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# Formulation and Optimization of PiroxicamNiosomes

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## ABSTRACT

The objective of this study was to investigate the bioavailability of piroxicam encapsulated niosomal formulation..Niosomes containing piroxicam were prepared by lipid film hydration technique using Tweens and Spans of varying molecular concentrations with cholesterol. The niosome with the highest entrapment was optimized using varying hydration volumes and time. The optimized niosome was evaluated for drug release using Franz diffusion cell. *In-vivo* performance of the niosomalpiroxicam was assessed using acute rat paw edema method. Results showed that Tween 60 had the highest entrapment efficiency of 68.5% and on optimization at varying hydration time and volume gave the optimal encapsulation efficiency of 70.2% at 60 s and 3 ml.*In-vitro* release study showed that the niosomal system gave a gradual release with the maximum release at 120 min.

**Key words**: Tween 60, niosome, piroxicam, edema, sustained release

## INTRODUCTION

Piroxicam is a poorly water-soluble, potent non-steroidal anti-inflammatory drug (NSAID) used for the treatment of rheumatoid arthritis or osteoarthritis. One of the major drawbacks in piroxicam delivery is its limited aqueous solubility. This problem could be overcome by entrapping the drug in a vesicular structure [1]. Although piroxicam has a strong therapeutic effect, it is associated with several side effects such as gastrointestinal irritation, edema, dizziness, and peptic ulceration when taken orally for a prolonged period. Encapsulation of the drug in a vesicular structure like niosomes could be expected to prolong the existence of the drug in the systemic circulation, enhance penetration into target tissue, and reduce toxicity, if selective uptake could be achieved [2]. Niosomes are formations of vesicles by hydrating mixtures of cholesterol and non-ionic surfactants [3]. These are formed by self-assembly of non-ionic surfactants in aqueous media as spherical, unilamellar or multilamellar vesicles that can entrap amphiphilic and hydrophobic solutes [4-8]. The process of vesicle formulation by self-assembly of non-ionic surfactants is rarely spontaneous and usually requires some input of energy through physical agitation, extrusion or heat [9]. Niosomes have shown advantages as drug carriers, such as being cheap and chemically stable alternatives to liposomes [10]. In the present study, the lipid film hydration technique was employed in the preparation of a piroxicam encapsulated niosomal formulation with the purpose of investigating its bioavailability.

## MATERIALS AND METHODS

### Materials

The following materials were used as procured from their manufacturers: piroxicam (Juhel, Nigeria), Tween 80 (Across Organics, Germany), Tween 60 (Merck, Germany), Span 20 (Sigma, USA), Span 60 (Sigma, USA), cholesterol (BDH, England). All other reagents and solvents were analytical grade and were used as such.

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**Animals**

Twenty-four white albino rats weighing between 190-230 g were used for the experiment. They were isolated and kept in clean conditions.

**Preparation of the niosomes using lipid film hydration method**

Four batches of niosomes were prepared using Tween 80, Tween 60, Span 60 or Span 20 as the non-ionic surfactant. Equimolar ratios of cholesterol and the non-ionic surfactant were used as shown in the table below.Multilamellar vesicles (MLV) were prepared using a modified technique based on lipid film hydration methodby dissolving cholesterol and the non-ionic surfactant in a 2:1 chloroform/methanol system in a 100 ml round bottom flask. The solvent mixture was evaporated to obtain a thin dry film on the wall of the flask. The film was hydrated with 4 ml of PBS (pH 7.4) containing 2.5 mg/ml of piroxicam with gentle shaking during which MLVs were formed. The vesicles were allowed to anneal for 30 min.

**5Table 1: Molecular combinations of the cholesterol and non-ionic surfactants**

| Batch | Non-ionic surfactant (mg) | Cholesterol (mg) | Piroxicam (mg) |
|---|---|---|---|
| **Tween 80A** | 131.0 | 38.6 | 10 |
| **Tween 80B** | 196.5 | 57.9 | 10 |
| **Tween 80C** | 262.0 | 77.2 | 10 |
| **Tween 60A** | 113.1 | 38.6 | 10 |
| **Tween 60B** | 169.7 | 57.9 | 10 |
| **Tween 60C** | 226.3 | 77.2 | 10 |
| **Span 60A** | 43.0 | 38.6 | 10 |
| **Span 60B** | 64.5 | 57.9 | 10 |
| **Span 60C** | 86.1 | 77.2 | 10 |
| **Span 20A** | 34.6 | 38.6 | 10 |
| **Span 20B** | 51.9 | 57.9 | 10 |
| **Span 20C** | 69.2 | 77.2 | 10 |

**Drug entrapment efficiency of the niosome**

A 4 ml volume of the different niosomes was centrifuged and the sediment introduced into test tubes. A 1 ml volume of propan-1-ol was added to lyse the niosomes. The solution was filtered and the clear solution separated and diluted 10-fold. The absorbance was read at 326 nm and the drug content was calculated from the calibration curve.

**Optimization of process variables**

The batch with the highest encapsulation efficiency was optimized by varying hydration volume and time as shown in the table below and the drug entrapment efficiency calculated

**Table 2: Optimization of the niosomesusing varying volumes and time of hydration**

| Niosome (batch) | Volume of hydration (ml) | Hydration time (min) |
|---|---|---|
| **A** | **2** | **30** |
| **B** | **3** | **30** |
| **C** | **4** | **30** |
| **D** | **3** | **60** |
| **E** | **4** | **60** |

***In-vitro* drug release studies**

The release of piroxicam from the niosomal preparation with the best encapsulation efficiency was compared with the marketed piroxicam using a Franz cell. A 20 ml volume of SIF was introduced into the Franz cell onto which a dialysis membrane has been fixed. Then 4 ml of the sample starting with batch A was introduced on the donor compartment of the Franz cell. The temperature was maintained at 37 $\pm$1$^{o}$C and 1 ml was withdrawn at predetermined time intervals and was replaced with 1 ml of fresh SIF. The collected samples were analyzed at 326 nm using UV-VIS spectrophotometer with SIF as blank. The same procedure was used for crushed piroxicam tablet.

**Rat paw edema test**

A modified rat paw edema method of Okoli*et al* was used [11]. Twenty-four albino rats were used for the study. The animals were divided into four groups of six rats. The first group received the 10 mg of a commercially available piroxicam. The second group received the 10 mg niosomalpiroxicam and the third and fourth group received 4 ml of 3% Tween 60 and PBS respectively. The various preparations above were orally administrered, 1h before 0.1 ml of 1% w/v in saline of the egg albumin was given. The egg albumin was injected in the subplantar surface of the left hind paw. The volume of paw oedema was measured at 0, 1, 2, 3, 4 and 5h. Oedema was assessed in terms of volume of water displaced after induction of inflammation.

**RESULTS AND DISCUSSION**

**Percent Drug Entrapment Efficiency and Optimization of Process Variables**

Non-ionic surfactants used in formation of niosomes are polyglyceryl alkyl ether, glucosyldialkyl ether, crown ether, polyoxyethylenealkyl ether, ester-linked surfactants, and steroid-linked surfactants.Niosome preparation is affected by processes variables, nature of surfactants, and presence of membrane additives and nature of drug

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to be encapsulated. The surfactants with alkyl chain length from C12-C18 are suitable for preparation of niosome [12,13]. In this study, twelve batches of niosomes were prepared by mixing four different non-ionic surfactants with cholesterol at varying molecular combinations. Tween 60 batches produced niosomes with the highest percent entrapment efficiencies of 68.5, 63.1 and 65.3 as shown in Figure 1. This was followed by the batches of Span 20 niosomes, then Span 60 niosomes and lastly Tween 80 niosomes. Tween 60 has a HLB value of 14 and acts as a good wetting agent as well as a solubilizer. This may have contributed to the high entrapment efficiency. As a generalization, it may be said that low concentrations of surface-active agents increase absorption, possibly due to enhanced contact of the drug with the absorbing membrane. The chain length and hydrophilic head of non-ionic surfactants also affect entrapment efficiency, such as stearyl chain C18 non-ionic surfactant vesicles which show higher entrapment efficiency than lauryl chain C12 non-ionic surfactant vesicles [14]. HLB value of surfactants affects entrapment efficiency. HLB values of 14 to 17 are not suitable for niosomes but HLB value of 8.6 -12 usually cause high entrapment efficiency and entrapment efficiency decreases with decrease in HLB value from 8.6 to 1.7 [15]. The entrapment efficiency can also be affected by phase transition temperature. [16]. The incorporation of cholesterol into bilayer composition of niosome induces membrane-stabilizing activity and decreases the leakiness of membrane [17]. On optimization of Tween 60A niosome by varying the hydration volume and time, the variable with 3 ml of hydration volume and 60 min of hydration time produced niosomes with 70.2% optimum entrapment efficiency as shown in Figure 2

**Fig. 1: Percent drug encapsulation efficiency of the niosomal batches**

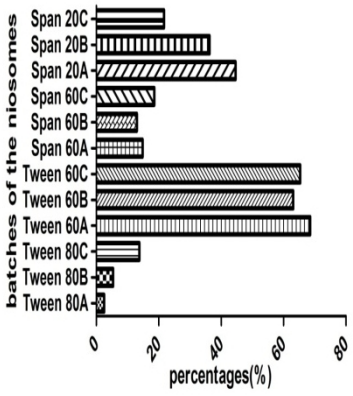
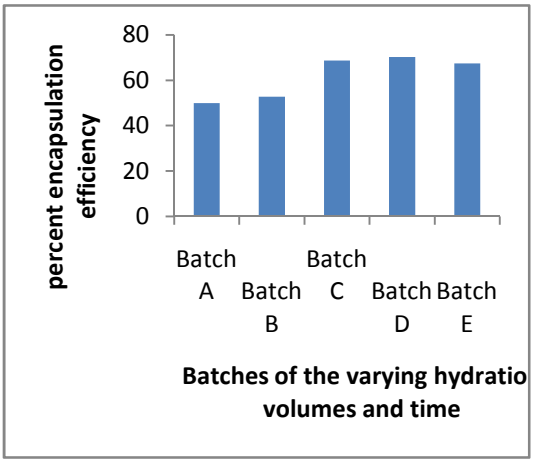



**Figure 2: Optimization of the niosomes by varying hydration volume and time**




Key: Batch A: 2 ml and 30 min; Batch B: 3 ml and 30 min; Batch C: 4 ml and 30 min; Batch D: 3 ml and 60 min; Batch E: 4 ml and 60 min.

**Drug release studies**

The drug release profile of the Tween 60 based niosome as compared with the crushed marketed piroxicam tablet showed a gradual but higher release which peaked at 2 h and then gradually declined. The *in-vitro* release of the niosome was gradually leaking the contents to the medium. The maximum percent of drug released after 24 h was 88.2% against the 76.0% of the crushed marketed piroxicam.

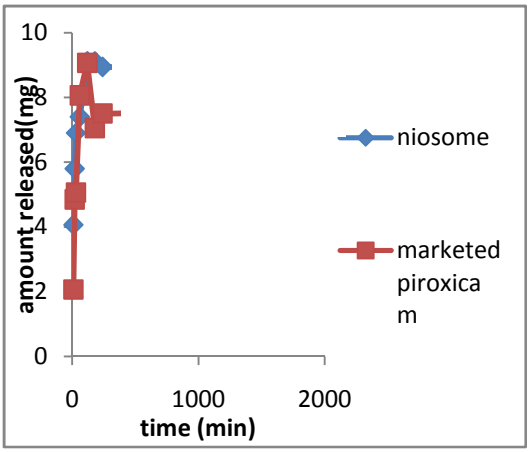



**Figure 3: *In-vitro* drug release of piroxicamfrom theniosome**

Most vesicular systems are known to follow zero order kinetics. The niosome acted like a reservoir system for continuous delivery. The slow release pattern of entrapped drug indicated the high stability of the niosomal membrane

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**Rat paw edema test**

The *in vivo* studies compared the percent edema inhibition of the marketed piroxicam tablet (positive control), the niosome, Tween 60 alone and phosphate buffer solution (negative control). The effects of the niosome are shown in Figure 4. There was overall higher suppression of edema by the niosome at 5h. Peak inhibitory effect was recorded at 5 h post egg-albumin injection. The marketed piroxicam gave an initial and immediate burst which declined sharply after 2 h while the niosome rose gradually and steadily till its maximum at 5 h.This result showed and confirmed the delayed and also sustained delivery of niosomes.

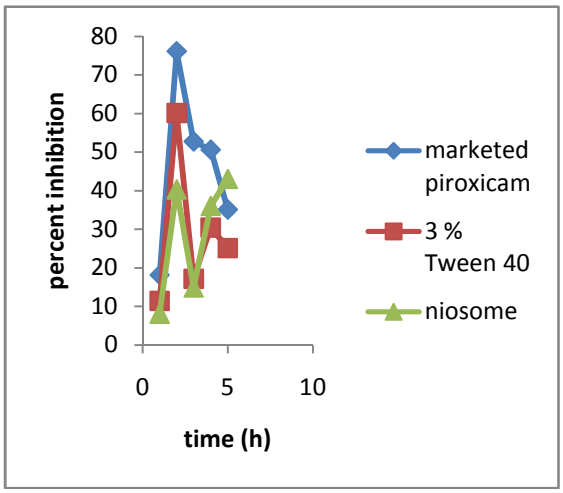


**Figure 4: Effect on rat paw edema induced by egg albumin**

## CONCLUSION

The results obtained from this work clearly showed that 100:100 µm concentrations of cholesterol and non-ionic surfactant is optimal for drug entrapment. Tween 60 niosome prepared using the lipid film hydration technique also showed good potential for high drug entrapment, stability and sustained drug release.

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