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# Formulation and Evaluation of Proniosomal Gel in Ocular Drug

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**ABSTRACT**

The objective of the present investigation was to formulate and evaluate levofloxacin ocular film with proniosomes to increase the efficiency of drug providing sustained release action. The Proniosomes are relatively easy to make and less costly as compared to other novel formulation approaches. Proniosomes are promising drug carrier system which not only increase the stability profile of drug but also provide additional convenience in transportation, distribution, storage and dosing. The development of levofloxacin ocular films with proniosomes were novel approach toward the formulation of such product in marked. The aim of the study was to develop a proniosomal carriers system for levofloxacin for the treatment of bacterial conjunctivitis that is capable of efficiently delivering entrapped drug over an extended period of time and levofloxacin ocular films in proniosomal form enhance the contact time and spreadibility in the eye and provide sustained release action and better availability of drug. The use of levofloxacin in the proniosome preparation permitted flexibility in the amounts of surfactants and other components, which greatly enhances the potential application of proniosomes.

**Keywords:** Proniosomal, Levofloxacin, ocular films, Sustained Release, Gel

## 1. INTRODUCTION

Proniosomes are dry formulation of water-soluble carrier particles that are coated with surfactant and can be measured out as needed and dehydrated to form niosomal dispersion immediately before use on brief agitation in hot aqueous media within minutes. The resulting niosomes are very similar to conventional niosomes and more uniform in size. The proniosome approach [3] minimizes these problems by using dry, free-flowing product, which is more stable during sterilization and storage. Ease of transfer, distribution, measuring, and storage make proniosomes a versatile delivery system with potential for use with a wide range of active compounds[1]. From early 1980s, niosomes have gained wide attention by researchers for their use as drug targeting agents, drug carriers to have variety of merits while avoiding demerits associated with the conventional form of drugs. Niosomes were studied as better alternatives to liposomes for entrapping both hydrophilic and hydrophobic drugs from a technical point of view, niosomes are promising drug carriers as they possess greater chemical stability and lack of many disadvantages associated with liposomes such as high cost and the variable purity problems of phospholipids [2]. The additional merits with niosomes are low toxicity due to non ionic nature, no requirement of special precautions and conditions for formulation and preparation. Moreover it is the simple method for the routine and large-scale production of niosomes without the use of unacceptable solvents. However, stability is a prime concern in the development of any formulation and even though, niosomes have shown advantages as drug carriers, such as being low cost and chemically stable as compared to liposomes[3]. They too, are associated with problems related to physical stability, such as fusion, aggregation, sedimentation, and leakage on storage.

In general a limited number of studies are available which deal with the preparation and evaluation of proniosomes these studies mostly focused on the utilization of proniosomes in transdermal drug delivery. This article briefly reviews the trends and the future perspective in the development of proniosomal drug delivery systems[4].

Colloidal particulate carriers such as liposomes or niosomes have been widely employed in drug delivery systems and producing them from proniosomes provides them a distinctive advantage[5]. These carriers can act as drug reservoirs and the rate of drug release can be controlled by modification of their composition[6]. These lipid vesicles can carry both hydrophilic drugs (by encapsulation) and hydrophobic drugs (in lipid domain). Due of their capability to carry a variety of drugs, these lipid vesicles have been extensively used in various drug delivery systems like drug targeting controlled release and permeation enhancement of drugs But there remains certain draw backs to be addressed and can be avoided if they are prepared in dry form. Proniosomes, prepared in dry form and hydrated by agitation in hot water to form niosomes provide an alternative with prospective for drug delivery via the transdermal route[7, 8, 9].

The Proniosomes are dry formulations of surfactant-coated carrier, which can be measured out as needed and rehydrated by brief agitation in hot water[10]. These "proniosomes" minimize problems of niosomes physical stability such as aggregation, fusion and leaking and provided additional convenience in transportation, distribution, storage and dosing. Proniosome-derived niosomes are superior to conventional niosomes in convenience of storage, transport and dosing. Stability of dry proniosomes is expected to be more stable than a pre-manufactured niosomal formulation. In release studies proniosomes appear to be equivalent to conventional niosomes. Size distributions of proniosome-derived niosomes are somewhat better that those of conventional niosomes so the release performance in more critical cases turns out to be superior. Proniosomes are dry powder, which makes further processing and packaging possible[11, 12]. The powder form provides optimal flexibility, unit dosing, in which the proniosome powder is provided in capsule could be beneficial. A proniosome formulation based on maltodextrin was recently developed that has potential applications in deliver of hydrophobic or amphiphilic drugs. The better of these formulations used a hollow particle with exceptionally high surface area[13, 14]. The principal advantage with this formulation was the amount of carrier required to support the surfactant could be easily adjusted and proniosomes with very high mass ratios of surfactant to carrier could be prepared. Because of the ease of production of proniosomes using the maltodextrin by slurry method, hydration of surfactant from proniosomes of a wide range of compositions can be studied[15, 16].

## 2. MATERIAL AND METHODS

### 2.1 Preformulation Studies Of Drug

*2.1.1 Solubility study*

The sample was qualitatively tested for its solubility in various solvents. It was determined by taking 2 mg of drug sample in 5 ml of solvent as water, methanol, ethanol, phosphate buffer, HCl, etc., in small test tubes and well solubilized by shaking.

*2.1.2 Melting point determination*

Melting point of Levofloxacin was determined by capillary method. A small quantity of powder was placed into a fusion tube. The tube was placed in the melting point determining apparatus. The temperature of the apparatus was gradually increased automatically and the temperature at which powder started to melt and the temperature when all the powder gets melted was recorded.

*2.1.3 Determination of partition coefficient*

The partition behavior of drug was examined in n-Octanol: PBS (pH 7.4). It was determined by taking 5 mg of drug in separating funnel containing 10 ml portions of each n-Octanol and 10 ml of PBS (pH 7.4). The separating funnels were shaken for 2 hrs in a wrist action shaker for equilibration. Two phases were separated and the amount of the drug in aqueous phase was analyzed spectophotometrically at 274 nm after appropriate dilution.

*2.2 Formulation of proniosomes*

Proniosomal gel was prepared by a coacervation-phase separation method. Precisely weighed amounts of surfactant, lecithin, 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. After warming, 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 Table 1**.**

Table 1. Composition of proniosomal formulations

| Proniosomal Code | Drug (mg) | Cholesterol | Lecithin | Span | | PEO mg |
|---|---|---|---|---|---|---|
| | | | | 40 | 60 | |
| PN1 | 10 | 20 | 50 | 10 | -- | 10 |
| PN2 | 10 | 20 | 20 | 20 | -- | 10 |
| PN3 | 10 | 20 | 20 | 30 | -- | 10 |
| PN4 | 10 | 20 | 20 | -- | 10 | 10 |
| PN5 | 10 | 20 | 20 | -- | 20 | 10 |
| PN6 | 10 | 20 | 20 | -- | 30 | 10 |

Table 2. Solubility Profile of Levofloxacin

| Sr. No. | Compound | Ratio | Solubility |
|---|---|---|---|
| 1 | Levofloxacin: Water | 1:10 | Slightly Soluble |
| 2 | Levofloxacin: Methanol | 1:10 | Completely soluble |
| 3 | Levofloxacin: Ethanol | 1:10 | Sparingly soluble |
| 4 | Levofloxacin: Dichloromethane | 1:10 | Slightly soluble |
| 5 | Levofloxacin: Glacial acetic acid | 1:10 | Completely soluble |
| 6 | Levofloxacin: 0.1N NAOH | 1:10 | Completely soluble |
| 7 | Lexvofloxacin : Ethyl acetate | 1:10 | Slightly soluble |

*2.3 Determination of Encapsulation Efficiency*

Percent encapsulation efficiency (EE) was determined by centrifugal method. The proniosomal gel was converted to a niosomal dispersion, which was centrifuged (18000 rpm) for 40 min at 5$^{o}$C in order to separate unentrapped drug. The supernatant was taken and diluted with PBS. The drug concentration in the resulting solution was assayed spectrophotometrically at 250 nm. The percentage of drug encapsulation was calculated by the following:

$$EE (\%) = [(Ct - Cf)/ Ct] \times 100,$$

where Ct is the concentration of total drug and Cf is the concentration of unentrapped drug.

Table 3: physicochemical characterization of proniosome batches of Levofloxacin

| S.No. | Proniosomal code | Encapsulation efficiency |
|---|---|---|
| 1. | PF1 | 95.67 |
| 2. | PF2 | 96.71 |
| 3. | PF3 | 97.12 |
| 4. | PF4 | 78.62 |
| 5. | PF5 | 81.45 |
| 6. | PF6 | 85.51 |

## 2.3 Characterisation of Levofloxacin Proniosomes

*2.3.1 Optical microscopy and vesicle size determination*

A drop of niosomal dispersion prepared from proniosomes was spread on a glass slide and examined for the vesicle structure and presence of insoluble drug crystals under the light microscope with varied magnification power. Photomicrographs were taken for niosomes using a digital camera with 5X optical 200 m. The proniosomal gel (10 mg) was hydrated with PBS (10 ml) in a small test tube by manual shaking for 5 min and the resulting niosomes were observed under optical microscope at 100 X magnification. The average size of vesicles was measured using calibrated ocular and stage micrometer in the microscope.

*2.3.2 Scanning electron microscopy*

The niosomes formed from the hydration of proniosomal gel were mounted on an aluminum stub with double-sided adhesive carbon tape. The vesicles were then sputter-coated with gold/palladium using a vacuum evaporator and examined with the scanning electron microscope equipped with a digital camera at 25kV accelerating voltage.

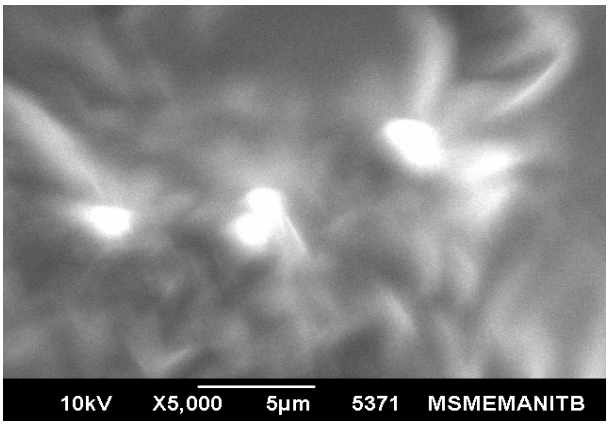


Figure 3: SEM of Levofloxacin Proniosomes

*2.3.3 In vitro release studies using dialysis cellophane membrane*

In vitro release studies on proniosomal gel were performed using Franz-diffusion cell. The capacity of receptor compartment was 15 ml. The area of donor compartment exposed to receptor compartment was 1.41cm$^2$. The dialysis cellophane membrane was mounted between the donor and receptor compartment. A weighed amount of proniosomal gel was placed on one side of the dialysis membrane. The receptor medium was STF (stimulated tear fluid) pH 7.4. The receptor compartment was surrounded by a water jacket to maintain the temperature at 37±1$^o$C. Heat was provided using a thermostatic hot plate with a magnetic stirrer. The receptor fluid was stirred by a Teflon-coated magnetic bead fitted to a magnetic stirrer. At each sampling interval, samples were withdrawn and were replaced by equal volumes of fresh receptor fluid on each occasion. Samples withdrawn were analyzed spectrophotometrically at 293 nm**.**

Table 4: In vitro release studies of levofloxacin proniosomes

| **Time (hour)** | **% of Drug release** | | | | | |
|---|---|---|---|---|---|---|
| | F1 | F2 | F3 | F4 | F5 | F6 |
| 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 2 | 10.608 | 8.328 | 11.564 | 9.508 | 9.890 | 10.958 |
| 4 | 16.469 | 13.256 | 17.256 | 15.386 | 16.534 | 15.503 |
| 6 | 28.249 | 25.352 | 28.958 | 27.554 | 28.527 | 28.063 |
| 8 | 34.305 | 30.262 | 32.963 | 31.371 | 34.458 | 32.379 |
| 10 | 53.567 | 48.368 | 49.530 | 51.467 | 52.439 | 52.745 |
| 12 | 60.698 | 58.528 | 60.528 | 59.638 | 61.275 | 58.864 |
| 14 | 72.576 | 69.312 | 71.536 | 70.535 | 71.674 | 71.649 |
| 16 | 75.750 | 72.548 | 73.374 | 72.824 | 76.128 | 73.860 |
| 18 | 81.425 | 77.925 | 79.648 | 78.734 | 83.146 | 81.647 |
| 20 | 86.153 | 82.183 | 84.486 | 83.813 | 87.648 | 85.953 |
| 22 | 88.343 | 85.475 | 87.529 | 86.368 | 88.480 | 88.649 |
| 24 | 92.130 | 91.046 | 92.092 | 91.845 | 92.437 | 92.438 |

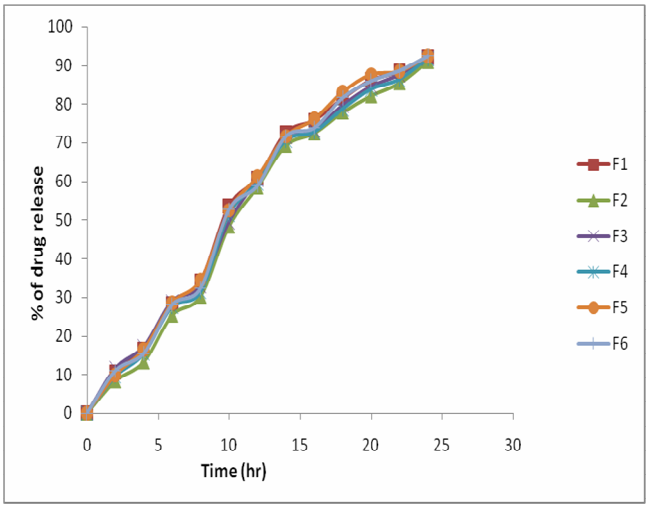


Figure 4. In-vitro release of all formulation

*2.3.4 Ocular Irritancy Test*

The optimized formulation was evaluated for in vivo performance in animal model (Albino rabbits). Three rabbits were used for this study. They were housed and maintained in the animal house at room temperature (27$^o$C) during the period of the study. They were fed with standard diet and water. The animals were placed in cages and the eyes were marked as test and control. The control group received no sample and the test eye received the formulation , and the eyes were observed for the ocular irritancy (includes the macroscopic observation of cornea, iris, and conjunctiva).

## 3. RESULTS AND DISCUSSION

The sample was qualitatively tested for its solubility in various solvents. It was determined by taking 2 mg of drug sample in 5 ml of solvent as water, methanol, ethanol, ethyl acetate etc., in small test tube and well solubilized by shaking. Solubility study in different solvents at room temperature revealed that it is soluble in, ethanol, methanol, 0.1N NAOH, Glacial acetic acid.Melting point of Levofloxacin was found at 218 $^o$C

The photomicrographs of hydrated PN1 and PN2 proniosomal formulations, composed of Span 40 and cholesterol in 1:1 and 2:1 ratios, are shown in Figure 1 and Figure 2 respectively. The photographs reveal that the niosomes are unilamellar vesicles having spherical shape and no aggregation or agglomeration is observed. Apparently, PN1 niosomal formulation gives vesicles of larger sizes.

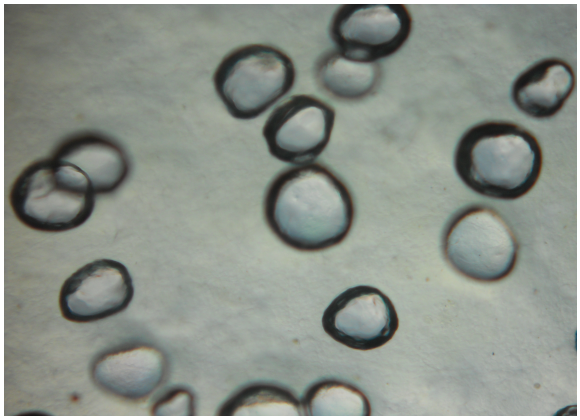

Figure 1. Photomicrograph of hydrated PN1 proniosomal formulation

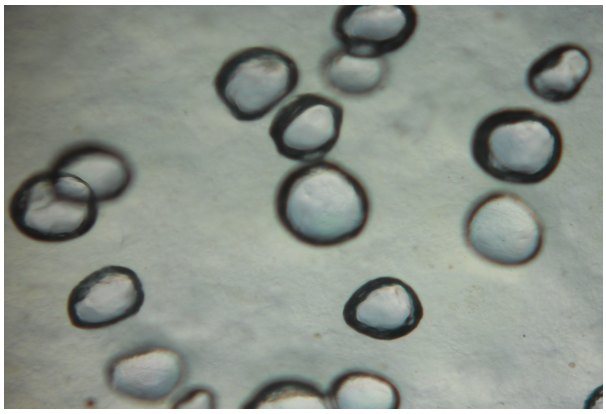

Figure 2. Photomicrograph of hydrated PN2 proniosomal formulation

Proniosomes prepared with non-ionic surfactants of alkyl ester including Span (sorbitan esters) and Tween (polyoxythylene sorbitan esters) were utilized to determine the encapsulation of associated Levofloxacin. The encapsulation efficiency of proniosomes formed from span 40 exhibits a very high value and the entrapment efficiency was found to be highest with the formulation F3. These Span surfactants give the least leaky proniosomes as have the highest phase transition temperature. This result was consistent with the entrapment efficiency of levofloxacin in proniosomes incorporated with Span 40. Most of the surfactants used to make non-ionic surfactant vesicles have a low aqueous solubility. However, freely soluble non-ionic surfactants such as Span can form the micelles on hydration in the presence of cholesterol .The encapsulation of levofloxacin can be entrapped into proniosomes composed of Tween; however, the encapsulation efficiency was relatively low as compared to those composed of Span. It shown in Table 4.

Soya lecithin was selected over egg lecithin because the former gives vesicles of larger size, possibly due to differences in the intrinsic composition of soya and egg derived lecithin. Preparations with a white semi-solid appearance were obtained with span and cholesterol. Incorporation of lecithin results in a gel-like appearance. The types of alcohol affect the size of niosomal vesicles as well; ethanol gave the largest size vesicles. The larger size with ethanol is due to the slower phase separation because of its greater solubility in water.

Proniosomes prepared by conventional method were subjected to scanning electron microscopy (SEM) (Figure 4A). It was observed that preparing proniosomes on dry powder base was easier, provided the powder is not over wetted during the process. Preparing proniosomes on levofloxacin was formulate in different surfactent like span 20 and span 40 also being include lecithin but it was necessary that the solution be incorporated in very small amounts and complete drying be ensured before further additions are made. The figure 4 shown the cross section Proniosomes.

A weighed amount of proniosomal gel was placed on one side of the dialysis membrane. The receptor medium was STF (stimulated tear fluid) pH 7.4. The receptor compartment was surrounded by a water jacket to maintain the temperature at 37±1$^{o}$C. Heat was provided using a thermostatic hot plate with a magnetic stirrer. The receptor fluid was stirred by a Teflon-coated magnetic bead fitted to a magnetic stirrer. At each sampling interval, samples were withdrawn and were replaced by equal volumes of fresh receptor fluid on each occasion. Samples withdrawn were analyzed spectrophotometrically at 293 nm. The release study was shown in table 5 and figure 5.

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