RB SCIENCE
ENTERPRISING BIOSCIENCE RESEARCH
E-ISSN: 2456-8244
www.rbscience.co.in

JOURNAL OF PHARMACOLOGY AND BIOMEDICINE

Tazarotene loaded niosomal gel for effective acne treatment

Meena Kumari Vaishya*, Virendra Sharma, J.P.Rai

School of Pharmacy, LNCT University, Bhopal, Madhya Pradesh (India)

*Corresponding author

Email- meenusingh251996@gmail.com

Abstract

Niosomes are the non-ionic surfactant-based vesicular system which may enhance anti-acne efficacy by improving bioavailability and intra-follicular penetration of the tazarotene. In the present study, we prepared tazarotene-loaded niosomes which were further incorporated in the carbopol 934 gel to alter the contact time to gain maximum benefits of the treatment. Prepared formulations of Niosome were optimized on basis of vesicle size, shape, surface charge and entrapment efficiency. % Entrapment efficiency and vesicle size of optimized Niosomes formulation (F-9) was found 78.85±0.25% and 212.4±0.32 nm respectively. Gel of F9 was formulated using carbopol 934 as the base. The *in vitro* anti acne activity of the niosomal gel was evaluated against *Propionibacterium acnes* using well diffusion method using clindamycin as the standard drug. The niosomal Gel NG-2 showed greater capacity (18.5±0.86, 19.0±0.7, 24.0±0.9) to inhibit bacterial growth than corresponding to plain gel (10.0±0.5, 12.0±0.8, 14.0±0.7) at the concentration level of 10µg/ml, 20µg/ml and 30µg/ml respectively.

Keywords: Tazarotene, noisome, gel, acne, stability, thin film hydration

Received 24/12/2020; Revised 06/01/2021; Accepted 11/01/2021

Introduction

Acne is a chronic inflammatory disease of the pilosebaceous follicle that affects about 85 % of adolescents. It is estimated that the prevalence of the disease is about 1-12 % in the adult males and 12-17% in adult females. It is more frequent and severe in males, but more persistent in women[1-2].

Topical preparations are used for the localized effects at the site of their application by virtue of drug penetration into the underlying layers of skin or mucous membranes. The main advantage of topical delivery system is to bypass first pass metabolism[3]. Niosomes are a novel drug delivery system, which entrapped the hydrophilic drug in the core cavity and hydrophobic drugs in the non-polar region present within the bilayer hence both hydrophilic and hydrophobic drugs can be incorporated into niosomes[4]. The main purpose of developing niosomal system is chemical stability, biodegradability, biocompatibility, chemical stability, low production cost, easy storage and handling and low toxicity.

Tazarotene, is 6-[2-(4,4-dimethylthiochroman- 6-yl)ethynyl] ethyl nicotinate, a member of a new generation of receptor-selective synthetic retinoids, indicated in the mild to moderate plaque psoriasis disease, acne vulgaris, and photoaging. Extremely low solubility limits incorporation of tazarotene into an acceptable vehicle and its tolerability results in either discontinuation of treatment or poor compliance in patients.

In the present study, we prepared tazarotene -loaded niosomes which were further incorporated in the carbopol 934 gel to alter the contact time to gain maximum benefits of the treatment.

Material and Methods

All the reagents and chemicals used the present study were used as obtained without any further purification.

Preparation of Niosomes

Niosomes were prepared by thin film hydration method[5]. Cholesterol and non-ionic surfactant were dissolved in 500ml of round bottom flask with 10ml chloroform and was allowed to rotate in a flash evaporator at 60°C. The flask was allowed to rotate at 125rpm for one hour to get a dry film. The film was hydrated with 5ml phosphate buffer saline (PBS) pH 7.2 containing 10mg Tazarotene was allowed to rotate for 60 minutes. All the formulations were sonicated for 60 sec. using bath sonicator.

Optimization of niosomes

Optimization of Cholesterol: surfactant ratio

The lipid: surfactant ratio was optimized by taking their different ratio such as 10:1, 10:1.5, 10:2 and 10:2.5 ratio and all other parameters were kept constant. The prepared formulations were optimized on the basis of average particle size and % entrapment efficiency.

Optimization of drug concentration

Drug concentration was optimized by taking different concentration of drug and prepared their formulation and all other parameter such as cholesterol, stirrer time kept constant. The formulation optimized on the basis of entrapment efficiency and average vesicle size.

Optimization of sonication time

Sonication time was optimized by sonicating the formulation for different time i.e 30, 60 and 90 sec at 40C in 3 cycles of 10 minutes with 5 sec rest between the cycles. The optimization was done on the basis of average particle size, and % Entrapment efficiency.

Evaluation of Tazarotene loaded niosomes

Surface charge and vesicle size

The vesicles size and size distribution and surface charge were determined by Dynamic Light Scattering method (DLS) (Malvern Zetamaster, ZEM 5002, Malvern, UK). For measurement of zeta potential, a zetasizer was used with field strength of 20 V/cm on a large bore measures cell. Samples were diluted with 0.9% NaCl adjusted to a conductivity of 50 lS/cm[6].

Entrapment efficiency

Entrapment efficiency was determined by dialysis method. The niosomes were filled into dialysis bags and the free drug dialyzed for 24 hours into 50 ml of buffer pH 7.2. The absorbance of the dialysate was measured at 351nm against blank buffer pH 7.2 and the absorbance of the corresponding blank was measured under the same condition. The concentration of free drug could be obtained from the absorbance difference based on standard curve. Standard curve was made by measuring the absorbance at 242.0.0 nm for known concentrations of drug solution[7].

$$\% EE = \frac{Theoretical\ drug\ content - Practical\ drug\ content}{Theoretical\ Drug\ content}\ X\ 100$$

Preparation of Niosomal Gel

Carbopol 934 (0.5-1.5%w/v) was accurately weighed and dispersed into double distilled water (80ml) in a beaker. This solution was stirred continuously at 800 rpm for 1 hour and then 10ml of propylene glycol was added to this solution. The obtained slightly acidic solution was neutralized by drop wise addition of 0.05 N sodium hydroxide solutions, and again mixing was continued until gel becomes transparent. Volume of gel was adjusted to 100 ml and then sonicated for 10 min on bath sonicator to remove air bubbles. Final pH of the gel base was adjusted to 6.5. Gel was also prepared with plain drug by adding 10mg of drug and dispersed properly by following same procedure given above. The same procedure was used to formulate noisome containing gel in which previously prepared niosomal cake was added in place of plain drug. Niosomes preparation corresponding to 0.1% w/w of drug was incorporated into the gel base to get the desired concentration of drug.

Evaluation of gel

Viscosity

Viscosity measurements of prepared topical niosome based gel were measured by Brookfield viscometer using spindle no. 63 with the optimum speed of 10rpm; viscosity of all formulation measured in cps[8].

pH measurements

pH of selected optimized formulations was determined with the help of digital pH meter. Before each measurement of pH, pH meter should be calibrated with the help of buffer solution of pH 4, pH 7 and pH 9.2. After calibration, the electrode was dipped into the vesicles as long as covered by the vesicles. Then pH of selected formulation was measured and readings shown on display were noted[9].

Drug content

Accurately weighed equivalent to 1 mg of topical Niosomal gel was taken in beaker and added 20 ml of methanol. This solution was mixed thoroughly and filtered using Whatman filter paper no.1. Then 2.0 mL of filtered solution was taken in 10 mL capacity of volumetric flask and volume was made upto 10 mL with methanol. This solution was analyzed using UV-Spectroscope at λmax 351nm[10].

Tube Extrudability

It was determined by applying weight on gel filled collapsible tube and recording the weight that was required to extrude the gel out of the tube.

Spreadibility

To determine spreadibility, 2-5 g of gel was placed between two slide and gradually weight was increased by adding it on the weight pan and time required by the top plate to cover a distance of 10 cm upon adding 80g of weight was noted[11].

$$Spreadability = \frac{Weight\ on\ top\ slide - Distance\ travelled\ by\ slide}{Time\ required\ to\ cover\ 10\ cm}$$

In vitro drug diffusion study

The *in-vitro* diffusion study was carried by using Franz Diffusion Cell. Egg membrane is taken as semi permeable membrane for diffusion. The Franz diffusion cell has receptor compartment with an effective volume approximately 60 mL and effective surface area of permeation 3.14cm^2. The egg membrane is mounted between the donor and the receptor compartment. A 2cm^2 size patch taken and weighed then placed on one side of membrane facing donor compartment. The receptor medium was phosphate buffer pH 7.2. The receptor compartment is surrounded by water jacket so as to maintain the temperature at 32 ± 0.5°C. Heat is provided using a thermostatic hot plate with a magnetic stirrer. The receptor fluid is stirred by Teflon coated magnetic bead which is placed in the diffusion cell. During each sampling interval, samples are withdrawn and replaced by equal volumes of fresh receptor fluid on each sampling. The samples withdrawn are analyzed spectrophotometrically at wavelength of drug at 351nm[12].

In-vitro anti acne activity

Well diffusion method was used to determine the antibacterial activity of the niosomal gel **NG2** prepared from Tazarotene using standard procedure[13]. Three concentrations (10, 20 and 30µg/ml) of gel were for antibiogram studies. The plates were incubated at 37°C for 24 h and then examined for clear zones of inhibition around the wells with particular concentration of drug.

Stability Study

Stability study was carried out for drug loaded niosomes at two different temperatures i.e. refrigeration temperature (4.0±0. 2°C) and at room temperature (25±2°C) for 3 month. The formulation subjected for stability study was stored in borosilicate container to avoid any interaction between the formulation and glass of container. The formulations were analyzed for any physical changes and drug content.

Results and Discussion

The optimization of process variables for formulation of the niosomes was performed as per the reported procedure and the optimized variables are presented in table 1. It was observed that the vesicles size of niosomes was increase with increasing the concentration of cholesterol and similarly vesicle size was decrease with increasing the concentration of span 80 due to its surfactant action. There was no significant difference in average vesicle size was observed with increasing the drug concentration. But in increasing the sonication time the size vesicle was decrease from 250.65±0.25 to 212.46±0.32 after 90 sec of sonication.

Table 1. Optimization of process variables for niosomes

Formulation Code	Soya PC: Span 80 (%w/v)	Drug (%w/v)	Sonication time (sec)	Vesicle Size (nm)	% EE
F1	10:1	1.0	30	245.5±0.45	71.25±0.21
F2	10:1.5	1.0	30	356.7±0.32	65.56±0.26
F3	10:2	1.0	30	262.7±0.42	63.12±0.25
F4	10:2.5	1.0	30	226.5±0.55	56.52±0.18
F5	10:1	0.5	30	255.6±0.45	68.89±0.32
F6	10:1	1	30	232.5±0.32	76.65±0.41
F7	10:1	1.5	30	225.6±0.15	40.36±0.32
F8	10:1	1	30	250.6±0.25	65.85±0.23
F9	**10:1**	**1**	**60**	**212.4±0.32**	**78.85±0.25**
F10	10:1	1	90	223.4±0.41	55.23±0.14

It was observed that the percent drug entrapment was decrease with increasing the concentration of surfactant and on increasing the time of sonication. F9 was selected as the optimized formulation with vesicle size 212.4±0.32 nm and 78.85±0.25 % EE.

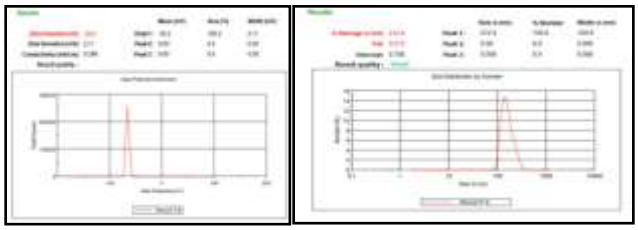

Figure 1. Vesicle size and zeta potential of F9

The zeta potential of F9 was obtained to be 35.2±0.1 suggesting penetrability into the lipophilic membrane.

The formulation F9 was incorporated into the gel base to obtain the niosomal gel formulation.

The results of the evaluation of the niosomal gel formulations are presented in table 2.

Table 2. Evaluation of niosomal gel formulations

Formulation code	Conc of carbopol (%)	Viscosity (cps)	pH	Drug content (%)	Extrudability	Spreadability	% drug released afer 12 h
NG1	0.5	3210.2 ±12	7.10 ±0.1	97.2± 0.49	182.23 ±1.12	6.12 ±0.41	85.32 ±0.57
NG2	1.0	3350.2 ±10	6.80 ±0.2	99.4± 0.23	175.65 ±0.35	5.65 ±0.12	96.65 ±0.32
NG3	1.5	3940.5 ±14	6.52 ±0.1	96.6± 0.78	170.45 ±0.23	5.12 ±0.40	90.35 ±0.41

Values are mean±SD, n = 3

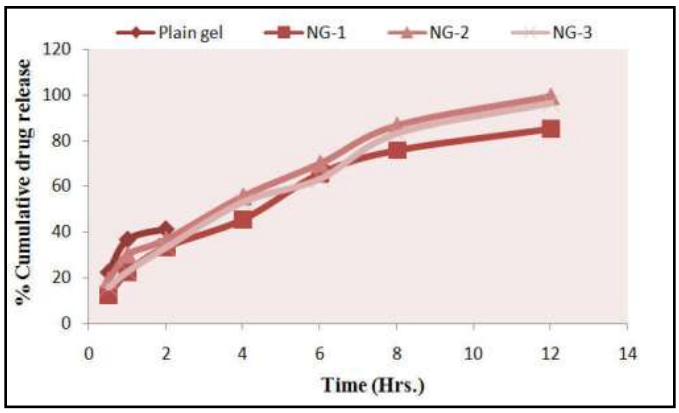

Figure 2. *In vitro* release of tazarotene from gel

The *in vitro* anti acne activity of the niosomal gel was evaluated against *Propionibacterium acnes* using well diffusion method using clindamycin as the standard drug. The niosomal Gel NG-2 showed greater capacity (18.5±0.86, 19.0±0.7, 24.0±0.9) to inhibit bacterial growth than corresponding to plain gel (10.0±0.5, 12.0±0.8, 14.0±0.7) at the concentration level of 10µg/ml, 20µg/ml and 30µg/ml respectively.

Table 3. Anti acne action of niosomal gel

Treatment	Zone of Inhibition (mm)		
	10 µg/ml	20µg/ml	30µg/ml
Clindamycin	18.0±0.5	20.0±0.9	23.0±0.8
Plain Drug Gel	10.0±0.5	12.0±0.8	14.0±0.7
Niosomal Gel	18.5±0.86	19.0±0.7	24.0±0.9

Table 4. Stability of noisome (F9)

Parameter	Time (months)					
Temperature (°C)	4.0 ±0. 2	25±2	4.0 ±0. 2	25±2	4.0 ±0. 2	25±2
Average particle size (nm)	212.1	245.5	215.4	265.4	218.8	285.5
% EE	78.12	74.45	78.05	70.65	77.85	65.58

Stability study data revealed that the optimized formulation (F-9) was stable after 3 month of storage at 4ºC whereas at 25±2°C the formulation was found unstable. Stability of formulation was observed on the basis of % EE andaverage vesicles.

Conclusion

From this study, we can conclude that encapsulation of tazarotene in carrier system, i.e. niosome gel is advantageous because it enhances the transdermal permeation of the drug, control the release of the drug and prevent the degradation of tazarotene by protecting it from the direct exposure to environment. Side effects of the drug may also reduce. In vitro study concluded that tazarotene-loaded NG-2 shows more anti acne effect than the plain gel.

References

1. Teixeira V, Vieira R, Figueiredo A. Impacto psicossocial da acne. Rev da Soc Port Dermatol Venereol. 2012; 70(3): 291–296.
2. Nast A, Dréno B, Bettoli V, et al. European evidence-based (S3) guidelines for the treatment of acne. J Eur Acad Dermatol Venereol. 2012; 26 (S1): 1–29.
3. Shah VP, Maibach HI, Topical Drug Bioavailability, Bioequivalence, and Penetration, 1, Springer, USA, 1993, 369-391.
4. Makeshwar K, Wasankar S. Niosomes: a novel drug delivery system. Asian J. Pharm. Res. 2013; 3: 16-20.
5. Goyal G, Garg T, Malik B, Chauhan G, Rath G, Goyal AK. Development and characterization of niosomal gel for topical delivery of benzoyl peroxide, Drug Delivery, 2015; 22(8): 1027–1042.
6. Balakrishnan P, Shanmugam S, Lee WS et al. Formulation and in vitro assessment of minoxidil niosomes for enhanced skin delivery. International Journal of Pharmaceutics. 2009; 377 (1-2), 1–8.
7. Firthouse PUM, Halith SM, Wahab SU, Sirajudeen M, Mohideen SK. Formulation and evaluation of miconazole niosomes. International Journal of PharmTech Research. 2011; 3 (2): 1019–1022.
8. Sathali AAH, Sangeetha T. Formulation and evaluation of ocular niosomal in situ gel of levofloxacin hemihydrates. Journal of Pharmacy Research. 2011; 4: 4331–4337.
9. Sathali AAH, Rajalakshmi G. Evaluation of transdermal targeted niosomal drug delivery of terbinafne hydrochloride. International Journal of PharmTech Research. 2010; 2 (3): 2081–2089.
10. Solanki AB, Parikh JR, Parikh RH, Patel MR. Evaluation of di erent compositions of niosomes to optimize aceclofenac transdermal delivery. Asian Journal of Pharmaceutical Sciences. 2010; 5 (3): 87–95.
11. Salih OS, Samein LH, Ali WK. Formulation and in vitro evaluation of rosuvastatin calcium niosomes. International Journal of Pharmacy and Pharmaceutical Sciences. 2013; 5 (4): 525–535.
12. Shinde UA, Kanojiya SS. Serratiopeptidase niosomal gel with potential in topical delivery. Journal of Pharmaceutics. 2014, Article ID 382959.
13. Nakatsuji T, Kao MC, Fang JY, et al. (2009). Antimicrobial property of lauric acid against Propionibacterium acnes: its therapeutic potential for inflammatory acne vulgaris. J Invest Dermatol 129: 2480–2488.