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Formulation and characterization of topical gel of erythromycin entrapped into niosomes

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Abstract: Erythromycin is macrolide antibiotic used as against various susceptible bacteria. In the present study erythromycin was entrapped into niosomes by thin film hydration technique with the optimization of various process parameters. The optimized batch of niosomes was incorporated into carbopol gel and extensively characterized for entrapment efficiency, particle size and in vitro drug release. The stability study was performed at different temperatures. The present study conclusively demonstrated that entrapment of drug into niosomes leads to prolongation of drug release, enhanced drug retention into skin and improved permeation across the skin after encapsulation.
Keywords: Niosomes, Erythromycin, Entrapment efficiency, carbopol gel, In vitro release.

INTRODUCTION

Drug delivery systems using vesicular carriers such as liposomes[1] and niosomes[2] have distinct advantages over conventional dosage forms because the vesicle can act as drug containing reservoirs. Modification of vesicle composition or surface can adjust the affinity for the target site and / or the drug release rate, and the slowing drug release rate may reduce the toxicity of the drug. Hence these carriers play an increasingly important role in drug delivery. Niosomes and liposome are unilamellar or multi-lamellar vesicles wherein an aqueous phase is encapsulated in highly ordered bilayer made up of nonionic surfactant (niosomes) or lipid (liposomes) with or without other components like, cholesterol (chol) and Dicetyl phosphate[3]. Both niosomes and liposomes show desired interaction with human skin when applied through topical preparation by improving especially the horny layer characteristics, which in turn due to reduction in transdermal water loss and increase in smoothness via replenishing skin lipids[4].Although niosomes and liposomes possess more or less same advantage, niosomes were preferred due to high cost and lower stability of lipids which have been replaced by non ionic surfactants. Niosomes loaded with drugs for dermal application show interactions with epidermal tissue without exerting immediate or strong systemic action[4]. Erythromycin is macrolide antibiotic which inhibits protein synthesis by binding reversibly to the 50S ribosomal subunits of susceptible microorganisms. Its action may be either bacteriostatic or bactericidal depending on the sensitivity of the microorganism and the concentration of the drug. Topical application of erythromycin has several disadvantages like unwanted side effects (skin redness, irritation, itching, etc.), need of frequent application (3-4 times a day) which leads to inconvenience and ignorance of therapy and results in no benefit or emergence of resistant strains of bacteria, some times. It was hypothesized that incorporation of erythromycin into niosomes will improve the amount and time of drug retention within the skin; which in turn will increase the therapeutic efficacy of the drug and reduce the toxicity.

MATERIALS AND METHODS

Materials

Erythromycin was obtained as gift sample from Recvina Pharmaceuticals Ltd. (Vadodara, India), Spans and Tweens were purchased from S.D. Fine Chemicals Ltd. (Mumbai, India), Cholesterol was purchased from Loba Chem (Mumbai, India). All the reagents were used without further purifications. Phosphate Buffer Saline pH 7.4 (PBS pH 7.4) and Phosphate Buffer Saline pH 6.8 (PBS pH 6.8) were prepared as described in the Indian Pharmacopoeia (1996) and necessary chemicals were obtained from the Loba Chem (Mumbai, India). All the chemicals used were of Analytical Reagent (AR) grade unless otherwise specified. Syni gel ® (5 % Erythromycin) was purchased from market.

Methods

Preparation and characterization of niosomes

Niosomes were prepared by thin film hydration technique[5]. Drug, Non ionic surfactant and cholesterol were taken in different proportion and dissolved in solvent system into a 500 ml round bottom flask. The assembly of rotary vacuum evaporator was set and solvent mixture was evaporated under a vacuum of up to 25 mm of Hg at a temperature of 60˚ C and the flask rotated until a thin, dry and smooth lipid film was obtained. The film was then hydrated with appropriate volume of PBS pH 7.4 for sufficient time at the same temperature and same speed of rotation without vacuum. The niosomal suspension was further kept in refrigerator (2-8˚ C) for rigidization of vesicles. Free drug was then separated by centrifugation at 2000 rpm for 10 minutes and niosomal cake was obtained by centrifugation at 10000 rpm for 10 minutes after the removal of free drug. Size of the vesicles was analyzed by using Olympus Microscope (40 x, 100 x).

The formulation was optimized for various process parameters, like type of surfactant (Table 1), surfactant:cholesterol ratio (Table 1), hydration volume (Table 2), hydration time (Table 2) and annealing time (Table 3), so as to get maximum drug entrapment. All the batches were prepared three times on different days to check the reproducibility.

The prepared niosomes loaded with erythromycin were analyzed for percentage drug entrapment (PDE) by colorimetric method using PDAB dye after separation of free drug. The method of analysis was developed by the author and validated for all necessary parameters.

Table 1. Composition of various niosomal batches containing Erythromycin.

Batch	Surfactant	Surf : Chol (Weight ratio)	Percentage drug entrapment (± S. D.)
First round of study with different surfactants taking two constant ratios			
N1	Span 20	1.0 : 1	29.23 % (± 0.96)
N2	Span 20	2.0 : 1	25.25 % (± 1.19)
N3	Span 60	1.0 : 1	32.55 % (± 1.65)
N4	Span 60	2.0 : 1	39.80 % (± 1.58)
N5	Span 80	1.0 : 1	38.73 % (± 0.54)
N6	Span 80	2.0 : 1	70.62 % (± 2.25)
N7	Span 85	1.0 : 1	37.51 % (± 1.35)
N8	Span 85	2.0 : 1	33.14 % (± 1.01)
Second round of study with selected surfactant (Span 80) and focusing on narrow range of concentrations			
N9	Span 80	1.25 : 1	47.05 % (± 0.98)
N10	Span 80	1.5 : 1	80.48 % (± 2.64)
N11	Span 80	1.75 : 1	59.05 % (± 0.98)
N12	Span 80	2.5 : 1	47.95 % (± 1.96)

The amount of the drug was kept fixed with drug:carrier(surfactant + cholesterol) ratio at 1:4.

Table 2. Study of effect of hydration volume and hydration time on PDE

Batch	Hydration volume	Hydration time	Percentage drug entrapment (± S. D.)
N13	03 ml	0.5 hr.	76.21 % (± 2.25)
N10	05 ml	0.5 hr.	80.48 % (± 2.64)
N14	07 ml	0.5 hr.	79.36 % (± 2.63)
N15	10 ml	0.5 hr.	78.11 % (± 2.69)
N16	07 ml	1.0 hr.	88.26 % (± 3.64)
N17	07 ml	1.5 hrs.	77.71 % (± 2.51)

Preparation of Carbopol gel: Sufficient quantity of Carbopol 934 (1% w/w) was weighed and sprinkled onto warm distilled water with continuous stirring. The dispersion was allowed to hydrate for 1-2 hours. Other ingredients like Propylene Glycol (10 % w/w) and Glycerol (30 % w/w) were added subsequently to the aqueous dispersion with continuous stirring. A required quantity of drug (2 % w/w) was added and properly dispersed. The dispersion was neutralized to pH 6 using 1 % w/v of Sodium Hydroxide solution and the final weight was adjusted with distilled water. The gel was sonicated for 15 minutes and kept overnight to remove air bubbles. (Batch C_1).

Niosomal gel was prepared by following the same procedure and adding niosomal cake containing an equivalent amount of drug.

Drug retention studies

Sufficient quantity of niosomal suspension (after removal of free drug from Batch SP 82) was sealed in 10 ml glass vial and the niosomal gel formulation (Batch C_2) was sealed in 10 gm collapsible aluminum tube in triplicate, and stored at refrigerated temperature (2-8˚ C) and room temperature (25 ± 2˚ C). Specimen (0.5 gm) from each sample was withdrawn at an interval of one week and analyzed for free drug content to determine the leakage rate. The results are recorded and presented in graphical form in figure 1.

Table 3. Effect of annealing time on entrapment efficiency.

Batch	**Annealing time in hours**	**Percentage drug entrapment (± S. D.)**
N18	1	77.79 % (± 3.05)
N16	**2**	88.26 % (± 3.64)
N19	3	81.45 % (± 3.36)
N20	4	78.77 % (± 2.98)

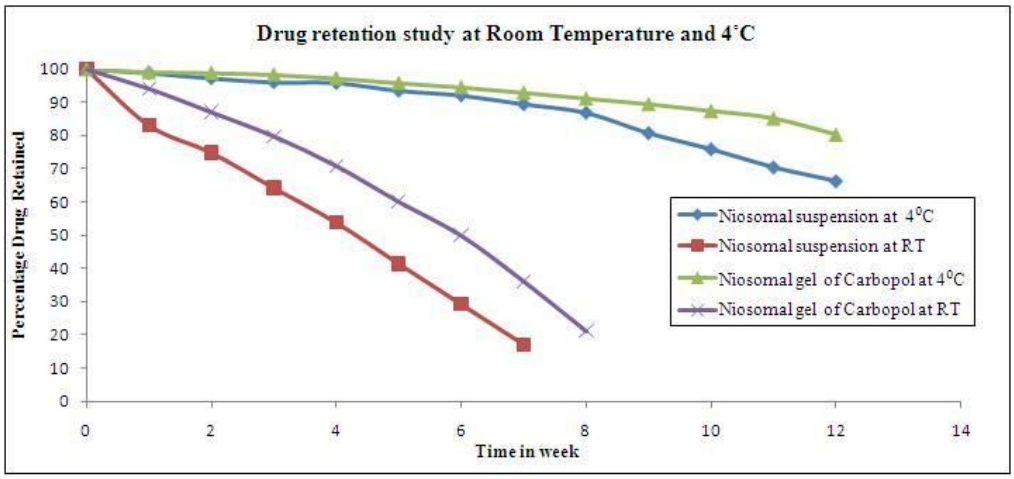

Figure 1. Drug retention study of niosomal suspension and niosomal gel at Room Temperature and 4˚C

In vitro permeation studies

Preparation of membrane for in vitro studies: Human cadaver skin (HCS) was obtained from medical college of the M. S. University and stored at 0°C. A full thickness HCS membrane was prepared by shaving the skin, punching out a tissue of approximately 2.5 cm^2 area with sharp blade, trimming away the excess fat and slicing to about 450µm thickness. These slices were hydrated in pH 6.8 phosphate buffer saline overnight prior to use.

The vertical type of franz diffusion cell was designed, fabricated and validated[6,7] prior to diffusion study. Fifty milligram of gel was applied on 2.00 cm^2 area of epidermal surface of HCS tied to the lower end of donor compartment. The volume of the receptor compartment was kept 20 ml. The cell was assembled in such a way that, the dermal surface was just flushed to the surface of permeation fluid (pH 6.8 PBS) maintained at 37 $\pm$ 1° C and stirred continuously on a magnetic stirrer at 50 rpm. Aliquots of 0.5 ml were withdrawn and analyzed for the drug content after suitable dilutions by colorimetric method. The volume of fluid was replaced with the same volume of fresh buffer after each sampling. The cumulative percentage drug diffused across the HCS was calculated at each sampling point and recorded in Table 4.

Amount of drug retained in the skin was calculated by subtracting the values of free drug content in the receptor compartment and the amount of drug remained on the epidermal surface of skin from the initial drug content of the formulation applied, and results were recorded in Table 4. All the determinations were carried out in triplicate and the data were compared by applying ANOVA (single factor) test.

RESULTS AND DISCUSSION

Amongst many reported methods for the preparation of niosomes, thin film hydration technique was selected as it is the most documented method with greater entrapment efficiency and smaller particle size.. Niosomes were prepared successfully by thin film hydration technique using different grades of polysorbates (Spans) as per the composition mentioned in table 1. Initially four grades of Spans i.e. Span 20, Span 60, Span 80 & Span 85, were taken into two fixed proportions of surfactant:cholesterol i.e. 1:1 & 1:2. It was observed in the initial study that only the Span 80 shows better result of percentage drug entrapment, hence Span 80 was selected and further batches were taken with narrowing the range proportions of surfactant and cholesterol. This observation shows that PDE increased as the HLB value was increased from 1.8 to 4.3 but poor entrapment results with HLB values beyond 4.3. The influence of HLB value suggests that the Critical Packing Parameter of potential niosomes system must take into account of presence of amphiphilic or hydrophobic drugs (erythromycin) as both these substances are incorporated into the vesicle membrane[8].

The data recorded in table 1 suggested that Span 80 and cholesterol at 1.5:1 proportion produced niosomes with PDE of 80.48 %, which highest among different batches under the same level of study and hence batch N10 was selected for further study. One of the important functions of cholesterol is to decrease the overall HLB value of the surfactant mixture used for the preparation and to help making suitable in situ environment for proper entrapment of drug into niosomes[8]. The experimental data revealed PDE increased, from 38.73% to 82.26%, when the proportion of cholesterol into surfactant:cholesterol ratio was decreased from 1:1 to 1.5:1, but the PDE decreased consistently beyond this proportion to 49.95% in 2.5:1. This may be due to the lipophilic behavior of the lipid bilayer of niosomes and crystallinity of the bilayer.

Table 4. Diffusion and percentage drug retained into human cadaver skin (HCS)

Time	Percentage drug release ($\pm$ S. D.)		
in hours	Batch C_1	Batch C_2	Market Prep.
2	5.45 ($\pm$ 0.16)	-	6.33 ($\pm$ 0.21)
4	9.63 ($\pm$ 0.36)	-	10.08 ($\pm$ 0.40)
6	14.13 ($\pm$ 0.48)	5.83 ($\pm$ 0.16)	15.23 ($\pm$ 0.47)
8	19.20 ($\pm$ 0.87)	8.31 ($\pm$ 0.28)	21.67 ($\pm$ 0.89)
24	30.80 ($\pm$ 1.03)	15.15 ($\pm$ 0.41)	40.32 ($\pm$ 0.55)
	Percentage drug retained into human cadaver skin (HCS) after 24 hours		
24	12.45 ($\pm$ 0.86)	62.53 ($\pm$ 2.75)	14.88 ($\pm$ 0.49)

After optimization of type of surfactant, proportion of surfactant and cholesterol, the hydration volume and hydration time were studied under robust trial and the data was recorded in table 2. Observation of data of table 2 suggested that hydration volume and hydration time, both have some impact on PDE, as the value of PDE was increased when hydration volume was increased from 5 ml to 7 ml but decreased a little at 10 ml of hydration volume and similarly the value of PDE was increased when hydration time was increased from 0.5 hr to 1 hr but decreased a little at 1.5 hr of hydration time. Study of effect of annealing time (table 3) suggested that there is significant impact of annealing time (when compared by ANOVA single factor) on PDE and 2 hrs was found to give highest PDE and hence was taken as optimized annealing time.

After the whole optimization exercise, optimized value of different variables was found as type of surfactant, Span 80; Surfactant:Cholesterol, 1.5:1; temperature of water bath, 60 ˚C; solvent system, methanol: chloroform 1:1; hydration volume, 7ml; hydration time, 1 hour; annealing time, 2 hours.

The drug retention study revealed that both the formulations were significantly more stable (when compared by F-test) at refrigerated temperature than room temperature, suggesting the storage in refrigerator is must. It was also observed that niosomal drug gel was significantly more stable (when compared by F-test) as compared to plain drug gel and market gel. The reason behind higher leakage at higher temperature may be the higher fluidity of lipid bilayer at higher temperature, and the improved stability of niosomes after incorporation into gel base may be due to prevention of fusion of niosomes[8].

The results of in vitro drug release study suggest Higuchi's diffusion controlled model for all the formulations. When the data was compared by ANOVA test (single factor) revealed no significant difference in drug release rate of plain drug gel and marketed gel but the significant difference was observed between plain drug gel and niosomal gel. The results of in vitro drug release study also suggested that 62.53 % of drug was retained into skin after 24 hours with niosomal gel (Batch C_2) as compared to only 12.45 % with plain drug gel and 14.88 % with marketed gel.

Prolonged drug release from niosomal erythromycin gel compared to plain drug gel and market preparation reflected in vitro diffusion study across human cadaver skin may due to slower diffusion of drug into the skin. The slower mean flux and lower diffusion co-efficient values of niosomal gel are suggestive of prolong drug release. The higher drug skin retention incase of niosomal gel may be due to, creation of reservoir effect for drug in skin due to deposition of other components of niosomes with drug into the skin and there by increasing the drug retention capacity into skin.

The findings of this investigation have conclusively demonstrated that encapsulation of erythromycin into niosomal gel formulation improves skin retention therapeutic response and considerably reduces the adverse symptoms. However, the role of niosomal erythromycin gel of this study can only be settled after clinical evaluation of the product with large number of patient with special focus on the adverse symptoms of the therapy.

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DECLARATION OF INTEREST

The authors report no declarations of interest.

REFERENCES

1. Betageri G. and Habib M., Liposomes as drug carriers, Pharm. Eng., 1994, 14, 76-77.
2. Schreier H. and Bouwstra J. A., Liposomes and niosomes as topical drug carriers: dermal and transdermal drug delivery, J. Con. Rel., 1994, 30, 1-15.
3. Namedo A. and Jain N. K., Niosomes as drug carriers. Indian J. Pharm. Sci., 58(2):41-46, 1996.
4. Junginger H. E., Hofland H. E. J. and Bouwstra J. A., Liposomes and niosomes interaction with human skin. Cosmet. Toil., 1991, 106, 45-50.
5. Azmin M. N., Florence A. T., Handjani-Vila R. M., Stuart J. F. B., Vanlerberghe G. and Whittaker J. S., The effect of non-ionic surfactant vesicle (niosome) entrapment on the absorption and distribution of methotrexate in mice; J. Pharm. Pharmacol., 1985, 37, 237–242.
6. Friend D. R., In vitro permeation techniques. J. Controlled Rel., 1992, 18, 235-248.
7. Tojo K., Design and calibration of in-vitro permeation apparatus. In: Transdermal Controlled Systemic Medications (Chien YW, Ed), Marcel Dekker, New York, 1987, 127-158.
8. Uchegdu I.F., Vyas S.P., Non ionic surfactant based vesicles in drug delivery, Int. J. Pharmaceutics, 1998, 172, 33-70.

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