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Fabrication and evaluation of Oxymetazoline Hydrochloride Proniosomal Gel as a Vesicular Drug Delivery System

Sunil Kumar[1*], Dr. Rakesh Kumar Jatav[2], Dr. Karunakar Shukla[3]

[1*]Research schlor, Dr. APJ Abdul kalam University Indore M.P.Sunil.sunilpharma.kumar@gmail.com,
[2]Professor Dr. APJ Abdul kalam University Indore M.P.
[3]Professor, Dr. APJ Abdul kalam University Indore M.P.

***Corresponding Author:**Sunil Kumar
*Research schlor, Dr. APJ Abdul kalam University Indore M.P. Sunil.sunilpharma.kumar@gmail.com, Contact no. 9598527644

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Abstract

The purpose of this research is to design proniosomal gel drug delivery system of oxymetazoline hydrochloridein a trial to overcome theadverse effects associated with oral administration of the drug. This can be overcome by the use ofvesicular drug deliverysystem. Encapsulation of a drug in vesicular structure can be predicted to prolong the existence of the drug in the systemiccirculation and thus enhance penetration into target tissue and reduce toxicity. Due to the limited solvent system present, theproniosomes formed were the mixture of many phases of liquid crystal, viz. lamellar, hexagonal and cubic phase liquidcrystals. The potential of proniosomes as a transdermal drug delivery system of oxymetazoline hydrochloride was investigated by encapsulatingthe drug in various formulations of proniosomal gel composed of various ratios of sorbitan fatty acid esters, cholesterol,prepared by coacervation-phase separation method. The formulated systems were characterized in vitro for size, vesicle count,drug entrapment, drug release profiles and vesicular stability at different storage conditions. Stability studies for proniosomalgel were carried out for 4 weeks. The method of proniosome loading resulted in an encapsulation yield of 30.6 – 75.4%. *In-vitro*studies showedprolonged release of entrappedoxymetazoline hydrochloride. At refrigeratedconditions, higher drug retentionwasobserved. It is evident from this study that proniosomes are a promising prolonged delivery system for oxymetazoline hydrochlorideand havereasonablygood stabilitycharacteristics.

Keywords: Proniosomes, oxymetazoline hydrochloride, *In-vitro* release, Gel System, Stability studies.

INTRODUCTION

Non-ionic surfactant vesicles known as niosomes are microscopic lamellar structures formed on admixture of a non-ionic surfactant, cholesterol and dicetyl phosphate with subsequent hydration in aqueous media. Proniosomes offer a versatile vesicle drug delivery concept with potential for delivery of drugs via transdermal route. This would be possible because proniosomes form niosomes upon hydration with water from skin following topical application under occlusive conditions. Proniosomes minimizes problems of niosomes physical stability such as aggregation, fusion and leaking and provide additional convenience in transportation, storage and dosing.

Oxymetazoline is a adrenergic alpha agonist, direct acting sympathomimetic used as a vasoconstrictor to relieve nasal congestion. The sympathomimetic action of oxymetazoline constricts the smaller arterioles of the nasal passage, producing a prolonged gentle and decongesting effect oxymetazoline relief of conjunctival hyperemia by causing vasoconstriction of superficial conjunctival blood vessels.This new topical use of oxymetazoline hydrochloride in the form of 1% cream for the treatment of facial erythema of rosacea has been approved by USFDA on 19 jan 2017[4].

Looking forward to exploit this new use of oxymetazoline hydrochloride in dermatology like to develop proniosomal drug delivery system for the treatment for Rosacea, a chronic disorder of the central face.

Based on the investigations provesicular systems appear to be an alternate drug carrier for various routes of drug administration. oxymetazoline hydrochloride, Oxymetazoline Hydrochloride is the hydrochloride salt form of oxymetazoline, an imidazole derivative with a direct acting sympathomimetic property. Oxymetazoline binds to and activates alpha-2 adrenergic receptors. Upon nasal or ocular administration, oxymetazoline constricts the arterioles in the nose and eye, resulting in decreased nasal and conjunctival congestion, respectively

It will be also affected through transdermal route because of its size, nature and chemistry, these systems give better drug permeability from biological bioavailability membranes and helps in solubilization of some practically insoluble

drugs and hence solve problems of many drug. To overcome the problem like gastric side effect, short half life and low bioavailability etc of Oxymetazoline Hydrochloride can be solved by developing the formulation of Oxymetazoline Hydrochloride as proniosome gel.

MATERIALS:

Oxymetazoline Hydrochloride was a gift from Lobachemie, Mumbai, cholesterol, and dialysis tubing was purchased from Hi-Media Laboratories (Mumbai, India). Span 20, 40, 60, 80 and BRIJ 35 were purchased from Central Drug House (Delhi, India). All other chemicals and solvents were of analytical grade and obtained from Central Drug House Delhi, India.

METHOD:

Proniosomal gel was prepared by a coacervation-phase separation method. Precisely weighed amounts of surfactant, cholesterol and drug were taken in a clean and dry wide mouthed glass vial of 5.0 ml capacity and alcohol (0.5 ml) was added to it. All the ingredients were mixed well with a glass rod; the open end of the glass bottle was covered with a lid to prevent the loss of solvent from it and warmed over water bath at 60-70°C for about 5 min until the surfactant mixture was dissolved completely. Then the aqueous phase (0.1% glycerol solution) was added and warmed on a water bath till a clear solution was formed which was converted into proniosomal gel on cooling. The gel so obtained was preserved in the same glass bottle in dark conditions for characterization. Compositions of proniosomal gel formulations are given in Table1.

CHARACTERIZATION OF PRONIOSOMAL GEL:

Optical microscopic examination:
Hydration of Oxymetazoline Hydrochlorideproniosomal gel (100mg) was done by adding saline solution (0.9% solution) in a small glass vial with occasional shaking for 10 min. The dispersion was observed under optical microscope at 100 x magnification. The sizes of 200-300 vesicles were measured using a calibrated ocular and stage micrometer (Erma, Tokyo) fitted in the optical microscope.[4]

Scanning Electron Microscopic Examination (SEM):
This technique was used to investigate the shape of the vesicles prepared and to assess the vesicle size of the formulation. Samples of formulations were applied and spread on a sample holder (thin carbon film). The samples were sputter-coated with Au/Pd under an argon atmosphere at 180 mA for 1 minute (Polaron E5100; VG MicroTech, West Sussex, UK).The samples were placed inside of the vacuum column of the microscope and the air was pumped out of the chamber. An electron gun placed at the top of the column emits a beam of high energy primary electrons. As the focused electron beam hits a spot on the sample, secondary electrons are emitted by the specimen through ionization. The vesicles of Proniosomes have been observed with a scanning electron microscope Fig. 2 (Zeiss DSM 982 Gemini; LEO Electron Microscopy Ltd, Cambridge, UK) at 5-20 kV.[5]

Transmission Electron Microscopic Examination:
For transmission electron microscopy (TEM) analysis, the proniosomal formulation samples were placed on a form war-coated copper grid. The samples were then negatively stained with 50 µl of 1.0% (w/v) phosphotungstic acid the staining process was allowed to proceed for 10 min at room temperature. Excess liquid was drained off with Whatman filter paper and the proniosomal samples were observed with a transmission electron microscope (FEI, Netherland) at 80 KV. The TEM photographs of selected formulations are shown in the Fig. 3, The selected and prepared Oxymetazoline hydrochloridegel was characterized for its shape by transmission electron microscopy (JEOL Model - JEM 2100–200KV, Tokyo, Japan), using a 300 mesh carbon-coated copper grid and phosphotungstic acid (1%; w/v) as a negative stain. After being stained, the samples were allowed to dry at room temperature for 10 min for investigation.[5]

Entrapment efficiency:
To evaluate the loading capacity of proniosomal systems for Oxymetazoline Hydrochloride, proniosomal gel (100mg) was dispersed in distilled water and warmed a little for the formation of niosomes. Then the dispersion was centrifuged at 18000 rpm for 40min at 5ºC (Remi CPR-24 centrifuge). The clear fraction was used for the determination of free drug at 247.0 nm spectrophotometrically. The percentage encapsulation efficiency was calculated from Equation 1
% Encapsulation Efficiency = [1- (Unencapsulated drug / Total drug)] x 100 [7, 9]

In-Vitro Release:
The *in vitro* drug release studies of Oxymetazoline Hydrochloride proniosomal gel were carried out by means of treated cellophane membrane. *In-vitro* release studies on proniosomal gel were performed using locally Fabricated Franz-diffusion cell. The capacity of receptor compartment was 15 ml. The area of donor compartment exposed to receptor compartment was 1.389cm2. The dialysis cellophane membrane (MMCO14KDC) was mounted between the donor and receptor compartment. A weighed amount of proniosomal gel was placed on one side of the membrane.
The receptor medium was saline phosphate buffer pH 7.4. The receptor compartment was surrounded by a water jacket to maintain the temperature at 37±1ºC. Heat was provided using a thermostatic hot plate with a magnetic stirrer.7, 14 The receptor fluid was stirred by a Teflon-coated magnetic bead fitted to a magnetic stirrer (Bio-Craft Scientific Systems Pvt. Ltd., Agra). At each sampling interval, (1 ml) were withdrawn and were replaced by equal volumes of fresh receptor fluid on each occasion. Samples withdrawn were analyzed spectrophotometrically (Shimadzu-1700) at 247 nm. [15]

Stability Studies:
The ability of vesicles to retain the drug (Drug Retention Behaviour) was assessed by keeping the proniosomal gel at three different temperature conditions, i.e., Refrigeration Temperature (4-80C), Room Temperature (25±20C) and oven (45±20C). Throughout the study, proniosomal formulations were stored in aluminum foil-sealed glass vials. [12]

Results and Discussion:

Proniosome gel containing Oxymetazoline Hydrochloridewere prepared by Coacervation phase separation method. Formation of vesicle mainly depends on the concentration of cholesterol and surfactant ratio.Table1 show that the entrapment efficiency of different optimized formulation. The entrapment efficiency of Oxymetazoline Hydrochloride within the formulation from 40.6% for span 80 vesicle (SKJ4) to high as 82.56 % for Brij 35 (SKK3) vesicle show higher entrapment efficiency for Brij 35 formulation can be attributed to its length of longer side chain, and it easily diffuse into receptor membrane integrity ,orientation and packaging ability. The entrapment efficiency of proniosomal gel was attributed due to the amphiphillic nature of the drug. The entrapment efficiency was found maximum for SKK3 formulation due to higher HLB value, of the formulation, which result in larger vesicle hence more entrapment of drug into the vesicle. The effect of cholesterol on Oxymetazoline Hydrochloride entrapment was varied according to the nonionic surfactant used, cholesterol was found to have little effect on the Oxymetazoline Hydrochloride entrapment. The results of analysis reports are shown in the (Table 2); it shows that vesicle size decrease with decrease in HLB value. Vesicle size decreases in the following manner span 20 > span 40 > span 60 > span 80 > Brij 35> span 80, i.e. higher the HLB value result in reduction in surface free energy which allow to form vesicle of larger and hence small area exposed to dissolution medium.

Table1: Compositionofproniosomalgel formulation

F.Code	Drug(mg)	Span20 (mg)	Span40 (mg)	Span60 (mg)	Span80 (mg)	Brij35 (mg)	CHL	Solvent ratio(ml)
SKG3	10	50	-	-	-	-	50	0.5
SKH3	10	-	50	-	-	-	50	0.5
SKI3	10	-	-	50	-	-	50	0.5
SKJ3	10	-	-	-	50	-	50	0.5
SKK3	10	-	-	-	-	50	50	0.5

Table:2 Variousresultofproniosomalgelformulation

S. No.	F. Code	Vesicle size(nm)	% DrugEntrapment	% drugreleased
1.	SKG3	375.2	57.3±1.97	57.78±0.45
2.	SKH3	330.6	62.6±1.65	62.75±0.87
3.	SKI3	305.6	76.0±1.98	68.12±0.86
4.	SKJ3	265.4	57.3±1.63	74.85±0.56
5.	SKK3	315.6	82.56±1.55	85.42±0.67

The proniosomal formulation having low cholesterol content was found to cause low entrapment efficiency, which might be because of leakage of vesicle. The higher entrapment may be explained by high cholesterol content (50 % of

the total lipid) reported that entrapment efficiency increase with increasing cholesterol content. It was also observed that very high cholesterol content had a lowering effect on drug entrapment to the vesicle. This could be due to the fact that cholesterol beyond a certain level starts disrupting the regular bilayer structure leading to loss of drug entrapment. [15]
This result are also observed and similar as study in the proniosomal formulation using Brij 35, span 60 and tween 80 surfactant. In this prepare vesicle using Brij 35: cholesterol show better drug release in Comparision to other surfactant.15 Our finding results are observed similar as study in Brij 35, Brij 58, and Brij 92 Brij 52 etc. The conclusion of this study show that Brij 35 did not form niosome in the absence of cholesterol, but in the presence of cholesterol niosome formed and vesicle shows more stable and less leaky.[16]
This result are also observed in a comparative study on the effect of some polyoxyethylene alkyl ether like (Brij 78, Brij 92, Brij 72, Brij 52) and sorbitan fatty acid ester surfactants on the performance of transdermal proniosomal gel using experimental design. In this the Brij 52 show similar property as compare to Brij 35.The findings of this study shows that the niosome formulation prepared with Brij 52 was better drug release in Comparision to other Brij Component.

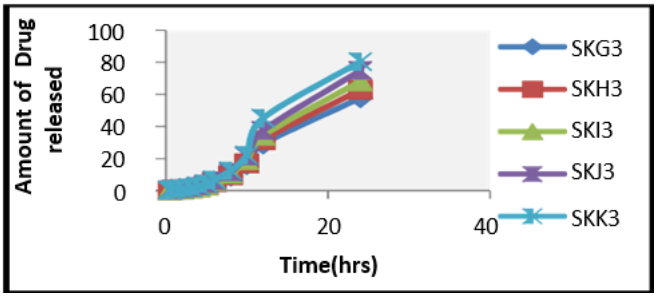

Figure: 1 In-vitro drug release profile of selected proniosomal gel formulation

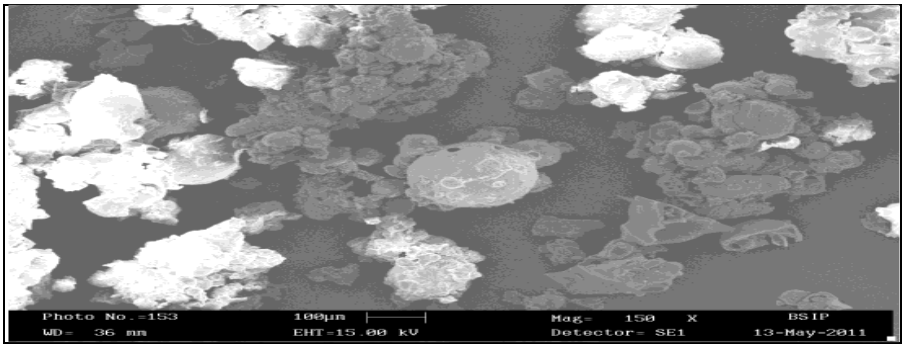

Figure 2: Scanning electron microscopy of formulation SKK3

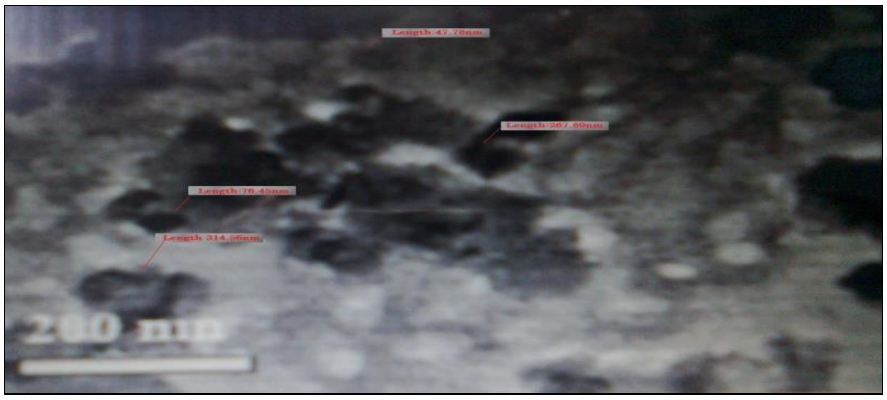

Figure 3: Transmission electron photomicrograph of SKK3

Most of the surfactant used to make non-ionic surfactant vesicle have low aqueous solubility, however freely soluble non-ionic surfactant such as Brij 35 can form micelles on hydration due to the presence of more polar head group in the chain, in the addition of cholesterol they abolish the more polar part present in surfactant mainly due to lipophillic in nature and help in formation of vesicle in the eqiumolar ratio of Brij 35 and cholesterol show better result (1:1). The proniosome formation takes place from Brij 35 with the presence of cholesterol, the length of alkyl chain show a crucial

factor of permeability, Brij 35 have long lauryl(c12) chain, thus the long chain influence the HLB of the surfactant and also lead to the higher drug entrapment efficiency and also show better stability of the proniosome using Brij 35. Cholesterol is one of the most important additives included in the formulation in order to prepare stable niosome. Cholesterol stabilizes bilayer, prevents leakiness and retards permeation of solutes enclosed in the aqueous core of these vesicle which could be able to effectively prevent leakage of drug from niosome. Cholesterol is thus included in 1: 1 molar ratio (non-ionic surfactant: cholesterol), show better result in the case of Brij 35. Thus it can be concluded that Brij 35 show highest drug release by the fact that niosome exhibit an alkyl chain length-dependent drug release.
In-vitro drug release was carried out by locally fabricated Franz diffusion cell. The ability of ethanolic lipid vesicles called ethosomes to deliver Oxymetazoline Hydrochloride was investigated by determining the flux of Oxymetazoline Hydrochloride. The data of percent cumulative amount of Oxymetazoline Hydrochloride permeated per unit area across dialysis membrane via various formulations was given. The amount of drug released from differentproniosomal gel formulation was found in order of SKK3 > SKJ3 > SKI3 > SKH3 > SKG3. It was found that SKK3 showed a controlled release property from 10-24 hrs. The result of cumulative % drug release 10th hour was found to be 45.12% & 80.42% at 24 hour .Thiss mins it show a better controlled property. The release profile was found constant between 10-24 hours. So the formulation was found to exhibit a zero order controlled release profile. Other Oxymetazoline Hydrochloride SKG3, SKH3, SKI3, SKJ3, also show good controlled release property. The *in-vitro* release of Oxymetazoline Hydrochlorideproniosomal gel was limited by two barriers, namely phospholipids bilayer & dialysis membrane.
The formulation SKK3 (50% Brij 35 and 50% cholesterol) showed highest amount of % drug released (80.42%). The enhanced % drug release obtained from the proniosomal gel system could be justified on the basis of dual function performed by ethanol present in the proniosomal formulations, length of side chain, i.e. fluidizing both the vesicular lipid bilayer and greater malleability to the vesicles and enhancing permeability of the skin.
Overall, the data clearly indicate that the proniosomal gel formulation SKK3 (50% w/w cholesterol and 50% w/w Brij-35) showed the highest entrapment efficiency (82.56 %), optimum size (315.6 nm), highest cumulative amount of % drug released (80.42%). Thus justifying itself as an optimized formulation and used for further skin *in-vivo* studies.

Stability study
In order to determine the percent drug remaining entrapped in vesicles and percent drug lost from Proniosome gel subjecting at temperature 4±2oC, 37±2oC and 45±2oC for 45 days, were determined drug lost at time interval of 15 days. On the basis of entrapment efficiency and controlled release property, formulation SKK3 selected for the stability studies. Stability study was carried out in term of % drug leacked.
Results showed that proniosomal gel formulation was quite stable at refrigeration and room temperature. In thiscondition not much leakage of drug was found at their temperature. Percent drug retained at 45ºC might have decreased due to the melting of surfactant and lipid present in the formulation to the proniosomal gel formulation can be stored at refrigeration and room temperature. Thus it can be concluded that the shelf life of proniosomal powder formulation is more than the proniosomal formulation. Because in dry surfactant can be avoided, by forming the suspension as needed, precipitation and aggregation can also be avoided.

CONCLUSION

Thus from above study it can be concluded that the proniosomes gel posses higher entrapment efficiency and utilizes alcohol, which itself act as penetration enhancer. The elicited an increase of the percutaneous permeation of Oxymetazoline Hydrochloride*in-vitro* and. In addition, In-*vitro* experiments showed that Oxymetazoline Hydrochloride proniosomes gel can ensure a sustained release of the drug and hence a prolongation of its therapeutic activity, which can be related to an accumulation of Oxymetazoline Hydrochloride in the skin.

These findings are very encouraging and confirm that proniosomes are a very promising carrier for the topical administration due to the enhanced delivery of drugs through the skin thus prompting various opportunities for the development of suitable therapeutic strategies through the topical route. The formulation is easy to scale up as the procedure is simple and do not involve lengthy procedure and unnecessary use of pharmaceutically unacceptable additives. It offers direct fabrication of transdermal patch and do not require dispersion of vehicle into polymer matrix.

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REFERENCES

1. Fang JY, Yu SY, Wu PC, Huang YB, Tsai YH. In-vitro skin permeation of estradiol from various proniosomes formulation. Int J Pharm 2001; 215: 91-99.
2. Hu C, Rhodes DG. Proniosomes: A Novel Drug Carrier Preparation. Int J Pharm 1999; 185(1): 23-35.
3. Shahiwala A, Misra A. Studies in topical application of niosomally entrapped nimesulide. J Pharm Pharm Sci 2002; 5(3): 220-225.
4. Khandare JN, Madhavi G, Tamhankar BM. Niosomes: Novel drug delivery system. East Pharm 1994; 37:61-64.
5. Wu PC, Huang YB, Chang JJF, Chang JS, Tsai YH. Evaluation of pharmacokinetics and
1. pharmacodynamics of captopril from transdermal hydrophilic gel in normotensive rabbit and
2. Spontaneously hypertensive rats. Int J Pharm 2000; 209: 87-94.
6. Abubkar O Nur, Zhang JS. Recent progress in sustained/controlled oral delivery of captopril: An overview. Int J Pharm 2000; 194: 139-146.
7. Yoshioka T, Sternberg B, Florence A. T. Preparation and properties of vesicles (Niosomes) of sorbitan monoesters (Span- 20, Span- 40, Span- 60 and Span- 80) and a sorbitan triester (Span- 85). *Int. J. Pharm*. 1994, 105, 1-6
8. Lusia D. Marianecci C. Novel pH sensitive non-ionic surfactant vesicles: comparison between Tween 21 and Tween 20. *colloids and surfaces B: Biointerfaces*2011,82, 18-24.
9. Tangri P, Khurana S. Niosomes: Formulation and evaluation. International journal of biopharmaceutics 2011, 2(1), 47-53.
10. Singh J, Robinson DH. Controlled release kinetics of captopril form tableted microcapsules. Drug Dev Ind Pharm 1988; 14(4): 545-560.
11. Leung SHS, Robinson JR. The contribution of anionic polymer structure feature to mucoadhesion. J Contrl Rel 1988; 5: 223-231.
12. Sheth PR, Tossounian J. The hydrodynamically balanced system (HBSTM): A novel drug delivery system for oral use. Dev Ind Pharm. 1984; 10(2): 313-339.
13. Abdelbary .G, El-gendy N. Niosome-Encapsulated Gentamicin for Ophthalmic Controlled Delivery. *AAPS PharmSciTech*, 2008, 105 8-9105.
14. Pardakhty A, Varshosaz J. In vitro study of polyoxyethylene alkyl ether niosomes for delivery of insulin. *International Journal of Pharmaceutics* 2007, 318, 130–141.
15. Zhou XH, Li Wan PA. Stability and in-vitro absorption of captopril, enalapril and lisinopril across the ratintestine. BiochemPharmacol 1994; 47: 1121-1126.
16. Tripathi KD. Essentials of Medical Pharmacology. New Delhi, India, Jaypee Brothers, 2003 pp 449-454.
17. Jain NK, Vora B, Khopade AJ. Proniosome based transdermal delivery of levonorgestrel for effective contraception. J Control Rel 1998; 54: 149-165.
18. Nagarsenker MS, Londhe VY, Nadkarni GD. Preparation and evaluation of liposomal formulation of tropicamide for occular delivery. Int J Pharm 1999; 190: 63-71.
19. Bhatia A, Kumar R, Katare OP. Tamoxifen in topical liposomes: Develoment, characterization and invitro evaluation. J Pharm Pharm Sci 2004; 7(2): 525-259.