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# Formulation and Evaluation of Tenoxicam Niosomes

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**This study aimed at investigating the influence of varying proportions of the surfactant span 60, cholesterol and dicetylphosphate on the morphology, particle size distribution, entrapment efficiency and *in vitro* drug release of encapsulated tenoxicam niosomes. *In vitro* stability studies of the niosomes were then performed using blood from albino rats. Drug release from the niosomes showed biphasic sustained release pattern of tenoxicam for a period of 24 h. The results obtained show that niosomes of tenoxicam can be prepared as carriers for transdermal therapy.**

**Key words:** Niosomes, Tenoxicam, Cholesterol, Span 60, Dicetylphosphate.

## INTRODUCTION

Niosomes are unilamellar or multilamellar vesicles in which an aqueous solution is enclosed in a highly ordered bilayer made up of nonionic surfactants with or without cholesterol (CHOL) and dicetyl phosphate (DCP) and which exhibit a behavior similar to liposomes *in vivo* [1]. The recent trends in the development of drug delivery systems focus on the concepts and techniques of targeting drugs to specific sites in the body using carriers and vehicles such as drug delivery devices [2-3]. To pursue optimal drug action, functional molecules could be transported by a carrier to the site of action where they are released [4]. Among the different carriers, liposomes and niosomes are well documented for transdermal drug delivery [5-9]. Niosomes in topical preparations improve the horny layer characteristics of the human skin, both by reducing trans-epidermal water loss and increasing smoothness through replenishing lost skin lipids [6].

Non-steroidal anti-inflammatory agents (NSAIDs) such as diclofenac, flurbiprofen, indomethacin and piroxicam have been reported, on topical application over an inflamed joint, to achieve synovial fluid drug concentrations which exceed plasma concentrations suggesting a direct penetration of the drug into the joint [10]. Tenoxicam is an NSAID belonging to the chemical class of oxicams. It is indicated in rheumatoid arthritis, osteoarthritis, ankylosing spondylitis, tendonitis, bursitis, periarthritis and acute gouty arthritis. The common side effects observed following oral administration were gastrointestinal disturbances, epigastric pain, nausea, heartburn and vomiting.

The drug is contraindicated in patients with active peptic ulcer disease or gastrointestinal bleeding. Tenoxicam is practically insoluble in water and very slightly soluble in ethanol but dissolves in solutions of acids and alkalis [11]. The solubility of NSAIDs like tenoxicam is improved by addition of surface-active agents and formation of water-soluble salts; and reduction of particle size has been reported to enhance its dissolution and absorptive rate [12]. The aim of this study was to encapsulate tenoxicam in niosomes and investigate the influence of varying proportions of the surfactant span 60, cholesterol and dicetylphosphate on the morphology, particle size distribution, entrapment efficiency and *in vitro* drug release of tenoxicam from the niosomes so as to improve its therapeutic index and solubility and avoid gastric side effects. The prepared niosomes could be used for transdermal therapy.

## EXPERIMENTAL

### *Materials*

Tenoxicam was donated by Bharat Pharmaceutical and Research Laboratories,

Bangalore, Karnataka State, India. Sigma Chemical Company (St. Louis, USA) supplied dicetyl phosphate, while Span 60 was obtained from National Chemicals (Mumbai, India). Cholesterol was a product of S.D. Fine Chem. Ltd. (Mumbai, India). Alkaline phosphate buffer solution pH 7.4 (PBS) was prepared according to U.S.P. XIX.

### *Equipment*

A Shimadzu Spectrometer UV-240 (Shimadzu Corp. Kyoto, Japan) was used for spetrophotometric analysis. An IEC Centra-CLD centrifuge (International Equipment Company, Bedfordshire, England) was used for centrifugation of blood samples.

### Preparation of the niosomes

The niosomes were prepared by the handshaking method described by Baillie *et al.* [13] and Azmin *et al.* [14]. Batches of niosomes were formulated using accurately weighed quantities of Span 60, cholesterol (CHOL) and dicetylphosphate (DCP) respectively as molar ratio mixtures (Table 1). The lipid ingredients namely Span 60, CHOL and DCP were dissolved in ether, in a 15 ml round bottomed flask. The ether was then removed under reduced pressure on a Rotavapor (Büchi RE 121, Flawil, Switzerland) and maintained at 25 ± 2 °C so as to form a lipid bilayer dry film on the inner wall of the flask. The dried film was then hydrated with 5 ml aqueous phase at 60 °C with gentle agitation for one hour to obtain a milky dispersion of niosomes. The aqueous phase used for the film hydration was either 5 ml of drug solution (2 mg/ml of tenoxicam) or 5 ml of PBS to give drug-loaded or empty batches of niosomes, respectively. The batches of niosomes were examined under a Carl Zeiss optical microscope with a stage micrometer at a magnification of X200. Niosomes with an optimum size distribution range (0.5-5 μm) were selected for further studies.

### Removal of free unentrapped drug

The unentrapped drug was removed by passing the dispersion through a sintered glass funnel (No. 3) under vacuum. The suspension containing the niosomes with the entrapped tenoxicam was transferred into a glass beaker while the filtrate was analyzed for the free drug. This was repeated until no tenoxicam was detectable in the recipient solution.

**Table 1. Batch design of niosomes**

| Batch no. | % Composition of niosomes | | |
|---|---|---|---|
| | Surfactant Span 60 | Cholesterol | Dicetyl phosphate |
| B1 | 50 | 50 | - |
| B2 | 60 | 40 | - |
| B3 | 70 | 30 | - |
| B4 | 80 | 20 | - |
| B5 | 90 | 10 | - |
| B6 | 60 | 35 | 5 |
| B7 | 47.5 | 47.5 | 5 |
| B8 | 100μmol | - | - |
| B9 | 200μmol | - | - |

### Analysis of percentage drug entrapment in the prepared niosomes

Assay for the amount of drug that was entrapped in the niosomes was carried out by lysis of the niosomes with acetone followed by the determination of absorbance at a wavelength of 362 nm against a PBS blank. The drug content was calculated as the ratio of the absorbance of sample versus the slope of the calibration curve. The amount of drug entrapped in the prepared niosomes was obtained by subtracting the amount of tenoxicam obtained in the washing after lysis from the initial amount of tenoxicam used to prepare the niosomes (20 mg).

### *In vitro* release studies

*In vitro* release of tenoxicam from niosomes was studied using a cellulose membrane [15], which was pretreated by boiling in distilled water for 15 min and then tied firmly on the end of a hollow glass tube with an internal diameter of 3.8 cm, fitted with a wire mesh. The niosomal suspension was placed inside the tube and suspended downward into the dissolution test equipment (USP XIX) containing 500 ml of PBS. The PBS was stirred with an overhead stirrer at 100 rpm and maintained at 30 °C ± 0.1 °C. 5ml aliquots were withdrawn every one hour and the volume

loss compensated by replacing with a fresh quantity of an equal volume of PBS into the beaker. The aliquots were then estimated for drug content spectrophotometrically at a wavelength of 362 nm against a PBS blank. All determinations were done in triplicates.

### *In vitro* stability studies

For testing *in vitro* stability of the tenoxicam niosomes, blood samples were withdrawn from the retinoovular plexus of the eyes of albino rats using hematocrit capillaries. The blood was kept undisturbed for a period of 1 h and the serum separated by centrifugation at 3000 rpm for 10 minutes. The *in vitro* stability of niosomes was assessed by incubating a 0.5 ml aliquot of the preparation in 0.5 ml of serum and determining the amount of drug leaked at various time intervals spectrophotometrically at a wavelength of 362 nm against a PBS blank. This was done in triplicates.

## RESULTS AND DISCUSSION

The optimum size distribution range of the niosomes was found to be between 0.5-5 μm for the five batches B1, B2, B3, B7 and B9. The major proportion of niosomes in these batches was observed to have multilamellar structured vesicles. The lipid bilayer film formed was observed to be crystalline in nature. It has been reported that multilamellar vesicles are more appropriate for local therapy and particles of 0.5-5 μm are retained more effectively in the joint cavity [16-17]. The hydrophilic-hydrophobic segments of non-ionic surfactants, and a balance between them, are of paramount importance for niosome vesicle formation [18]. Hence, the influence of the ratio of surfactant: CHOL: DCP was studied on tenoxicam entrapment in niosomes. The percentage entrapment efficiencies (mean± SD) of the different batches of niosomes were11.42 % ± 0.018 for B1, 12.24 % ± 0.016 for B2, 17.45 % ± 0.011 for B3, 19.63 % ± 0.008 for B7 and 23.76 % ± 0.012 for B9. The highest efficiency was obtained in niosomes prepared using Span 60 alone (HLB 4.7). The influence of the HLB value suggests that critical packing parameter of a potential niosome system must take into account the presence of amphipathic or hydrophobic drugs (tenoxicam) as both these substances are to be incorporated into the vesicle membrane [19]. The increase in entrapment may be due to an increase in the lipophilic behavior of the lipid bilayer of niosomes and cthe rystalline nature of the bilayer [20].

The *in vitro* release profile of tenoxicam from niosomes showed that release occurred in two distinct phases: an initial burst release which lasted for 2-4 h, followed by a sustained but reduced release which was maintained for at least 24 h (Figure 1).

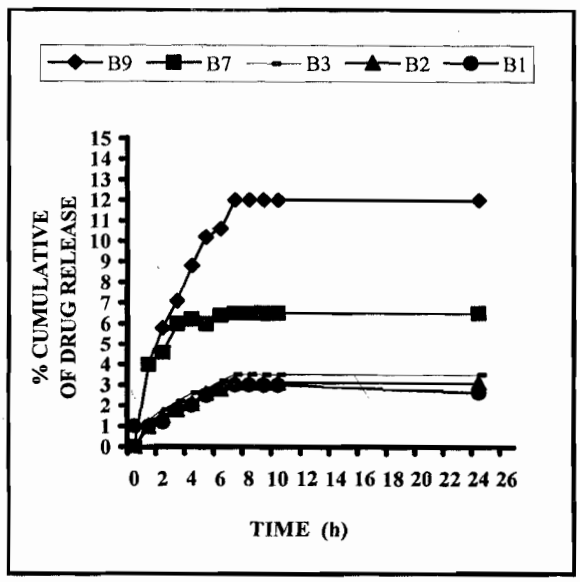


**Figure 1. *In vitro* release profile of tenoxicam from niosomes in phosphate buffer solution pH 7.4**

This biphasic release pattern might be due to size heterogeneity of the niosomes. On incorporating CHOL and DCP, the release of tenoxicam from the niosomes decreased. CHOL and DCP are retained within the niosomal membrane and decrease the diffusion of the tenoxicam through the cellulose membrane (Figure1). From the *in vitro* stability studies, it appears that the presence of CHOL in niosomes is essential for the maintenance of their stability in serum. After 24 h, 30.09 % of tenoxicam was retained in niosomes prepared using surfactant Span 60 alone while niosomes prepared using Span 60 and cholesterol combinations retained 53.7 % to 67.46 % of the drug (Table 2).

**Table 2. *In vitro* stability of tenoxicam in niosomes**

| Time hrs. | % Drug retained in niosomes (mean± SD) | | | | |
|---|---|---|---|---|---|
| | (Span 60t:CHOL: 50:50) B1 | (Span 60: CHOL 60:40) B2 | (Span 60: CHOL 70:30) B3 | (Span 60:CHOL: DCP 47.5:47.5:5) B7 | (Only Span 60 200µmol) B9 |
| 1 | 72.08± 0.10 | 69.18± 0.01 | 60.91± 0.02 | 71.04± 0.04 | 48.53± 0.02 |
| 2 | 69.81± 0.02 | 66.09± 0.01 | 57.66± 0.01 | 69.44± 0.02 | 41.22± 0.03 |
| 3 | 68.53± 0.01 | 64.43± 0.01 | 56.02± 0.01 | 68.74± 0.02 | 38.41± 0.01 |
| 4 | 67.96± 0.04 | 64.94± 0.03 | 55.58± 0.03 | 67.36± 0.02 | 34.14± 0.05 |
| 5 | 67.17± 0.01 | 63.14± 0.04 | 55.15± 0.01 | 67.14± 0.01 | 30.35± 0.02 |
| 6 | 67.04± 0.01 | 62.94± 0.01 | 54.12± 0.01 | 67.09± 0.03 | 31.01± 0.01 |
| 7 | 66.97± 0.01 | 62.57± 0.02 | 54.32± 0.01 | 67.46± 0.01 | 30.90± 0.03 |
| 8 | 65.90± 0.02 | 62.23± 0.02 | 53.93± 0.01 | 67.46± 0.01 | 30.90± 0.02 |
| 24 | 66.73± 0.01 | 61.89± 0.01 | 53.65± 0.01 | 30.90± 0.04 | 67.46± 0.02 |

n= 3

## CONCLUSION

The results from the studies done on the niosomes suggested that the niosomes prepared might be suitable carriers of tenoxicam for transdermal therapy. By varying the vesicle composition when preparing the niosomes one can obtain stable vesicles with optimum size range, entrapment efficiency and drug release properties that can result in a formulation with optimum pharmacokinetic and pharmacotherapeutic characteristics.

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