Journal of Chemical Health Risks

www.jchr.org

JCHR (2024) 14 (03), 2512-2519 | ISSN: 2251-6727

Formulation and Evaluation of Proniosomal Gel of Neomycin Sulphate

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(Received: 04 February 2024 Revised: 11 March 2024 Accepted: 08 April 2024)

KEYWORDS
Proniosomes, Neomycin Sulphate, Sorbitane monostearate (span 60), Lecithin and Cholesterol

ABSTRACT:

Objective: The objectives of present investigation were to prepare and evaluate proniosomes of neomycin sulphate (NS) by coacervation phase separation method by using sorbitan monostearate (span 60) and lecithin as a surfactant to increase the penetration through the skin and study the effect of concentration of the same.

Methods: Proniosomes of neomycin sulphate (NS) were prepared by coacervation phase separation method by using span 60 and lecithin. The effect of concentration of span 60 and lecithin was studied by factorial design. The prepared proniosomes were converted to gel by using carbopol as a gelling agent. The prepared formulations were evaluated for entrapment efficiency, in vitro drug diffusion, in vitro antibacterial activity and in vivo skin irritation test etc.

Results: All Formulation showed the percentage entrapment efficiency in the range 38.31±0.05% to 77.96±0.06%, good homogeneity and gel was easily spreadable with minimal of shear. Optimized formulation showed enhanced rate of diffusion in vitro, increase in zone of inhibition against staphylococcus aureus, no skin irritation and showed good stability.

Conclusion: The results of present study indicates that proniosomal gel formulated by using combination of span 60, Lecithin, cholesterol can be used to enhance skin delivery of NS because of excellent permeation of drug. Developed proniosomal gel formulation was promising carrier for NS.

1. Introduction

Oral route of administration is most accepted route for majority of drugs but still faces challenges as compared to other routes. Transdermal route gives better control of blood level, reduce systemic toxicity, it protects drug from the hepatic first pass metabolism, better patient compliance and can be a potential option for oral dosage forms. Human skin is the important target site for the application of drug especially in the treatment of local disease. Penetration enhancement with special formulation approaches is mainly based on the usage of colloidal carriers [1]. Colloidal carrier have distinct advantages over conventional drug delivery as it act as drug containing reservoirs, modification of the particle composition or surface can adjusts the release rate to the target site [2]. These carriers accumulate in stratum corneum or other upper skin layers and are not expected to penetrate into viable skin. The penetration enhancement is the most critical factor in the transdermal drug delivery [3]. Hence it is necessary to increase the flux through skin membrane by using different approaches of penetration enhancement. Vesicular systems have been

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widely studied as vehicles for dermal and transdermal drug delivery. A number of vesicles systems such as liposomes, niosomes, ethosomes, emulsomes and transfersomes have been developed. The vesicular carrier such as niosomes has distinct advantage over conventional dosage forms because these particles can act as drug reservoir [1-2].

Compounds having high molecular weight cannot cross skin, need some amendment owing to the availability of novel methods that might enhance the transport of large molecular weight compounds into or through the skin. Vesicular systems especially niosomes and liposomes are well established system for the transdermal route but the stabilityis the major problem of these carriers. Liposome exhibits some difficulties such as instability of aqueous dispersions on storage and the leakage of the encapsulated drugs. Also the high cost of synthetic phospholipids.

An alternative approach i.e. niosomes that overcomes several of these problems associated with liposomes [3]. Non-ionic surfactant vesicles obtained on hydration of synthetic non-ionic surfactants, with or without incorporation of cholesterol or other lipid [4]. But the proniosomes are more advantageous than nonionic surfactant vesicles i.e., niosomes, in terms of physical stability such as aggregation, fusion and leaking, and provide additional convenience in transportation, distribution, storage, and dosing [5]. Proniosomes encloses both hydrophilic and lipophilic drugs. Proniosomes reduce the toxicity related to drug because of their non-ionic nature of surfactant [6]. Neomycin Sulphate is a bactericidal aminoglycoside antibiotics (or antibacterial agent) is categorized as a BCS class-III i.e. high solubility and poor permeability, and generally it used as topical agent in skin infection. Physicochemical properties of NS like highly polar nature and high molecular weight (908.87D). Poor skin permeability (<3%) of Neomycin sulphate reduces its deeper penetration in skin [7]. That aminoglycoside antibiotic works by binding to the bacterial 30S ribosomal subunit, causing misreading of t-RNA, leaving the bacterium unable to synthesize proteins vital to its growth [8-10]. In present investigation proniosomal gel of NS was prepared by using span 60 and lecithin to increase the skin penetration.

2. Materials and Methods

Materials

Neomycin Sulphate was obtained as a gift sample from Encube Ethical Pvt. Ltd. Goa. Span 60, Cholesterol and carbopol 934 were purchased from Loba Chemicals, Mumbai. Lecithin was purchased from Research lab Mumbai. All other ingredients were of analytical grade.

Formulation of proniosomal gel

Proniosomes were prepared by the modified literature method reported by Fang et al. 2001 [11]. Proniosomes prepared by co-acervation phase separation method. Precisely weighed amount of drug, surfactant, cholesterol, lecithin, and organic solvent (Ethanol) taken in wide mouth container. After mixing all ingredients, the open end of glass tube was covered with a lid to prevent loss of solvent from it and warmed on water bath at 60-70 °c for about 10 min, until the surfactants were dissolved completely. Then aqueous phase Phosphate buffer pH 7.4 was added and the mixture was further warmed in the water bath for about 2 min. so that a clear solution was obtained. The mixture was allowed to cool to room temperature until the dispersion was converted to proniosomal gel. Carbopol 934 was added in formulation in 1:1 ratio.

Effect of variables

To study the effect of variables by using 3^2 factorial designs was applied for

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optimization of proniosomal gel formulation shown in table 1. In this study two factors were evaluated each at three levels and experimental trials were performed at all nine possible combinations. The amount of surfactant and lecithin were selected as independent variables. The percentage entrapment efficiency and percentage drug release was selected as dependent variables. Here the cholesterol concentration was kept constant at 10% because of the concentration of cholesterol above 10 % then it might lead to decreases % entrapment efficiency and forms more rigid bilayer and also decrease % drug release [12].

Physical evaluation of gels

The prepared gel formulations were evaluated for physical appearance, pH, homogeneity, spread ability and viscosity by using conventional digital R/S plus Rheometer Brookfield Engineering Lab Inc. (USA)

Microscopic evaluation

In glass tube, 0.2 gm proniosomal gel of all formulation was diluted with 10 ml of pH 7.4 phosphate buffer; few drop of formed niosomal dispersion were spread on a glass slide and examined for the vesicles structure using ordinary light microscope with varied magnification power. Photomicrographs were taken for niosomes at 100X.

Entrapment efficiency

It was determined indirectly i.e. by calculating the amount of un-entrapped drug as shown in equation 1. To 0.2 g of proniosome gel 10 ml of phosphate buffer pH 7.4 was added. The niosomes were separated from untrapped drug by centrifugation at 5000 rpm for 30 min and evaluating the supernatant spectrophotometrically (Shimadzu–1601, Tokyo) at 277 nm with suitable dilution.

Entrapment efficiency was calculated by using following formula,

%%Entrapment Efficiency = (Ct-Cr)/Cr x100 …… 1

Where,Ct = Concentration of total drug, Cr = Concentration of free drug

Vesicle size analysis

Particle size and zeta potential of formulation was determined by using Malvern Zetasizer instrument v2.1.

In vitro permeation study

These studies were performed using locally Fabricated Keshary-Chientype diffusion cell with an area of 2.1 cm^2 (Sigma, Inc. Mo. USA). The capacity of receptor compartment was 20 ml. The dialysis membrane and human cadaver skin 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 containing phosphate buffer pH 7.4 with continuous rotation speed of 50 rpm by using magnetic stirrer. The receptor compartment was surrounded by a water jacket to maintain the temperature at 37±0.5°C. At each sampling interval during 24 h, 1 mlwas withdrawn and was replaced by equal volumes of fresh receptor fluid on each occasion. Samples withdrawn were analyzed by UV-visible double beam spectrophotometer (Shimadzu-1601, Tokyo) at 277 nm by using furfural assay of NS [5].

Drug release kinetics

The release data obtained from various formulations werestudied further for their fitness of data in different kinetic models like zero order, first order, Higuchi model, Hixoncrowell and Korsemeyer peppa's model.

In vitro antimicrobial activity

This study was carried out by using Agar-cup diffusion method and Staphylococcus Aureus culture was used. The nutrient agar broth was used as a media. The subculture of Staphylococcus Aureus culture was spread

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in petri-dish. (Bacterial strains were obtained from faculty of allied sciences, Microbiology and Biotechnology KIMSDTU, Karad) On solidification, 1 cm holes were made and filled with a formulation. The one plate hole filled with pure NS solution, second plate hole filled with marketed NS cream formulation

Stability studies

The ability of vesicles to retain the drug was assessed by keeping the proniosomal gel at ambient temperature. Throughout the study, proniosomal formulations were stored in stoppered glass vials. Entrapment efficiency of formulations were determined before and after completion of one month and measurement were made by spectrophotometer(Shimadzu-1601, Tokyo) at 277 nm

3. Results and Discussion

The physical appearance of the gel was observed by eyes. All proniosomal gel formulations were yellowish in colour as shown in table 2. It might be due to brown colour of soya lecithin [7].The pH of all formulations was found in ranged from 6.5-7.1 given in table 2 and that pH suitable to skin pH [13]. It reveales that the formulations will be compatible with skin. All developed proniosomal gel formulations showed good homogeneity with absence of lumps. The value of spreadability of all proniosomal formulations ranged from 4.5±0.01 to 6.9±0.02 (g. cm/sec) shown in table 2. The value of spreadability indicate that the gel is easily spreadable with minimal of shear [14].

Viscosity determination

All proniosomal gel formulations were semisolid in consistency as required for skin application except the PG9 formulation, which had softer consistency (table 2). The consistency of the gel increased because of the molecular interaction of polar head groups of surfactant with the solvent and permeation of solvent into the bilayers. The solvent diffusion into the bilayers did not disturb the liquid crystalline structure. It results in complete bilayer formation due to the saturation of the lipid polar heads. This might have lead to an increase in bilayers distance resulting in an overall increase in consistency [15]. One another reason of increased consistency due to the loss of alcohol upon storage [14, 15].

S. No.	Formulation code	Appear-ance	pH*	Homoge-neity	Spreadability* (g. cm/sec)	Viscosity* (cP)
1	AF1	Yellowish	6.1±0.02	Good	5.3±0.03	354.82±0.03
2	AF2	Yellowish	6.4±0.02	Good	4.7±0.01	251.48±0.04
3	AF3	Yellowish	6.2±0.02	Good	5.5±0.03	391.68±0.01
4	AF4	Yellowish	6.9±0.02	Good	4.4±0.01	251.48±0.04
5	AF5	Yellowish	6.4±0.02	Good	5.1±0.03	354.82±0.03
6	AF6	Yellowish	6.8±0.02	Good	4.3±0.01	391.68±0.01

Microscopic determination

After hydration of proniosomes by using phosphate buffer the niosomal suspension was formed which was seen under the microscope. The photomicrographs of all formulations are shown in fig. 1. Most of vesicles are well identified; spherical in shape having large internal aqueous space niosomes were observed. Addition of water with polar groups of surfactant. In presence of excess of water there was complete hydration leading to formation of niosomes [16].

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Percentage entrapment efficiency

Entrapment efficiency of proniosomes formulations ranged from 38.31% to 77.96%. The drug encapsulation efficiency of all nine formulations is shown in table 3. PG3 formulation had showed good encapsulation efficiency. The effect of concentration of lecithin on entrapment efficiency is shown in table 3. Increase in lecithin concentration increases percentage entrapment efficiency. This might be due to lecithin containing long hydrocarbon chain of lecithin [15, 17]. Therefore they increase the vesicle size of niosomes and increase percentage entrapment efficiency [17]. Effect of amount of surfactant on entrapment efficiency. Initially increase the concentration of surfactant i.e. span 60 might be increased number of niosomes formed because of the volume of hydrophobic domain increases, hence increasing entrapment efficiency. However further increase in the concentration of surfactant decrease entrapment efficiency.

It might be due to formation of mixed micelles along with niosomal vesicles which leads fewer amount of drug might be entrapped inside the vesicle. This leads to lower entrapment efficiency [18].

Entrapment efficiency of proniosomes formulations

S. No	Formulation code	%EE*
1	AF1	78.96±0.06
2	AF2	39.31±0.05
3	AF3	66.67±0.01
4	AF4	35.31±0.05
5	AF5	77.96±0.06
6	AF6	68.67±0.01

Vesicle size analysis

The particle size was found in the ranged from 153.1±0.03 to 435.9±0.04 as shown in table 4. proniosomes prepared by using combination of span 60 and lecithin showed increase in particle size at the optimum concentration. These results suggest that addition of lecithin leads to increase in particle size. This might be due to the lecithin contains long hydrocarbon chains [17, 19]. The relationship observed between proniosomes size and hydrophobicity has been attributed to the decrease in surface energy with increase in hydrophobicity, resulting in the smaller vesicles. The larger vesicles size of proniosomes prepared with high concentration of lecithin which has much lower hydrophobicity than the high concentration of span 60. Because increase in amount of span 60, increase in hydrophobicity which leads to decrease in vesicle size. The differences in vesicle size among the proniosomes prepared with different ratio of span 60 and lecithin [20]. All formulations showed low value of polydispesity index. Polydispesity index is the ratio of standard deviation to the mean particle size and it signifies the uniformity of particle size within the formulation. The polydispesity values of formulation were found<1 (0.428-0.746). It indicates that narrow distribution and uniformity of particle size within formulation [7, 15].

Zeta potential determination

Zeta potential of optimized formulation i.e. PG3 formulations is shown in fig. 3. The value of zeta potential was found to be-31.18 mV and hence it indicates that the prepared formulations was stable [20].

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Particle size analysis of proniosomes formulations

S. No	Formulation code	Particle size* (nm)	PDI
1	AF1	335.8±0.01	0.479
2	AF2	431.9±0.01	0.673
3	AF3	423.9±0.04	0.744
4	AF4	365.3±0.05	0.478
5	AF5	445.9±0.01	0.746
6	AF6	362.2±0.08	0.673

In vitro permeation study

The AF1 and AF6 formulation showed a more permeation of drug78.39±0.05 % and 80.06±0.01 % respectively over the period of 24 h through dialysis membrane as compared to other formulations and carbopol gel containing pure NS as it contains more concentration of lecithin [3, 15, 17]. But this formulation was less viscous as shown in table 2. The PG3 formulation showed optimum drug release due to the optimum concentration of span60 and lecithin. On the basis of entrapment efficiency, in vitro drug diffusion study through the dialysis membrane, spreadability, and viscosity, the formulation PG3 (combination of span 60 and lecithin) was concluded as optimized formulation. Hence PG3 formulation was further subjected to ex-vivo permeation studies. The cumulative drug diffusion of NS carbopol gel and PG3 formulation through human cadaver skin in phosphate buffer pH 7.4 was found to be 17.93±0.04 % and 63.76±0.01 respectively over the period of 24 h.

4. Conclusion

Conclusion revealed that the proniosomal gel formulation had highest cumulative amount of drug diffusion as compared to carbopol containing pure Neomycin Sulphate. It might be due to the vesicles acts as penetration enhancers to reduce the barrier properties of stratum corneum [7, 21]. The possible reason for niosomes to enhance the permeability of drug through the skin is depends upon structure of skin. Formulation contains surfactant and phospholipid. The phospholipid is fluidizing both the vesicle and the SC lipids such as ceramides etc. and surfactant i.e. span 60 forms the vesicles are more flexible. Phospholipid disturbs structure of the intracellular lipid bilayers in stratum corneum (SC) and increases their fluidity. Niosomes are entered into disturbed SC bilayers through the skin lipids. Thus, resulting the drug release from niosomal vesicles in skin due to the fusion vesicles with skin lipids [7, 14, 21, 22].

Acknowledgement: I would like to special thanks to HIMT College of pharmacy, their support and help in data analysis and interpretation.

Conflict of interest: Nill

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JCHR (2024) 14 (03), 2512-2519 | ISSN: 2251-6727

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