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Anti-inflammatory drug (proniosome) based medication conveyance arrangement of piroxicam

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Abstract

Piroxicam is a widely used potent non-steroidal anti-inflammatory drug, with due potential for dermal delivery. Permeation of piroxicam from proniosome based reservoir type transdermal gel formulation across excised rat abdominal skin was investigated using Keshery Chein diffusion cell. There was considerable improvement in flux over the control gel formulation. The lipid vesicles were evaluated for entrapment efficiency and vesicle size of niosomes formed. It was observed that Span 60 based formulations produced vesicles of smallest size and higher entrapment efficiency while those of Span 80 produced vesicles of least entrapment efficiency. Incorporation of lecithin further enhanced entrapment efficiency. Proniosomes were prepared by conventional technique and employing maltodextrin and sorbitol as base. The morphology of the proniosomes was studied by scanning electron microscopy. Maximum flux achieved was 35.61 g/cm^2/h, an enhancement of 7.39 times was achieved for transdermal system based on proniosomal gel as compared to control gel. Anti-inflammatory studies revealed that proniosome based transdermal drug delivery system of piroxicam were promising carriers for delivery of piroxicam. There was significant reduction in carrageenan induced rat paw inflammation compared to control.

Keywords: Piroxicam, niosomes, permeation enhancement, dermal delivery.

INTRODUCTION

Colloidal particulate carriers such as liposomes (Mishra et al., 2007) or niosomes (Shahiwala and Misra, 2002) have been widely employed in drug delivery systems and producing them from proniosomes provides them a distinctive advantage. These carriers can act as drug reservoirs and the rate of drug release can be controlled by modification of their composition. 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 (Puglia et al., 2004) like drug targeting (Gupta et al., 2005), controlled release (Barber and Shek, 1993) and permeation enhancement of drugs (Verma et al., 2003). 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 (Vora et al., 1998; Hu and Rhodes, 1999).

The transdermal route of drug delivery has many advantages for administration of drugs in local and systemic therapy. But, skin is widely recognized for its effective barrier properties compared with other biological membranes. The low permeability of the skin makes it a minor port of entry for drugs. The vesicular drug delivery is thus potentially beneficial as vesicles tend to fuse and adhere to the cell surface; this is believed to increase the thermodynamic activity gradient of the drug at vesicle-stratum corneum interface thus leading to enhanced permeation rate.

Piroxicam, a non- steroidal anti- inflammatory drug (NSAID), are used in the treatment of dysmenorrheal, various acute and chronic musculoskeletal disorders like rheumatoid arthritis, osteoarthritis etc., and also as potent analgesics (Andersson et al., 1998). However, the use of piroxicam has been associated with a number of gastrointestinal disorders (Schiantarelli and Cadel, 1981). Dermal delivery is an alternative route, but requires a formulation which ensures the deep skin penetration. Several researchers have successfully delivered piroxicam via organogel by Agrawal et al. (2004) , buccal

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Table 1. Composition of proniosomal formulations of piroxicam.

Proniosomal code	Span 20 (mg)	Span 40 (mg)	Span 60 (mg)	Span 80 (mg)	Cholesterol (mg)	Lecithin (mg)
S2	360	-	-	-	40	-
S4	-	360	-	-	40	-
S6	-	-	360	-	40	-
S8	-	-	-	360	40	-
S6L	-	-	180	-	40	180
S6LM	-	-	180	-	40	180
S6LS	-	-	180	-	40	180

gel by Attia et al. (2004), mucoadhesive system by Cilurzo et al. (2005), microspheres based drug delivery by Raman et al. (2005), Berkland et al. (2004), Georgeta et al. (2004), iontophoresis was attempted by Curdy et al. (2001), cyclodextrin based enhancement was carried out Murthy et al. (2004) and gel based formulation by Shin et al. (2000) and Santoyo et al. (1995).

Thus the study encompasses the ability of lipid vesicles to deliver piroxicam across skin in order to evaluate it transdermal delivery potential. Moreover greater stability can be accorded by proniosomal formulation as compared to niosomes and access their potential towards dermal delivery.

MATERIALS AND METHODS

Piroxicam was procured from Torrent Pharmaceuticals, Ahemdabad, India. Span 20, 40, 60 and 80, chloroform, isopropyl alcohol, maltodextrin, sorbitol, cholesterol, Carbopol 934 was purchased from Central Drug House, New Delhi, India. Dialysis membrane-150 and egg lecithin was purchased from Himedia, Mumbai, India. Due permission was obtained from Institutional Animal Ethics Committee for conduct of animal experimentation (Registration number 716/02/a/CPCSEA)

Preparation of proniosomes

Various proniosomal preparations were formulated using surfactant, cholesterol, lecithin, and piroxicam. The compositions of different proniosomal formulations prepared are listed in Table 1.

Preparation of conventional niosome

Proniosomes were prepared using the method reported (Perrett et al., 1991) with slight modification. The formulations S2, S4, S6 and S8 prepared comprised of 360 mg of surfactant (Span 20, 40, 60 and 80), 40 mg of cholesterol, and 20 mg of piroxicam in isopropyl alcohol were taken in a wide-mouth glass vial. The open end of the glass vial was covered and the tube was warmed in a water bath at 65°C till the surfactant mixture dissolved completely. However the formulation S6L comprised of 180 mg of Span 60, 40 mg of cholesterol, 180 mg of egg lecithin and 20 mg of piroxicam and was prepared in a similar manner.

Preparation of maltodextrin based proniosome

The formulation S6LM comprised of 180 mg of Span 60, 40 mg of cholesterol, 180 mg of egg lecithin and 20 mg of piroxicam dissolved in chloroform and iso propyl alcohol mixture (4:1) . The mixture was added to a 100 ml round bottom flask containing 0.5 g of maltodextrin powder. The flask was attached to the rotary evaporator (Hicon Grover Enterprises, New Delhi, India) maintained at a temperature of 65°C using water bath and the flask was rotated at 60 rpm under vacuum until the powder appeared to be dry and free flowing. The dried material (S6LM) was removed from the evaporator and kept under vacuum overnight.

Preparation of sorbitol based proniosomes

0.5 g of sorbitol was placed in a 100 ml round bottom flask attached to a rotary evaporator. Span 60 180 mg, cholesterol 40 mg, lecithin 180 mg and piroxicam 20 mg mixture in chloroform and isopropyl alcohol mixture (4:1) was added slowly on to sorbitol powder bed. Care was taken not to over wet the powder base. The rotary evaporator (Hicon Grover Enterprises, New Delhi, India) was maintained at a temperature of 65°C using water bath and the flask was rotated at 60 rpm under vacuum so as to dry the powder base before further addition of surfactant mixture. The dried material (S6LS) was finally removed and kept under vacuum overnight.

Preparation of piroxicam niosomal gel

Proniosome powder was weighed into screw cap vials to which was added water at 80°C. The vials were vortex mixed for complete and uniform hydration. The niosomal preparations were then converted into gel by appropriately diluting the proniosomes and adding Carbopol 934 (1%w/w) for ease of handling. The final drug concentration achieved was 0.5%w/w.

Preparation of piroxicam carbopol gel

0.5% w/w piroxicam was dissolved/suspended in saline phosphate buffer pH 7.4 and to it was added carbopol 934 (1% w/v). The gel was finally obtained by addition of triethanolamine.

Fabrication of reservoir type patch of niosomal gel of piroxicam

Transdermal patches (reservoir type) of piroxicam were fabricated by encapsulating niosomal gel preparation of piroxicam within a shallow compartment made of drug impermeable backing membrane (laminated aluminum foil) and a hollow ring shaped compartment. A micro porous tape of a larger area was stuck onto the impermeable backing membrane to bring the transdermal patch in close contact with the skin. The device was closed by a release liner on the open side (Figure 1).

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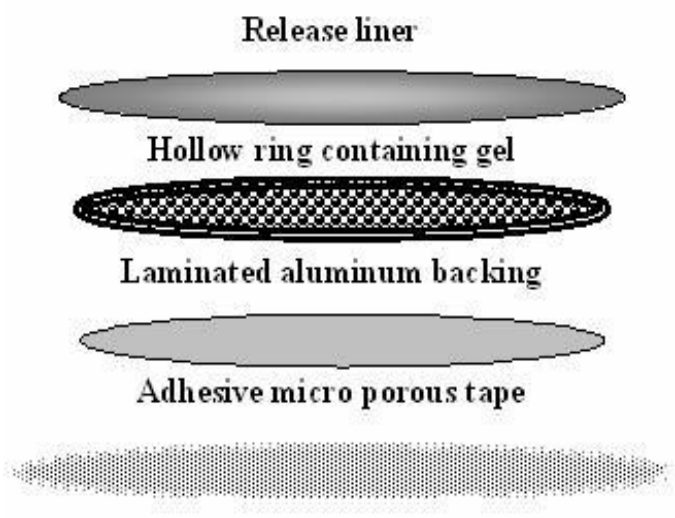

Figure 1. Fabrication design of reservoir type transdermal drug delivery system for piroxicam niosomal gel formulation.

Evaluation

Encapsulation efficiency

Weighed quantity of proniosomal gel (S2, S4, S6 and S8) were hydrated with saline and placed in a glass tube to which a Hi Media dialysis membrane was securely attached and dialyzed into 600 ml of saline (0.9%w/v) for 8h with three washings. The niosomes were collected and lysed using ethanol and suspended in 30% PEG 400 – phosphate buffer saline pH 7.4. The resulting solution was filtered and analyzed spectrophotometrically at 356 nm. Encapsulation efficiency (%EE) was calculated by the following equation:

$\%EE = [(C_t - C_f)/C_t] \times 100\%$

Where C_t is the total concentration of drug and C_f is the concentration of free drug.

Scanning electron microscopy (SEM)

Proniosome powders were affixed to double-sided carbon tape, positioned on an aluminum stub and excess powder removed. The stubs were stored under vacuum overnight. The samples were sputter-coated with gold. Electron micrographs were obtained using scanning electron microscope operating at 15 kV accelerating voltage (LEO 435 VP 501B Electron Microscopy Ltd, UK).

***In vitro* skin permeation studies**

The full-thickness abino Wistar rat skin was used for the permeation experiments. After removing the hair with a clipper, the skin was rinsed with physiological saline and clamped between the donor and the receptor chamber of Keshary Chien diffusion cell with the stratum corneum surface facing the donor compartment of vertical diffusion cell. The effective diffusion area of the cell was 2.0 cm^2 and had a receptor volume of 22 ml. The receptor chamber was filled with 30%v/v PEG 400 in phosphate buffer saline pH 7.4. The diffusion cell was maintained at 37 ± 1°C and the solution in the receptor chambers was stirred continuously at 600 rpm with the help of magnetic bead. 2 g of niosomal gel of piroxicam was gently placed in the donor chamber and spread evenly. 2 ml of the solution in the acceptor chamber was removed for drug content determination and replaced immediately with an equal volume of receptor media. Drug concentration was determined UV spectrophotometrically at 356 nm ($y = 0.0316x + 0.0066$, $R^2 = 0.9997$).

***In vivo* anti-inflammatory studies**

Experiments were approved by the Animal Ethics Committee of the University. Male Wistar rats (220 – 250 g) were assigned to weight-balanced groups (n = 6). The experimental groups received the different formulations, while the control group was treated with placebo only. In the experiment, 2 g of the different formulations (S6L, S6LM and S6LS) were applied over 9 cm^2 as transdermal patch on the dorsal skin after removing the hair with a clipper. After 2 h, 0.05 ml of a 0.5% carrageenan suspension was injected into the subplantar area of the left hind paw. The activity was measured by measuring the changes in paw volumes with a screw gauge (Mitutoyo, Kanagawa, Japan) 4 h after carrageenan injection. The right hind paw served as control was treated with physiological saline solution without carrageenan (Alol, 1993). The degree of paw swelling and inhibition in inflammation was calculated as:

$$Swelling\ (\%) = \frac{V_t - V}{V} X\ 100$$

Where, V_t is the volume of the carrageenan-treated paw, V is that of the non-treated paw.

$$Inibition\ (\%) = \frac{S_c - S_t}{S_c} X\ 100$$

Where, S_c is the swelling of the control paw, S_t is that of the test formulation treated paw.

Statistical analysis

The results were analyzed by Student's t- test using Statistica for Windows (Version 5.0) from StatSoft, Inc., USA. The results were evaluated at probability level of $p < 0.05$.

RESULTS AND DISCUSSION

Among the niosomes prepared with spans, S4 and S6 showed maximum percentage entrapment that is, 90.4 and 94.8% respectively. The particle size analysis revealed that niosomes from S4 were larger as compared to those of S6 (Figure 2). Vesicles with smaller diameter are believed to better permeate through the skin as smaller vesicles tend to fuse readily. Niosomes of S6 were smaller in size, demonstrated higher entrapment efficiency and higher surface area as compared to that of S4. Niosomes of S2 demonstrated low entrapment efficiency and was not taken up for further study. It was observed that niosomes of Span 40 produced niosomes of larger size but in case of piroxicam vesicles from Span 60 had higher entrapment efficiency (Figure 3) and were

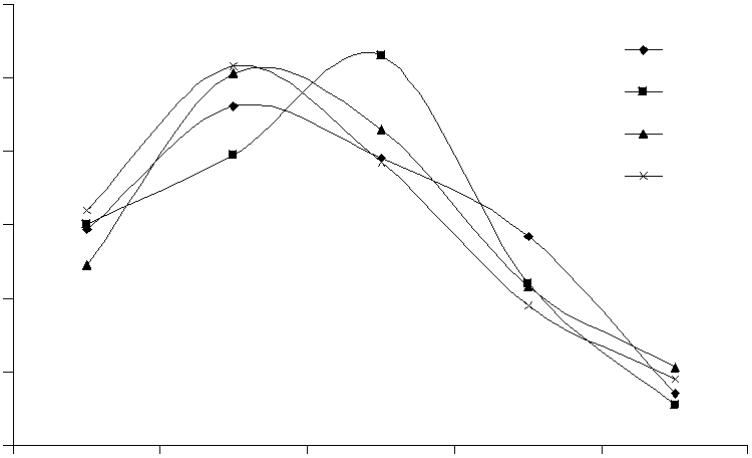

Figure 2. Mean size distribution of piroxicam niosomes prepared from S2, S4, S6 and S8.

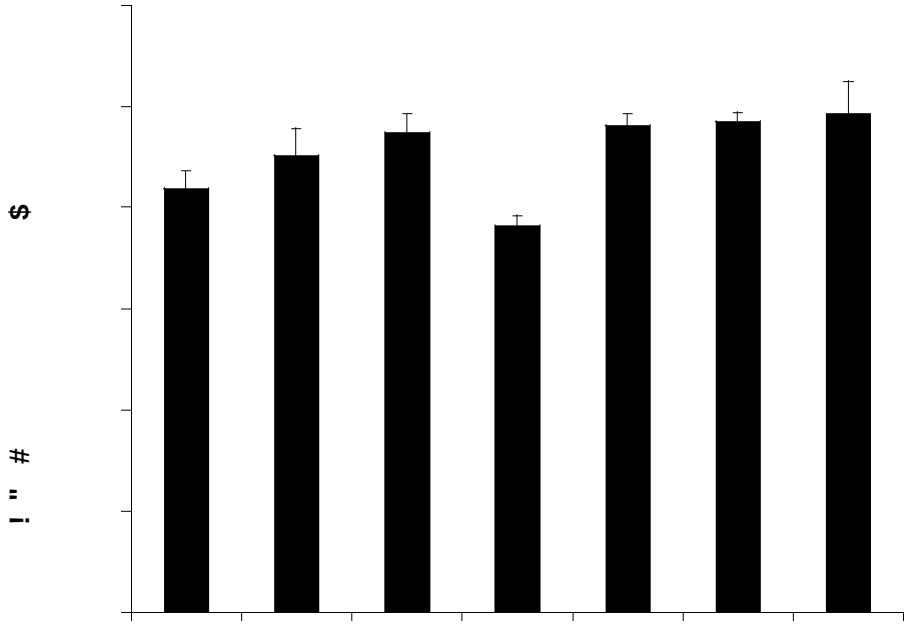

Figure 3. Encapsulation efficiency of various piroxicam niosomes (%EE±S.D.).

therefore selected, this probably could be credited to its high transition temperature and low permeability**.** Attempt was also made to incorporate lecithins. Egg lecithin was added and it was found to enhance drug entrapment. Incorporation of lecithin is also justified as it acts as permeation enhancers. Incorporation of lecithin further enhanced the percent drug entrapment to 96.1%. Incorporation of lecithin leads to vesicles of smaller size due to increase in hydrophobicity which results in reduction of vesicle size (Alsara et al., 2005). There is probably for-

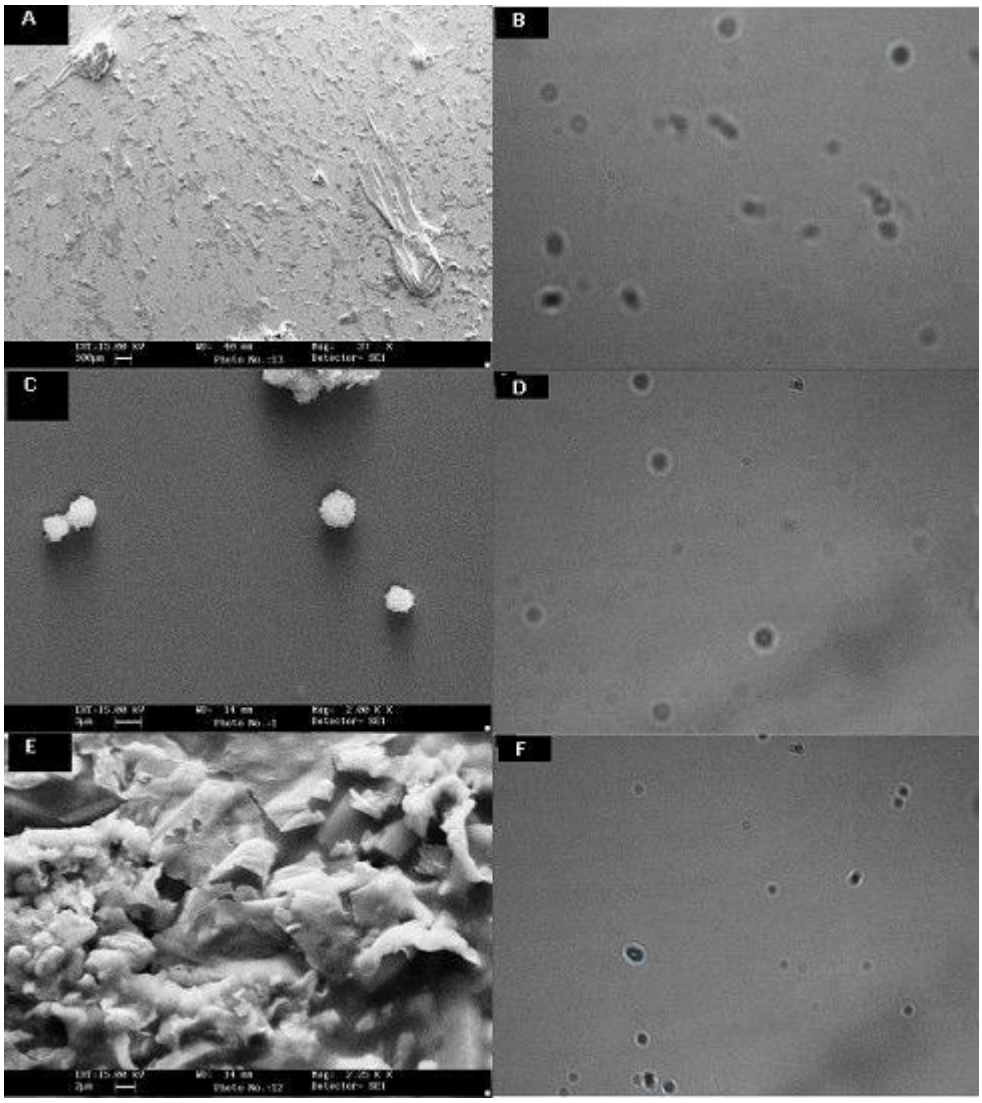

Figure 4. SEM images of proniosomes and polarized microscopic view of niosomes (40x): conventional (A & B), maltodextrin based (C & D), sorbitol based (E & F).

mation of more compact and well organized bilayers which prevents the leakage of drug (Fang et al.,2001).

In the present study attempt was also made to formulate niosomes from proniosomes formed on maltodextrin and sorbitol. Maltodextrin and sorbitol provided a base for preparing proniosomes. Percentage entrapment (Figure 3) observed was 97.2 and 98.6% respectively. 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 maltodextrin was comparatively easy as compared to sorbitol but it was necessary that the solution be incorporated in very small amounts and complete drying be ensured before further additions are made.

Maltodextrin is a polysaccharide; it has minimal solubility in organic solvents. Thus, it is possible to coat maltodextrin particles by simply adding surfactant in organic solvent to dry maltodextrin and evaporating the solvent. The maltodextrin particle morphology is preserved (Figure 4C), circular maltodextrin particles can be used for a significant gain in surface area. The higher surface area results in a thinner surfactant coating, which makes the rehydration process more efficient. The use of maltodextrin as the carrier in the proniosome preparation permitted flexibility in the ratio of surfactant and other components which can be incorporated.

Coating sorbitol results in a solid cake like mass (Figure 4E). It was necessary that the sorbitol bed be completely dry before further additions are made and making proniosomes with a reduced amount of sorbitol

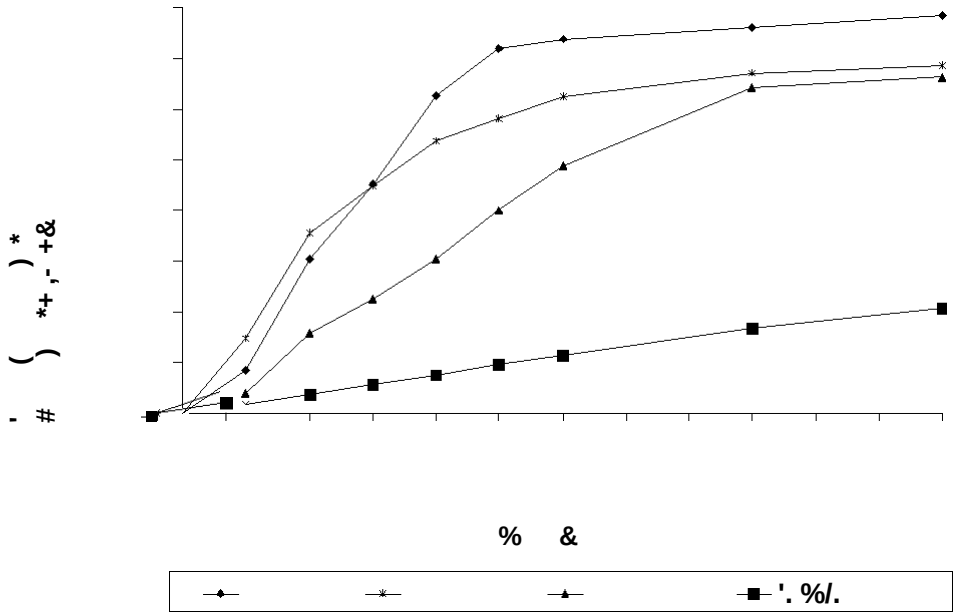

Figure 5. Cumulative amount of drug permeated through rat abdominal skin from different piroxicam niosomes.

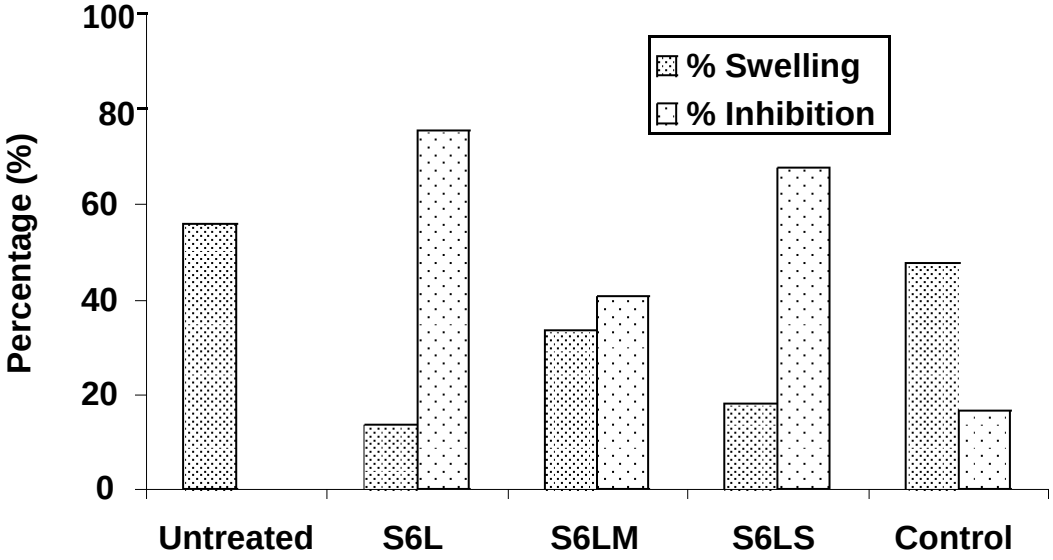

Figure 6. Anti-inflammatory studies of niosomal gel based piroxicam transdermal system.

was not only tedious but lead to niosomes with larger vesicle size. Addition of water leads to swelling of bilayers as well as vesicles due to interaction of water with polar groups of surfactant. In presence of excess of water there was complete hydration leading to formation of niosomes. Niosomes formed from conventional proniosomes, maltodextrin based and sorbitol based proniosomes are shown in (Figure 4B, 4D and 4F) respectively. The niosomes were visualized by polaroid microscope (Nikon HFX-DX, Labophot Microscope, Germany).

The amount of drug permeated across rat abdominal skin was determined for piroxicam carbopol gel and niosomes formulated using span 60 and lecithin (S6L) and on maltodextrin (S6LM) and sorbitol as base (S6LS) is shown in Figure 5. The flux attained at the end of 24 h was 4.82, 35.61, 20.14 and 19.05 µg/cm^2/h respectively and their enhancement ratios were 1.00, 7.39, 4.18 and

3.95 respectively. The incorporation of maltodextrin and sorbitol retarded drug release. There was significant ($p<0.05$) increase in flux over control preparation.

The adsorption and fusion of niosomes onto the surface of skin and the role played by the constituents of niosomes might facilitate drug permeation across skin. The interaction of niosomes with skin probably alters the barrier properties of stratum corneum thus enhancing permeation.

Lecithins and surfactants present in proniosomes have been reported to alter the structure of the stratum corneum. The intercellular lipid barrier in the stratum corneum gets fluidized and becomes permeable (Barry, 2001; Ogiso et al., 1996) thus increasing the permeation of drugs. Fusion of noisome vesicles to the surface of skin, results in higher flux of the drug due to direct transfer of drug from vesicles to the skin (Barry, 2001).

The concentration of cholesterol and lecithin are important as they tend to affect the morphology of the vesicles. An alteration in their composition leads to disruption of vesicles which leads to leakage of free drug before fusion of the vesicles with the skin.

The results of the anti-inflammatory studies (Figure 6) revealed that span 60 based lecithin vesicles (S6L) showed significant ($p<0.05$) reduction in paw swelling. The percent inhibition was found to be more than that of piroxicam carbopol gel. It is probable that there is enhanced drug delivery from lipid vesicles. The short fall seen with maltodextrin and sorbitol based formulations account for the slow release observed in in-vitro studies.

Conclusions

The *in vitro* permeation of piroxicam from proniosomes of various compositions and types of nonionic surfactants have been studied and evaluated. Piroxicam was successfully entrapped within the lipid bilayers of the vesicles with high efficiency. The experimental results suggest that either the vesicles fuse with the intercellular lipid of the stratum corneum and transfer the drug from vesicles to the skin and/or there might be penetration enhancement due to surfactants. Presence of lecithin probably aids the process. Proniosomes thus are capable of delivering piroxicam and probably other drugs also.

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