International Journal of Pharmaceutics 405 (2011) 142–152

Contents lists available at ScienceDirect

International Journal of Pharmaceutics

journal homepage: www.elsevier.com/locate/ijpharm

Pharmaceutical Nanotechnology

Proniosomes as a carrier system for transdermal delivery of tenoxicam

H.O. Ammar[a,*], M. Ghorab[b], S.A. El-Nahhas[c], I.M. Higazy[a]

[a] National Research Centre (NRC), Pharmaceutical Technology, Dokki, Cairo, Egypt
[b] Faculty of Pharmacy, Cairo University, Department of Pharmaceutical Sciences, Egypt
[c] 6th October University, Department of Pharmaceutical Sciences, Egypt

ARTICLE INFO

Article history:
Received 7 June 2010
Received in revised form 27 October 2010
Accepted 5 November 2010
Available online 1 December 2010

Keywords:
Tenoxicam
Transdermal
Proniosomes
Anti-inflammatory

ABSTRACT

Tenoxicam is a non steroidal anti-inflammatory drug (NSAID) widely used in the treatment of rheumatic diseases and characterized by its good efficacy and less side effects compared to other NSAIDs. Its oral administration is associated with severe side effects in the gastrointestinal tract. Transdermal drug delivery has been recognized as an alternative route to oral delivery. Proniosomes offer a versatile vesicle delivery concept with the potential for drug delivery via the transdermal route. In this study, different proniosomal gel bases were prepared, characterized by light microscopy, revealing vesicular structures, and assessed for their drug entrapment efficiency, stability, their effect on *in vitro* drug release and *ex vivo* drug permeation. The lecithin-free proniosomes prepared from Tween 20:cholesterol (9:1) proved to be stable with high entrapment and release efficiencies. The *in vivo* behaviour of this formula was studied on male rats and compared to that of the oral market product. The investigated tenoxicam loaded proniosomal formula proved to be non-irritant, with significantly higher anti-inflammatory and analgesic effects compared to that of the oral market tenoxicam tablets.

1. Introduction

Tenoxicam (TX) is a long-acting non steroidal anti-inflammatory drug (NSAID) (Bird et al., 1984) of the oxicam family (Woolf and Radulovic, 1989), characterized by its potent anti-inflammatory (Morof et al., 1988), antipyretic and marked potent analgesic effect (Todd and Clissold, 1991). Thus, TX is widely used in the treatment of rheumatic diseases (González and Todd, 1987), acute gout (Waterworth and Waterworth, 1987), enkylosing spondylitis, primary dysmenorrhea (Thadikonda et al., 1995), extra-articular diseases (Huang et al., 2002), in addition to renal colics, postoperative and back pains (Sporn and Suh, 2000) as well as postpartum uterine contraction pain (Huang et al., 2002). TX is, as well, known as a very good anti-oxidant (Vartiainen et al., 2001).

The pharmacological and metabolic behaviour of TX is induced by blocking prostaglandins' (PGs) biosynthesis, inhibiting phagocytosis and leukocyte migration, scavenging active oxygen spieces at inflammatory sites (Vartiainen et al., 2001) and inhibiting human metalloproteinases which induce cartilage breakdown (González and Todd, 1987).

TX is practically administered in several dosage forms: oral (tablets, capsules), rectal (suppository) and parenteral (IV, IM, IA) (Huang et al., 2002). However, there has always been inconvenience accompanying each of the mentioned routes of administration (Information for Health Professionals, 2006).

TX side effects' profile appeared similar to that of other NSAIDs; affecting GIT causing epigastric pain, nausea, dyspepsia, indigestion, vomiting and GI ulceration (González and Todd, 1987) and increasing the risk of renal failure or bleeding (Al-Obaid and Mian, 1993). It also has severe effects on the liver and biliary tract that may lead to hepatitis in high doses as well as increasing liver enzyme activity (Information for Health Professionals, 2006).

Lately, there has been a continuous interest towards transdermal drug delivery (TDD) (Prausnitz et al., 2004), as it would avoid problems associated with the other routes of administration (Thong et al., 2007). A timely warning challenge to TDD formulators was issued by Hadgraft and Guy (Hadgraft and Guy, 1987); represented in the lipid matrix of the skin stratum corneum, which presents the rate limiting barrier of drug permeation (Elias and Friend, 1975). Thus, several technological advances have been made in the recent decades to overcome skin barrier properties and enhance percutaneous drug penetration (Thong et al., 2007).

Drug delivery systems using colloidal particulate carriers such as liposomes (Betageri and Habib, 1994), niosomes (Schreier and Bouwstra, 1994) or proniosomes proved to have distinct advan-

* Corresponding author. Tel.: +20 23 3335456; fax: +20 23 3370931.
E-mail addresses: husseinammar@hotmail.com (H.O. Ammar), mkghorab@yahoo.com (M. Ghorab), soheir.elnahhas@hotmail.com (S.A. El-Nahhas), imane.higazy@hotmail.com (I.M. Higazy).

0378-5173/$ – see front matter © 2010 Elsevier B.V. All rights reserved.
doi:10.1016/j.ijpharm.2010.11.003

tages over conventional dosage with an increasingly important role in drug delivery; as particles can act as drug containing reservoir, and modification of the particle composition or surface can adjust the drug release rate and/or the affinity for the target site (Hu and Rhodes, 1999).

Proniosomes are liquid crystalline-compact niosomal hybrid which could be converted into niosomes upon hydration with water offering a versatile vesicle delivery concept with potential for drug delivery via transdermal route (Blazek-Welsh and Rhodes, 2001). Upon skin application proniosomes get hydrated with water from skin under occlusion (Alsarra et al., 2005). Proniosomes should be hydrated to form niosomal vesicles before the drug is released and permeates across the skin. Previous experimental results and supportive theoretical analysis suggested several mechanisms to explain the ability of niosomes to modulate drug transfer across skin; it is thought that structure modification of stratum corneum is one of the most probable reasons for enhancing the permeability of drugs: the intercellular lipid barrier in stratum corneum would be dramatically changed to be more loose and permeable by treatment with liposomes and niosomes (Barry, 2001). Both phospholipids and non-ionic surfactants in proniosomes can act as penetration enhancers, since it was found that some phospholipids are able to fluidize the stratum corneum lipid bilayers and diffuse through them (Kirjavainen et al., 1996).

Proniosomes; provide additional convenience of transportation, distribution, storage and dosing. They are known to avoid many of the problems associated with either the aqueous niosome dispersion, as problems of physical stability (aggregation, fusion, leaking), or liposomes, as degradation by hydrolysis (Frfkjaer et al., 1984) or oxidation (Hunt and Tsang, 1981), as well as sedimentation, aggregation or fusion during storage (Wong and Thompson, 1982), in addition to its high cost, difficulties in sterilization, variable purity problems of phospholipids (Vora et al., 1998) and in large scale production (Frfkjaer et al., 1984) of a product with adequate physical and chemical stability. Proniosomes not only do they offer a promising means of drug delivery, but also could enhance the recovery rate of the skin barrier (Hatziantoniou et al., 2000). All this make proniosomes; "dry niosomes", a promising industrial product (Hu and Rhodes, 1999).

Since both hydrophilic and hydrophobic substances can be embedded in niosomal vesicles (Niemiec et al., 1995), thus, it is known that sparingly soluble drugs can be entrapped in vesicles (Arunothayanun et al., 2000). Consequently, proniosomes are expected to offer a special advantage for tenoxicam which is lipophilic with relative hydrophilicity. Thereby, the present study aims at designing a new transdermal formulation for tenoxicam characterized by safety and high therapeutic efficacy, through designing an optimum proniosome gel formulation so as to reduce the daily administered dose of tenoxicam with a subsequent improvement in patient compliance and drug safety.

2. Materials

Tenoxicam was kindly donated by Epico (Egypt). Ethyl alcohol absolute and Tween 80 were obtained from Adwic (Egypt). Egg yolk from chicken, Span 80, Tween 20 and Tween 60 were supplied from Sigma Chemical Company (USA). Cholesterol from lanolin and Span 60 were purchased from Fluka (Switzerland). Cellulose membrane was purchased from Sigma–Aldrich (USA). Glycerin 99.5% was obtained from TVV (Egypt). Lecithin-soyabean was obtained from ICN Biomedicals (USA). Carbomer 934P, Potassium dihydrogen phosphate, disodium hydrogen phosphate, acetic acid and formaldelyde were supplied from El Nasr Pharmaceutical Chemicals (Egypt), all of analytical grade.

3. Methods

3.1. Preparation of proniosomes

Proniosomes were prepared by the method reported by Perrett et al. (1991). Precisely, hundred milligrams surfactant mixture, surfactants: alcohol (1:1), and drug were weighed in a clean and dry, wide mouth small glass tube. After mixing all the ingredients, the open end of the glass tube was covered with a lid to prevent loss of solvent, and then warmed on a water bath at 60–70 °C for about 5 min, until the surfactants were dissolved completely. The aqueous phase was then added and warmed on a water bath till clear solution was formed. The mixture was allowed to cool to room temperature until the dispersion was converted to proniosomal gel. Proniosomal gel was then mixed with 1% Carbopol® gel in 1:1 ratio. The final ratio of surfactant: alcohol: aqueous phase was 5:5:4 by weight (Fang et al., 2001). The gel obtained was preserved in dark until characterization. In case of incomplete dissolution of the drug in the prepared formulations, the drug and surfactants were dissolved first in chloroform or ether, followed by vacuum evaporation of the solvent (Alsarra et al., 2005).

In order to optimize and evaluate various tenoxicam proniosomal formulations, different grades of two types of non-ionic surfactants: Span® (sorbitan esters) as Span 20, Span 60 and Span 80, and Tween® (polyoxyethylene sorbitan esters) as Tween 20, Tween 60 and Tween 80 were used in addition to cholesterol. Three different aqueous phases (phosphate buffer pH 7.4, distilled water or 0.1% aqueous glycerol solution) were also used (Table 1).

3.2. Characterization of proniosomal gel bases

3.2.1. Light microscopy

A thin layer of proniosomal gel was spread in a cavity slide, and then a cover slip was placed. Slide was observed under microscope with and without polarized light (Leica Q 500 MC Image Analyzer System, Germany). A drop of water was added through the side cover slip into the cavity slide while under microscope and observed again. Photomicrographs were taken at suitable magnifications after addition of water (Vora et al., 1998).

3.2.2. Transmission electron microscopy (TEM)

The morphology of the prepared proniosome formulations was determined by TEM (JEOL 100 CX transmission electron microscope at 80 KV): a drop of the dispersion was diluted 10-fold using deionized water, then a drop of the diluted dispersion was applied to a carbon-coated 300 mesh copper grid and left for 1 min to allow some of the proniosomes to adhere to the carbon substrate. The remaining dispersion was removed by absorbing the drop with the corner of a piece of filter paper. After twice rinsing the grid (deionized water for 3–5 s) a drop of 2% aqueous solution of uranyl acetate was applied for 1 s. The remaining solution was removed by absorbing the liquid with the tip of a piece of filter paper and the sample was air dried (Hu and Rhodes, 1999).

3.2.3. Entrapment studies

To 0.2 g of proniosome gel, weighed in a glass tube, 10 ml of the aqueous phase (phosphate buffer pH 7.4, distilled water or 0.1% aqueous glycerol solution) were added; the aqueous suspension was then sonicated. Niosomes containing tenoxicam were separated from untrapped drug by centrifugation at 9000 rpm for 45 min at 4 °C. The supernatant was recovered and assayed spectrophotometrically using Shimadzu UV spectrophotometer (240 j/PC, Japan), at 358 nm for phosphate buffer pH 7.4 and 351 nm for distilled water and 0.1% aqueous glycerol solution. The encapsulation percentage of drug (EP) was calculated by the following equation

Table 1
Composition of different proniosome formulations.

Formula	Surfactant:phospholipid:cholesterol								
	Span 20	Span 60	Span 80	Tween 20	Tween 60	Tween 80	SBL[a]	EYL[a]	Cholesterol
S1A	9	–	–	–	–	–	–	–	1
S1B	4.5	–	–	–	–	–	4.5	–	1
S1C	4.5	–	–	–	–	–	–	4.5	1
S2A	–	9	–	–	–	–	–	–	1
S2B	–	4.5	–	–	–	–	4.5	–	1
S2C	–	4.5	–	–	–	–	–	4.5	1
S3A	–	–	9	–	–	–	–	–	1
S3B	–	–	4.5	–	–	–	4.5	–	1
S3C	–	–	4.5	–	–	–	–	4.5	1
T1A	–	–	–	9	–	–	–	–	1
T1B	–	–	–	4.5	–	–	4.5	–	1
T1C	–	–	–	4.5	–	–	–	4.5	1
T2A	–	–	–	–	9	–	–	–	1
T2B	–	–	–	–	4.5	–	4.5	–	1
T2C	–	–	–	–	4.5	–	–	4.5	1
T3A	–	–	–	–	–	9	–	–	1
T3B	–	–	–	–	–	4.5	4.5	–	1
T3C	–	–	–	–	–	4.5	–	4.5	1

[a] SBL, soya bean lecithin; EYL, egg yolk lecithin.

(Alsarra et al., 2005):

$$\mathrm{EP} = \left[\frac{C_t - C_r}{C_t}\right] \times 100,$$

where C_t, concentration of total tenoxicam; C_r, concentration of free tenoxicam.

3.2.4. Particle size analysis

The average size of the prepared proniosomes was performed by laser diffraction particle size analysis (LD) using Mastersizer X, Laser Diffraction Particle Size Analyzer (Malvern Instruments Ltd., Malvern, UK). Before measurement, samples were dispersed in distilled water.

3.3. Assessment of physical stability for proniosomes

Aggregation or fusion of the vesicles as a function of temperature was determined as the change in entrapment efficiency after storage. The vesicles were stored in glass vials at room temperature or kept in refrigerator (4–8 °C) for 3 months. The retention of entrapped drug was measured 72 h after preparation and then after 1, 2 and 3 months of storage in selected formulations (Pardakhty et al., 2007). Stability for each formulation was defined in terms of retaining its initial entrapment efficiency for three months duration. Stable formulations were defined as those showing high entrapment efficiency (>60%) and high tenoxicam retention value (>90%), at each time interval. By the end of each month only stable formulations were selected to proceed through the following month stability investigation.

$$\text{Tenoxicam retained in proniosomes} = \left(\frac{\text{Entrapped tenoxicam after storage}}{\text{Entrapped tenoxicam before storage}}\right) \times 100$$

3.4. In vitro release studies

Release experiments were assessed for the selected proniosomal gel formulations showing highest drug entrapment level. The paddle method was applied using phosphate buffer (pH 5.5) and temperature was adjusted at 32 ± 0.5 °C to simulate both, human skin pH and temperature.

Two hundred milligrams of each preparation (containing 10 mg of TX) were accurately weighed and placed in a cellulose bag closed from both sides. The assembly was placed at the bottom of the USP dissolution tester (Erweka Apparatebau GmbH, model DT-D, Germany). The vessel contained 1000 ml buffer solution and the speed was adjusted to 50 rpm (Rao et al., 1989). Aliquots of 5 ml were withdrawn from the release medium at different time intervals (every hour for 6 h) and replaced by equivalent volume of the buffer solution. The amount of drug released from the bases was determined spectrophotometrically at 380.5 nm (Murthy et al., 2004).

Mean cumulative amount of drug released was plotted against time. The data obtained from the release studies were kinetically analyzed and the order of drug release from different formulations was determined. Release efficiency (RE) was also computed by calculating the AUC-values using the trapezoidal method. It is expressed as a percentage of the area of the rectangle corresponding to 100% release for the same total time (6 h) (Csóka et al., 2005). The release rate was determined from the slope of the line obtained on plotting cumulative amount of drug released versus time.

Each experiment was carried three times in order to reach the formulation providing the highest release rate of TX.

3.5. Ex vivo permeation studies

Permeation of TX, through excised rat skin, from the selected proniosomal preparations was assessed. The abdominal hair of male Wistar rats (150 ± 50 gm) was removed carefully. After the animals were sacrificed, the abdominal skin was excised and the adhering fat eliminated. The whole skin was equilibrated in phosphate buffer solution (pH 7.4, the human blood pH) for 1 h before the experiment. This membrane was mounted on a vertical Franz type diffusion cell with the dermis facing the receptor compartment. The donor side was charged with 200 mg of the investigated preparation containing 5% TX. The membrane surface area available for diffusion was 3.14 cm^2. The receptor compartment was filled with the buffer. Temperature was maintained at 37 ± 0.5 °C to simulate human blood temperature. The receptor compartment was constantly stirred at 300 rpm (Escribano et al., 2003).

Samples from the receptor fluid (2 ml) were withdrawn at various time intervals up to 24 h and replaced immediately by fresh buffer solution; to maintain the "sink" conditions constantly and a constant volume as well. The samples were then assayed spectrophotometrically at 358 nm.

Data was analyzed by plotting mean cumulative amount of drug permeated versus time to investigate the best fit to distinct kinetic model (zero, first or Higuchi order) to elucidate the drug permeation mechanism. Flux was calculated from slope of the line obtained on plotting mean cumulative amount permeated per area versus time. Permeation rate was determined; being the slope of the line obtained on plotting cumulative amount of drug permeated versus time. Also, the enhancing ratio (ER: the relationship between the flux from a certain gel and that from the control gel) was calculated from the following equation (Larrucea et al., 2001):

$$\mathrm{ER} = \frac{\text{flux in presence of enhancer}}{\text{flux in absence of enhancer}}$$

3.6. Skin irritancy test

Irritancy test was carried out to determine possible localized reaction of the selected formula on the skin since skin safety is of prior consideration for transdermal delivery systems. A single dose of 200 mg of the selected medicated formulations (10 mg TX) was applied to the left side of the shaved back of male albino rabbits (1.5 ± 0.5 kg) and the right side was considered as control. The control area was further divided into two sub areas, one receiving the selected formulation unloaded with the drug (positive control) and the other receiving no treatment (negative control). The development of erythema was monitored daily for 6 days.Extents of development of erythema were indicated on the basis of the following (Van-Abbé et al., 1975).

0: No erythema development; 2: barely visible few blood vessels and light erythema development; 4: main blood vessels visible and slight erythema development; 6: main blood vessels more obvious and slight erythema development.

Irritation potential was calculated using the following equation:

$$\text{Resultant index} = \frac{A \cdot B}{\text{number of observation days}},$$

where A and B represent erythema value and corresponding day, respectively.

3.7. In vivo studies

3.7.1. Assessment of anti-inflammatory effect

Experimental arthritis was induced in male Wistar rats (200 ± 50 gm) according to the method described by Selye (1949). The animals were divided into four groups, each containing 6 rats. One group received no medication (negative control group) and the second received the oral market tenoxicam tablets (Tilcotil® tablets, Roche) (standard group). The rats' backs in the remaining two groups were shaved: one group served as placebo (positive control group), while the other received the TX transdermal formulation. The anti-inflammatory effect of the four groups was simultaneously monitored. Localized inflammation was induced by subplantar injection of 0.1 ml of 4% formaldehyde solution into the left footpad of the rat's hind paw, 30 min before drug administration where maximum oedema was reached. The initial paw size was then determined using plethysmometer. Each group received its medication and the oedema volume was then assessed at different time intervals using plethysmometer. Readings were taken every hour for 6 h in the first day and then at 24, 48 and 72 h (Moura et al., 2005).

3.7.2. Assessment of antinociceptive activity

In this test, three groups, each comprising 6 mice weighing 25 ± 5 gm, were used. The first group received no treatment (negative control), the second received the oral market product (standard), while the third received the medicated transdermal formulation. The backs of the mice in the third group were shaved.

Analgesic activity was evaluated on the acetic acid-induced abdominal constriction according to Koster et al. (1959). The test was used with local modification as described by Adzu et al. (2001). 10 ml/kg of 0.7% aqueous solution of acetic acid was injected to mice through intra-peritoneal route (i.p.) 30 min after applying the medicated transdermal drug delivery systems (TDDS). Each mouse was placed in a transparent observation cage. Abdominal constriction consisting of contortions of the abdominal muscles (stretching of hind limbs) that occur between 5 and 15 min after acetic acid injection was cumulatively counted. Activity was expressed as percent inhibition of nociception (reduction in episodes of writhing) between standard, control and treated groups (Young et al., 2005).

3.8. Statistical analysis

- Data were collected and coded prior to analysis.
- All data were expressed as mean ± SD.
- For all continuous data that were normally distributed, one way analysis of variance test (ANOVA) followed by the least significant difference test (LSD) or unpaired t-test were performed to compare two or more groups (Pardakhty et al., 2007).
- Statistical analysis was performed using SPSS® software, USA.

4. Results and discussion

4.1. Characterization of proniosome gel formulations

4.1.1. Light microscopy

After adding water to the examined samples; the microscopic examination of the prepared proniosome gel formulations revealed proniosomal vesicular structure with entrapped tenoxicam (Fig. 1).

4.1.2. Transmission electron microscopy (TEM)

The examined proniosomes appeared as spherical nano vesicles under the transmission electron microscope (Fig. 2).

4.1.3. Entrapment studies

4.1.3.1. Proniosomes prepared using phosphate buffer (pH 7.4). Fig. 3 shows that, the lecithin-free formulation S3A showed the highest EP (91.95%), while the formulation T3A showed the lowest EP (40.23%) ($p < 0.05$). It is also evident that incorporation of EYL has a superior effect on enhancing TX entrapment in the prepared proniosomes compared to the effect of adding SBL ($p < 0.05$). It was, as well, found that using Tween 60 either alone or in the presence of SBL or EYL yield a preparation entrapping tenoxicam efficiently compared to Tween 20 and Tween 80 ($p < 0.05$).

4.1.3.2. Proniosomes prepared using distilled water. Fig. 4 clarifies that, the lecithin-free formulation T2A showed a significantly high EP (95.16%), compared to the other prepared proniosomes ($p < 0.05$), followed by the formulations T3A and S3A (93.38, 93.33%, respectively). On the other hand the formulation S2C showed the lowest EP (61.38%) ($p < 0.05$). In addition, it is generally clear that proniosomes prepared with different grades of Tween were able to entrap TX more efficiently than those prepared with different Spans ($p < 0.05$).

4.1.3.3. Proniosomes prepared using 0.1% glycerol. From Fig. 5 it is noticed that the formulation T2B possessed the highest EP (72.91%), followed by S2C (72.07%), compared to the other proniosomal preparations ($p < 0.05$), whereas, the formulation T1B showed the

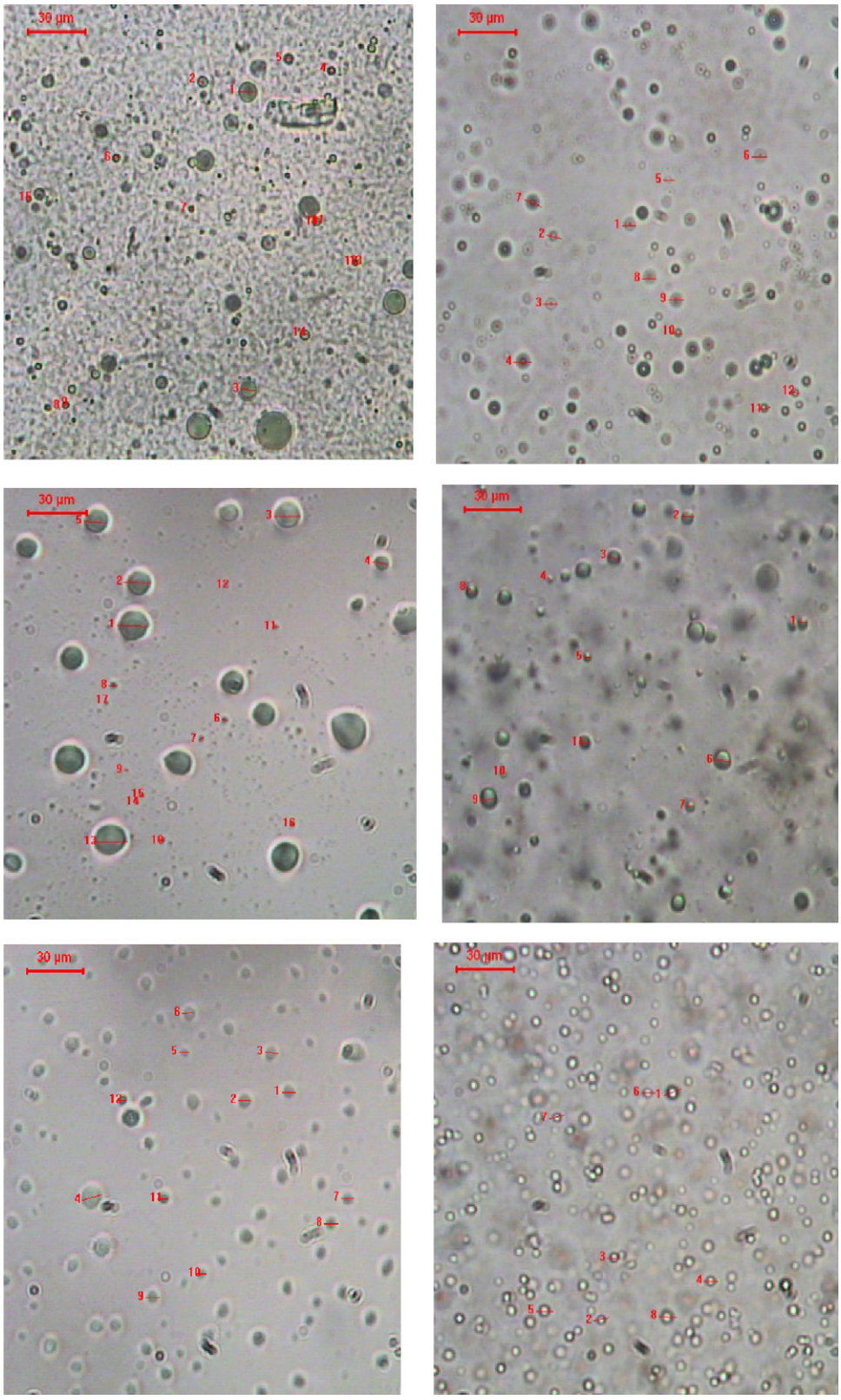

Fig. 1. Light Microscopy of some proniosome formulations entrapping TX.

lowest EP (22.35%) ($p < 0.05$). It was also observed that conjugation of EYL resulted in a significant reduction in the EP of all formulations, compared to their lecithin-free analogues, with the exception of S2C, where a significant increase was observed ($p < 0.05$). Considering the influence of different non-ionic surfactants used, it is evident that various Tween grades yield proniosomes which can entrap TX more efficiently than those prepared using different Span types ($p < 0.05$). It was also found that combining Span 60 with EYL resulted in a formulation with a significantly high EP compared to other Span preparations. On the other hand, mixing Tween 60 with

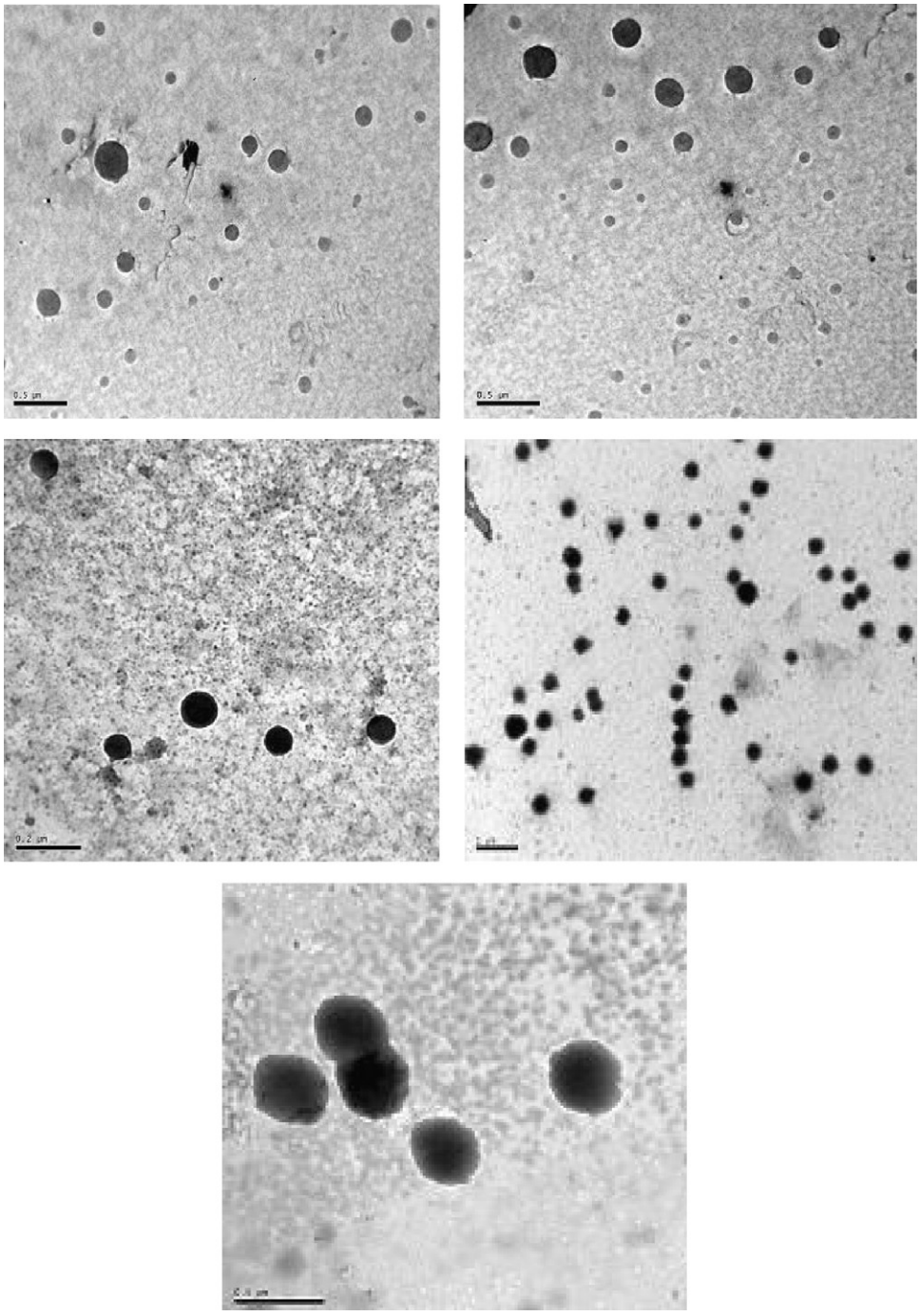

Fig. 2. TEM of some proniosome formulations showing different sizes of spherical nano vesicles.

SBL produced the most efficient formulations compared to other Tween preparations ($p < 0.05$).

Briefly; the EP of proniosomes prepared using 0.1% glycerol is much lower than those prepared using either distilled water or phosphate buffer ($p < 0.05$). Of all the prepared formulations, T2A (distilled water) has the highest EP: 95.16% ($p < 0.05$), followed by T3A (distilled water): 93.38% and then S3A (distilled water): 93.33%. It is evident that Tweens, as non-ionic surfactants, had better effect on TX entrapment than Spans. This could be explained on the basis that the highly lipophilic portion of the drug is expected to be housed almost completely within the lipid bilayer of the niosomes (Gulati et al., 1998), and since it is known that most of the surfactants used to make nonionic-surfactant vesicles have a low aqueous solubility, it has been suggested from previous studies that low drug EP, in general, may be attributed to the small number of niosomes produced by dilute surfactant (Hao et al., 2002). However, more soluble nonionic surfactants as Tweens especially the freely soluble ones as Tween 20 can form micelles on hydration in the presence of cholesterol (Uchegbu and Vyas, 1998). As for the effect of phospholipids; it is clear that it is highly influenced by the type of non-ionic surfactant and the aqueous medium used, since variable results were obtained in each case. In this means, the obtained conclusion goes with that previously reported by Fang et al.: both the type and content of non-ionic surfactants in proniosomes are crucial factors affecting the efficiency of drug delivery (Fang et al., 2001).

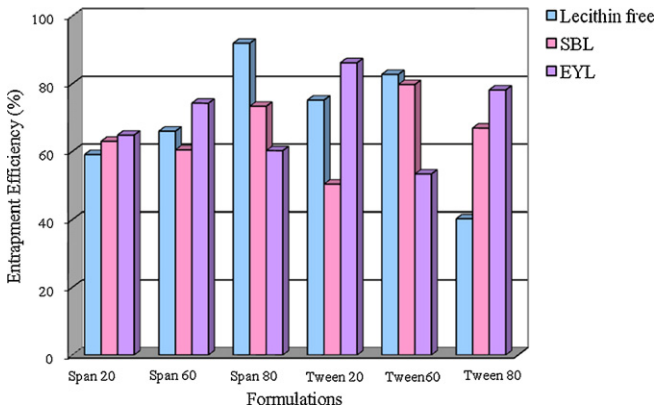

Fig. 3. Entrapment efficiency of proniosomes prepared using phosphate buffer (pH 7.4).

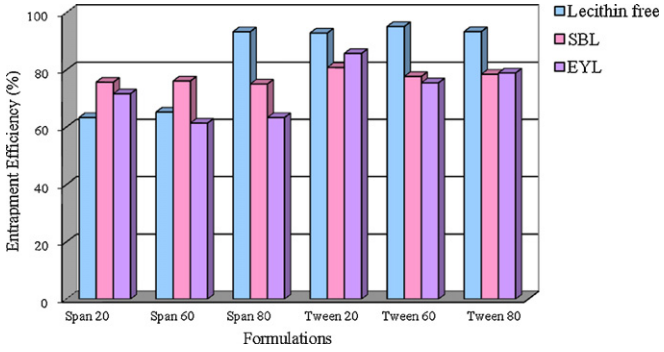

Fig. 4. Entrapment efficiency of proniosomes prepared using distilled water.

4.1.4. Particle size analysis

Determining the particle size of the prepared proniosomes; it was found that size range lied between 50 and 980 nm, with a mean particle size 479.17, 380.8 and 627.86 nm for proniosomes prepared using phosphate buffer, distilled water and 0.1% glycerol, respectively. Some scattered micro particles appeared, as well, in the field with mean size 2 ± 0.5 μm. Such micro particles are rather thought as aggregations of nano particles.

On studying the effect of particle size on entrapment efficiency, it was noticed that an inversely proportional relationship correlated them; formulations with smaller mean particles' size possessed higher EP value.

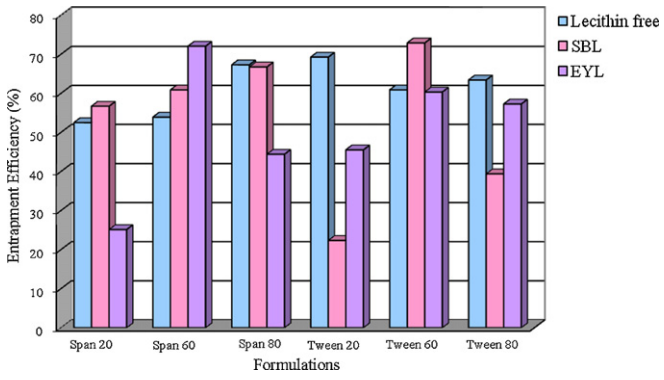

Fig. 5. Entrapment efficiency of proniosomes prepared using 0.1% glycerol.

4.2. Assessment of physical stability for proniosomes

4.2.1. At refrigeration temperature (4–8 °C)

After three months of stability monitoring, it is concluded that, among the formulations prepared using phosphate buffer (pH 7.4), the formulation T2B proved to be the most stable. On the other hand, the formulations S3B, T1A, T1B, T2B and T3C are the most stable, compared to other formulations prepared using distilled water. It is also clear that the formulation S3B prepared using 0.1% glycerol is the most stable among other similar formulations. All the previously mentioned formulations showed, both, high initial entrapment efficiency (>60%) and high tenoxicam retention value (>90%), by the end of each month (Tables 2–4).

4.2.2. Storage at room temperature

Studying the stability of different proniosomal formulations prepared with different aqueous phases for three months at room temperature revealed that the formulations S3B, T1A and T2B prepared using distilled water are the most stable, since they were the only showing both, high initial entrapment efficiency (>60%) and high tenoxicam retention value (>90%), throughout the three months. However, those prepared using phosphate buffer (pH 7.4) or 0.1% glycerol, showed certain stability till either the first or second month of storage but none was stable by the end of the third month (Tables 2–4).

Comparing stability of different proniosomal formulations at both refrigeration and room temperatures; the following is observed: (1) proniosomes are generally more stable at low temperature, (2) distilled water, not only did it provide the optimal entrapment conditions for TX, but also, yield the most stable proniosomes, followed by buffer preparations and finally, those prepared using glycerol. This would suggest that both storage conditions and type of aqueous medium might influence the tactness of proniosomes, thus, affecting their entrapment efficiencies. Formulations S3B, T1A and T2B, (distilled water), were the only ones proving stability at both room and refrigeration temperatures.

As a result of stability studies; stable formulations were subjected to further *in vitro* release studies: T2B (phosphate buffer pH 7.4), S3B, T1A, T1B, T2B and T3C (distilled water), and S3B (0.1% glycerol).

4.3. In vitro release studies

The release of TX from the investigated stable formulations followed zero order kinetics with an immediate release of TX (no lag time). The lecithin-free formula T1A, (distilled water), showed both the highest release rate and efficiency; 0.85 mg/h and 31.77%, respectively ($p < 0.05$), while, T2B, (phosphate buffer pH 7.4), showed the lowest values for both; 0.25 mg/h and 7.61%, respectively ($p < 0.05$). Formulations T3C and S3B (distilled water) showed the same release rate as T2B, (phosphate buffer pH 7.4), but a higher release efficiency 14.82 and 15.85 mg/h, respectively ($p < 0.05$). Formulation S3B, (0.1% glycerol), showed release rate: 0.59 mg/h and release efficiency: 25.31% (Figs. 6 and 7
).

Comparing the release profile of formulation T1A to that of Carbopol® gel and other formulations; higher release rate and efficiency were observed, revealing that this particular proniosomal structure improved TX release properties (Table 5). This may be attributed to the hydrophilic nature of Tween 20 which makes it act as a solubilizing agent for the drug, thus, facilitating drug release from the gel base (Vora et al., 1998).

The formulation T1A (Tween 20, cholesterol, distilled water), selected as the most efficient and most stable formulation, was subjected to further investigations.

Table 2
Stability studies of proniosomes prepared using phosphate buffer pH 7.4.

Formula	EP_0* (%)	Stability under refrigeration						Stability at room temperature					
		EP_1* (%)	EP_2* (%)	EP_3* (%)	TXR* (%)			1st month	2nd month	3rd month	TXR* (%)		
					1st month	2nd month	3rd month				1st month	2nd month	3rd month
S1A	59.08	53.10	–	–	89.88	–	–	46.90	–	–	79.38	–	–
S1B	62.99	46.90	–	–	74.45	–	–	60.92	–	–	96.72	–	–
S1C	64.83	61.49	58.16	–	94.86	89.72	–	35.17	–	–	54.26	–	–
S2A	65.98	62.87	59.77	57.63	95.30	90.59	87.35	34.02	–	–	51.57	–	–
S2B	60.46	39.31	–	–	65.02	–	–	60.44	55.76	50.22	99.97	92.23	83.06
S2C	74.25	43.68	–	–	58.82	–	–	54.48	–	–	73.37	–	–
S3A	91.95	83.22	61.84	–	90.50	67.25	–	74.48	–	–	81.00	–	–
S3B	73.33	43.22	–	–	58.93	–	–	56.78	–	–	77.43	–	–
S3C	60.23	58.28	56.32	51.96	96.76	93.51	86.27	39.77	–	–	66.03	–	–
T1A	75.17	57.70	–	–	76.76	–	–	42.30	–	–	56.27	–	–
T1B	50.35	48.74	–	–	96.80	–	–	49.66	43.66	–	98.63	86.71	–
T1C	86.21	58.93	–	–	68.36	–	–	51.03	–	–	59.20	–	–
T2A	82.76	52.30	–	–	63.19	–	–	74.02	–	–	89.44	–	–
T2B	79.77	78.74	77.70	77.60	98.70	97.41	97.28	20.23	–	–	25.36	–	–
T2C	53.33	47.70	–	–	89.44	–	–	46.67	–	–	87.50	–	–
T3A	40.23	33.45	–	–	83.14	–	–	39.77	–	–	98.86	–	–
T3B	66.90	51.03	–	–	76.29	–	–	48.97	–	–	73.20	–	–
T3C	78.16	58.85	–	–	75.30	–	–	64.37	–	–	82.35	–	–

EP_0*, EP_1*, EP_2* and EP_3*: entrapment percentage at zero time (72 h), after 1st, 2nd, and 3rd months, respectively, TXR*: tenoxicam retained = (entrapped tenoxicam after storage/entrapped tenoxicam before storage) × 100.

Table 3
Stability studies of proniosomes prepared using distilled water.

Formula	EP_0* (%)	Stability under refrigeration						Stability at room temperature					
		EP_1* (%)	EP_2* (%)	EP_3* (%)	TXR* (%)			1st month	2nd month	3rd month	TXR* (%)		
					1st month	2nd month	3rd month				1st month	2nd month	3rd month
S1A	63.33	43.50	–	–	68.69	–	–	56.50	–	–	89.23	–	–
S1B	75.69	61.83	–	–	81.69	–	–	74.20	25.80	–	98.03	34.08	–
S1C	71.64	54.05	–	–	75.45	–	–	36.46	–	–	50.89	–	–
S2A	65.25	62.90	59.59	55.64	96.41	91.32	85.28	37.10	–	–	56.86	–	–
S2B	76.12	75.16	68.96	63.59	98.74	90.59	83.54	74.20	70.58	49.42	97.48	92.72	64.93
S2C	61.38	52.99	–	–	86.32	–	–	57.78	48.19	–	94.14	78.51	–
S3A	93.33	40.09	–	–	42.95	–	–	59.91	–	–	64.19	–	–
S3B	75.05	71.64	68.22	67.98	95.46	90.91	90.58	68.91	68.23	67.59	91.81	90.91	90.06
S3C	63.33	53.09	–	–	83.84	–	–	46.91	–	–	74.07	–	–
T1A	92.88	91.89	91.02	90.76	98.93	98.00	97.72	91.22	90.97	89.71	98.21	97.94	96.59
T1B	80.81	78.25	75.42	74.33	96.84	93.33	91.98	21.75	–	–	26.91	–	–
T1C	85.75	70.68	–	–	82.43	–	–	78.47	62.90	–	91.51	73.35	–
T2A	95.16	84.40	82.30	–	88.69	86.49	–	90.88	87.15	84.36	95.50	91.58	88.65
T2B	77.70	76.38	75.95	75.05	98.30	97.75	96.59	75.69	74.41	73.57	97.42	95.77	94.69
T2C	75.48	55.33	–	–	73.30	–	–	35.18	–	–	46.62	–	–
T3A	93.38	84.99	80.76	–	91.02	86.49	–	92.20	70.58	–	98.73	75.58	–
T3B	78.47	58.64	–	–	74.73	–	–	38.81		–	49.46	–	–
T3C	78.89	76.76	74.63	74.21	97.30	94.60	94.07	74.63	21.11	–	94.59	26.76	–

EP_0*, EP_1*, EP_2* and EP_3*: entrapment percentage at zero time (72 h), after 1st, 2nd, and 3rd months, respectively; TXR*: tenoxicam retained = (entrapped tenoxicam after storage/entrapped tenoxicam before storage) × 100.

Table 4
Stability studies of proniosomes prepared using 0.1%glycerol.

Formula	EP_0* (%)	Stability under refrigeration						Stability at room temperature					
		EP_1* (%)	EP_2* (%)	EP_3* (%)	TXR* (%)			1st month	2nd month	3rd month	TXR* (%)		
					1st month	2nd month	3rd month				1st month	2nd month	3rd month
S1A	52.51	36.59	–	–	69.68	–	–	52.11	50.00	44.35	99.23	95.21	84.46
S1B	56.70	55.87	–	–	98.52	–	–	56.70	55.87	43.30	100.00	98.52	76.35
S1C	25.14	12.57	–	–	50.00	–	–	24.97	–	–	99.32	–	–
S2A	53.91	41.90	–	–	77.72	–	–	47.30	–	–	87.74	–	–
S2B	60.89	53.63	46.38	–	88.07	76.15	–	39.11	–	–	64.22	–	–
S2C	72.07	57.26	–	–	79.46	–	–	27.93	–	–	38.76	–	–
S3A	67.32	59.92	52.52	–	89.00	78.01	–	32.68	–	–	48.55	–	–
S3B	66.76	65.36	63.62	63.28	97.91	95.29	94.79	55.12	–	–	82.57	–	–
S3C	44.41	42.74	–	–	96.23	–	–	43.76	–	–	98.53	–	–
T1A	69.27	62.74	62.29	59.84	90.57	95.29	86.39	56.42	–	–	81.45	–	–
T1B	22.35	11.73	–	–	52.50	–	–	22.01	–	–	98.50	–	–
T1C	45.53	41.34	–	–	90.80	–	–	45.23	–	–	99.34	–	–
T2A	60.89	57.96	55.03	53.77	95.18	90.37	88.30	39.11	–	–	64.22	–	–
T2B	72.91	58.10	–	–	79.69	–	–	70.67	69.33	29.33	96.93	95.10	40.23
T2C	60.34	52.37	50.42	–	86.79	83.56	–	39.66	–	–	65.74	–	–
T3A	63.41	33.52	–	–	52.86	–	–	60.34	59.66	39.67	95.15	94.10	62.56
T3B	39.39	24.30	–	–	61.70	–	–	35.70	–	–	90.64	–	–
T3C	57.26	19.55	–	–	34.15	–	–	55.45	39.55	–	96.83	69.07	–

EP_0*, EP_1*, EP_2* and EP_3*: entrapment percentage at zero time (72 h), after 1st, 2nd, and 3rd months, respectively; TXR*: tenoxicam retained = (entrapped tenoxicam after storage/entrapped tenoxicam before storage) × 100.

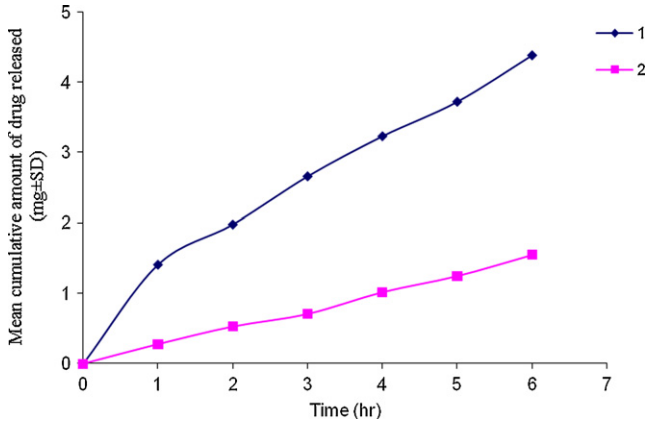

Fig. 6. Release profile of TX from certain proniosome formulations. 1. (T2B) using phosphate buffer (pH 7.4) as aqueous phase; 2. (S3B) using 0.1% glycerol as aqueous phase.

4.4. Effect of cholesterol:Tween 20 ratio on the entrapment efficiency

Generally, no significant difference is noticed in the entrapment efficiency of the formulation T1A on changing cholesterol:Tween 20 ratio. This might be attributed to a previous report stating that a fixed amount of lipid in the formula produces constant number of niosomes and has a definite encapsulating capacity; increasing this amount has no beneficial effect. It has, as well, been reported that proniosomal formulation with molar ratio (cholesterol:non-ionic surfactant) of 1:9 is, relatively, the most beneficial for the efficient encapsulation, and extra Cholesterol is unfavorable (Hao et al., 2002).

4.5. Effect of drug content on entrapment efficiency

Increasing the amount of TX added, from 0.1 mg to 10 mg, led to 100% entrapment and any further increase in amount of TX added (20 or 40 mg) had no further effect.

4.6. Ex vivo permeation studies

Fig. 8 shows that permeation of TX entrapped in the proniosomal formula T1A through the skin follows zero order kinetics. It is also evident that the mentioned formula effectively enhanced tenoxicam permeation, showing high ER (6.24) reflecting a high flux value (0.11 mg/cm^2/h), and relatively slow permeation rate (0.34 mg/h). It has been suggested that the significantly high flux value of the drug, compared to that of Carbopol® gel (0.017 mg/cm^2/h), might

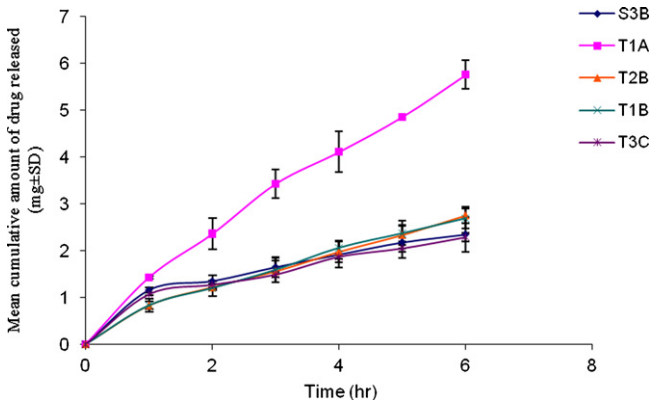

Fig. 7. Release profile of TX from proniosomes prepared with distilled water.

Table 5
Release properties of TX from proniosome formulations compared to Carbopol® gel.

Formulation	Aqueous phase	Release rate (mg/h)	Release efficiency (%)
T2B	Phosphate buffer (pH 7.4)	0.25	7.61
S3B		0.25	15.85
T1A		0.85	31.77
T1B	Distilled water	0.38	15.66
T2B		0.38	15.52
T3C		0.25	14.82
S3B	0.1%Glycerol	0.59	25.31
Carbopol®		0.61	28.56

probably be attributed to fusion of noisome vesicles to the surface of skin (Barry, 2001), due to direct transfer of drug from vesicles to the skin (Alsarra et al., 2005). All these factors would help achieving a sustained release of tenoxicam into the blood stream.

In order to verify the predominant driving force for tenoxicam proniosomes permeating across skin, some efforts were made to clarify these mechanisms. The release rate of the drug across cellulose membrane was previously determined and compared to its flux through rat skin. It was found that the release rate was significantly higher than its flux across skin. This is thought to indicate the barrier properties of skin for the drug, as well as, indicating that different mechanisms of drug transport across skin from niosomes and proniosomes might be involved. Also, it has been suggested that both penetration enhancing effect of non-ionic surfactant and vesicle-skin interaction may contribute to the enhancing mechanisms for proniosome permeation (Fang et al., 2001).

4.7. Skin irritancy test

The selected proniosome formulation (T1A) showed an irritation potential of 0.33, thus proving to be non-irritant as it was mentioned by Van-Abbé et al., that a value between 0 and 9 in an irritancy test indicates that the applied formulation is generally non-irritant to human skin (Van-Abbé et al., 1975). No obvious erythema, oedema or inflammation was observed on rabbits' skin after one week of application of the selected formulation.

4.8. In vivo studies

4.8.1. Assessment of anti-inflammatory effect

Fig. 9 reveals maximum increase in oedema in rats receiving no treatment (negative control) after 6 h with mean increase value equivalent to 96.02%, and then a sharp decrease followed reaching 53.67% after 72 h. Oedema size in rats receiving oral tenoxicam tablets (Standard) increased rapidly till the rat paw reached a maximum size with mean increase in oedema equivalent to 80.78% at 6 h, followed by a slightly sharp decrease reporting a mean increase

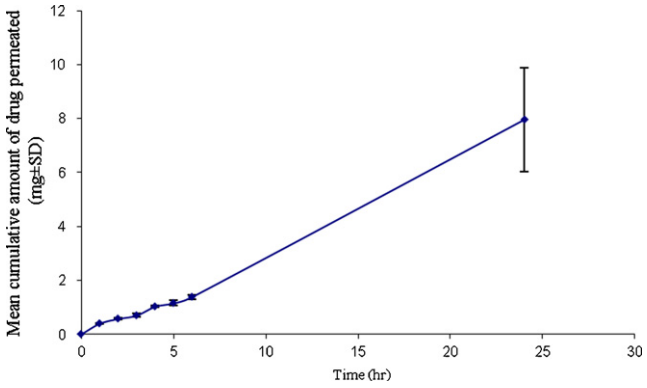

Fig. 8. Permeation profile of TX from the formula T1A through rat skin.

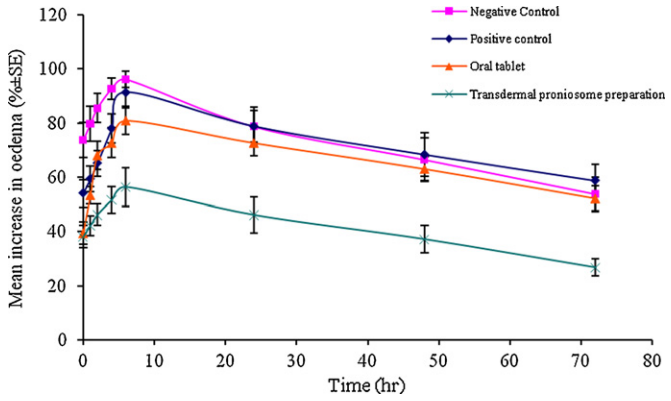

Fig. 9. Effect of the transdermal tenoxicam proniosome gel formulation on inhibition of oedema in the hind paw of rats.

in oedema size 52.04% after 72 h. Rats receiving the non-medicated transdermal proniosome preparation (positive control) possessed an increase in oedema with maximum value after 6 h (91.18%) and then it decreased to 58.57% after 72 h. Rats receiving the medicated transdermal proniosome preparation showed maximum increase in oedema size after 6 h with a mean increase of 56.30%, which then started to decrease till 26.59% after 72 h.

On comparing the anti-inflammatory efficiency of the selected transdermal tenoxicam formulation to the efficiency of the oral market tenoxicam tablets of the same dose (20 mg), it was found that the medicated proniosome gel formula significantly inhibited the induced oedema ($p < 0.05$) showing the least AUC value (49.51), whereas the market product showed a higher value (79.75). It was also noticed that both the negative control group and the placebo group showed, more or less, the same AUC value (87.91 and 87.35, respectively). This might indicate that the non-medicated preparation has no effect on its own.

4.8.2. Assessment of antinociceptive effect

Results compiled in Table 6 show the number of writhes noticed and the degree of inhibition achieved by the investigated formulation in comparison with the oral tenoxicam market product.

The examined transdermal tenoxicam proniosome formulation significantly reduced the number of writhes, by 68.15%, compared to the oral market product ($p < 0.05$), which inhibited the acetic acid induced writhes by 63.06%. This goes exactly with the results obtained from formalin induced oedema test, where the proniosome formulation proved to be more efficient as well.

Table 6
Effect of different formulations on acetic acid-induced writhing in mice.

Preparation	Number of writhes (mean ± SE)	Percent inhibition (%)
Negative control	52.33 ± 2.04	–
Oral market product	19.33 ± 0.71	63.06
Proniosome gel	16.67 ± 1.86	68.15

Although it has been suggested that this pain mechanism is believed to involve, in part, local peritoneal receptors (Bentley et al., 1983), it caused peritoneal fluid concentration of PGE2α and PGF2α (Deraedt et al., 1980), it was found that the intraperitoneal administration of acetic acid induces the liberation, not only, of PGs but also sympathetic nervous system mediators (Neto et al., 2005). Thus, this explains the similarity in results obtained from both the writhing and oedematogenic test. Consequently, this clarifies the fact that an anti-inflammatory substance may also be involved in the peripheral analgesic activity (Vasudevan et al., 2007).

5. Conclusion

From the presented study, it is clear that the lecithin-free proniosomal gel formulation T1A (Tween 20:cholesterol, 9:1 and distilled water as aqueous phase) provides both the highest entrapment efficiency and stability among other proniosome formulations, in addition to highest release efficiency. T1A has, as well, showed a significantly higher therapeutic efficacy ($p < 0.05$) – revealed from monitoring both the anti-inflammatory and antinociceptive effects – compared to the oral market tenoxicam tablets of the same dose. Thus, announcing a more promising tenoxicam dosage form.

References

Adzu, B., Amos, S., Wambebe, C., Gamaniel, K., 2001. Anti-nociceptive activity of the aqueous extract of *Zizyphus spina-christi* root bark. Fitoterapia 72, 344–350.

Al-Obaid, A.M., Mian, M.S., 1993. In: Brittain, H.G. (Ed.), Analytical Profiles of Drug Substances and Excipients, vol. 22. Academic Press, New York, pp. 431–453.

Alsarra, I.A., Bosela, A.A., Ahmed, S.M., Mahrous, G.M., 2005. Proniosomes as a drug carrier of ketorolac. Eur. J. Pharm. Biopharm. 59, 485–490.

Arunothayanun, P., Bernard, M.-S., Craig, D.Q.M., Ucheghu, I.F., Fluorence, A.T., 2000. The effect of processing variables on the physical characteristics of non-ionic surfactant vesicles (niosomes) formed from a hexadecyl diglycerol ether. Int. J. Pharm. 201, 7–14.

Barry, B.W., 2001. Novel mechanisms and devices to enable successful transdermal drug delivery. Eur. J. Pharm. Sci. 14, 101–114.

Bentley, G.A., Newton, S.H., Starr, J., 1983. Studies on the antinociceptive action of α-agonist drugs and their interaction with opioid mechanisms. Br. J. Pharmacol. 79, 125–134.

Betageri, G., Habib, M., 1994. Liposomes as drug carriers. Pharm. Eng. 14, 76–77.

Bird, H.A., Hill, J., Lowe, J.R., Wright, V., 1984. A double-blind comparison of tenoxicam (Tilcotil, Mobiflex) at two doses against ibuprofen in rheumatoid arthritis. Eur. J. Rheumatol. Inflamm. 7, 28–32.

Blazek-Welsh, A., Rhodes, D.G., 2001. Maltodextrin based proniosomes. AAPS Pharm. Sci. 3, 1–8.

Csóka, I., Csányi, E., Zapantis, G., Nagy, E., Fehér-Kiss, A., Horváth, G., Blazso, G., Eros, I., 2005. In vitro and in vivo percutaneous absorption of topical dosage forms: case studies. Int. J. Pharm. 291, 11–19.

Deraedt, R., Jouquey, S., Delevallee, F., Flahaut, M., 1980. Release of prostaglandins E and F in an algogenic reaction and its inhibition. Eur. J. Pharmacol. 61, 17–24.

Elias, P.M., Friend, D.S., 1975. The permeability barrier in mammalian epidermis. J. Cell Biol. 65, 180–191.

Escribano, E., Calpena, A.C., Queralt, J., Obach, R., Doménech, J., 2003. Assessment of diclofenac permeation with different formulations: anti-inflammatory study of a selected formula. Eur. J. Pharm. Sci. 19, 203–210.

Fang, J.-Y., Yu, S.-Y., Wu, P.-C., Huang, Y.-B., Tsai, Y.-H., 2001. In vitro skin permeation of estradiol from various proniosome formulations. Int. J. Pharm. 215, 91–99.

Frfkjaer, S., Hjorth, E., Wfrtis, O., 1984. Stability testing of liposomes during storage. In: Gregoriadis, G. (Ed.), Liposome Technology, vol. 1. CRC Press, Boca Raton, FL, pp. 235–245.

González, J.P., Todd, P.A., 1987. Tenoxicam: a preliminary review of its pharmacodynamic and pharmacokinetic properties and therapeutic efficacy. Drugs 34, 289–310.

Gulati, M., Grover, M., Singh, M., 1998. Lipophilic drug derivatives in liposomes. Int. J. Pharm. 165, 129–168.

Hadgraft, J., Guy, R.H., 1987. Transdermal drug delivery: a perspective. J. Control. Rel. 4, 237–251.

Hao, Y., Zhao, F., Li, N., Yang, Y., Li, K., 2002. Studies on a high encapsulation of colchicine by a niosome system. Int. J. Pharm. 244, 73–80.

Hatziantoniou, S., Rallis, M., Demetzos, C., Papaioannou, G.Th., 2000. Pharmacological activity of natural lipids on a skin barrier disruption model. Pharmacol. Res. 42, 55–59.

Hu, C.J., Rhodes, D.G., 1999. Proniosomes: a novel drug carrier preparation. Int. J. Pharm. 185, 23–35.

Huang, Y.-C., Tsai, S.-K., Huang, C.-H., Wang, M.-H., Lin, P.-L., Chen, L.-K., Lin, C.-J., Sun, W.-Z., 2002. Intravenous tenoxicam reduces uterine cramps after Cesarean delivery. Can. J. Anesth. 49, 384–387.

Hunt, C., Tsang, S., 1981. α-Tocopherol retards auto-oxidation and prolongs the shelf-life of liposomes. Int. J. Pharm. 8, 101–110.

Information for Health Professionals, 2006. Data Sheet: Tilcotil®, http://www.medsafe.govt.nz/Profs/Datasheet/t/Tilcotiltabsuppinj.htm.

Kirjavainen, M., Urtti, A., Jääskeläinen, I., Suhonen, T.M., Paronen, P., Valjakka-Koskela, R., Kiesvaara, J., Mönkkönen, J., 1996. Interaction of liposomes with human skin in vitro-the influence of lipid composition and structure. Biochim. Biophys. Acta 1304, 179–189.

Koster, R., Anderson, M., De-Beer, E.J., 1959. Acetic acid analgesic screen. Federation Proc. 18, 418–420.

Larrucea, E., Arellano, A., Santoyo, S., Ygartua, P., 2001. Combined effect of oleic acid and propylene glycol on the percutaneous penetration of tenoxicam and its retention in the skin. Eur. J. Pharm. Biopharm. 52, 113–119.

Morof, O., Herrmann, T., Roth, M.J., 1988. Effect of tenoxicam and two of its main metabolites-5'-OH tenoxicam and 6'-OH tenoxicam-on prostaglandin H synthase and release of leukotriene B4 from human PMNLs. In: Presented at the Sixth South East Asia and Pacific League against Rheumatism Congress of Rheumatology, pp. 5–10.

Moura, A.C.A., Silva, E.L.F., Fraga, M.C.A., Wanderley, A.G., Afiat Pour, P., Maia, M.B.S., 2005. Anti-inflammatory and chronic toxicity study of the leaves of *Ageratum conyzoides* L. in rats. Phytomedicine 12, 138–142.

Murthy, S.N., Hiremath, S.R.R., Paranjothy, K.L.K., 2004. Evaluation of carboxymethyl guar films for the formulation of transdermal therapeutic systems. Int. J. Pharm. 272, 11–18.

Neto, A.G., Costa, J.M.L.C., Belati, C.C., Vinholis, A.H.C., Possebom, L.S., Da Silva Filho, A.A., Cunha, W.R., Carvalho, J.C.T., Bastos, J.K., Silva, M.L.A., 2005. Analgesic and anti-inflammatory activity of a crude root extract of Pfaffia glomerata (Spreng) Pedersen. J. Ethnopharmacol. 96, 87–91.

Niemiec, S.M., Ramachandran, C., Weiner, N., 1995. Influence of non-ionic liposomal composition on topical delivery of peptide drugs into pilosebaceous units: an in vivo study using the hamster ear model. Pharm. Res. 12, 1184–1188.

Pardakhty, A., Varshosaz, J., Rouholamini, A., 2007. In vitro study of polyoxyethylene alkyl ether niosomes for delivery of insulin. Int. J. Pharm. 328, 130–141.

Perrett, S., Golding, M., Williams, W.P., 1991. A simple method for the preparation of liposomes for pharmaceutical application and characterization of liposomes. J. Pharm. Pharmacol. 43, 154–161.

Prausnitz, M.R., Mitragotri, S., Langer, R., 2004. Current status and future potential of transdermal drug delivery. Nat. Rev., Drug Discov. 3, 115–124.

Rao, R., Giridhar, R., Avadhanulu, A.B., Pantulu, A.R.R., 1989. Gas liquid chromatographic determination of terbutaline sulfate and guiaphenesin in combined dosage forms. East. Pharm. 32, 131–135.

Schreier, H., Bouwstra, J., 1994. Liposomes and niosomes as topical drug carriers: dermal and transdermal drug delivery. J. Control. Rel. 30, 1–15.

Selye, H., 1949. Further studies concerning the participation of the adrenal cortex in the pathogenesis of arthritis. Br. Med. J. 2, 1129–1135.

Sporn, M.B., Suh, N., 2000. Chemoprevention of cancer. Carcinogenesis 21, 525–530.

Thadikonda, K.P., Lan-Cam, C.A., Thadikonda, V.L., Theofanopoulous, V., 1995. The intranasal absorption of the quaternary ammonium compounds neostigmine bromide and tubocurarine chloride in the rat. Drug Dev. Ind. Pharm. 21 (4), 461–473.

Thong, H.-Y., Zhai, H., Maibach, H.I., 2007. Percutaneous penetration enhancers: an overview. Skin Pharmacol. Physiol. 20, 272–282.

Todd, P.A., Clissold, S.P., 1991. Tenoxicam—an update of its pharmacology and therapeutic efficacy in rheumatic diseases. Drugs 41 (4), 625–646.

Uchegbu, I.F., Vyas, S.P., 1998. Nonionic surfactant based vesicles (niosomes) in drug delivery. Int. J. Pharm. 172, 33–70.

Van-Abbé, N.J., Nicholas, P., Boon, E., 1975. Exaggerated exposure in topical irritancy and sensitization testing. J. Soc. Cosmet. Chem. 26, 173.

Vartiainen, N., Huang, C.Y., Salminen, A., Goldsteins, G., Chan, P.H., Koistinaho, J., 2001. Piroxicam and NS-398 rescue neurones from hypoxia/reoxygenation damage by a mechanism independent of cyclooxygenase inhibition. J. Neurochem. 76, 480–489.

Vasudevan, M., Gunnam, K.K., Parle, M., 2007. Antinociceptive and anti-inflammatory effects of *Thespesia populnea* bark extract. J. Ethnopharmacol. 109, 264–270.

Vora, B., Khopade, A.J., Jain, N.K., 1998. Proniosome based transdermal delivery of levonorgestrel for effective contraception. J. Control. Rel. 54, 149–165.

Waterworth, R.F., Waterworth, S.M., 1987. An open assessment of tenoxicam (Tilcotil) in the treatment of acute gout in general practice. N. Z. Med. J. 100, 744–745.

Wong, M., Thompson, T., 1982. Aggregation of dipalmitoyl–phosphotidyl–choline vesicles. Biochemistry 21, 4133–4139.

Woolf, T.F., Radulovic, L.L., 1989. Oxicams: metabolic disposition in man and animals. Drug Metab. Rev. 21, 255–276.

Young, J.M., Luo, Y., Cheng, H., Hsieh, W., Liao, J., Peng, W., 2005. Analgesic and anti-inflammatory activities of 6-gingerol. J. Ethnopharmacol. 96, 207–210.