

Research paper

Proniosomes as a drug carrier for transdermal delivery of ketorolac

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Abstract

Niosomes are nonionic surfactant vesicles that have potential applications in the delivery of hydrophobic and hydrophilic drugs. Permeation of a potent nonsteroidal anti-inflammatory, ketorolac, across excised rabbit skin from various proniosome gel formulations was investigated using Franz diffusion cells. Each of the prepared proniosomes significantly improved drug permeation and reduced the lag time ($P < 0.05$). Proniosomes prepared with Span 60 provided a higher ketorolac flux across the skin than did those prepared with Tween 20 (7- and 4-fold the control, respectively). A change in the cholesterol content did not affect the efficiency of the proniosomes, and the reduction in the lecithin content did not significantly decrease the flux ($P > 0.05$). The encapsulation efficiency and size of niosomal vesicles formed by proniosome hydration were also characterized by specific high performance liquid chromatography method and scanning electron microscopy. Each of the prepared niosomes achieved about 99% drug encapsulation. Vesicle size was markedly dependent on the composition of the proniosomal formulations. Proniosomes may be a promising carrier for ketorolac and other drugs, especially due to their simple production and facile up.

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1. Introduction

Drug delivery systems using colloidal particulate carriers such as liposomes [1] or niosomes [2] have distinct advantages over conventional dosage forms. These carriers can act as drug reservoirs, and modification of their composition or surface can adjust the drug release rate and/or the affinity for the target site.

Liposomes or niosomes can carry hydrophilic drugs by encapsulation or hydrophobic drugs by partitioning of these drugs into hydrophobic domains. Liposomes are unilamellar or multilamellar spheroid structures composed of lipid molecules, often phospholipids, assembled into bilayers. Because of their ability to carry a variety of drugs, liposomes have been extensively investigated for their potential application in pharmaceutics; such as drug

delivery [3,4], drug targeting [5]; controlled release [6] or increased solubility [3].

However, there remain significant problems in the general applications of liposomes for drug delivery. In a dispersed aqueous system, liposomes have problems associated with degradation by hydrolysis or oxidation [7], as well as sedimentation, aggregation, or fusion of liposomes during storage [8]. Other problems associated with clinical applications of liposomes include difficulties in sterilization and in large-scale production to obtain a product with adequate physical and chemical stability [9].

One alternative involves formation of liposome-like vesicles from the hydrated mixtures of cholesterol and nonionic surfactant such as monoalkyl or dialkyl polyoxyethylene ether [10]. Niosomes are unilamellar or multilamellar vesicles capable of entrapping hydrophilic and hydrophobic solutes [11]. From a technical point of view, niosomes are promising drug carriers as they possess greater stability and lack of many disadvantages associated with liposomes, such as high cost and the variable purity problems of phospholipids [12]. Another advantage is

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the simple method for the routine and large-scale production of niosomes without the use of unacceptable solvents.

Proniosomes, hydrated by agitation in hot water for a short period of time, offer a versatile vesicle delivery concept with the potential for drug delivery via the transdermal route [13,14]. This would be possible if proniosomes form niosomes following topical application under occlusive conditions, due to hydration by water from the skin itself. The aim of this study is to investigate the feasibility of using proniosomes as a transdermal drug delivery system for ketorolac.

Ketorolac tromethamine (KT) is a nonsteroidal agent with potent analgesic and moderate anti-inflammatory activity [15,16]. The drug is currently administered intramuscularly and orally in divided multiple doses for short-term management of post-operative pain (30 mg q.i.d. by IM injection and 10 mg q.i.d. as oral tablets). This frequent dosing, which results in unacceptable patient compliance, is required due to the short half-life of the drug (4–6 h). Therefore, an alternative noninvasive mode of delivery of the drug is needed. Transdermal delivery certainly appears to be an attractive route of administration to maintain the drug blood levels of KT for an extended period of time.

2. Materials and methods

2.1. Materials

Span 60 was supplied from Koch-light laboratories Ltd (Colebrook Bucks, England). Tween 20, acetonitrile HPLC grade, potassium dihydrogen phosphate, and disodium hydrogen phosphate were purchased from BDH Laboratory Supplies (BDH Chemicals Ltd, Poole, UK). Lecithin was obtained from Merck Company (Darmstadt, Germany). Cholesterol, acetic acid and ethanol were supplied from Riedel Dehaën (Darmstadt, Germany). Hydroxypropyl methylcellulose (HPMC K100 Premium CR grade) was obtained from Dow Chemical Company (Midland, MI, USA). Carbopol 934 was supplied by Serva Company, (Heidelberg, Germany). Sodium carboxymethylcellulose (CMC) was obtained from Winlab Company (Maidenhead, Berkshire, England). Ketorolac tromethamine was generously donated from Amriya Pharmaceuticals Ind. (Alexandria, Egypt).

2.2. Preparation of proniosomes

Proniosomes were prepared using a modified literature method [17]. The compositions of different proniosomal formulations are listed in Table 1. Using a wide-mouth glass tube, 100 mg of KT with surfactant, lecithin, and cholesterol was mixed with 2.5 ml of absolute ethanol. Then the open-end of the glass tube was covered with a lid and the tube was warmed in a water bath at 65 ± 3 °C for 5 min. Then 1.6 ml

Table 1
Compositions of various proniosomal formulations (mg)

| Proniosome code (formula) | Ketorolac | Span 60 | Tween 20 | Lecithin | Cholesterol |
|---------------------------|-----------|---------|----------|----------|-------------|
| S | 100 | 1800 | – | 1800 | 200 |
| T | 100 | – | 1800 | 1800 | 200 |
| SL | 100 | 1800 | – | 900 | 200 |
| SC | 100 | 1800 | – | 1800 | 400 |

of pH 7.4 phosphate buffer was added and the mixture was further warmed in the water bath for about 2 min so that a clear solution was obtained. The mixture was allowed to cool to room temperature until the dispersion was converted to proniosomal gel. The proniosomal gel was then mixed with one of several 2% polymeric gels (HPMC, CMC, or Carbopol) to give a final concentration of 0.5% KT.

2.3. Ketorolac encapsulation efficiency

To 0.2 g of proniosome gel, weighed in a glass tube, was added 10 ml of pH 7.4 phosphate buffer. The aqueous suspension was sonicated in a sonicator bath (Transonic T460/H, Elma, Germany). The KT-containing niosomes were separated from untrapped drug by centrifugation at 25,000 rpm ($32,000 \times g$) at 20 °C for 30 min. The supernatant was recovered and assayed by an HPLC method for KT content. The percentage of drug encapsulation (EP (%)) was calculated by the following equation:

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

where C_t is the concentration of total KT and C_f is the concentration of free KT.

2.4. Vesicle physical analysis

The shape, surface characteristics, and size of the niosomes were observed by scanning electron microscopy. Once again, 0.2 g of the proniosome gel in a glass tube was diluted with 10 ml of pH 7.4 phosphate buffer. The niosomes were mounted on an aluminum stub using double-sided adhesive carbon tape. Then the vesicles were sputter-coated with gold palladium (Au/Pd) using a vacuum evaporator (Edwards) and examined using a scanning electron microscope JSM-5510 (Jeol Ltd, Tokyo, Japan) equipped with a digital camera, at 20 kV accelerating voltage.

2.5. In vitro release study

One gram of proniosomal gel of different compositions was spread on glass circular disk (5.04 cm² diameter), then covered by cellophane dialyzing membrane with molecular weight cut-off of 8000 (Spectrum Medical Inc., Los Angeles, CA, USA) which was securely mounted on the disk by a rubber band. The disk was placed on the bottom of

a glass tube large enough to accommodate the disk diameter and 50 ml of pH 7.4 phosphate buffer was poured onto the membrane surface. The whole assembly was immersed in a water bath maintained at 37 °C. The buffer solution was continuously circulated over the membrane surface in a closed circle at a rate of 5 ml/min using a Watson–Marlow peristaltic pump. The drug release was monitored using an automated monitoring system, which consisted of an IBM computer and PU 8605/60 dissolution software, a Philips Vis/UV/NIR single beam eight cell spectrophotometer model PU 8620. For each formula, drug release was studied in triplicate, absorbance at 320 nm was recorded automatically over 6 h, and the percentage of drug release was calculated.

2.6. *In vitro* permeation study

The permeation of KT from proniosomal formulations was determined by using Franz diffusion cell. The shaved dorsal skin of albino rabbit (0.8 ± 0.1 mm thickness and 3.14 cm^2 exposed surface area) was mounted on the receptor compartment with the stratum corneum side facing upwards towards the donor compartment. The receptor compartment was filled with 15.0 ml of pH 7.4 phosphate buffer maintained at 37 °C and stirred by a magnetic bar at 600 rpm. One gram of proniosomal gel formulation was placed on the skin and the top of the diffusion cell was covered with paraffin paper. At appropriate time intervals (3, 6, 9, 12, 18, 21, 24, 27, and 30 h), 1 ml aliquots of the receptor medium were withdrawn and immediately replaced by an equal volume of fresh receptor solution to maintain sink conditions. The samples were analyzed by the HPLC method described below.

2.7. Sample preparation and the HPLC analytical method

To each 0.5 ml sample, 0.5 ml of 2% zinc sulphate solution, a protein precipitant, was added and the mixture vortexed (Scientific Industries, Inc., NY, USA) for 1 min., and then centrifuged at 13,000 rpm ($22,500 \times g$) for 10 min. The supernatant was directly injected into HPLC system. The ketorolac content of these various samples was analyzed by a modified literature HPLC method [18]. The HPLC system consisted of a Waters pump Model 515, Waters autosampler Model 717 plus (Waters Inc., Bedford, MA, USA), a Shimadzu variable wavelength UV detector Model SPD-10A (Shimadzu Corporation, Kyoto, Japan) controlled by a microcomputer running Millennium[®] version 32 software. The detector wavelength was set at 320 nm. Separation was achieved by isocratic elution with a mobile phase consisting of 70:30 v/v water/acetonitrile with the pH adjusted to pH 4.0 using glacial acetic acid, delivered at a flow rate of 1 ml/min at ambient temperature through a μ -Bondapak C₁₈ analytical column, 150×3.9 mm ID, 5 μm particle size (Waters Inc., Bedford, MA, USA).

2.8. Statistical analysis

Data were expressed as the mean of three experiments \pm the standard deviation (SD) and were analyzed using one-way analysis of variance (ANOVA), followed by Tukey's multiple comparison post test. Statistical differences yielding $P < 0.05$ were considered significant.

3. Results and discussion

3.1. Encapsulation efficiency and vesicle size

As shown in Table 2, niosomes formed from Span 60 (formula S) and Tween 20 (formula T) exhibited a very high encapsulation efficiency. 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. Similar observations have been previously reported [19]. The results are also consistent with the high entrapment efficiency of levonorgestrel in proniosomes incorporating Span 40 [12]. Most of the surfactants used to make nonionic surfactant vesicles have a low aqueous solubility. However, freely soluble nonionic surfactants such as Tween 20, can form micelles on hydration in the presence of cholesterol [20]. The Tween 20 formulations in the present study were also able to entrap ketorolac efficiently.

The mean vesicle sizes of the niosomes formed from ketorolac proniosome formulations are presented in Table 2. The differences in vesicle size among the niosomes prepared with Span were not significant. On the other hand, niosomes prepared with Tween 20 were significantly larger than those prepared with Span ($P < 0.05$). The relationship observed between niosome size and Span hydrophobicity has been attributed to the decrease in surface energy with increasing hydrophobicity [11], resulting in the smaller vesicles. This would also explain the large vesicle size of niosomes prepared with Tween which has a much lower hydrophobicity than does Span. Increasing the cholesterol content or reducing the lecithin content also contributed an increase in the hydrophobicity, with a subsequent slight reduction in vesicle size. The scanning electron microscopy images of the niosomes prepared from different proniosomal formulations are shown in Fig. 1. Most of the vesicles are spherical and discrete with sharp boundaries.

Table 2
Characterization of proniosomal formulations by encapsulation and vesicle size after dilution with buffer

| Proniosome code | Encapsulation (%) | Vesicle size (μm) |
|-----------------|-------------------|--------------------------------|
| S | 98.9 ± 6.2 | 6.6 ± 0.71 |
| T | 99.0 ± 4.7 | 27.5 ± 2.3 |
| SL | 99.2 ± 5.1 | 4.4 ± 0.21 |
| SC | 99.0 ± 3.3 | 4.4 ± 0.16 |

Each value represents the mean \pm SD ($n = 3$).

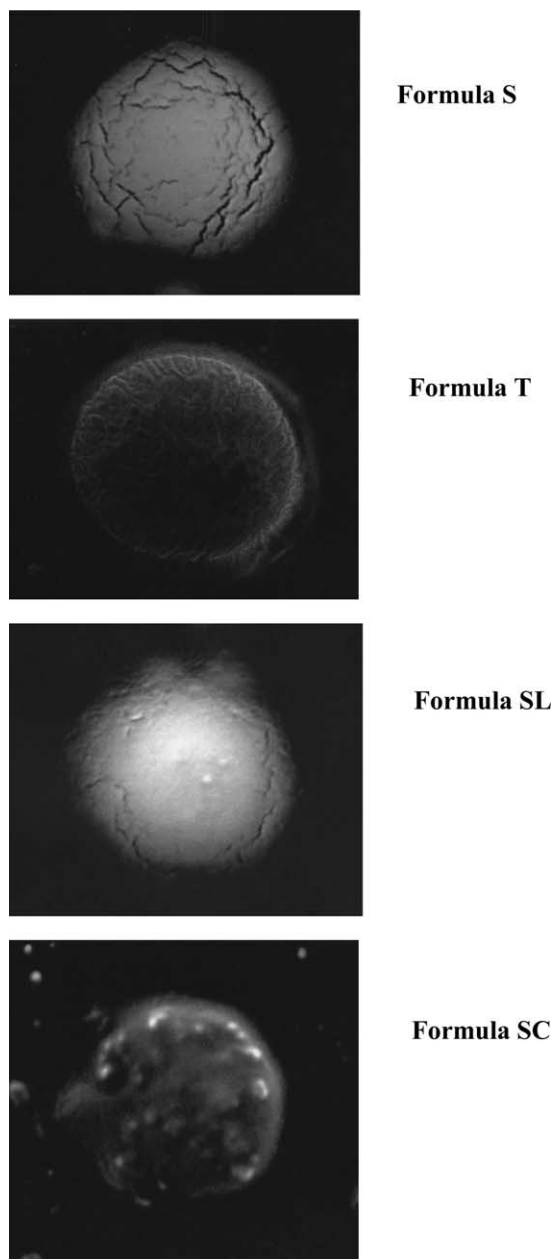


Fig. 1. Scanned electron microscopy (SEM) images of different proniosomal formulations: formula S (contains Span), formula T (contains Tween), Formula SL (contains half amount of lecithin), and formula SC (contains double amount of cholesterol). Formulas S, SL, and SC experimental conditions: magnification= $\times 9500$, Acc. V 20 kV, signal SEI, WD 16 mm, --- 2.11 μm ; formula T experimental condition: magnification= $\times 2000$ --- 10.00 μm .

3.2. In vitro release study

Fig. 2 shows the in vitro release profile of ketorolac from different polymeric gels compared to its release from the proniosomal gel. It is obvious that there is no significant difference between the drug release rates from HPMC and proniosomal gels in the first 3 h of their profiles ($P > 0.05$). This means that the drug is exposed to the same hydration

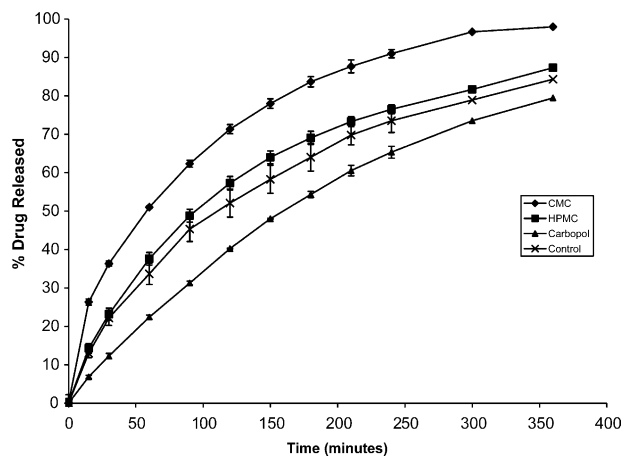


Fig. 2. In vitro release of ketorolac from different polymeric gels and proniosomes.

level, and the inclusion of the proniosomes into an HPMC gel should not affect the drug release rate. On the other hand, incorporation of proniosomes into CMC or Carbopol gels resulted in slight enhancement and retardation of the drug release rate, respectively. Therefore, HPMC gel was selected as a suitable base for the comparative study of in vitro drug release from different proniosomal formulations.

Table 3 illustrates that a higher drug release ($P < 0.05$) is seen from niosomes prepared with Span 60 than from the control (drug in the HPMC gel). This could be due to the emulsification effect of the surfactant after the hydration of the proniosomes by the dissolution medium and formation of elution channels within the gel structure due to loss of lipid bilayers [21]. The faster release of the drug from niosomes with a reduced amount of lecithin may indicate the disrupted structure of the vesicles. Increasing the cholesterol content resulted in a more intact lipid bilayer as a barrier for drug release and decreased its leakage by improving the fluidity of the bilayer membrane and reducing its permeability, which led to lower drug elution from the vesicles [21,22]. Drug release from proniosomes prepared with Tween showed no significant difference from the control ($P > 0.05$).

Table 3

Cumulative amount, release rate (through a cellophane membrane), and permeation flux of ketorolac across excised rabbit skin

| Formula | Release rate ($\mu\text{g}/\text{cm}^2 \text{h}^{-1}$) | Permeation | | |
|---------|--|---|--|------|
| | | Cumulative amount ($\mu\text{g}/\text{cm}^2$) | Flux ($\mu\text{g}/\text{cm}^2 \text{h}^{-1}$) | ER |
| S | 165.0 ± 1.70 | 459.4 ± 3.0 | 17.74 ± 2.9 | 6.97 |
| T | 149.2 ± 1.2 | 321.3 ± 2.9 | 11.12 ± 2.8 | 4.37 |
| SL | 203.1 ± 1.6 | 392.8 ± 6.9 | 14.4 ± 3.0 | 5.66 |
| SC | 138.0 ± 1.7 | 464.2 ± 5.9 | 16.91 ± 3.1 | 6.64 |
| Control | 152.4 ± 1.2 | 74.1 ± 5.5 | 2.54 ± 0.32 | – |

ER, enhancement ratio, each value represents the mean \pm SD ($n = 3$).

3.3. In vitro permeation study

Proniosomes should be hydrated to form niosomal vesicles before the drug is released and permeates across the skin. Several mechanisms could explain the ability of niosomes to modulate drug transfer across skin [3,12,20,23], including (i) adsorption and fusion of niosomes onto the surface of skin would facilitate drug permeation, (ii) the vesicles act as penetration enhancers to reduce the barrier properties of the stratum corneum, and (iii) the lipid bilayers of niosomes act as a rate-limiting membrane barrier for drugs.

As shown in Fig. 3, the release rate of ketorolac across the cellulose membrane, calculated in the linear portion of the plot (1–3 h) is significantly higher than its flux across the skin ($P < 0.05$), indicating the barrier properties of skin for the drug. Since there were great discrepancies between the permeation profiles of the drug proniosomal formulations across skin and across cellulose membrane, interaction between skin and proniosomes may be an important contribution to the improvement of transdermal drug delivery.

One of the possible mechanisms for niosomal enhancement of the permeability of drugs is structure modification of the stratum corneum. It has been reported that the intercellular lipid barrier in the stratum corneum would be dramatically looser and more permeable following treatment with liposomes and niosomes [3,24]. Both phospholipids and nonionic surfactants in the proniosomes can act as penetration enhancers, which are useful for increasing the permeation of many drugs. Fusion of niosome vesicles to the surface of skin, demonstrated in a previous report [3], results in higher flux of the drug due to direct transfer of drug from vesicles to the skin.

Increasing the cholesterol content in the vesicles did not affect the transdermal delivery of the drug as shown in Table 3. This result is in agreement with other

reports [17], although another study suggested that reducing the cholesterol content increased the permeation of estradiol [25]. This may indicate different mechanisms of drug transport across skin depending on the composition of the niosomal formulation and the drug used. On the other hand, reducing lecithin content resulted in a lower flux (Table 3), although this lower flux was not statistically significant. This could be attributed to the slight disruption of vesicles due to the reduction in the lecithin content which leads to leakage of free drug before fusion of the vesicles with the skin. These results suggest that inclusion of lecithin at a certain level may play an important role in drug permeation. In addition, lecithin is reported to have only weak permeation enhancement ability [26]. Therefore, lowering its concentration in the formula resulted in a slight decrease in drug permeation. Proniosomes prepared with Tween 20 showed a significantly lower enhancement effect than those prepared with Span 60 ($P < 0.05$). This was expected due to the larger size of the vesicles and the less lipophilic nature of the former, which makes it more difficult for these vesicles to penetrate or fuse with the skin.

4. Conclusions

The in vitro permeation of ketorolac from proniosomes of various compositions and types of nonionic surfactants have been studied and evaluated. Ketorolac was successfully entrapped within the lipid bilayers of the vesicles with high efficiency. The experimental results and supportive theoretical analysis suggest that either fusion of the vesicles with the intercellular lipid of the stratum corneum and direct transfer of drug from vesicles to the skin and/or the penetration enhancement effect of the nonionic surfactants may contribute to the mechanism of drug permeation enhancement by proniosomal formulations. No significant difference was observed between the enhancement effect of proniosomes prepared with different cholesterol contents. On the other hand, inclusion of an optimum ratio of surfactant/lecithin in the vesicles may play a more important role than cholesterol plays in modulating drug permeation. Proniosomes may be a promising carrier for ketorolac and other drugs, especially due to their simple production and facile scale up.

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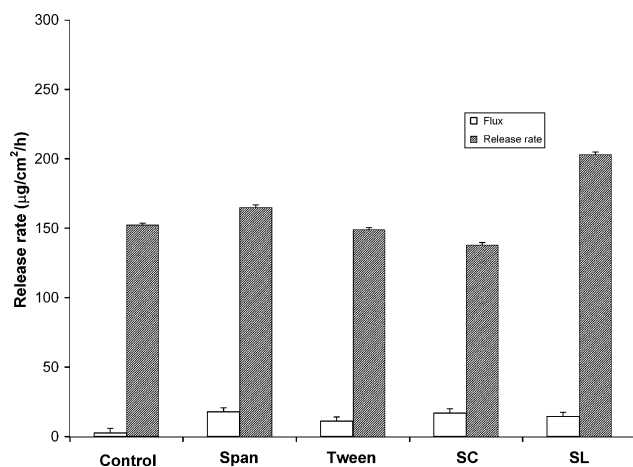


Fig. 3. The flux and release rate of ketorolac from various proniosomal formulations across excised rabbit skin and cellulose membrane ($n = 3$).

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