Available online at http://ajprd.com

Asian Journal of Pharmaceutical Research and Development

(An International Peer-Reviewed Journal of Pharmaceutical Research and Development)

Open Access to Pharmaceutical and Medical Research

Open Access Research Article

PREPARATION AND EVALUATION OF BETA SITOSTEROL NANOGEL: A CARRIER DESIGN FOR TARGETED DRUG DELIVERY SYSTEM

Inamdar Yashashri *, Rane Bhushan, Jain Ashish

Department of Pharmaceutics,Shri D.D. Vispute College of Pharmacy and Research Center, New-Panvel 410206, Navi Mumbai, Maharashtra, India.

ABSTRACT

Transdermal drug delivery system is promising but challenging system available for local as well as systemic effect of the drug. The prolonged residence of drug formulation in the skin is important for transdermal drug delivery. Nanogel drug delivery has remained as one of the most challenging task. The objective of the investigation was to develop a nanogel with reduced particle size in order to improve the bioavailability of the hydrophobic drug. The objective of the controlled and sustained delivery is to provide and maintained adequate concentration of drugs at the site of action. Nanogels based materials have high drug loading capacity, biocompatibility and biodegradability which are key points to design the drug delivery system effectively. Drug molecules loaded into the nanogel need to be retained and not to be transported out or leak prematurely while circulating in order to provide maximum therapeutic effects and minimum toxicity or side effects. The present study is to formulate nanosizes dispersion of Beta sitosterol by nanoprecipitation method and incorporating it into the gelling agent to produce nanogel by dispersion method. 1% of carbopol 934 shows better in-vitro drug release than the other concentrations of carbopol 934.

Keywords: *Transdermal, Nanogel, Carbopol 934, Novel drug delivery system, Dispersion method*

Cite this article as:

Inamdar Yashashri , Preparation And Evaluation of Beta Sitosterol Nanogel: A Carrier Design For Targeted Drug Delivery System,Asian Journal of Pharmaceutical research and Development.**2018;6 (3): 81-87**

DOI: http://dx.doi.org/10.22270/ajprd.v6.i3.390

***Address for Correspondence**

Yashashri Inamdar, Department of Pharmaceutics,Shri D.D. Vispute College of Pharmacy and Research Center, New-Panvel 410206, Navi Mumbai, Maharashtra, India

INTRODUCTION

Nanogels are the cross linked polymers of sub-micrometer size made up of hydrophilic polymers. They are soluble in water, but have properties different from linear macromolecules of similar molecular weight. Such structures along with their bigger analogues. As a family of nanoscale particulate materials, hydrogel nanoparticles (recently referred as nanogels) have been the point of convergence of considerable amount of efforts devoted to the study of these systems dealing with drug delivery approaches. Interestingly hydrogel nanoparticulate materials would demonstrate the features and characteristics of hydrogels and NPs separately possess at the same time. Therefore it seems that pharmacy world will benefit from both the hydrophilicity, flexibility, versatility, high water absorptivity and biocompatibility of these particles and all the advantages of the nanoparticles, mainly long life span in circulation and the possibility of being actively or passively targeted to the desired bio phase, eg Tumor sites. Different methods have been adopted to prepare nanoparticles of hydrogel consistency. Besides the commonly used synthetic polymers. Active research is focused on the preparation of nanoparticles using naturally occurring hydrophilic polymers. [1, 2]

Nanogels are been prepared for the encapsulation of hydrophobic drug so it shows better bioavailability and increase efficacy of the drug. Nanogels acts as the carrier mediated drug delivery system to deliver the drug to the site of action. Nanogels when applied to the skin, even if the gel above gets evaporated from the skin the nanoparticles present gets deep penetrated inside the skin at the site of action and shows the action at the specific site of the skin.[13]

Features of Nanogels [3]

- **Size control:** Nanogel size and surface properties can be chemically controlled to limit the rate of clearence by phagocytic cells as well as to enable either passive or active cell targeting. Nanogels must be small enough to traverse capillaries and penetrate tissues through either paracellular or transcellular pathways.
- **Ease of synthesis:** The scalability of laboratory based nanogel development to industrial scale production for clinical markets and the use of "green" approaches to nanogel manufacturing are important considerations for cost and environmental impact.
- **High encapsulation stability:** Drug molecules loaded into the nanogel need to be retained and not to be transported out or leak prematurely while circulating in order to provide maximum therapeutic effects and minimum toxicity or side effects.
- **Controlled and sustained drug release:** Drug transport should occur at the target site, thereby providing both therapeutic efficacy and reduced side effects. Drug loading should be sufficiently high to achieve therapeutic goals.
- **Response to stimuli:** Nanogels are designed to respond to specific stimuli must retained high drug encapsulation efficiency stability while circulating to reach target site and drug should release readily to the appropriate stimulus.
- **Targetting:** Site specific delivery of nanogels carriers can be achieved via either coupling to their surface affinity ligands binding to target determinants or using responsiveness to local factors as above, or via "passive" targeting approaches including extravasation in the pathological sites and retention in the microvasculature.
- **Low toxicity:** The nanogels themselves should be highly biocompatible and free from toxicity, and should be biodegradable with non-toxic degradation products that are readily cleared from the body.

MATERIALS AND METHODS

Materials

Carbopol 934, Propylene glycol, Glycerin and Triethanolamine was a kind of gift sample from Research lab chemicals.

Preformulation study:

Preformulation study is the first step in the rationale development of any pharmaceutical dosage form of a new drug. Preformulation study focuses on those physiochemical properties of the new drug performance and development of an efficacious dosage form. The preformulation investigations confirms that there are no significant barriers to the compounds development. Melting point of Beta sitosterol was determined using Thiele's tube technique. Drug-excipients compatibility was carried out using Fourier-transform infrared spectroscopy, it was found that there are no incompatibilities between the drug and the excipients.

Method of preparation:

Beta sitosterol nanoparticles were prepared using eudragit RL100 and Poloxamer 407 as a stabilizer. Drug and polymer are taken in ratio 1:10 as this ratio shows more stability and no flocculation or sedimentation is been observed, also the required size to produce nanogel is obtained by this ratio. So nanoparticles were prepared for 1.10 drug to polymer ratio by nanoprecipitation method. The gel is been prepared by dispersion method by dispersing carbopol 934 in water for 2 hours for swelling. Once the carbopol is been swelled it is been kept on magnetic stirrer for stirring and the prescribed amount of nanoparticles dispersion or the separated nanoparticles is been added in the carbopol mixture, along with it propylene glycol which acts as the penetration enhancer and glycerin which acts as the humectant and viscosity modifier is ben added into the mixture. pH is been maintained using triethanolamine.

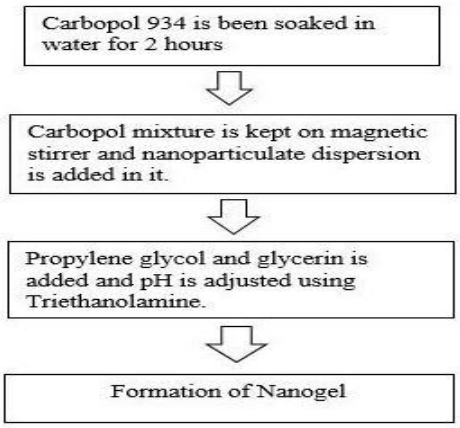

Fig 1- Flowchart for preparation of nanogel

Table 1: Optimization of nanogel using different concentrations of carbopol 934

Name of the ingredients	Formulation Code			
Ingredients	**F1**	**F2**	**F3**	**F4**
Carbopol 934	0.5%	1%	1.5%	2%
Propylene glycol	10 ml	10 ml	10 ml	10 ml
Glycerin	30 ml	30 ml	30 ml	30 ml

Physical characteristics:

The prepared nanogel formulations were inspected visually for their color. Appearance and consistency. **Table no 2** tells about the physical characteristics.[1, 2]

pH determination:

The pH values of 1% aqueous solutions of the prepared nanogels were measured by a pH meter (Digital pH meter). Refer **Table no 3**.[1, 2]

Viscosity study:

The viscosity of the formulated batches was determined using a Brookfield viscometer with spindle 64 at 10 rpm. The assembly was connected to a thermostatically controlled circulating water bath maintained at 25˚C. The formulation whose viscosity was determined was

added to a beaker covered with a thermostatic jacket. The spindle was allowed to move freshly into the nanogel and the reading was noted. The **Table no 4** tells about the viscosities of the four formulations.[1, 2]

Extrudability study:

After gels were set in the container the formulations were filled in the collapsible tube. The extrudability of the formulation was determined in terms of weight in grams required to extrude a 0.5 cm ribbon gel in 10 seconds.[1, 2]

Spreadability coefficient:

It consists of a wooden block which is attached to a pulley at one end. Spreading coefficient is measured on the basis of 'slip' and 'drag' characteristics of nanogel. A ground glass slide is fixed on the wooden block. An excess of nanogel under study was placed on the glass slide. The nanogel formulation was sandwitched between the two slides having the same dimensions that of fixed ground slide. The weight of 100 grams was placed on the top of the two slides for few seconds to provide uniform film of nanogel between the two slides. A measured quantity of weight was placed in the pan attached to pulley with the help of hook. The time (in seconds) required by the top slide to cover a distance of 7 cm was noted. A shorter interval indicates better spreading coefficients. Refer **Table no 6** for spreading coefficients.[1, 2]

Drug Content determination:

The drug concentration in nanogel was measured by high performance thin layer chromatography. Beta Sitosterol content in nanogel was measured by dissolving known quantity of nanogel in Phosphate buffer (pH-7.4) by sonication. Area under curve was measured after suitable dilution at 550 nm in HPTLC.[5]

***In vitro* drug release:**

The *in-vitro* drug release studies were carried out using Franz diffusion cell. The formulation was applied on dialysis membrane which was placed between the donor and receptor compartment of the franz diffusion cell. The temperature of the cell was maintained at 37˚C by circulation jacket. This whole assembly was kept on a magnetic stirrer and the solution was stirred continuously using a magnetic bead. A similar blank set was run simultaneously as a control. Sample 5 ml was withdrawn at suitable time intervals and replaced with equal amounts of fresh dissolution media. Samples were analysed chromatographically at 550 nm and % drug release and control was used as the actual reading in each case. % drug release of each is shown in **table no 8.**[1, 2]

Drug release kinetics[9, 10]

Several drug release data on mathematical models can be obtained, hence drug release profile can be correlated with drug release kinetic models.

Zero order release kinetics

According to the principles of pharmacokinetics, drug release from the dosage form can be represented by the below equation

$$\mathbf{Co - Ct = K_0 t} \text{ …….. Eq.1}$$

$$\mathbf{Ct = Co + K_0 t} \text{……..Eq.2}$$

Ct is the % amount of drug released at time t,

C0 is the% CR drug at time t=0

K_0 is the zero order rate constant

Graph is plotted against % cumulative drug release vs. time.

First order release kinetics

The release of drug which follows first order kinetics is given below, it is the first order process whose rate is directly proportional to the concentration of drug undergoing reaction

$$\mathbf{Log\ C = log\ C_0 - K_1 t / 2.303} \text{……..Eq.3}$$

K_1 is the first order rate equation expressed in time^{-1} or per hour.

C_0 is the initial concentration of drug

C is the percent of drug remaining at time t

Graph is obtained by log % of CR vs. Time.

Higuchi model

Today it is considered as one of widely used and most well-known controlled release equation, which is represented below

$$\mathbf{Q = K_H \times t^{1/2}} \text{……Eq.4}$$

K_H is the Higuchi constant

Graph is plotted as %CR vs. square root of time

Korsmeyer peppas

Once it has been ascertained that the prime mechanism of drug release is diffusion controlled from Higuchi plot then it comes the release of drug follows which type of diffusion, to understand the dissolution mechanisms from the matrix the release data were fitted using the empirical equation.

$$\mathbf{F = (M_t / M) = K_m t^n} \text{…… Eq.5}$$

F = Fraction of drug released at time't'

M_t = Amount of drug released at time't'

M = Total amount of drug in dosage form

K_m = Kinetic constant

n = Diffusion or release exponent

t = Time in hours

Graph is plotted log % CDR vs log time in hours

Hixson-Crowell model

The Hixon-Crowell cube root law describes the release from systems where there is a change in surface area and diameter of particles or tablets. Hence, particles of regular area are proportional to cube root of its volume. The relationship between the time and drug release can be described as

$$\mathbf{W_0^{1/3} - W_t^{1/3} = K_{HC} t} \text{…… Eq.6}$$

W_0 is initial amount of drug in pharmaceutical dosage form at time t

K_{HC} Hixon-Crowell constant

Graph is plotted Cube root of %CDR vs Time

***In-vitro* antibacterial activity:**

Cup plate method is been used for studying various bacterial species. Muller Hinton agar medium is been used for the study. Make a saline solution and add loop of bacterial species in each of it. Add saline solution containing bacterial species and also add Muller Hinton agar in it which is allowed to cool and solidify. Later on make a disc using the cylinder and pour nanogel solution in the disc and incubate them for 24 hours and calculate area of inhibition.

RESULTS AND DISCUSSION

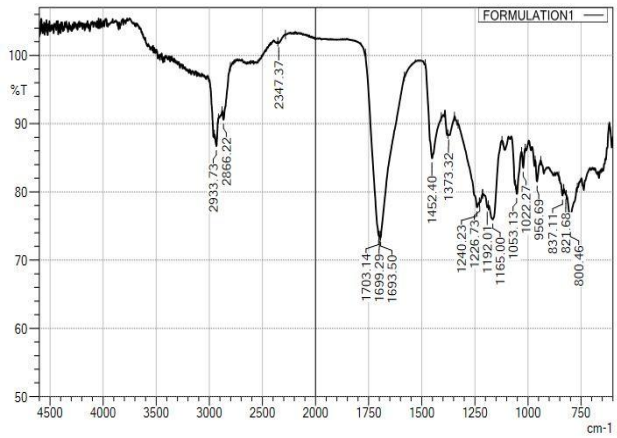

Fig 2: IR spectra of formulation

The above figure tells that there are no incompatibilities between the drug-excipients. It shows the presence of various functional groups like C-H stretching, C=O shows the presence of carboxylic acids and S=O shows the presence of sulfones, sulfates and sulfonamides.

Physical Appearance:

F1, F2, F3, F4 formulations show clear white appearance, all the 4 formulations were homogenous and free of grittiness

Table 2: Physical appearance of nanogel formulations

Formulation code	Appearance	Homogeneity	Grittiness
F1	White	Homogenous	No
F2	White	Homogenous	No
F3	White	Homogenous	No
F4	White	Homogenous	No

pH:

The pH values of all prepared formulations were ranged between 6.40- 6.84 which is been shown in (**Table no 3)** which is considerable to avoid the skin irritation of the skin after application.

Table 3: pH of nanogel formulations

Formulation code	pH
F1	6.40 (± 0.09)
F2	6.68 (± 0.05)
F3	6.80 (± 0.05)
F4	6.84 (± 0.12)

Viscosity studies:

The measurement of viscosity of the prepared nanogel was done using the Brookfield viscometer F2 formulation showed better viscosity as compared to other formulations.

Table 4: Viscosity of nanogel formulations

Formulation code	Viscosity (cps)
F1	29,406 (± 58.02)
F2	35,956 (± 5.50)
F3	42,510 (± 96.43)
F4	51,814 (± 98.73)

Extrudability studies:

The extrudability studies is been determined and F2 Formulation showed better results that is 0.5 cm of ribbon is been extrude in 10 seconds from the collapsible tube.

Table 5: Extrudability of nanogel formulations

Formulation code	Extrude ribbon (cm)
F1	0.9 cm
F2	0.5 cm
F3	0.3 cm
F4	0.2 cm

Spreadability coefficient:

The spreadability of various concentrations of carbopol 934 is been, out of which F2 showed better spreadability than the other formulations.

Table 6: Spreadability of nanogel formulations

Formulation code	Spreadability (g.cm/s)
F1	28.10 (± 0.09)
F2	20.35 (± 0.05)
F3	15.49 (± 0.18)
F4	11.84 (± 0.06)

Drug Content determination:

The drug content of Beta Sitosterol from its various nanogel formulations are represented in the (**Table no 7)** F2 and F3 showed better drug content as compared to other formulations. The percent drug content of these formulations was 95.06% and 94.11% respectively.

Table 7: Drug content of nanogel formulations

Formulation code	Drug content (%)
F1	90.04 (±0.51)
F2	95.06 (±0.28)
F3	94.11 (±0.23)
F4	89.12 (±0.27)

***In-vitro* diffusion study:**

The *in-vitro* release profiles of Beta Sitosterol from various nanogel formulations are represented in **(Table no 8)**. The better release of the drug from all nanogel formulation can be observed and nanogel formulation can be ranked in the following descending order F2> F3> F4>F1. Where the amounts of the drug released after 8 hours were 78.10%, 88.29%, 86.59% and 82.19% respectively. The higher drug release was observed with the formulations F2 and F3. Carbopol 934 of concentrations 1% and 1.5% show better release than 0.5% and 2%.

Table 8: Drug release of nanogel formulations

Time (hours)	Formulation code and their % drug release			
	F1	F2	F3	F4
0	0.06	0.14	0.11	0.09
1	16.17	22.96	21.35	20.55
2	32.45	38.91	36.5	33.54
3	47.95	52.22	51.21	47.50
4	53.60	60.31	58.14	55.60
5	59.62	65.48	64.6	63.78
6	64.60	75.6	73.54	72.27
7	73.40	81.88	79.34	78.16
8	78.10	88.29	86.59	82.19

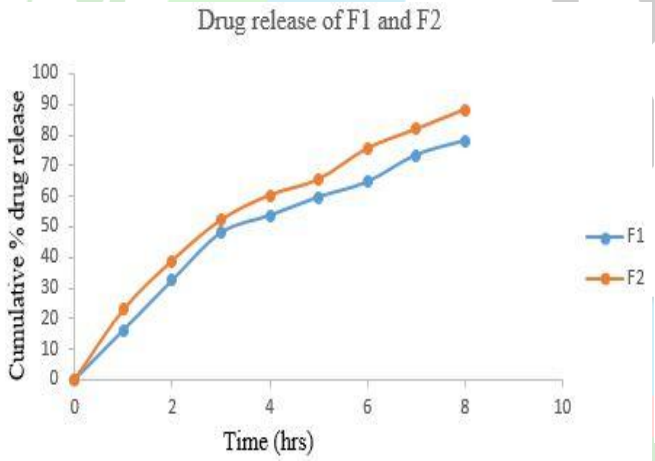

Fig 3- Drug release of F1 and F2 formulation

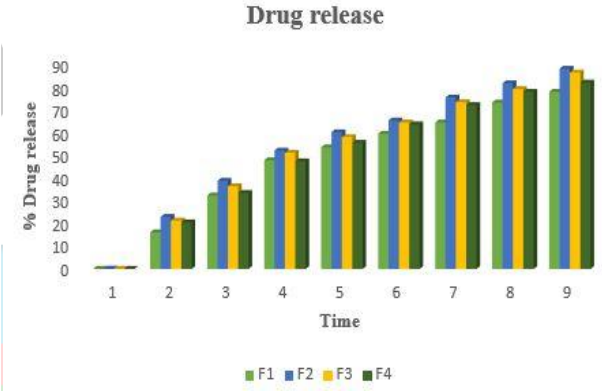

Fig 5: Graphical representation of % Drug release

Drug Release Kinetics

For the zero order kinetics graph is been plotted against % Cumulative drug release vs. Time

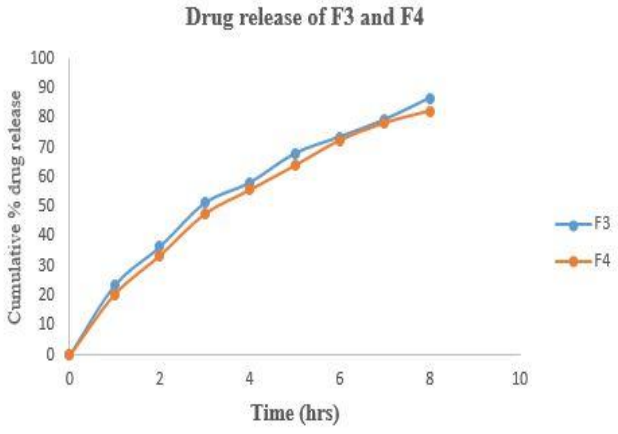

Fig 4- Drug release of F3 and F4 formulation

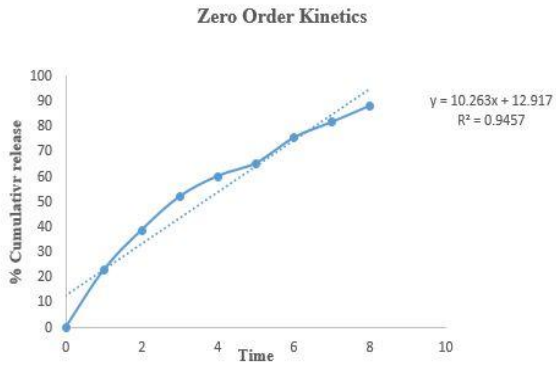

Fig 6: Zero order release kinetics

For the first order release kinetics the graph is been plotted against log % Cumulative release vs. Time.

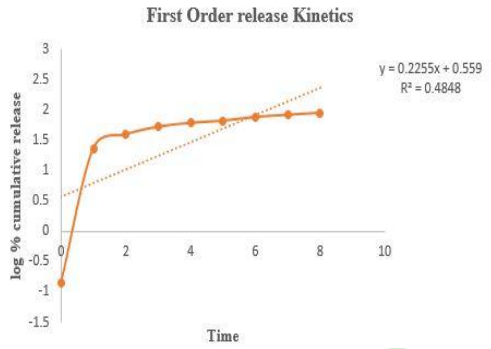

Fig 7: First order release kinetics

For the Higuchi model the graph is been plotted against % Cumulative release vs. Square root of time.

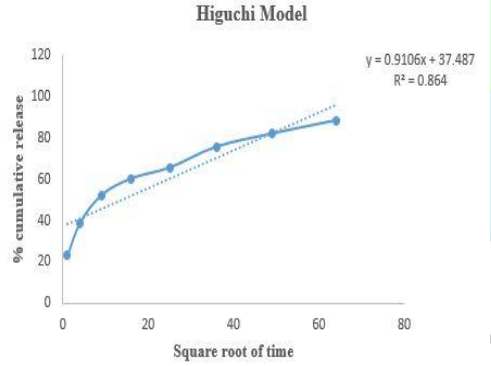

Fig 8: Higuchi Model

For Korsmeyer peppar graph is been plotted as log %CDR vs log time in hours.

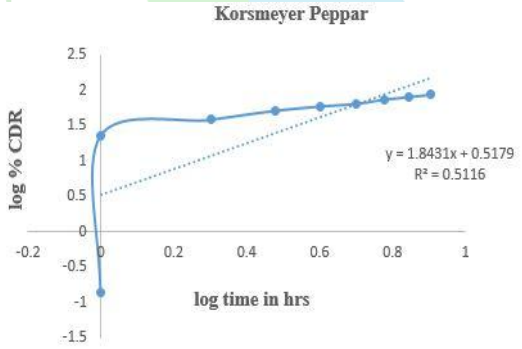

Fig 9: Korsmeyer Peppar

For Hixon-Crowell graph is been plotted as Cube root of %CDR vs Time in hours

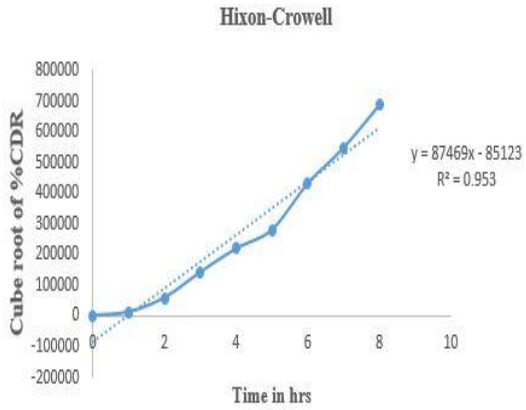

Fig 10: Hixon-Crowell

From the above drug release kinetics it was found that it follows Hixon-Crowell model of drug release kinetics as the value of R^2 was found to be near 1 that is 0.953.

Table 9- Drug release models

Model Name	Slope	Intercept	R^2
Zero order	10.263	12.917	0.9457
First order	0.2255	0.559	0.4848
Higuchi model	0.9106	37.487	0.864
Korsmeyer	1.8431	0.5179	0.5116
Hixon-Crowell	87469	85123	0.953

5.3.8 *In-vitro* antibacterial activity:

The antibacterial activity of Beta Sitosterol against E.coli, Bacillus subtilis, P. vulgaris and S. aureus in its different nanogel formulations is shown in the **Table no.10** in which percentage inhibition was taken as a measure of the drug antibacterial activity. Thus the highest activity was observed with F2 and F3 formulation against S. aureus and P. vulgaris bacterial stains where the percentage for F2 was found to be 73.45 % for S. aureus and 65% for P. vulgaris and in F3 69.37% for S. aureus and 64.80% for P. vulgaris respectively, while the lowest activity was found with F1 where the percentage inhibition was least in all 4 bacterial stain.

Table 10: Percentage inhibition of different bacterial species for antibacterial activity of beta sitosterol in different nanogel formulations

Formulation code	% Inhibition of bacterial species			
	E. Coli	P. vulgaris	B. subtilis	S. aureus
F1	45	62.50	56.25	65
F2	58.45	65	63.30	73.45
F3	55	64.80	62	69.37
F4	50	60	58	67

DISCUSSION:

Beta sitosterol nanogel is been prepared using 1% carbopol 934 as a gelling agent as it shows better drug release compared to others i.e 88.29% and the formulation follows Hixon-Crowell model of drug release kinetics. Appearance of the gel was found to be homogenous with no grittiness and white in color. The pH was found to be 6.68, Viscosity 35,956, Spreadability 20.35, drug content 95.06%. The gel was found to be more effective against P. vulgaris and S.aureus showing greater zone of inhibition.

CONCLUSION:

Nanogel of Beta Sitosterol is been prepared which shows a good drug release, increased bioavailability of medium to moderate water soluble drugs. Nanogels is been helpful in providing the better action or efficacy of the drug due to its small particle size, as less the particle size the more is the surface area and hence more is the action. It also becomes a solution for encapsulating the hydrophobic drug in water soluble gel base for long term stability.

FUTURE PROSPECTIVE:

Nanogel containing beta sitosterol is been prepared and proved for *In-vitro* anti-bacterial activity. Anti-cancer activity will be done using animal study and proved in future.

Conflicts of interest:

There are no conflicts of interest

Acknowledgement:

The authors are grateful to the Shri D.D Vispute College of Pharmacy for providing all the necessary help like technical support, guidance for instruments, library, net surfing etc

REFERENCES:

1. *Adhikari B, Cherukuri S, Reddy SC, Chinthaginjala H, Bhatta H, Naidu R., Inturi. Recent Advances in Nanogels in Drug Delivery System. World J Pharm Pharm Sci. 2016; 5(9):505-530.*
2. *Talele S, Nikam P, Ghosh B, Deore C, Jaybhave A, Jadhav A. A Research Article on Nanogel as Topical Promising Drug Delivery for Diclofenac Sodium. Indian Journal of Pharmaceutical Education and Research.2017:51(4S):5680-5685.*
3. *Eckmann DM, Composto RJ, Tsourkas A, Muzykantov VR. Nanogel Carrier Design for Targeted drug Delivery. J Mater Chem B Mater Bio Med. 2014;2(46):8085-8097.*
4. *Aiyalu R, Govindarjan A, Ramasamy A. Formulation and evaluation of topical herbal gel for the treatment of arthritis in animal model. Brazilian journal of Pharmaceutical Sciences.2016;52(3).*
5. *Kaur LP, Guleri T.K. Topical Gel: A Recent Approach for Novel Drug Delivery. Asian Journal of Biomedical and Pharmaceutical Sciences. 2013;3(17):01-05.*
6. *Thampanchira D, Krishnakumar, K., John A., Dineshkumar B. Hydrogel and Nanohydrogel- a Review. Int J Pharm. 2016;6(2):78-81.*
7. *Jamadar MJ, Shaikh RH. Preparation and Evaluation of Herbal Gel Formulation. J Pharm Sci Educat. 2017;1(2)201-224*
8. *Phatak A, Chaudhari PD. Development and Evaluation of Nanogel as a Carrier for Transdermal Delivery of Aceclofenac. Asian. J. Pharm. Tech. 2012;2(4):125-132.*
9. *Gouda R, Baishya H, Qing Z. Application of Mathematical Models in drug Release Kinetics of Carbidopa and Levodopa ER Tablets. J Develop Drugs. 2017;6(2):1-8.*
10. *https://www.slideshare.net/sagarsavale1/drug-release-kineticsaccessed on dated February 12, 2018.*
11. *https://www.slideshare.net/FarazaJaved/gels-33285405 (accessed on February 1, 2018)*
12. *Rafia B. Phytosterols in Human Nutrition. Int. J. of Scientific Research and Reviews. 2013;2: 01-10*
13. *Patel HA, Patel JK. Nanogel as a Controlled Drug Delivery System. Int. J. Pharm Sci Rev Res. 2010;4(2):37-40.*
14. *Kumar L, Verma R. In vitro evaluation of topical gel prepared using natural polymer. International Journal of Drug Delivery 2. 2010:58-63.*
15. *Nayak BS, Rout PK, Nayak U, Bhowmik BB. Development and characterization of Bio adhesive gel of Microencapsulated Metronidazole for Vaginal Use. Iran J Pharm Res. 2010;9(3):209-219.*
16. *Khan AW, Kotta S, Ansari HS, Sharma RK., Kumar A., Ali. J. Formulation development, optimization and evaluation of aloevera gel for wound healing. Pharmacogn Mag. 2013*