
A5	6.7	**	Good	19.42	92.53	*
A6	7.0	***	Good	25.12	88.42	*
B1	6.04	***	Good	22.42	87.24	**
B2	6.02	***	Good	20.52	89.24	**
B3	6.5	***	Good	21.42	88.04	**
B4	6.8	***	Good	23.34	87.12	**
B5	6.87	***	Good	20.43	88.06	**
B6	7.00	***	Best	25.24	93.02	***
C1	6.01	**	Good	17.10	76.27	*
C2	6.05	**	Good	18.1	88.53	*
C3	6.02	**	Good	18.4	87.14	*
C4	6.05	**	Good	17.10	87.14	*
C5	6.45	**	Good	19.2	88.05	*
C6	6.90	**	Best	24.02	93.06	**

Symbol: (*) Acceptable, () Good, (***) Excellent.**

Drug Content or Entrapment Efficiency (%)

Entrapment efficiency (EE %) or loading efficiency of drug was determined by following steps: Accurately weighed about 10 mg of nanosponges (F1) and stirred properly with analytical grade solvent Methanol (10ml) after stirring the attained solution was kept overnight. Same phenomenon followed for other proportion (F2 to F5) of nanosponge formulation. Then, filtered the all F1 to F6 formulation and diluted with methanol. For calculating the attentiveness of free drug (unentrapped) in aqueous medium detected the formulation spectrophotometrically at 292nm. The nanosponges, since it influences the release characteristics of drug molecule [17]. The amount of drug encapsulated per unit weight of nanosponges is determined after separation of the entrapped drug from the Nanosponges formulation:

$$\text{Entrapment Efficiency} = \frac{\text{Total drug(assay)} - \text{Free drug}}{\text{Total Drug}} \times 100$$

Such procedure follows for 5-6 times for 8 hrs. Maintain the drug diffusion graph [18,19].

RESULTS AND DISCUSSION:

***in-vitro* drug release study:**

For drug diffusion release study of Ketoconazole loaded nanosponge based topical gel formulation modified Franz diffusion cell was used and for simulation cellophane membrane was used. Such process performed in following steps:

Membrane was soaked in 0.1 N HCl for 18 hr. In receptor compartment of Franz diffusion cell was filled with 6.8 pH phosphate buffer solution about 7 ml. In donor compartment a measured quantity about (1g Of gel contains 100mg of dug loaded nanosponges) was applied uniformly on the cellophane membrane surface. The prepared membrane mounted over the modified Franz diffusion cell cautiously to evade air bubbling underneath the membrane. Entire assembly was conserved at 37°C for 08 hrs and stirring also be at constant speed about 600-700 rpm. Then, from the receptor compartment 1ml sample was withdrawn every 1hr and equal amount 1 ml fresh sample was deposited. All the analysis results were computed in following way.

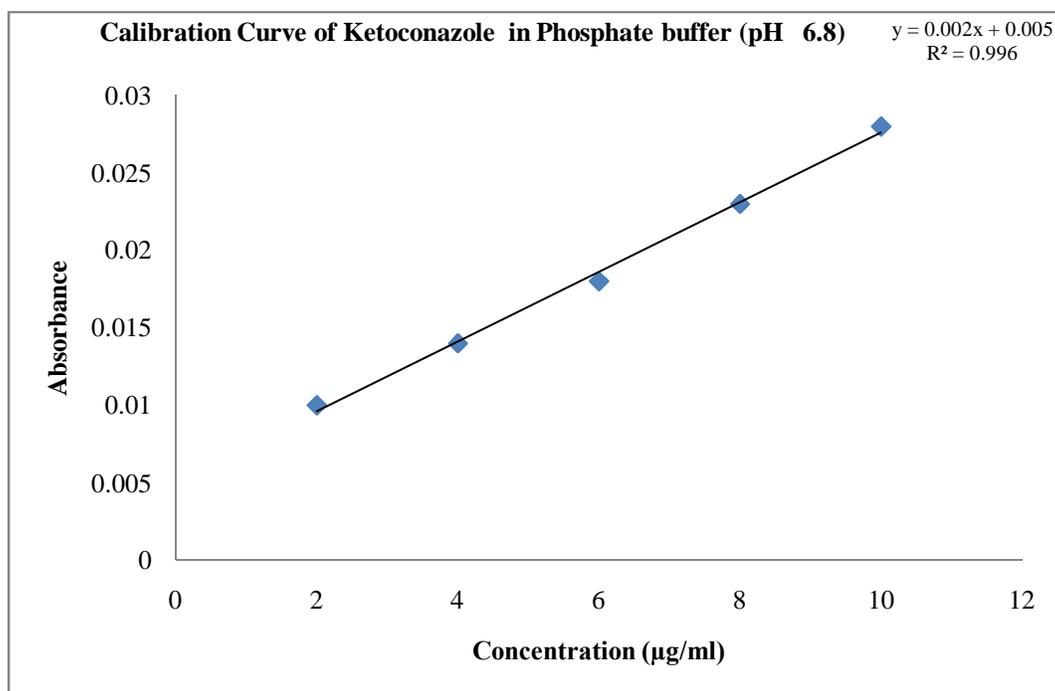
- **Physical properties analysis data** for quality determination of drug given below in following figure report:

Table: 1.3 Tabulated representations of physical properties of Ketoconazole

S. No	Parameters	Inferences
1)	Appearance (colour, nature and taste)	White, crystalline powder and unpleasant taste.
2)	Melting Point	149 ⁰ C
3)	Solubility	Insoluble in water, soluble in methanol

UV Spectrometric measurement:**Table: 1.4** Tabulated and graphical representation of the standard curve of Ketoconazole in Phosphate Buffer (pH 6.8).

Concentration ($\mu\text{g/ml}$)	Absorbance
0	0
2	0.010
4	0.014
6	0.018
8	0.023
10	0.028

**Fig 1.7** Graphical Representation of standard curve of Phosphate buffer pH

- **Drug Content or Entrapment Efficiency (%)**

Table: 1.5 Tabulated and graphical representation of Entrapment efficiency

Formulation	Drug content (%)
F1	16.7
F2	25.81
F3	35.36
F4	37.63
F5	40.1
F6	42.0

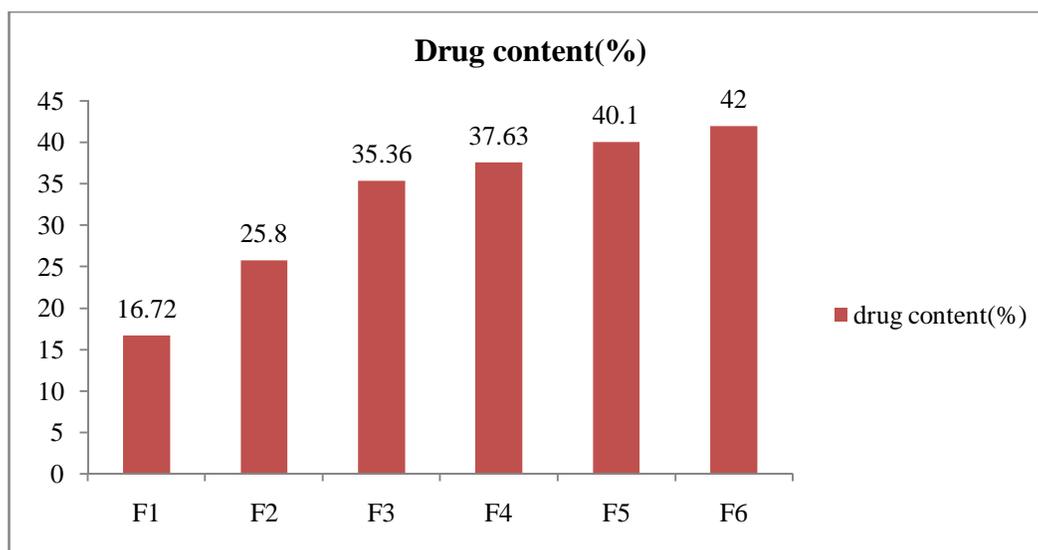


Fig.1.8 Graphical representation of Drug content

- ***In-vitro* drug release study**

Table: 1.6: Kinetic study of drug release data

Sr no.	Time ▼	%CDR ▼	Model Fitting	R ²	k
1	0.0	0.00			
2	1.0	2.64	Zero order	0.9837	3.6687
3	2.0	5.45			
4	3.0	8.48	1st order	0.9685	-0.0431
5	4.0	11.58			
6	5.0	15.29	Higuchi Matrix	0.9123	5.6105
7	6.0	19.76			
8	7.0	24.31	Peppas	0.9958	1.4841
9	8.0	29.92			

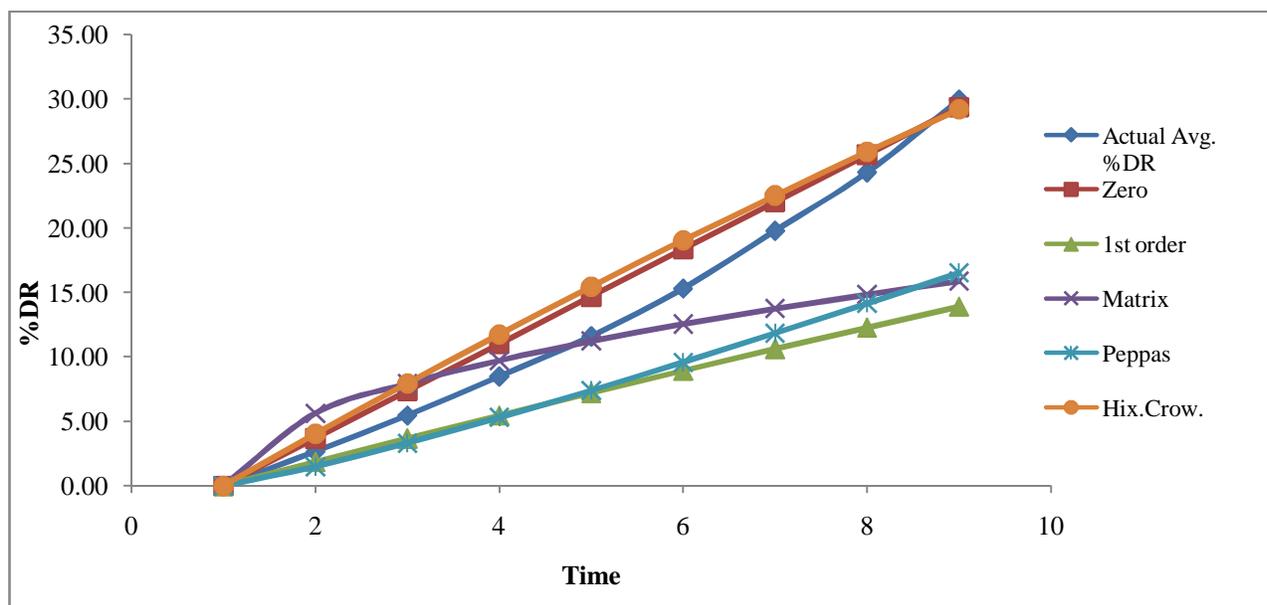


Fig no. 1.9 graphical representation of kinetic study of drug release data

Estimation and discussion of *in-vitro* release study:

The drug release data was formfitting in different kinetic models using BIT-SOFT software where data was premeditated for zero order, first order and Higuchi kinetics. It was conclude that the drug discharge data followed

the zero order kinetics more closely in comparison to first order and Higuchi kinetics. Further the data was best fitted to Peppas-Korsmeyer equation as the correlation coefficient was found to be 0.9958. The exponent value was 1.1581 which revealed that drug diffusion followed super case II transport mechanism.

Particle Size and Polydispersity Index of different prepared Nanosponge

Table: 1.7:Tabulated representation of Particle Size and Polydispersity Index

Formulation	Particle size (nm)	Polydispersity index
F1 (50:100)	579.8	0.682
F2 (50:150)	882.7	0.777
F3 (50:200)	306.1	0.487
F4 (50:250)	363.2	0.599
F5 (50:300)	269.2	0.403
F6 (50:350)	205.6	0.585

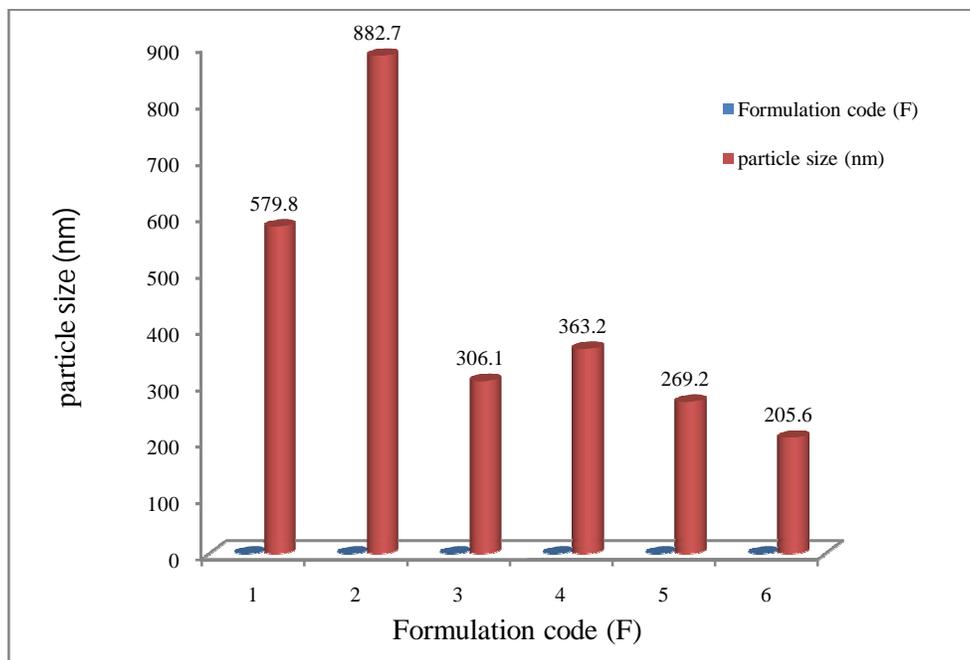


Fig 2.0 Bar Diagram of Particle size analysis

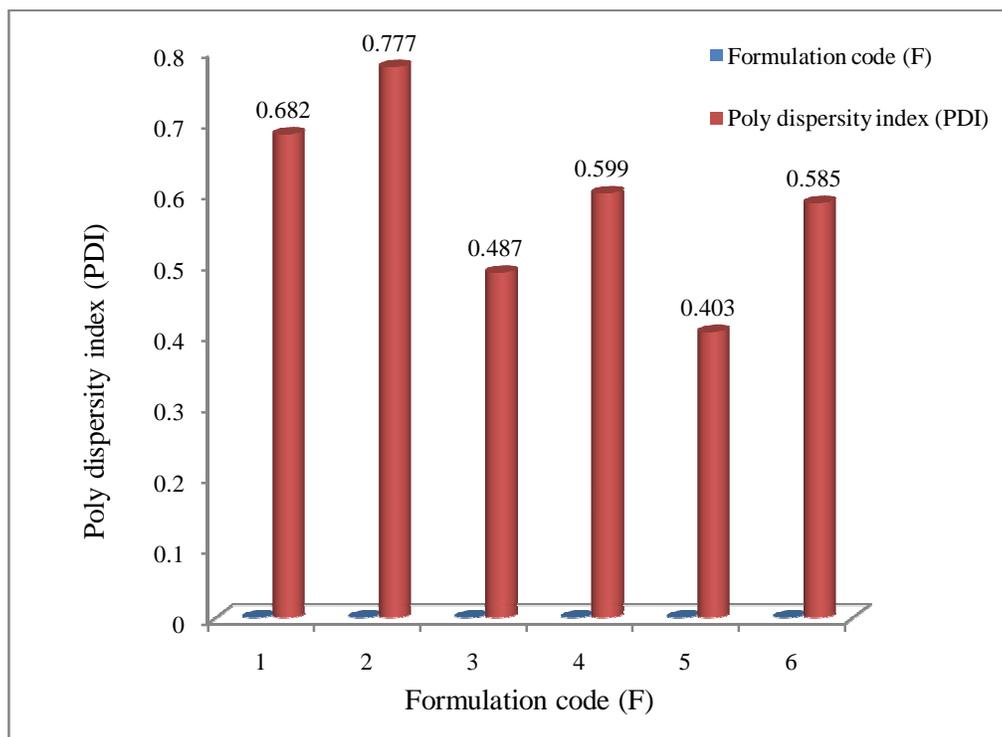


Fig.2.2 Bar Diagram of Polydispersity index

Scanning Electron Microscopy (SEM) analysis of different proportion of nanosponge formulation

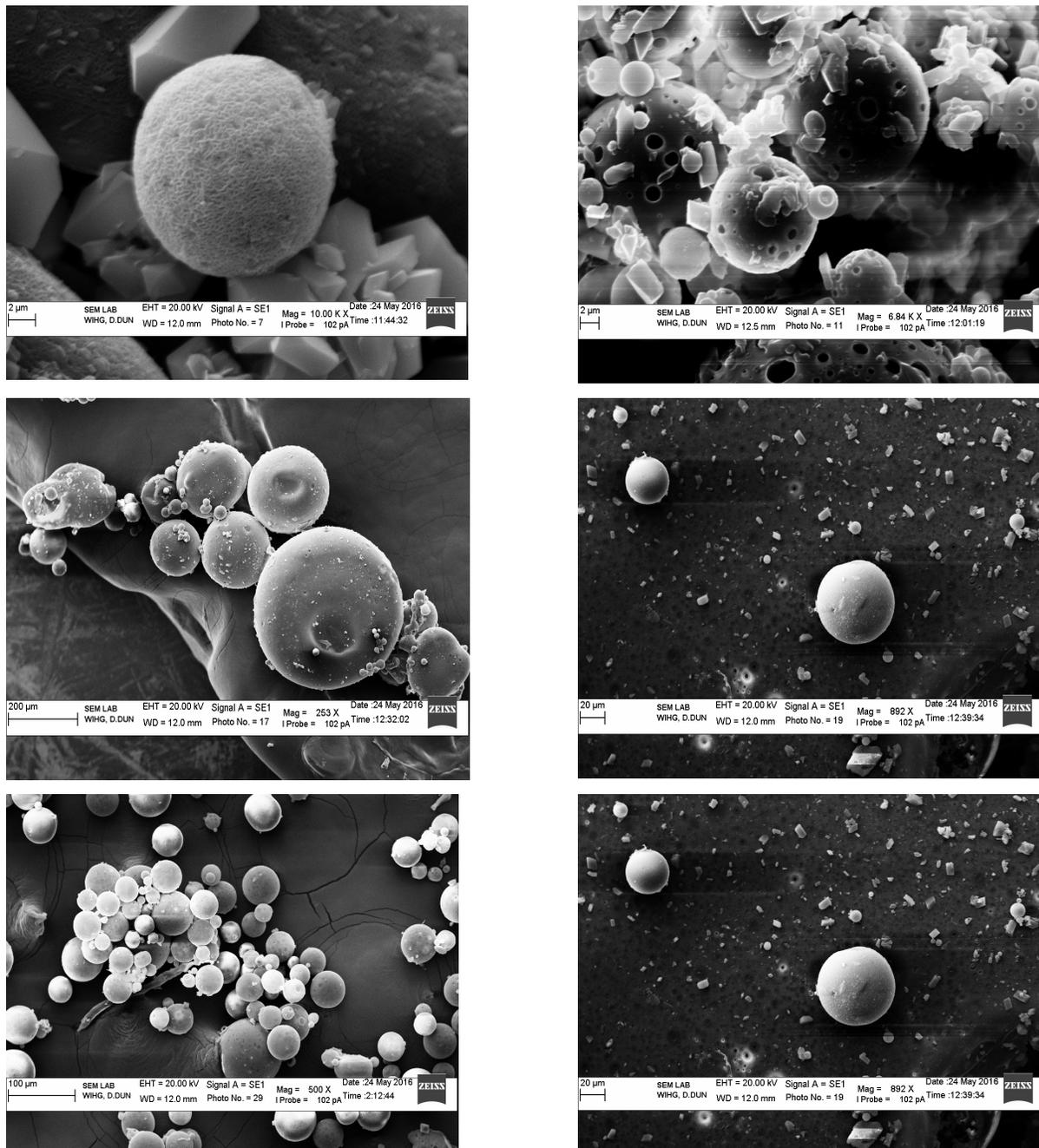


Fig 2.4 SEM of different proportion of nanosponge based topical gel formulation (F1, F2, F3, F4, F5 and F6 respectively)

Discussion

From the in-vitro diffusion release study and kinetic modeling following observation was estimated: The drug release data was fitted in different kinetic models using BIT-SOFT software where data was estimated for zero order, first order and Higuchi kinetics. It was concluded that the drug release data followed the zero order kinetics more closely in comparison to first order and Higuchi kinetics. Further the data was best fitted to Korsmeyer-peppas equation as the correlation coefficient found to be 0.9958. The exponent value was 1.1581 which revealed that drug diffusion followed super case II transport mechanism.

Conclusion

Topical route is most prominent and effective route for controlled and targeted drug delivery. The aim of this work is to produce controlled release ketoconazole (KTZ) loaded nanosponge based gel for topical delivery and to treat cutaneous fungal infection in short period of time.

Such formulation act by enhancing solubility of ketoconazole due to which controlled and prolonged drug release attained. Nanosponges loaded with ketoconazole drug prepared by using ethyl cellulose as polymer, polyvinyl alcohol as surfactant and dichloromethane as crosslinking agent by solvent evaporation method.

The physical characterization like effect of drug and polymer ratio, surfactant and excipient concentration, stirring speed and time, drug entrapment efficiency were calculated.

For determination of particle size and surface morphology performed by particle size analysis by malvern zeta sizer and SEM and result shows that the drug particle are nano (200-600nm) in range and spherical-spongy in nature.

The nanosponge based gel formulation prepared by using carbopol 934, carbopol 940 and sodium carboxy methyl cellulose and estimated for pH, viscosity, spreadability, extrudability, in-vitro drug release.

The optimized formulation shows efficient, standard and good result which exhibit that the nano technology was a better choice as future aspects for topically targeted and controlled drug release in promising field of health and life sciences.

Acknowledgement

This work was performed in partial fulfillment of the requirements for a M. Pharm. of Ms. Jyoti Pandey, Research Scholar, Uttarakhand Technical University.

References

1. Mark AM, Przemyslaw R, Greg C, Greg S, Akram S, Jonathan F *et al.* *In vivo* human time exposure study of orally dosed commercial silver nanoparticles.

- Nanomedicine: Nanotechnology, Biology, and Medicine. 2014, Vol.10, pp. 1-9.
2. Vyas SP, Khar RK. Novel carrier systems: Targeted and controlled drug delivery, 2002, 1st ed, New Delhi: CBS publishers & distributors, pp. 332-413
 3. Panda S, Vijayalakshmi Sv, Pattnaik S, Prasad R. Nanosponges: a novel class of drug delivery system-review. *International Journal of Pharma Tech Research*, 2015, Vol. 8, Issue. 7, pp. 213-224.
 4. Baburao et al. Nanosponges: A review on Novel Drug delivery system, 2015, Vol.330, pp. 1054-1055.
 5. Swaminathan S, Pastero L, Serpe L, Trotta F, Vavia P, Aquilano D *et al.* Cyclodextrin based nanosponges encapsulating camptothecin: Physicochemical characterization, stability and cytotoxicity. *European Journal of Pharmaceutical sciences.*, 2010, Vol. 74, pp. 193-201.
 6. Richhariya Neha, Dr. Prajapati S.K., Dr. Sharma U.K. Nanosponges: an innovative drug delivery system. *World Journal of Pharmaceutical Research.*, 2015, Vol. 4, Issue. 7, pp. 1747-1759.
 7. Subramanian S, Singireddy A, Krishnamoorthy K *et al* Nanosponges: a novel class of drug delivery system-review. *Journal of Pharmaceutical Sciences.*, 2012, Vol. 15, Issue. 1, pp. 103-111.
 8. Shinde G, Rajesh KS, Bhatt D, Bangale G, Umalkar D, Virag G. Current status of colloidal system (nano range). *International Journal of Drug Formulation and Research.*, 2011, Vol.2, Issue.6, pp. 39-54.
 9. Trotta F, Zanetti M, Cavalli R. Cyclodextrin-based nanosponges as drug carriers. *Beilstein J Organic Chemistry*, 2012, Vol. 8, pp. 2091-2099.
 10. Swaminathan S, Vavia PR, Trotta F, Cavalli R, Tumbiolo S, Bertinetti L, et al. Structural evidence of differential forms of nanosponges of beta-cyclodextrin and its effect on solubilization of a model drug. *Journal Including Phenominal Macrocyclic Chemistry.*, 2012, Vol. 76, pp. 201-211.
 11. Patel EK, Oswal RJ. Nanosponge and microsponges: a novel drug delivery system. *International journal of Pharmaceutical Chemistry and Research.*, 2012, Vol.2, Issue.2, pp.237-244.
 12. Vrushali Tamkhane, P.H. Sharma. Nanosponge-A Novel Drug Delivery System. *International Current Pharmaceutical Journal of Research.*, 2014, Vol. 4, Issue. 3, pp. 1186-1193.
 13. B.V. Mikari, K.R.Mahadik, Formulation and evaluation of topical liposomal gel for fluconazole. S.A. Korde, *Indian Journal of Pharmaceutical Sciences.*, 2010. (2007), Vol. 44, Issue. 4, pp. 324-325.
 14. Kaur Loveleen Preet, Guleri Tarun Kumar, Topical Gel: A Recent Approach for Novel Drug delivery. *Asian Journal of Biomedical and Pharmaceutical Sciences.*, 2013. Volume. 3, Issue 17, pp. 1-2.
 15. K. Saroha, S. Singh, A. Aggarwal, S. Nanda, Transdermal Gels- An alternative vehicle for drug delivery, *International Journal Pharmaceutical Research and Biosciences.*, 2013, Vol.3, Issue.3, pp. 495-503.
 16. S. Roy chowdhary, D. H. Singh, R. Gupta, D. Manish, A Review on Pharmaceutical gel, *International Journal Pharmaceutical Research and Biosciences.*, 2012, Vol. 1, Issue. 5, pp. 21-36.
 17. Shah VP: Transdermal drug delivery system regulatory issues. In: Guy R.H. and had graft J. (eds.), *Transdermal drug delivery.* Marcel Dekker, New York, 2003, pp. 361-367.
 18. Wilson and Ross, Waugh A. & Grant A., *Anatomy and Physiology in Health and Illness*, Churchill Livingstone Elsevier Limited, 10th edition, 2006. pp. 358.
 19. Bolmol U. B., Manvi F. V., Kotha Rajkumar, Palla S. S., Paladugu A, Reddy K R, Recent advances in nanosponge as drug delivery system, 2013, Vol. 6, pp. 3.