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Research Article

# FORMULATION AND EVALUATION OF VANCOMYCIN LOADED NIOSOMAL GEL

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## ABSTRACT

The present study was aimed to investigate the delivery potential of vancomycin containing topical niosomal gel. Niosomal formulations were prepared by thin film hydration method at various ratios of Soya PC: Span 80 and were evaluated with respect to particle size, shape, entrapment efficiency, and *in vitro* characteristics. Average vesicle size of optimized formulation F9 was 158.85 nm. The Zeta potential was found 38.25%. Entrapment efficiency of optimized Niosome formulation (F9) was found as 78.85%. Further, niosomes were incorporated into gel base and characterized for viscosity, % entrapment, extrudability, spreadability and drug release study. It was found that viscosity of prepared gel was 3310±25 cps, % assay was 98.95±0.2 %, extrudability was 175±4 g and spreadibility (g/cm/sec) was found that 8.56±0.25 (g/cm/sec) respectively. *In vitro* drug release from niosome gel was carried out using franz diffusion cell method. The percentage drug release of optimized formulation was found to be 96.65±0.14 after 12 hrs.

**Keywords:** Vancomycin, Formulation, Niosomes, *In vitro* drug release studies.

## 1. INTRODUCTION

Niosomes (non-ionic surfactant vesicles) are microscopic lamellar structures obtained on an admixture of non-Ionic surfactant of the alkyl or dialkyl polyglycerol ether class and cholesterol (CHO) with subsequent hydration in aqueous media [1]. Niosomes attracts much attention because of its advantages in many aspects, such as chemical stability, high purity, content uniformity, low cost, convenient storage of non-ionic surfactants, and large numbers of surfactants available for the design of niosomes [2]. Niosomes are promising vehicle for drug delivery. The encapsulation of drugs in niosomes can minimize drug degradation and inactivation after administration; prevent undesirable side effects, increase drug bioavilability and targeting to the pathological area [3]. Surfactants also act as penetration enhancers as they can remove the mucus layer and break functional complexes. The non-ionic surfactants form a closed bilayer vesicle in aqueous media based on its amphiphilic nature using some energy, for instance, heat and physical agitation to form this structure. Their effectiveness is strongly dependent on their physiological properties, such as composition, size, charge, lamellarity and application conditions [4]. Hence, in the present investigation, an attempt is made to develop and characterize niosomal gel formulation of Vancomycin. Vancomycin is selected as a drug candidate for the present study to treat bacterial infections of the skin more efficiently. Vancomycin, a branched tricyclic glycosylated peptide antibiotic, is a last-line defense against serious infections caused by staphylococci, enterococci and other Gram-positive bacteria.

## 2. MATERIAL AND METHOD

### 2.1. Materials

Span 80, Soya PC, Chloroform, Carbopol 934, Propylene Glycol and ethanol were procured from S. D. Fine Chem. Ltd., Mumbai. The phosphate Buffer of pH 7.4 (PBS pH 7.4) were made as mentioned in Indian Pharmacopoeia and necessary chemical were obtained from Qualigens Fine Chemicals, Mumbai. All the chemicals used were of Analytical Reagent (AR) grade.

### 2.2. Preparation of Vancomycin loaded Niosomes

Niosomes were prepared by thin film hydration method. Cholesterol and non-ionic surfactant were dissolved in 500 ml of round bottom flask with 10ml chloroform and was allowed to rotate in a flash evaporator at 60°C [5]. The flask was allowed to revolve at 125rpm for 1 hour to obtain a dry film. The film was hydrated with 5ml phosphate buffer saline (PBS) pH 7.4 containing 10mg

Vancomycin was allowed to revolve for 60 minutes. All the preparation was sonicated for 60 sec. using bath sonicator (EI, Mumbai).

### 2.3. Preparation of Gel Base

Carbopol 934 (0.5-1.5 % w/v) was accurately weighted and poured into double distilled water (80ml) in a beaker [6]. This solution was stirred continuously at 800 rpm for 1 hr. and then 10ml of propylene glycol was added to this solution. The obtained slightly acidic solution was neutralized by adding drop wise 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 minute 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 added into the gel base to get the desired concentration of drug.

### 2.4. Characterization of Vancomycin loaded niosomes

#### *2.4.1. Surface charge and vesicle size*

The vesicles size and size distribution & surface charge were obtained by Dynamic Light Scattering method (DLS) (SAIF RGPV Bhopal, Malvern Zetamaster, ZEM 5002, Malvern, UK) [7]. Zeta potential measurement of the Niosomes was based on the zeta potential that was estimated according to Helmholtz-Smoluchowsky from electrophoretic mobility. 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.

#### *2.4.2. Entrapment efficiency*

The entrapment efficiency of the drug was defined as the ratio of the mass of formulations associated drug to the total mass of drug. Entrapment efficiency was determined by dialysis method. Niosomes entrapped drug were isolated from the free drug using dialysis method. The above said formulations were filled into dialysis bags and the free drug dialyzed for 24 hr. into 50 ml of buffer pH 7.4. The absorbance of the dialysate was measured at 282.0 nm against blank buffer pH 7.4 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 obtained by the absorbance at 282 nm for known concentrations of drug solution.

### 2.5. Characterization of Niosomes containing Gel

#### *2.5.1. Measurement of 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].

#### *2.5.2. Drug content*

Accurately weighed equivalent to 1 mg of topical Niosomal gel was taken in beaker and 20 ml of methanol was added. The solution was mixed thoroughly and filtered using Whatman filter paper. 2.0mL of filtered solution was then poured in 10mL capacity of volumetric flask and made upto 10mL volume with methanol. The solution was analyzed using UV-Spectroscope at $\lambda_{max}$ 282nm.

#### *2.5.3. Extrudability study*

The niosomal gel formulations were filled in the collapsible tubes after the gels set in the container. Extrudability of gel required 175 grams of weight to extrude a 0.6cm ribbon of gel in 6 seconds [9].

#### *2.5.4. pH*

A 2.5 g of gel was accurately weighed and dispersed in 25 ml of distilled water. The pH of the dispersion was measured by using a digital pH meter [10].

#### *2.5.5. Spreadibility*

Spreadibility of formulation is necessary to provide sufficient dose available to absorb from skin to get good therapeutic response [11]. An apparatus in which a slide fixed on wooden block and upper slide has movable and one end of movable slide tied with weight pan. Determination of spreadibility was done by placing 2-5 g of gel between two slides 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. Good spreadibility show lesser time to spread.

Spreadability (g.cm/sec) = {(Weight tide to upper slide x Length moved on the glass slide)/ Time taken to slide}

### 2.6. *In -Vitro* drug diffusion study

The *in-vitro* diffusion study was carried by using Franz Diffusion Cell. Egg membrane was taken as semi permeable membrane for diffusion [12]. The Franz diffusion cell has receptor chamber with a potent volume approximately 60mL and effective surface area of permeation 3.14 sq.cms. The receptor medium was phosphate buffer (pH 7.4). The receptor compartment is surrounded by water jacket so as to control the temp at 32±0.5°C. By using a thermostatic hot plate with a magnetic stirrer heat was provided. The receptor fluid was stirred with the help of Teflon coated magnetic bead which was kept in the diffusion cell. During each sampling interval, samples were taken and replaced by same volumes of fresh receptor fluid on each sampling. The samples withdrawn were analyzed spectro-photometrically at wavelength of drug 282nm.

### 2.7. Stability Studies

Stability studies were carried for drug loaded Niosomes at two different temperatures i.e. refrigeration temperature (4.0 ± 0.2°C) and at room temperature (25-28±2°C) and elevated temperature (45 ±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.

## 3. RESULTS AND DISCUSSION

Prepared formulations of Niosome were optimized on basis of vesicle size, shape, surface charge and entrapment efficiency. Vesicle size of Niosome were examined under trinocular microscopic (magnification 400X) and also determined by light scattering method (Malvern Zetasizer, ZEM 5002, and UK) and found that average vesicle size of optimized formulation F9 was 158.85 nm. The Zeta potential was 38.25. It was observed that the vesicles size of Niosome was increased with increasing concentration of phosphotidylcoline and similarly vesicle size was decreased with increasing concentration of span 80 due to its surfactant action. There was no significant difference in average vesicle size was observed with increasing drug concentration. But in increasing the time of sonication, the size of vesicle was decreased from 221.21 to 142.23 sec. after 90 sec of sonication.

**Table 1: Results of Optimization of formulation development**

| Formulation code | Soya PC: Span 80 (% w/v) | Drug (% w/v) | Average Vesicle size (nm) | % entrapment efficiency |
|---|---|---|---|---|
| **Optimization of lipid: surfactant concentration** | | | | |
| F1 | 10:0.5 | 1.0 | 315.56 | 75.56 |
| F2 | 10:1.0 | 1.0 | 254.65 | 78.12 |
| F3 | 10:1.5 | 1.0 | 341.74 | 65.54 |
| F4 | 10:2.0 | 1.0 | 365.65 | 65.45 |
| **Optimization of drug concentration** | | | | |
| F5 | 10:1 | 0.5 | 254.56 | 55.65 |
| **F6** | **10:1** | **1** | 262.12 | 75.65 |
| F7 | 10:1 | 1.5 | 345.45 | 62.21 |
| **Optimization of sonication time** | | | | |
| F8 | 10:1 | 1 | 30 | 221.21 |
| **F9** | **10:1** | **1** | **60** | 158.85 |
| F10 | 10:1 | 1 | 90 | 142.23 |

Percent entrapment efficiency of optimized Niosome formulation (F9) was found to be 78.85%. It was observed that the percent drug entrapment was decreased with increasing conc. of surfactant and upon increasing the time of sonication. It was clearly shown when formulation was sonicated for 30 sec then the % EE was 72.23 (F8) and when it sonicated for 60 and 90 sec then the % EE was found as 73.03±2.39 (F9) and 65.58 (F10) respectively. The 60 sec was selected as optimized time for sonication because it provided the required size of vesicle 158.85 nm and good % EE *i.e.* 78.85. The F9 formulation was selected as optimized formulation.

Stability study data revealed that the optimized formulation (F9) was stable after 3 month of storage at 4°C while at 25-28±2°C, the formulation was found unstable. Stability of formulation was observed on the basis of % drug remain, average vesicles size and

physical appearance. There was no significant changes in % drug remain and physical appearance in niosomal formulation was observed after 3 month of storage at 4°C. Prepared gel was characterized for viscosity, % entrapment, extrudability, spreadability and drug release study. It was found that viscosity of prepared gel was 3310±25 cps, % assay was 98.95±0.2 %, extrudability was 175±4 g and spreadibility (g/cm/sec) was found as 8.56±0.25 (g/cm/sec) respectively. *In vitro* drug release from Niosome gel was carried out using franz diffusion cell method. The percentage drug release of optimized formulation was found to be 96.65±0.14 after 12 hrs. Niosomal formulation was successfully prepared by thin film hydration technique using different ratios of Soya PC: Span 80. It was found that niosomal formulation F9 having Soya PC: Span 80 (10: 1) showed better entrapment efficiency and *in vitro* release profile. Selected niosomal formulation was further incorporated in Carbopol gel base to prepare niosomal gel. Niosomal gel was found to be stable at cold condition. Niosomal gel was prepared, exhibiting better skin permeation study when compared with plain gel.

**Table 2: Optimized formulation Niosomes**

| Formulation code F9 | |
|---|---|
| Soya PC: Span 80 (%w/v) | 10:1 |
| Drug (% w/v) | 1 |
| Sonication time (Sec.) | 60 |

**Table 3: Characterization of Optimized formulation of Niosomes**

| Characterization | Average vesicle size (nm) | % Entrapment efficiency | Zeta Potential (mV) |
|---|---|---|---|
| F-9 | 158.85 | 78.85 | -38.25 |

**Table 4: Characterization of gel based formulation of Niosomes**

| | Parameters | | | | | |
|---|---|---|---|---|---|---|
| F. code | Viscosity (cps) | % Drug content | % Release after 12 hr | Extrudability (g) | Spreadibility (g/cm/sec) | pH |
| **FG-9** | 3310±25 | 98.95±0.2 | 96.65±0.14 | 175±4 | 8.56±0.25 | 6.82±0.2 |

*FG* Optimized gel formulation*

**Table 5: *In-vitro* drug release study of prepared gel formulation**

| S. No. | Time (hr) | % Cumulative Drug Release | |
|---|---|---|---|
| | | Plain gel | Niosomal Gel |
| 1 | 0.5 | 33.65 | 11.25 |
| 2 | 1 | 55.65 | 22.32 |
| 3 | 2 | 78.89 | 36.65 |
| 4 | 4 | 96.12 | 42.23 |
| 5 | 6 | - | 65.65 |
| 6 | 8 | - | 88.85 |
| 7 | 12 | - | 96.65 |

**Table 6: Characterization of optimized formulation of Niosomes formulation**

| Characteristics | Time (Months) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | 1 Month | | | 2 Months | | | 3 Months | | |
| **Temperature (°C)** | 4.0±0.2 | 25-28±2 | 45±2 | 4.0±0.2 | 25-28±2 | 45±2 | 4.0±0. 2 | 25-28±2 | 45±2 |
| **Average vesicle size (nm)** | 158.23 | 165.23 | 198.85 | 159.23 | 210.25 | 265.87 | 162.12 | 265.54 | 325.65 |
| **% EE** | 78.85 | 75.65 | 70.23 | 77.23 | 65.56 | 62.23 | 75.65 | 60.23 | 58.85 |
| **Physical Appearance** | Normal | High turbid | High turbid | Normal | High turbid | High turbid | Normal | High turbid and agglomeration | High turbid |

## 4. CONCLUSION

Niosomes are versatile, easy to prepare, biocompatible nanocarriers with sizes change from tens of nanometers to few micrometers. The niosomes are utilized for drug and gene delivery to both anterior and posterior segments of the eye with increased corneal permeation, higher ocular bioavailability of the drug, and prolonged drug release. It was found that viscosity of prepared gel was 3310±25 cps, % assay was 98.95±0.2 %, extrudability was 175±4 g and spreadibility (g.cm/sec) was found as 8.56±0.25 (g.cm/sec) respectively. *In vitro* drug release from Niosome gel was carried out using franz diffusion cell method. The percentage drug release of optimized formulation was found to be 96.65±0.14 after 12 hrs.

### *Conflict of interest*

None declared

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