Original Research Article

Comparative Characterization of Gel Loaded Ketoprofen Nanosponges for Topical Delivery

Viresh K Chandur[1], Thrishna TK[1], Ramkrishna Shabaraya A[2]

[1]Associate Professor, Department of Pharmaceutics, Srinivas College of Pharmacy, Valachil, Farangipete Post, Mangalore, Karnataka -574143
[2]Professor and HOD, Department of Pharmaceutics, Srinivas College of Pharmacy, Valachil, Farangipete Post, Mangalore, Karnataka -574143

Corresponding Author: Viresh K Chandur

ABSTRACT

Nanosponges are tiny mesh-like nano porous particular structure in which a large variety of substances can be encapsulated or suspended, and then be incorporated into a dosage form. The aim of the present study was to develop and characterize an optimal stable nanosponges of Ketoprofen by emulsion solvent diffusion method using Ethyl Cellulose as polymer and Polyvinyl Alcohol as surfactant with different concentration and aimed to increase its bioavailability and release the drug in a controlled manner. Formulated nanosponges were evaluated for percentage yield, drug entrapment efficiency, surface morphology, particle size analysis and zeta potential. The FTIR spectra showed stable character of Ketoprofen in mixture of polymers and revealed the absence of drug polymer interactions. Selected F5 formulation showed, Percentage Yield (88 ± 0.16), %EE (86 ± 1.23%), Particle Size (320.7nm) and Zeta Potential of (-24.3mV) respectively and from SEM analysis its concluded that nanosponges are spherical, discrete with smooth surface. Best formulation F5 was loaded into the 1% carbopol 934 P gel which was evaluated for viscosity, spreadability, pH, drug content, in-vitro drug diffusion study, release kinetics, comparative study and stability studies. The release kinetics of the optimized formulation shows zero-order drug release. The stability study indicates no significant change in the in vitro diffusion profile of optimized formulation.
Finally we concluded that prepared Ketoprofen nanosponge topical gel showed promising drug release and stability.

Keywords: Ketoprofen nanosponge, Topical gel, Zeta Potential, Zero order, Carbopol 934 P.

INTRODUCTION

Topical drug delivery is the application of pharmaceutical dosage form to the skin for direct treatment of cutaneous disorder or the cutaneous manifestation of the general disease, with the intent of confining the pharmacological or other effect of the drug to the surface of the skin[1].It can penetrate deeper into skin and hence give better absorption. Topical application has number of advantages over the conventional dosage forms. In general, they are deemed more effective less toxic than conventional formulations due to the bi-layered composition and structure. In the formulation of topical dosage forms, attempts has being made to utilize drug carriers that ensure adequate localization or penetration of the drug within or through the skin in order to enhance the local and minimize the systemic effect [2]. A brand-new class of materials called nanosponges is formed of small particles with voids that are only a few nanometers across and can contain a vast range of different substances. These particles have the capacity to transport both hydrophilic and lipophilic materials, as well as to increase the solubility of weakly water-

soluble compounds. By minimising repeated doses and adverse effects, topical nanosponge can increase patient compliance while still offering enough advantages for the patient [3].

NSAIDs are the best choice to be administered through Topical drug delivery system in the management of diseases like Osteoarthritis, Rheumatoid Arthritis and similar inflammatory disorders. Oral administration of NSAID shows side effects like Nausea, Vomiting, Heartburn, Gastric Ulceration, Epigastria. These drawbacks can be overcome by developing sustained release non-steroidal anti-inflammatory topical gel which is able to provide constant drug concentration at the site of administration and also avoids first pass effect, with increased patient compliance and ease of application [4].

Ketoprofen (KP; 2- (3-benzoylphenyl-propionic acid)) is a non-steroid anti inflammatory drug, most often used for the treatment of muscular-skeletal aches. It possesses perfect physico-chemical properties - a suitable distribution coefficient, low molecular weight and high permeability. Its plasma levels are kept relatively constant for approximately 24 hours after transdermal application.

The present study was aimed towards the formulation and evaluation of gel loaded with nanosponges of Ketoprofen for topical delivery using ethyl cellulose as polymer by emulsion solvent diffusion method. Based on the characterization, nanopsonges with high entrapment efficiency and least particle size (F5) was selected for hydro gel formulation. Hydro gels were prepared by using Carbopol 934P as gelling agent [5].

MATERIALS & METHODS
Materials
Ketoprofen was kindly provided by Yarrow Chem Products, Mumbai. Ethyl Cellulose, Poly Vinyl Alcohol and Dichloromethane were purchased from HI MediA Labortories . All the reagents were analytical grade.
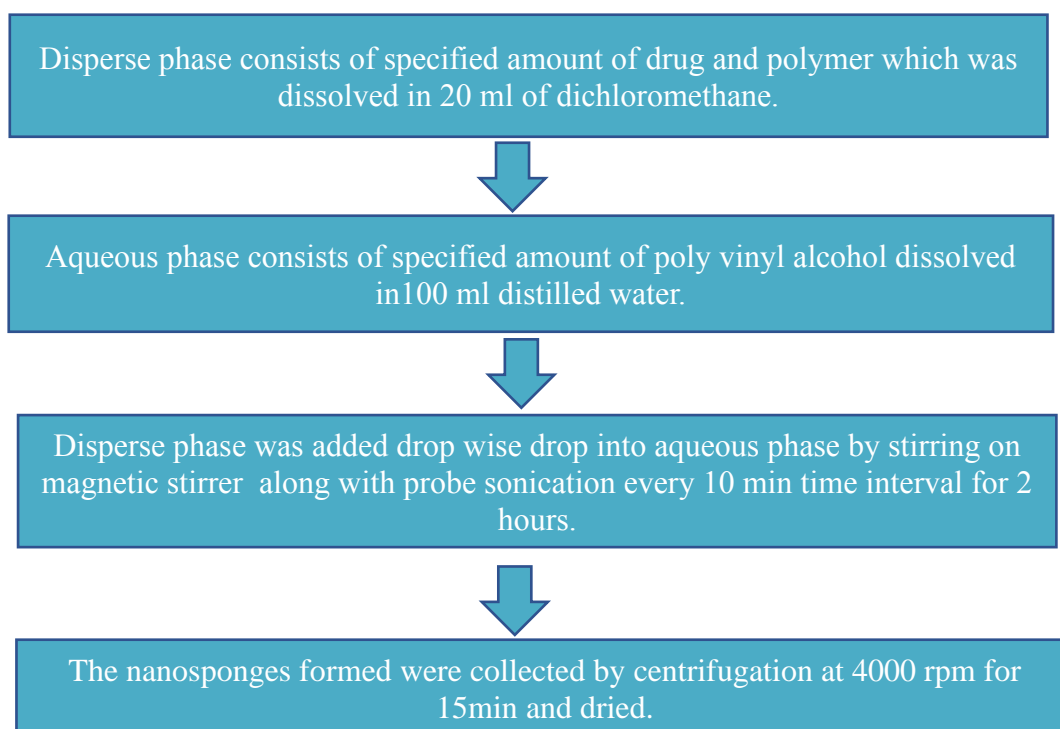
METHODS
Formulation of Ketoprofen Nanosponges [6-7]

Figure 1: Emulsion solvent diffusion method of nanosponges

Table 1: Formulation chart of Ketoprofen loaded nanosponges

INGREDIENTS	FORMULATIONS					
	F1	F2	F3	F4	F5	F6
Ketoprofen (mg)	250	250	250	250	250	250
Ethyl Cellulose (mg)	200	200	300	300	400	400
Polyvinyl alcohol (mg)	200	300	200	300	200	300
Dichloromethane (ml)	20	20	20	20	20	20
Distilled water (ml)	100	100	100	100	100	100

Figure 2: Prepared Ketoprofen nanosponges.

Formulation of Ketoprofen nanosponges loaded gel [8-9]

Accurately weighed amount of carbopol 934 was moistened in solvent for 2 hrs.

↓

Dispersed by constanting at 600 rpm with the aid of magnetic stirrer to get smooth dispersion.

↓

The stirring is stopped and dispersion is allowed to stand for 15 min to expel entrapped air.

↓

To this solution, prepared drug loaded nanosponges were added, propylene glycol is added as penetration enhancer, Methyl paraben is added as preservative and make up the volume with distilled water.

↓

Slowly add drop wise drop of triethanolamine to the prepared gel to neutralize pH.

Fig 3: Simple dispersion method to prepare Ketoprofen nanosponges loaded gel

Fig 4: Photograph of prepared Ketoprofen Nanosponges Gel

PREFORMULATION STUDIES OF PURE DRUG

Construction of calibration curve of ketoprofen[10-11]

Determination of absorbance maximum (λmax)

The standard solution of Ketoprofen (10 µg / ml) in phosphate buffer of pH 7.4 is scanned in the wavelength region of 200-400 nm and the λmax is found.

Preparation of stock solution of Ketoprofen using phosphate buffer of pH 7.4

Stock solution A:

Stock solution of ketoprofen were prepared by dissolving 100 mg of the drug in 10 ml of 7.4 pH Phosphate buffer and the volume was made up to 100 ml with same buffer . This gives drug concentrations 1000 µg / ml (Stock A).

Stock solution B:

10 ml of stock A were transferred to 100 ml volumetric flask and the volume made up to 100 ml with phosphate buffer of pH 7.4 to get concentration of 100µg / ml (Stock B).

Standard calibration curve:

0.2, 0.4ml, 0.6ml,0.8ml and 1.0ml of the stock solution B was diluted with phosphate buffer of pH 7.4 to make the concentration of 2,4,6,8 and 10 µgm/ml. absorbance of each solution were measured at λ max using UV spectrophotometer. Standard curve was generated for the entire range from 2 to 10µgm/ml.

Compatibility studies [12-13]

FTIR can be used to investigate and predict any physicochemical interaction between different excipients. IR spectra matching approach was used for detection of any possible chemical interaction between the drug and excipients. The mixture of drug and excipients was scanned in FTIR spectrophotometer in the range of 4000 to 400 Cm^{-1}. The IR spectrum of the mixture was compared with that of pure ketoprofen and peak matching is done to detect any appearance or disappearance of peaks.

EVALUATION OF NANOSPONGES CONTAINING KETOPROFEN

1. Percentage yield:[13]

The percentage yield of the nanosponge can be calculated by following equation. Accurate initial weight of the raw materials taken and final weight of the nanosponge obtained was calculated.

$$Percentage\ Yield\ (PY) = \frac{(Practical\ mass\ of\ nanosponges\ x\ 100)}{(Theoretical\ mass\ (drug + polymer)}$$

2. Determination of drug entrapment efficiency:[14-15]

The entrapment efficiency was determined by measuring 10 mg Ketoprofen loaded Nanosponge in 10 ml of 7.4 pH phosphate buffer and then the dispersion were centrifuged at 4000 rpm for 30minutes in order to separate entrapped from the un entrapped drug. The free drug concentration in supernatant layer after centrifugation is determined at λmax (260nm) using UV Spectrophotometer.

The percentage entrapment efficiency (%EE) was calculated by following formula:

$$\%EE = \frac{Weight\ of\ Initial\ drug - Weight\ of\ free\ drug.}{Weight\ of\ Initial\ drug\ x\ 100}$$

3. Particle size :[16]

The particle size of Ketoprofen nanosponges was determined using Malven zeta sizer. From this, the mean diameter was measured. Measurements were made at the fixed angle of 90^0 for all the samples .The samples were suitably diluted with water for every measurement.

4. Zeta potential[17]

Zeta potential analysis was performed to estimate the stability of the Nanosponges. It is performed to estimate the stability of the Nanosponges. Zeta potential is a measure of effect of electrostatic charges. This is the basic force that causes the repulsion between adjacent particles. The nanosponges was diluted to 10 times with distilled water and analysed by zeta sizer using laser Doppler micro electrophoresis.

5. SEM analysis:[18]

For the evaluation of the surface morphology of nanosponges, the sample was analysed in a scanning electron microscope after preparing the sample by lightly sprinkling on a double adhesive tape stuck to an aluminium stub. The stubs were then coated with platinum. The stub containing the coated sample was placed in a scanning electron microscope. The samples were then randomly scanned and photomicrographs were taken at the acceleration voltage of 20 kV. From the resulting image, average particle size was determined.

CHARACTERIZATION OF GEL CONTAINING KETOPROFEN
1. Physical Examination:

Gels should have a pleasant appearance with respect to colour, consistency etc. The prepared nanosponge gel was inspected visually for their colour, homogeneity, consistency, and grittiness.

2. Determination of viscosity :[19-20]

The measurement of viscosity of the prepared gel was done using Brookfield viscometer (Brookfield DV-II+Pro). The reading was taken at 0.6 rpm using spindle no 64.

3. Determination of pH :[21-22]

pH of the various gel formulations were determined using digital pH meter. The measurement of pH of each gel was done in triplicate by dissolving1 gm of gel in 50 ml water and average values were calculated.

4. Spreadability :[23-24]

The spreadability of the gel formulation was determined by taking two glass slides of equal length. On one of the glass slide, 1 gm gel was applied and the other slide was kept over it and weights were added on it and the time taken in the seconds for the glass slide to slip off from the first glass slide was determined. A shorter interval indicates better spreadability. Spreadability was calculated by using the following formula.

$$S = \frac{M \times L}{T}$$

S= Spreadability, M= Weight tied to upper slide, L= Length of glass slide,T=Time taken to separate the slide completely from each other.

5. Drug content :[25]

The amount of drug contained in the prepared nanosponge gel was determined by dissolving 100 mg of prepared gel in 5 ml of methanol and volume was made uo to the mark with phosphate buffer (pH 7.4). The mixture was analysed by spectrometrically at λ-max against same blank.

6. *In- Vitro* drug diffusion studies :[26]

In- Vitro drug diffusion study of gel loaded ketoprofen nanosponges was carried out by using Franz diffusion cell. The formulation was taken in the donor compartment. Treated cellophane membrane was placed between

the donor and receptor compartment. 1g of the formulation was spread uniformly on the cellophane membrane, which is in contact with receptor medium. The whole assembly was placed on the thermostatically controlled magnetic stirrer with continuous stirring and the temperature of the medium was maintained at $37 \pm 0.5\,^0$C. At specific intervals, 1ml of sample was withdrawn from the receptor compartment and replaced it with equal volume of phosphate buffer pH 7.4. After suitable dilutions, the absorbance of the sample was determined by UV - Visible spectrophotometer λ-max.

7. Drug release kinetic studies[27-28]

To analyse the mechanism of drug release the release data was fitted to Zero order, First order, Higuchi's, and Korsmeyers peppa's equations to investigate the mechanism of drug release from the topical gel.

8. Comparison with conventional marketed product[29]

In order to compare the drug release from nanosponge gel loaded Ketoprofen and a marketed product in-vitro diffusion study was carried out in phosphate buffer pH 7.4 by using Franz diffusion cell and the samples were analysed spectrophotometrically at λ max

9. Stability studies[30-31]

Ketoprofen nanosponge gel were subjected to stability studies for 3 months at an accelerated condition at $40\pm2°C$ / $75\pm5\%$ RH as per ICH guidelines. Gel was evaluated for Physical appearance, drug content and *in-vitro* drug dissolution studies.

RESULT
Determination of absorption maximum (λmax)

This was performed by using UV spectrophotometer by using 7.4 pH phosphate buffer as medium. The spectrum of Ketoprofen (10 µg/ml) in 7.4 pH phosphate buffer showed the absorption maximum at 260 nm .
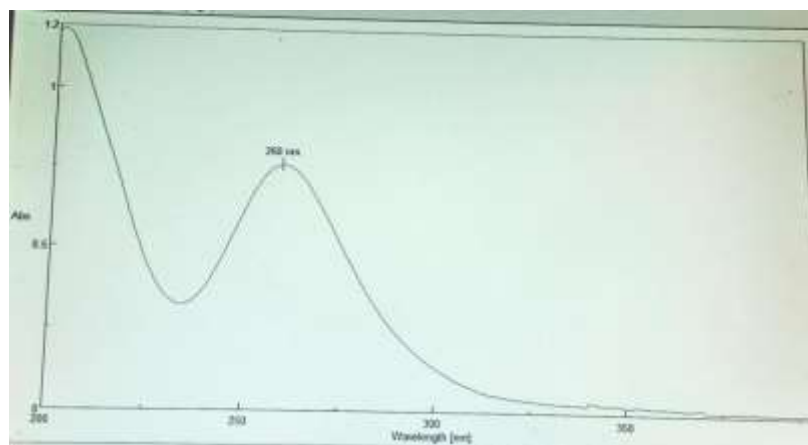
Figure 5: UV spectrum of Ketoprofen

Standard graph of Ketoprofen

Table No. 2: calibration data of Ketoprofen in phosphate buffer of pH 7.4

S No.	Concentration (µg/ml)	Absorbance *
1	0	0
2	2	0.156 ± 0.012
3	4	0.3122±0.017
4	6	0.4641±0.010
5	8	0.6065±0.011
6	10	0.7612±0.015

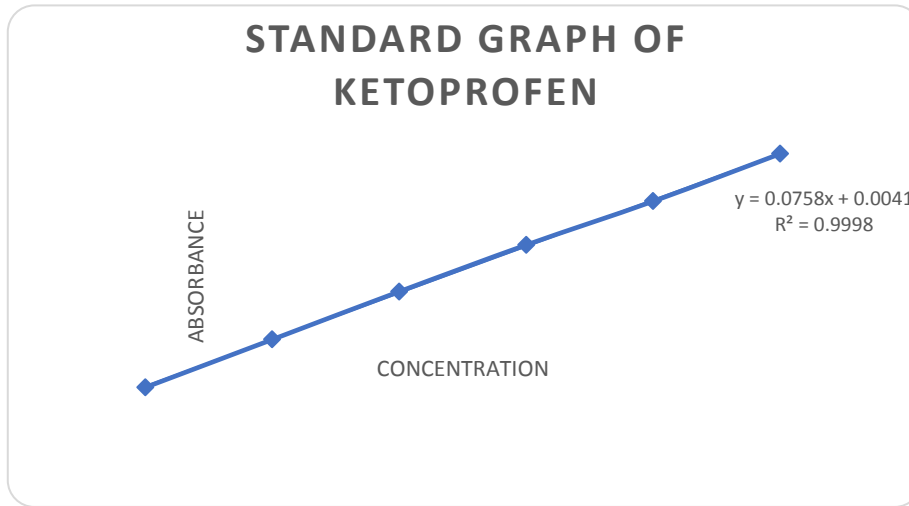* Data expressed as a mean ± SD, n= 3

Fig 6: Standard calibration curve of Ketoprofen in Phosphate buffer pH 7.4

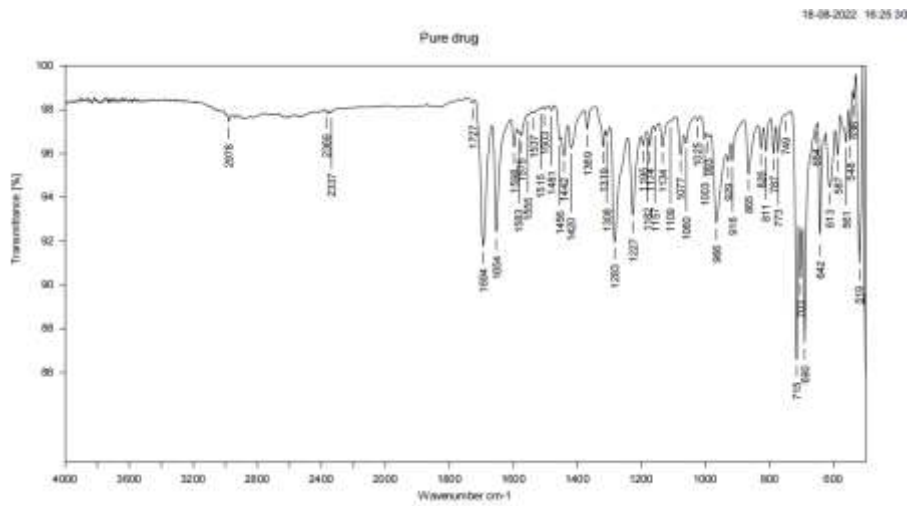## Drug-polymer compatibilty studies
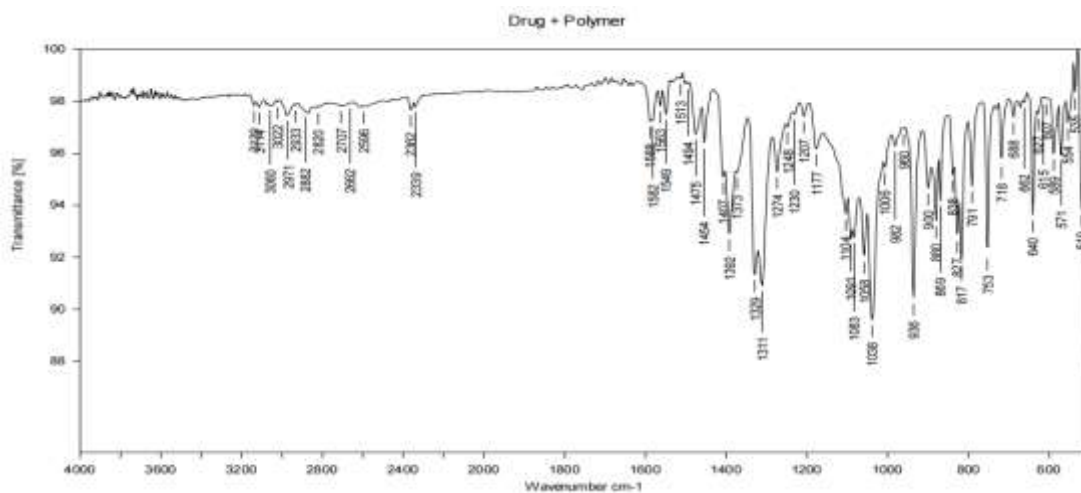

Figure7: IR-spectrum of pure drug Ketoprofen

Figure 8: IR-spectrum Ketoprofen+Ethyl cellulose

Comparison of FTIR spectra of Ketoprofen and Ketoprofen -Ethylcellulose

Table 3: Comparison of FTIR spectra of Ketoprofen and Ketoprofen -Ethylcellulose

Sr. No.	Functional groups	Reported frequency (cm⁻¹)	Observed frequency (cm⁻¹)	
			Drug	Drug - Polymer
1	C=C Stretching	1400-1600	1515	1513
2	C-H Stretching	3100	2978	2971
3	C=O stretching	1697	1583	1582
4	O-H stretching	3400-2300	2337	2339

The IR spectra of the Ketoprofen was compared with the mixture of drug and polymer and the characteristic peaks associated with specific functional groups and bonds of the molecule and their presence/ absence were noted in table No. 6 and the overlay of pure drug and mixture was shown in fig 7 & 8.

The prominent peaks associated with C=C stretch (1400-1600), C-H stretch (2900-3100), C=O stretch (1500- 1697), O-H stretch (3400-2300) were analysed. The range of peak values were found to be the same indicating that there were no interaction of Ketoprofen with polymer conforming the compatibility of drug with polymer.

Percentage yield

The percentage yield of different batches was determined by weighing the nanosponges after drying are tabulated in Table 7 and formulation F5 shows highest yield that is $88 \pm 0.16\%$. It was revealed that all formulation have good production yield.

Table 4: Percentage yield of Ketoprofen nanosponge

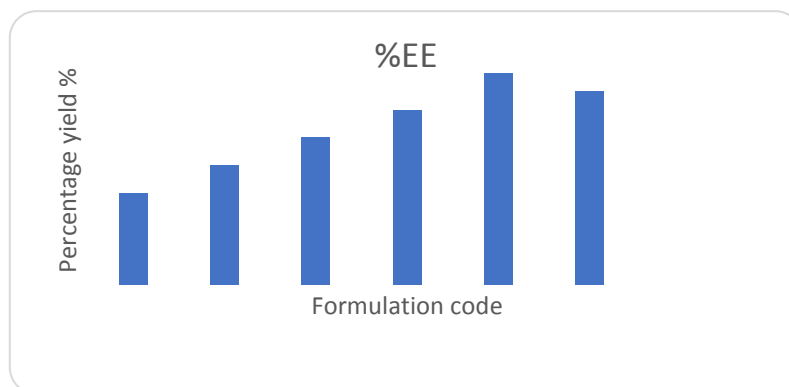| SI.NO	Formulation code	% Yield *
1	F1	75±0.03
2	F2	78±0.07
3	F3	81±0.2
4	F4	84±0.06
5	F5	88±0.01
6	F6	86±0.04

Figure 9: Percentage Yield of Nanosponge

Entrapment efficiency (EE)

Table 5: percentage entrapment efficiency (%EE) of nanosponges (F1- F6)

FORMULA	% EE *
F1	52 ± 0.05
F2	64±0.07
F3	69±0.01
F4	75±0.05
F5	86±0.02
F6	84±0.04

The EE of all the formulations F5>F6> F4 >F3> F5 > F6 is in range of 86 ±0.02 % to 52 ±0.05 % as shown in the Table No.8 an Figure No. 12. The percentage yield was

minimum for formulation F1 (52±0.05%) and maximum for formulation F5 (86±0.02%). From the results we can conclude that as the concentration of polymer increases the entrapment efficiency also increases. As the polymer concentration increases the viscosity of innerface also increases , decrease in the mobility. Hence more entrapment efficiency. Higher concentration of polymer leading to higher entrapment in nanosponge. This result was in agreement with Sharma *et al* (Sharma and pathak, 2011).
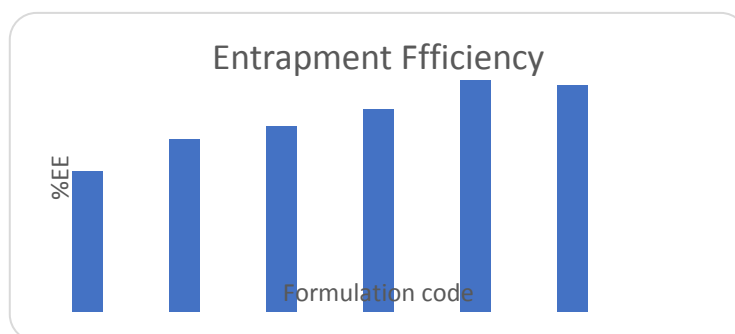

Figure 10: Percentage Entrapment Efficiency of Nanosponge

Average particle size and size distribution

Table 6: Average particle size of Nanosponge

Formula	Particle size (nm)
F1	435.6
F2	415.2
F3	380.3
F4	440.5
F5	280.7
F6	333.3
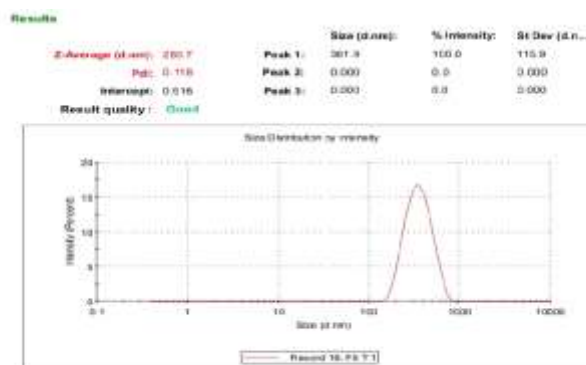
Figure 11: particle size of F5 by malvern zeta size

Particle size analysis of nanosponge was determined using Malvern zeta sizer instrument. The Change in the concentration of polymer results variation in particle size of nanosponges.

The average particle size of formulation batch F1 showed maximum particle size ie, 435 nm while formulation batch F5 showed minimum particle size ie. 320.7 nm.

An increase in the concentration of polymer leads to decrease in the particle size of nanosponges.

Zeta Potential

Table 7: Zeta Potential of F5 formulation of nanosponge.

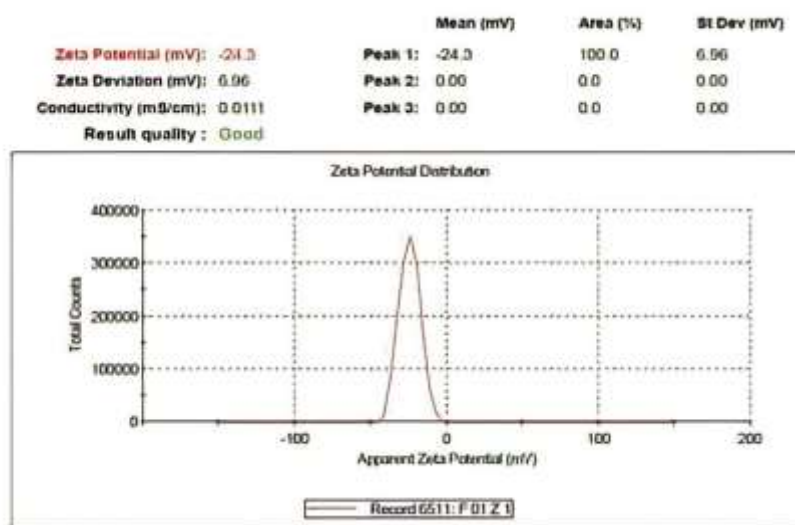| Formula	Zeta potential
F5	-24.3

Figure 12: Zeta Potential of nanosponge formulation F5

Zeta potential of the Ketoprofen nanosponge F5 was determined by Malvern nano zeta sizer instrument. It was found that zeta potential of F5 formulation was negative i.e. -24.3 Mv. Negative potential indicates that the particles stay in separate entity making the whole system stable.

The data of the zeta potential and PDI exhibits that all the nanosponges were negatively charged with sufficient interparticle repulsive force with the narrow size distribution as the PDI value was found to be less than ≤0.7, the results interrelated with the statement presented in the review of Danaei *et al* (Danaei *et al*.,2018).

Surface morphology
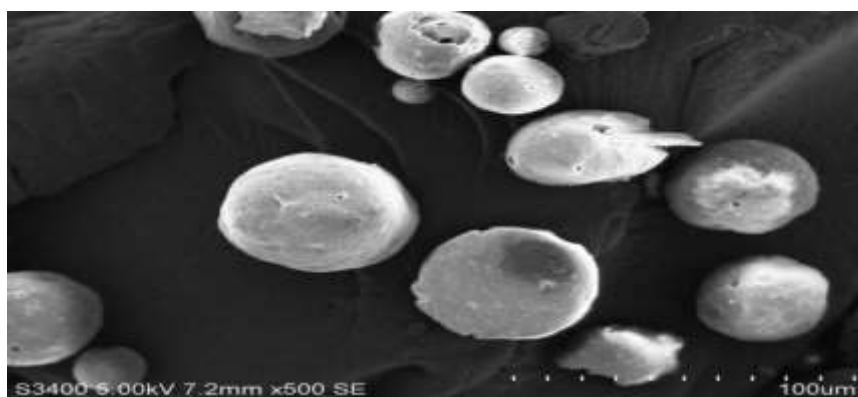
Fig 13: SEM image of F5 formulation of nanosponge

The shape and morphology of prepared nanosponge were observed by scanning electron microscopy. The SEM image showed the porous, spongy feature of nanosponge and it could be due to the in-ward diffusion of DCM in the EC polymeric surface of NS during the fabrication (Al-Suwayeh et al., 2014).

EVALUATION TEST FOR FORMULATED NANOSPONGE GEL
Physical characteristics of Ketoprofen nanosponge gel

Table 8: Physical characteristics of ketoprofen nanosponge gel

Formulation code	Appearance	pH	Spreadability (gms.cm/sec)	Consistency	Drug content (%)	Viscosity (cps)
F5	White- gel	6.45±0.03	11.84±0.04	Good	91.25±00.4	1255±00.7

The prepared gel containing nanosponge (F5) was evaluated for the physicochemical properties such as pH (6.45), Spreadability (11.84±0.04) , Viscosity (1255±0.75), and the drug content (91.25±0.46). Obtained data tabulated in Table. No.11 the values indicates that formulation showed good physicochemical properties.

This complies with physiology of skin pH, better retention, good permeation and improved bioavailability. Hence less dose maximum utility of the drug.

In vitro **diffusion study**

Table 9: In vitro diffusion study of nanosponge gel.

Time(hr)	% Cumulative drug release
0	0
1	15.03 ±0.05
2	20.74±0.07
3	31.73±0.02
4	39.95±0.06
5	52.75±0.03
6	63.13±0.01
8	82.05±0.07

*Data expressed as a mean ±SD, n=3
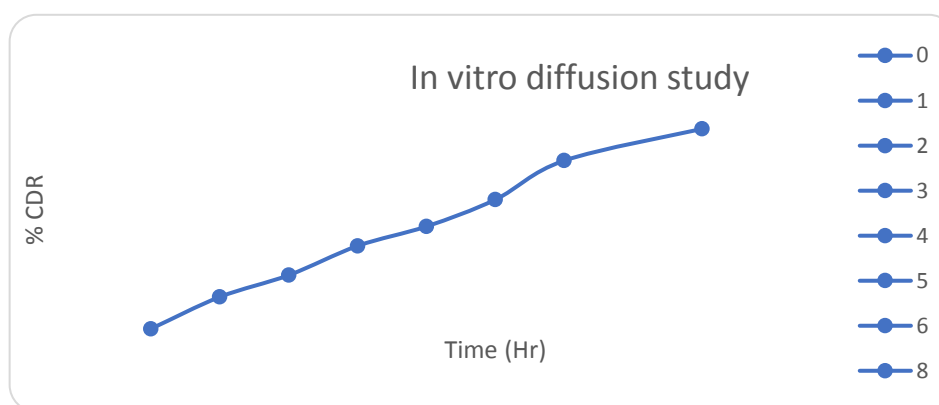
Figure 14: Release profile of nanosponge gel

Kinetics of drug release of nanosponge gel

Table 10: Kinetic release study of nanosponge gel

Time	Log time	Square root of time	% Cumulative release of F5	Log % cumulative release of F5	% Cumulative remaining	Log % cumulative remaining
0	0	0	0	0	100	2
1	0.000	1.000	15.03	1.176	84.97	1.929
2	0.301	1.414	20.74	1.316	79.26	1.899
3	0.477	1.732	31.73	1.501	68.27	1.834
4	0.602	2.000	39.95	1.601	60.05	1.778
5	0.698	2.236	52.75	1.722	47.25	1.674
6	0.778	2.449	63.13	1.800	36.87	1.566
8	0.903	2.828	82.05	1.914	17.95	1.254

In-vitro **drug release kinetics**

Drug release data for the nanosponge formulation fitted into various release kinetic equations to find out order of drug release. The various release kinetic curves are shown in figure. 15-18. The correlation coefficient showed that the release profile followed the zero-order drug release (R2=0.9964) from

these results it is apparent that the regression coefficient value closer to unity in case of zero order kinetic model. The data indicate more linearity when plotted by the zero-order kinetic model. Hence, it can be concluded that the major mechanism of drug release follows zero-order kinetics
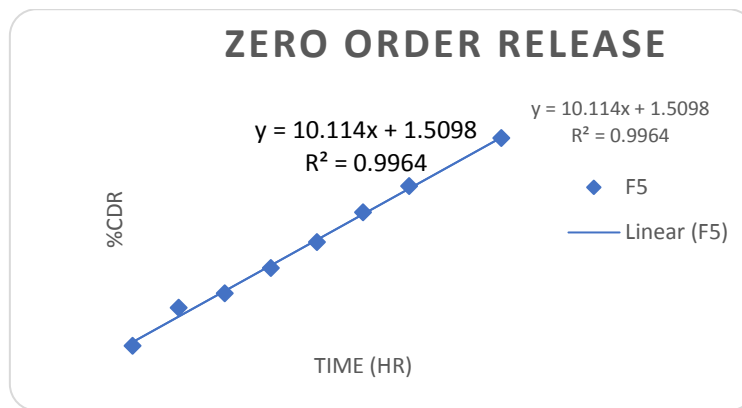
Zero order kinetics release

Figure15: Plot of Percentage CDR v/s Time (Zero order kinetics)
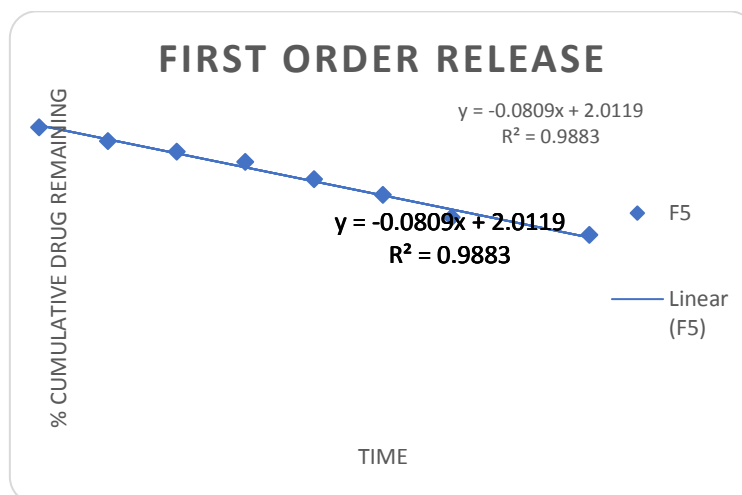
First order kinetics release

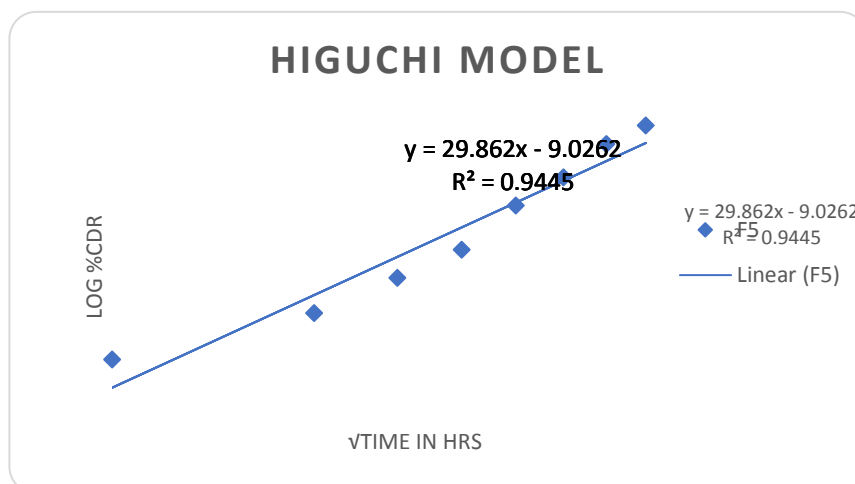**Figure16: Plot of Log Percentage CDR v/s Time (First order kinetics)**

Higuchi Model

Figure17: Plot of Percentage CDR v/s √ t (Higuchi model)
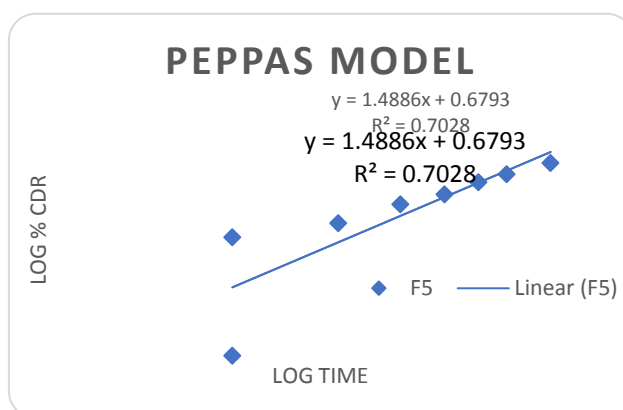
Peppas Model

Figure 18: Plot of Log Percentage CDR v/s Log Time (Peppas exponential equation) Release kinetics data

Table 11: Release Kinetics Data

FORMULATION	ZERO ORDER	F IRST ORDER	HIGUCHI PLOT	PEPPA'S PLOT
	R^2	R^2	R^2	R^2
F5	0.9964	0.9883	0.9445	0.7028

Comparison of *in vitro* diffusion study of formulations with Marketed product

Table: 12 Comparison of invitro diffusion study of Ketoprofen formulation and Marketed product.

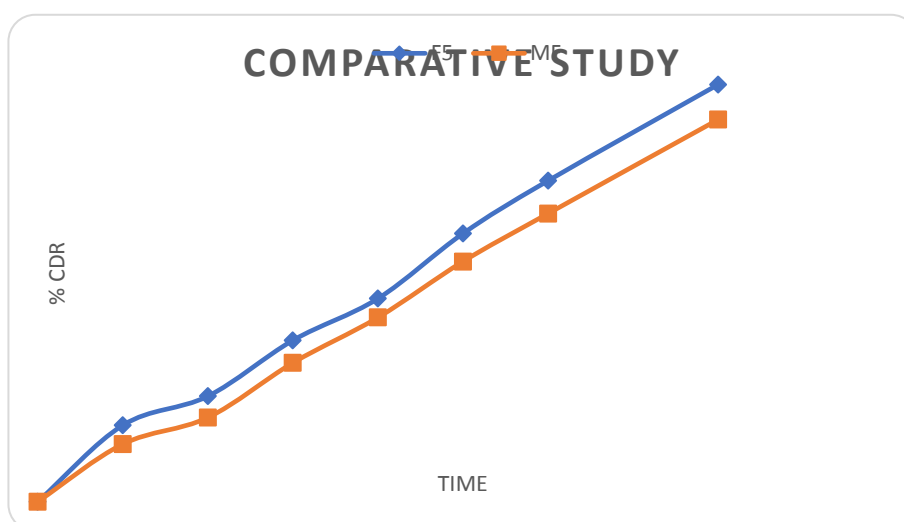| Time (Hrs)	% Cumulative drug release	
	F5	Marketed Product
0	0	0
1	15.03 ±0.05	13.64 ±0.03
2	20.74 ±0.07	21.54 ±0.01
3	31.73 ±0.02	29.53 ±0.07
4	39.95 ±0.06	45.73 ±0.05
5	52.75 ±0.03	51.34 ±0.09
6	63.13 ±0.01	63.59 ±0.06
8	82.05 ±0.07	81.65 ±0.08

Figure: 19 Comparison of invitro diffusion study of ketoprofen formulation and the Marketed product

At the end of 8 hr formulation F5 containing 0.25 % maintained uniform concentration up to 82.05±0.036% whereas Marketed formulation (2.5%) showed less (81.65±0.08 %). It can be concluded that with lesser amount of drug better treatment can be achieved.

Stability Studies

Table 13: Evaluation of F5 for stability study

Evaluation parameters	Time (days) Accelerated condition 40±2ºC at (75±5%RH)			
	0 day	**30 days**	**60 days**	**90 days**
Colour	White	White	White	White
Drug content (%)	91.45	91.16	91.01	90.11
In vitro diffusion in 8hr	82.05%	81.43%	81.25%	80.54%

The results of the stability studies indicates that the nanosponge gel did not show much difference in the physical appearance, drug content and percentage cumulative drug release (80.54)after 90 days the end of 8 hrs indicating no significant changes and the results obtained were depicted in Table . 13.

CONCLUSION

Preformulation studies of Ketoprofen comply with the reported literature limits.
The IR spectra revealed that, there was no interaction between Ketoprofen and polymer, thus indicating the compatibility of drug and polymers used.
The nanosponges were evaluated for percentage yield, drug entrapment efficiency, surface morphology, Particle size and Zeta potential. Based on the results obtained from these parameters F5 formulation is considered as best.
Nanosponges of formulation F5 were incorporated into gel and evaluated for pH, viscosity, spreadability, drug content, drug diffusion study, release kinetics and stability study.
The %CDR of gel was found to be 80.54% at the end of 8 hrs. The formulation shows zero order kinetics. Comparative study of the prepared nanosponge gel F5 was carried out against marketed Formulation (Fastum gel). The diffusion study shows that the drug release of prepared nanosponge gel F5 was found to be 10 fold more as compared to that of marketed formulation.
The stability studies were carried out for the gel, the results of Physical appearance, drug content and in-vitro drug release studies showed no significant changes and indicates the good stability.

REFERENCES

1. Patil PB, Datir SK, Saudagar RB. A review on topical gels as drug delivery system. J. of drug deliv. and Ther. 2019;15;9(3):989-94.
2. William AC, Barry BW. Penetration enhancers. Adv Drug Deliv Rev 2016;56:603-18.
3. Ghurghure S, Pathan MS, Surwase P. Nanosponges: A novel approach for targeted drug delivery system. Int.J. chem stud. 2018; 2(6):15-23
4. Swetha T, Chakraborty T. Nanosponges: New colloidal drug delivery system for topical drug delivery. 2019; 6(2):4263-76.
5. Thorat KR, Laware RB. Formulation and evaluation of Lornoxicam loaded Lyotropic liquid crystalline gel. J.drug deliv.ther. 2019; 9(6):116-25.
6. Ahmed A. A New Approach for Drug Targeting. Int J Pharm Pharm Res. 2016;7(3):381-96.
7. Patel B, Bagade O, Kuldeep Ramteke RP, Varsha Awsarkar.An Assessment on Preparation, Characterization and PolesApart Appliance of Nanosponge. Int. J. Pharma Tech Research.2014;6(6):1898-907.
8. Pandey J, Amandeep S. Formulation and Evaluation of Nanosponge Based Controlled Release Topical Gel Preparation of Ketoconazole. Int. J. Pharm.Pharm.Res. 2018; 12(3):367-82.
9. Vijetha S L, Shabaray A R, K Vineetha. Formulation and evaluation of patch containing proniosomes for transdermal delivery of Metformin Hydrochloride. Int.J.res. Pharm. Nano sci. 2021; 10(1): 1-12.
10. Mishra J, Nayak SK, Sahoo SK. Development, validation and stability study of UV spectrophotometric method for determination of Repaglinide in bulk and

pharmaceutical dosage forms. J.Indian Assoc.Pediatr.surg.2016; 1(3):10-16.

11. Arun DK, Kishanta PK, Ranjita D. Murthy PN, Amithesh PK. Method development, validation and stability study of Repaglinide in bulk and pharmaceutical dosage form by spectrophotometric method. Indian J .Biotech.Pharm.Res .2011; 2(1):7-10.

12. Sahu M, Bhowmick M, Rathi J. Preformulation screening of Repaglinide for transdermal anti-diabetic therapy. J. drug deliv . ther. 2017; 7(4):103-09.

13. Ahmed A. A New Approach for Drug Targeting. Int.J .Pharm Pharm Res. 2016;7(3):381-96.

14. Abbas N, Parveen K, Hussain A, Latif S, Zaman S, Shah PA, Ahsan M. Nanosponge based hydrogel preparation of fluconazole for improved topical delivery. Trop .J .Pharm. Res. 2019;18(2):215-22.

15. Shivani S, Poladi KK. Nanosponges-novel emerging drug delivery system: A review. Int. J . Pharm .Sci .Res. 2015; 6(2): 529-40.

16. Sharma R, Pathak K. Polymeric nanosponges as an alternative carrier for improved retention of econazole nitrate onto the skin through topical hydrogel formulation. Pharm .Dev. and Technol. 2011; 16(4):367-76.

17. Renuka Sharma, Roderick BW, Kamla Pathak. Evaluation of kinetics and mechanism of drug release from econazole nitrate nanosponge loaded carbapol hydrogel. Ind. J. Pham. Edu .Res.2011; 45(1):25-31.

18. Patil TS, Nalawade NA, Kakade VK, Kale SN. Nanosponges: A novel targeted drug delivery for cancer treatment. Int. J. Adv. Res. Dev. 2017;2(4):55-62.

19. Sharma R, Roderick B. Walker, Kamla P. Evaluation of kinetics and mechanism of drug release from econazole nitrate nanosponge loaded carbapol hydrogel. Ind .J. Pham. Edu. Res.2011;45(1):25-31.

20. Kumar PS, Hematheerthani N, Ratna JV, Saikishore V. Design and characterization of miconazole nitrate loaded nanosponges containing vaginal gels. Int. J. Pharm .Ana. Res. 2016;5(3):410-7.

21. Shirsand SB, Para MS, Nagendra KD, Kanani KM, Keerthy D. Formulation and evaluation of Ketoconazole niosomal gel drug delivery system. Int. J. pharm. investig. 2012;2(4):201-7.

22. Prathima S, Sreeja k. Formulation and evaluation of voriconazole loaded nanosponges for oral and topical delivery. Int.J. drug develop. res. 2012;5(1):55-69.

23. Raja NV, Kumar G, Anusha K. Fabrication and evaluation of ciprofloxacin loaded nanosponges for sustained release. Int. J .Res. Pharm. Nano Sci. 2013;2(1):1-9.

24. Sowmyashree, Shripathy D, Shabaraya AR. Nanosponges- a novel nanocarrier approach for targeted drug delivery: a brief review. World JPharm PharmSci. 2021; 10(5): 417-35.

25. Murlidhar Targe B, Patil M.P., Jahagirdar A.C., Khandekar B.D. Nanosponges - An Emerging Drug Delivery System. Int J Inst Pharm Life Sci. 2015; 5(6):160-74.

26. Sumana G, Shabaraya AR. Nanosponges : a novel carrier for drug delivery .World J PharmPharm Sci. 2021; 10(5):287-95.

27. Shameem S, Nithish N. Bhavitha M, Kumar S, Sahithya K. Formulation and Evaluation of Lawsone Loaded Nanosponge Gel for Topical Delivery. Future J.Pharm. Sci. 2021 ;1(1):29-36.

28. Gadakh PP, Geevarghese R. Evaluation of kinetics and mechanism of drug release from clotrimazole microsponge loaded carbopol gel. J Pharm Res. 2012;5(9):4648-51.

29. Moglad H, Fatima F, Muqtader M, Devanathad, Khalid Anwer, Mohammed F*et al.*, Development of Topical Antibacterial Gel Loaded with Cefadroxil Solid Lipid Nanoparticles: In vivo Wound Healing Activity and Epithelialization Study. Int. J. Pharmacol 16:298-309.

30. Raja NV, Kumar G, Anusha K. Fabrication and evaluation of ciprofloxacin loaded nanosponges for sustained release.Int. J. Res.Pharm. Nano Sci. 2013;2(1):1-9.

31. Jadho UT, Sayali RP, Gunesh DN ,Shital SD, Sneha LS. Formulation and evaluation of nanosponge gel containing Ketoconazole . Innov. Pharm. Pharm ther. 2021;9(1):15-24.

******