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Performance of Meloxicam Niosomal Gel Formulations for Transdermal Drug Delivery

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Authors' contributions

This work was carried out in collaboration between all authors. Author TEF designed the study and wrote the protocol. Author GF performed the statistical analysis, managed the analyses of the study and wrote the first draft of the manuscript. Author AU managed the literature searches and conducted most of the practical work. All authors read and approved the final manuscript.

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ABSTRACT

Niosomes have been reported as a possible approach to improve low skin permeation shown by conventional vehicles. In this study, a noisome-based delivery system of meloxicam (MX) was developed and characterized for in vitro performance.
Niosomes were prepared by reverse-phase evaporation method (REV) using different non ionic surfactants and cholesterol in different molar ratios (1:1, 2:1, 3:1,1: 2 and 1:3) and different drug loading (5, 10 and 15 mg). The used surfactants included Tweens (20, 40 and 80), Brij (35 and 58) and Myrj 52. The prepared systems were characterized for entrapment efficiency, and in-vitro release. Accordingly, selected systems were evaluated for vesicle size, and formulated into different hydrogel bases (sodium carboxymethyl cellulose, hydroxypropyl cellulose, and sodium alginate). In-vitro drug release from the different formulations was studied over a period of 8 hr. Effect of formulation additives on drug release was also investigated. The anti-inflammatory activity of the selected formulations was evaluated by the paw edema test.
Results showed high encapsulation efficiency which ranged from about 81.93% to 99.23%. The highest entrapment efficiency was obtained with 1:1 surfactant: cholesterol ratio and 15 mg drug loading, so niosomes prepared by this ratio were selected for further studies. Particle size ranged

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from 4.047 to 12.334 µm for different niosomal systems. In vitro drug release from different gel formulations containing 0.3% MX was compared to that from the same formulations containing 0.3% niosomally entrapped drug. In all formulations the drug release was more sustained in case of niosomally entrapped drug. Incorporation of glycerol and propylene glycol as formulation additives into gel formulations markedly enhanced the drug release, but the release from gels containing niosomally entrapped drug was still delayed.

Keywords: Meloxicam; niosomes; non-ionic surfactants; niosomal gel.

1. INTRODUCTION

Meloxicam (MX), 4-hydroxy-2-methyl-*N*-(5-methyl-2-thiazolyl)-2H-1,2-benzothiazine-3-carboxamide-1,1-dioxide, is a potent, newer nonsteroidal antiinf lammatory drug (NSAID) approved by FDA in 2000 and is used in treatment of reheumatoid arthritis, osteoarthritis and degenerative joint disease [1]. Although MX preferentially inhibits COX-2 (cyclooxgenease-2) over COX-1 (which is responsible for physiological processes in the stomach and kidney), it still has 10-20% incidence of gastrointestinal side effects [2,3]. In order to avoid the irritation of gastrointestinal tract and minimize systemic side effects, one promising method is to administer the drug via skin [4]. In addition it has been demonstrated that NSAIDs promote local analgesia when administered locally through the skin [5]. Meloxicam also possesses appropriate physiochemical properties for potential transdermal delivery such as low molecular weight, low polarity, low melting point and low daily therapeutic dose. The molecular weight (354.1) of meloxicam is appropriate but the aqueous solubility is poor. The oral dose (7.5-15 mg/day) of MX is the lowest among NSAIDs [6]. However the barrier properties of intact skin limit the permeability of a wide variety of substances, including pharmaceutical active agents. In recent years many researchers reported that vesicular structures such as liposomes and niosomes are acting as carriers for administration of drugs across the skin and help to overcome the barrier properties of the skin [7]. However, liposomes have significant problems regarding applications for drug delivery. Some of their major disadvantages include degradation by hydrolysis or oxidation, sedimentation, leaching of drugs, and aggregation or fusion of liposomes during storage [8]. Problems associated with clinical applications of liposomes include difficulties in sterilization and large-scale production to obtain a product with adequate physical and chemical stability [9]. Moreover, tedious conditions in handling liposomes under cryogenic atmosphere have prompted the use of nonionic surfactant vesicles or niosomes as an alternative to liposomes [10]. So, niosomes had been developed in order to overcome the previously mentioned problems associated with sterilization, large-scale production and stability. Niosomes can be sterilized by membrane filtration, autoclaving, and gamma irradiation [11]. They possess greater stability and they improve the bioavailability of poorly soluble drugs and can enhance skin permeation of drugs [12].

Liposomes are usually not efficient for transdermal delivery across the skin, because they do not deeply penetrate the skin, but rather remain on the upper layer of stratum cornium [13]. Niosomes are supposed to give desirable interactions with human skin when applied in topical preparations by improving especially the horny layer characteristics, both by reducing trans-epidermal water loss and by increasing smoothness via replenishing lost skin lipids [14]. Non-ionic surfactant based vesicles (niosomes) are formed from the self-assembly of non-ionic amphiphiles in aqueous media resulting in closed bilayer structures [15] which can entrap both hydrophilic and lipophilic drugs either in an aqueous layer or in vesicular membrane [16].

Niosomes in topical delivery are preferred over other vesicular systems because: they are chemically stable; they have low toxicity because of their non-ionic nature; they handle surfactants with no special precautions or conditions; they can improve the performance of the drug via better availability and controlled delivery at a particular site; they are biodegradable, biocompatible and non-immunogenic [17].

Previous trials to formulate MX niosomes have been reported [18-20], in which niosomal formulations generally showed superior results compared to non niosomal formulations. However, in all the previously reported studies Spans and Tweens were used for formulation of noisomes, in our study, Myrj and Brij were

employed in niosomal MX formulations in addition to Tweens.

The objective of the present study was to formulate niosomal formulations of MX for topical administration to overcome the problems related to oral administration of the drug. These formulations were evaluated for the in vitro characteristics. Selected niosomal formulations were incorporated into different gel bases and were evaluated for the in vitro and in vivo performance.

2. EXPERIMENTAL

2.1 Materials

Meloxicam (MX) was kindly provided by Medical Union Pharmaceuticals (MUP) Co., Abu-Sultan, Ismailia, Egypt. Cholesterol (Ch), Pluronic F-127, diethyl ether, Brij35, Brij58, Myrj52, carrageenan and stearylamine (SA), were purchased from Sigma Chemicals Co., (St. Louis, USA,). Chloroform, sodium alginate and sodium carboxymethyl cellulose (BDH Chemicals, Ltd, Poole, U.K.). Acetone, methanol, potassium dihydrogen orthophosphate, sodium hydroxide, sodium carboxymethyl cellulose, Tweens (20, 40 and 80), Spans (60, 65, and 85), glycerol, propylene glycol, were purchased from Adwic, El-Nasr Chemical Co., Cairo, Egypt. Carbopol 934 (B.F. Goodrich Co., Germany). Hydroxypropylcelluloose MF (Kolmar Company, California,USA). All reagents were of analytical grade and 99% pure.

2.2 Methods

2.2.1 Preparation of MX niosomes

Niosomes were prepared by the reverse-phase evaporation method (REV) [21]. Niosomes were prepared using Tween (20, 40 and 80) and cholesterol, in different molar ratios, viz. 1:1, 2:1, 1:2, and 3:1, and different drug loading: 5, 10, and 15 mg of meloxicam.

Briefly, mixtures of surfactant (Tween 20, 40 and 80) and cholesterol, in different molar ratios were accurately measured into a long necked quick fit round-bottom flask and dissolved in 6 ml of a chloroform/methanol mixture (2:1, v/v). The organic solvents were slowly evaporated under reduced pressure, using a rotary evaporator (Rotavapor type R 110, Buchi, Switzerland) at 50ºC such that a thin dry film of the components was formed on the inner wall of the rotating flask. The film was re-dissolved in 10 ml diethyl ether and a solution containing drug dissolved in acetone together with 5 ml distilled water was added. The mixture was then sonicated for 2 min, swirled by hand, and resonicated again for another 2 min in a bath sonicator (Crest Ultrasonics Corp., Trenton, USA). The resultant opalescent dispersion was rotary evaporated for 5-10 minutes duration to ensure the removal of residual diethyl ether. The niosomal suspension was left to mature overnight at 4℃. The selected (surfactant: cholesterol) ratio and the selected drug loading was applied to (Span 60, 65 and 80, Brij 35, 58 and Myrj52) surfactants. The composition of different niosomal formulations is listed in Table 1.

For the separation of non-entrapped drug, the niosomal dispersion was subjected to centrifugation in a cooling centrifuge (Centurion Scientific Ltd., W. Sussex, U.K) at 14000 rpm for 60 minutes at 4℃. After centrifugation, the supernatant was siphoned off carefully to separate the non-encapsulated MX leaving behind the niosomes with the entrapped drug in the sediment. The sediment was re-suspended in adequate amount of fresh distilled water to get 3 mg/ml final concentration of MX. The niosomal dispersion (free from the non-encapsulated MX) was stored at 4℃ in glass vials.

2.2.2 Characterization of MX niosomes

2.2.2.1 Determination of entrapment efficiency [22]

Certain volume of the niosomal dispersion was subjected to centrifugation. The niosomes were separated from the supernatant and 1 ml of the supernatant was diluted and adjusted to volume with methanol in a 10-ml volumetric flask, and the amount of drug was determined spectrophotometrically at λ 362 nm.

The entrapment efficiency was calculated by the following at equation:

$$EE\ (\%) = [(C_t - C_f)/C_t] \times 100 \quad (1)$$

Where:

C_t is the concentration of total MX,
C_f is the concentration of free MX.

Tween (20, 40 and 80) were chosen to study the effect of drug loading (5, 10, and 15 mg) on

entrapment efficiency. Cholesterol was used to increase the rigidity and stability of the bilayer. The effect of increasing cholesterol content on drug entrapment in niosomes, prepared by reverse-phase evaporation method, was also investigated. Tween (20, 40 and 80) and cholesterol, in different molar ratios (1:1, 2:1, 1:2, and 3:1) were prepared and the entrapment efficiencies for all these formulae were assessed.

2.3 Photomicroscopic Analysis

Samples of MX niosomal preparations, prepared by reverse phase evaporation method were examined microscopically at magnification of 1000X with a binocular microscope (Zeiss Optical Co-LTD Model Carl Zeiss, fitted with camera, Zeiss Model MC 80, Germany). A drop of niosome suspension placed on microscopic slide was examined and photographed for morphological evaluation.

2.3.1 Determination of vesicle size

A small aliquot of freshly prepared niosome dispersion sample was used to characterize the particle size and size distribution, by light scattering based on laser diffraction technique (Horiba's LA-300 Laser Diffraction Particle Size Distribution Analyzer).

2.3.2 Infrared absorption spectroscopy (IR)

Infrared absorption spectroscopic (IR) analyses were performed using a Hitachi 295 spectrophotometer (Hitachi, Tokyo, Japan) using the KBr disc method. The samples (selected freeze dried niosomes, MX powder, and the physical mixture of MX, cholesterol and Myrj 52) were scanned over the range of 4000 to 400 cm^{-1}.

2.3.3 Differential scanning calorimetry (DSC)

Thermograms of the samples (selected freeze dried niosomes, MX powder, and the physical mixture of MX, cholesterol and Myrj 52) were obtained using differential scanning calorimetry (DSC-60, Shimadzu, Japan). Thermal analysis data were recorded using a TA 50I PC system with Shimadzu software programs. Indium standard was used to calibrate the DSC temperature and enthalpy scale. The samples (3–5 mg) were sealed in aluminum pans and heated at a constant rate of 10℃ /min, over a temperature range of 20–380℃.

2.3.4 *In vitro* drug release from different niosomes

The *in vitro* release of MX from different niosomes was investigated using semipermeable cellophane membrane. The membrane was stretched over the open end of a glass tube and made water tight by rubber band. The surface area of tube opening was 2.21 cm^2. Niosomal suspension equivalent to 3 mg MX (1 ml) was introduced in the tubes after separation of free drug. Three mg of free drug was suspended into 1 ml distilled water inserted into another tube and used as a control. The tubes were immersed in a 100 ml beaker containing 50 ml of phosphate buffer pH 7.4 and were maintained in a thermostatically controlled shaker (shaked at 50 stroke /min in a water bath maintained at 37±1℃). At predetermined time intervals for up to 8 hr; 5 ml aliquot of the release medium were withdrawn for analysis and replaced with equal volume of fresh phosphate buffer solution to maintain constant volume. The absorbance of the collected samples was measured spectrophotometrically at λ 362 nm using phosphate buffer pH 7.4 as blank. Experiments were carried out in triplicate and the average values were calculated.

2.3.5 Preparation of gels

Selected niosomal formulations which showed the highest drug release were incorporated into different gel bases. Gels containing 0.3% (w/w) MX either free or niosomally entrapped were prepared (for comparison) using three different polymers with or without formulation additives such as glycerol (30% w/w) and propylene glycol (10% w/w). The polymers used were, sodium alginate (8%w/w), sodium carboxymethyl cellulose (3%w/w) and hydroxy propylmethyl cellulose (15%w/w).

Sodium alginate, sodium carboxymethyl cellulose, and hydroxy propylmethyl cellulose gels were prepared by dispersing the required quantity of polymer in small quantity of distilled water to prepare an aqueous dispersion. The dispersion was allowed to hydrate for 4-5 hours. Propylene Glycol (10%w/w) and Glycerin (30%w/w) were added subsequently to the aqueous dispersion. 0.3%w/w of drug was added and properly dispersed then the final weight of the gel was adjusted to 10 g with distilled water.

Niosomal gels were prepared using the same formula. For this purpose calculated amount of

niosomal suspension containing drug equivalent to 0.3%w/w was centrifuged and the pellets obtained were incorporated instead of drug.

2.4 *In vitro* Drug Release from Different Gels

In vitro drug release from different gels was investigated using semipermeable cellophane membrane as previously mentioned in niosomes release study under the same conditions. 0.5 gm of gel formulations, equivalent to 1.5 mg of MX based on the mean percent of encapsulation, was used in the study.

2.5 Determination of Niosomal Gel pH

The pH of niosomal gels selected for permeability study was determined. One g MX niosomal gel was mixed with 10 ml distilled water with homogenizer. Then the electrode was immersed in the prepared gel solution and readings were recorded using digital pH meter, (Jenway, U.K.) in triplicate and average value was calculated.

2.6 Viscosity Measurement

The viscosity of niosomal gels selected for permeability study was determined at room temperature using a Brookfield DV+II model LV viscometer (Brookfield, Middleboro, MA, USA). Measurements were made using spindle S-96 at 1.5 rpm.

2.7 *In vitro* Skin Permeation Study

In vitro permeation of MX in different gel formulations through rat hairless skin was performed using abdominal skin of male albino rats as permeation by the same procedure employed for in-vitro release study with the epidermal surface upward; the stratum corneum was facing the donor side.

The cumulative amount of permeated drug (µg/cm^2) was plotted versus time (h) and Meloxicam steady state flux (J_{ss}) was calculated as the slope of linear regression line at the steady state phase for each experimental run. Apparent permeability coefficient (P_{app}) was calculated according to the following equation [23]:

$$P_{app} = J_{ss}/C_o \quad (2)$$

Where, C_o is the initial drug concentration.

2.8 *In vivo* anti-inflammatory Activity of MX Gels

Experiments were carried out according to the animal ethics guidelines of Assiut University, Egypt. Principles of laboratory animal care (NIH publication No. 85-23, revised 1985) were followed. All experiments have been examined and approved by the ethics committee of the Faculty of Pharmacy, Assiut University.

In this part, the formulations showed the highest drug permeation were evaluated for their anti-inflammatory activity using the rat paw edema test. Male albino rats (3 per group) weighing 200±20 g were used. The rats in each group were selected so that the average body weight among the groups was as close as possible. Rats were fasted with free access to water for 24 hours prior to the test. For the induction of edema, a volume of 50 µl of 3% (p/v) carrageenan suspension in 0.9% saline solution was injected into the subplantar region of the right hind paw of the rat [24]. rats were divided into three groups, the first group (control) received 1.0 g of sodium carboxymethyl cellulose 3.0% gel base with 30.0%glycerol and 10.0%propylene glycol, the second group received 1.0 g of sodium carboxymethyl cellulose 3.0% gel base with 30.0% glycerol and 10.0% propylene glycol containing 0.3% free drug, the last group received 1.0 g of sodium carboxymethyl cellulose 3.0% gel base with 30.0% glycerol and 10.0% propylene glycol containing 0.3% niosomally entrapped drug (myrj52) niosomes.

The thickness of right paw each rat was measured by Vernier Caliper (SMEC, China) before carrageenan injection and immediately after carrageenan injection (0 time) and then at (1, 2, 3, 4, 6 and 8 hr).

The percent swelling of the paw was calculated using the following equation [25]:

$$\% \text{ Swelling} = [(V - V_i)/V_i] \times 100 \quad (3)$$

Where, V is the paw thickness at each time interval, and V_i the initial paw thickness before carrageenan injection.

The average paw swelling in treated rats was compared with that of control rats and the percent inhibition of oedema was determined using the following equation [26]:

$$\% \text{ inhibition} = [1 - (\% \text{ swelling}_{\text{treated}} / \% \text{ swelling}_{\text{control}})] \times 100 \quad (4)$$

Where, $\text{swelling}_{\text{treated}}$ is the mean value observed in the treated group, and $\text{swelling}t_{\text{control}}$ is the mean value observed in the control group.

2.9 Statistical Analysis

Statistical analysis of the obtained results was carried out by the Student *t*-test

3. RESULTS AND DISCUSSION

3.1 Entrapment Efficiency

For Tween (20, 40, and 80), increasing drug loading from 5 mg to 15 mg, increased the entrapment efficiency by about (5-8%) at different surfactant: cholesterol ratios. This can be attributed to that, saturating the media with MX forcing it to be encapsulated into the niosomes. Prepared niosomes showed high encapsulation efficiency which ranged from about 81.93% to 99.23%. The highest entrapment efficiency was obtained with 1:1 surfactant: cholesterol ratio and 15 mg drug loading (Table 1), so this formula was applied for preparation of niosomes using other surfactants such as Brij 35, Brij 58 and Myrj 52. The entrapment efficiency of these niosomes ranged from 94.8% to 95.8%.

3.2 Photomicroscopic Analysis

The photomicrographs of MX niosomes composed of (1:1) surfactant: cholesterol molar ratios are shown in Fig. 1. The MX vesicles are gravel-like with sharp edges in nature, having large internal aqueous core and exist in either disperse or aggregate collections.

3.2.1 Vesicle size

Particle size ranged from 3.69 to 12.33 µm for the selected niosomal systems (Table 2).

3.2.2 Infrared absorption spectroscopy (IR)

IR spectroscopy was performed to determine the interaction of drug in presence of excipients. The IR spectrum of pure meloxicam (MX) showed characteristic peaks at 1620 cm^{-1} (amide carbonyl), 3290 cm^{-1} (– N – H stretching of secondary amine, and prominent bands such as at 848–570 cm^{-1} corresponding to (–CH aromatic and heteroaromatic ring bending) and 1531, 1551 cm^{-1} (O =S= O stretching) as shown in Fig. 2. The IR spectrum of Myrj-52 showed characteristics peaks at 3446 cm^{-1} (–OH stretching), 1734 cm^{-1} (– C=O of ester), and 2889 cm^{-1} (–C–H stretching).The IR spectrum of the physical mixture of MX with Myrj 52 (PM) showed the additive spectra of both. However, in the spectrum of niosomes (N), the intensity of the peak of (–N–H stretching of secondary amine) of meloxicam at 3290 cm^{-1} was markedly decreased and was shifted to lower wave number (3127) cm^{-1}. These results indicated intermolecular hydrogen bonding between MX and the other components.

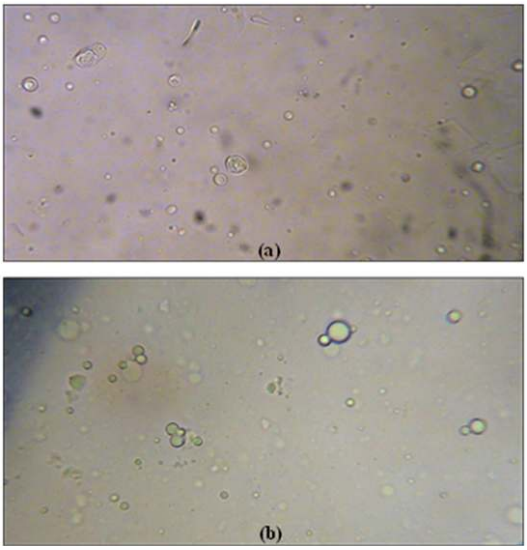

Fig. 1. MX niosomes prepared with: (a) 1:1 Tween 80: cholesterol and 15 mg MX loading, (b) 1:1 Brij 58: cholesterol and 15 mg MX loading

3.2.3 Differential scanning calorimetry (DSC)

The differential scanning thermograms presented in Fig. 3 show that, in the physical mixture (PM), there is marked decrease in intensity and also shifting of the peak of MX from 256.17℃ to 239.7℃. This shift probably results from the partial reduction in crystallinity. The DSC thermogram of MX loaded Myrj 52 niosomes (MjN) showed two broad endothermic peaks at 232.2-235.4℃ and a shifting of the endotherms of surfactant bilayer components including Myrj 52 (from 52.25 to 47.7℃) and cholesterol (from 127.29 to 129.37℃). This suggests significant interaction of MX with bilayer components and can account for the enhanced entrapment of MX into these formulations [27,28].

Table 1. Composition and entrapment efficiencies of different MX niosomal formulations

Surfactant used	Code	Surfactant (µmol)	Cholesterol (µmol)	Meloxicam (mg)	EE (%)
Tween 20	T20-1	75	75	5.0	94.85±1.500
	T20-2	75	75	10	98.56±0.708
	T20-3	75	75	15	99.23± 0.04
	T20-4	75	150	5.0	96.64±0.710
	T20-5	75	150	10	95.36±0.313
	T20-6	75	150	15	95.88±0.865
	T20-7	150	75	5.0	88.89±0.644
	T20-8	150	75	10	96.01±0.251
	T20-9	150	75	15	96.83±0.305
	T20-10	225	75	5 .0	90.87±2.386
	T20-11	225	75	10	96.73±0.427
	T20-12	225	75	15	96.62±0.162
Tween 40	T40-1	75	75	5.0	93.38±0.495
	T40-2	75	75	10	97.34±0.700
	T40-3	75	75	15	97.17±0.301
	T40-4	75	150	5 .0	95.96±0.553
	T40-5	75	150	10	97.20±0.320
	T40-6	75	150	15	97.31 ±.024
	T40-7	150	75	5 .0	90.91±0.783
	T40-8	150	75	10	95.72±0.438
	T40-9	150	75	15	96.27±0.318
	T40-10	225	75	5.0	88.29±1.824
	T40-11	225	75	10	92.87±0.621
	T40-12	225	75	15	94.66±0.465
Tween 80	T80-1	75	75	5.0	88.84±1.916
	T80-2	75	75	10	96.24±0.768
	T80-3	75	75	15	98.11± 0.29
	T80-4	75	150	5.0	92.22±0.970
	T80-5	75	150	10	95.99±0.565
	T80-6	75	150	15	95.24±0.986
	T80-7	150	75	5.0	84.56±0.493
	T80-8	150	75	10	90.17±0.757
	T80-9	150	75	15	93.84±0.366
	T80-10	225	75	5.0	81.93±3.802
	T80-11	225	75	10	92.41±0.730
	T80-12	225	75	15	94.15±0.480
Brij 35	B35-1	95	95	15	94.82±0.187
Brij 58	B58-1	90	90	15	95.77±0.511
Myrj 52	M52-1	45	45	15	95.84±0.280

Mean±SD, n=3

Table 2. Average particle size of selected niosomal formulations

Code	Mean particle size (µm)
T20-3	12.334±2.480
T40-3	6.640±0.579
T80-3	9.422±0.361
B35-1	6.193±1.187
B58-1	3.694±0.799
M52-1	04.047±1.420

Mean±SD, n=3

3.2.4 *In vitro* drug release from different niosomal suspensions

Fig. 4 shows the release pattern of MX from different niosomal suspensions prepared from Tween 20, 40, 80, Brij 35, 58 and Myrj 52 with surfactant to cholesterol ratio of (1:1) and 15 mg MX loading. Also, the release pattern of free MX from drug suspension is presented in Fig. 4 for comparison. All the studied niosomal formulations showed significantly higher release (P < 0.01) of MX compared to free MX suspension.

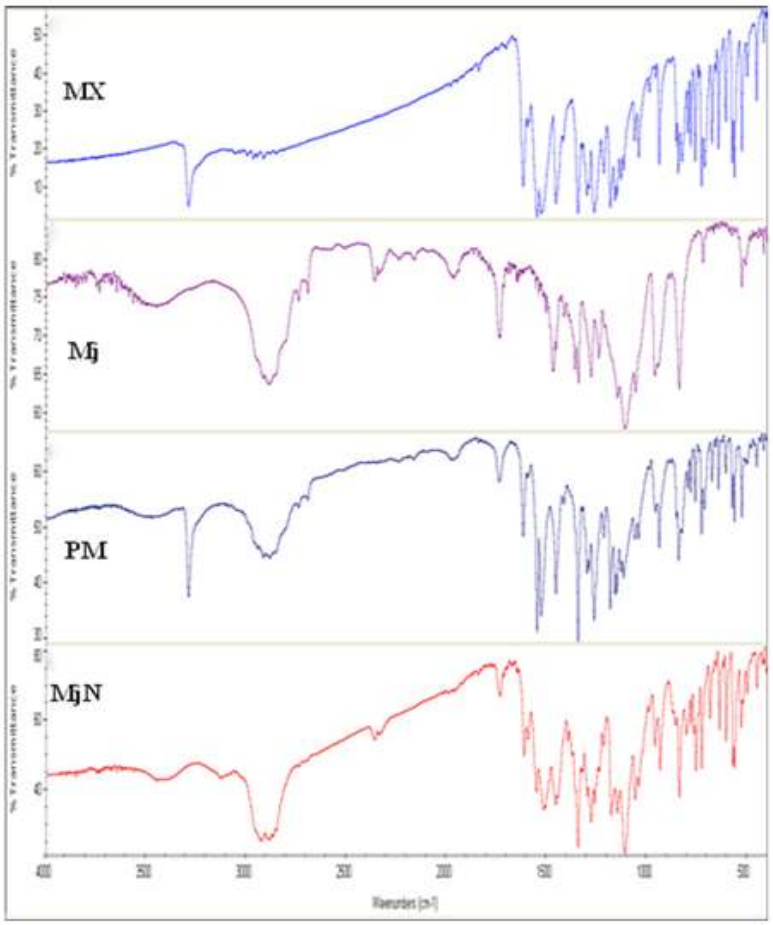

Fig. 2. The infrared spectra of MX (MX), Myrj 52 (Mj), physical mixture of MX with Mrj 52 (PM) and MX niosomes prepared with Myrj 52 as a surfactant (MjN)

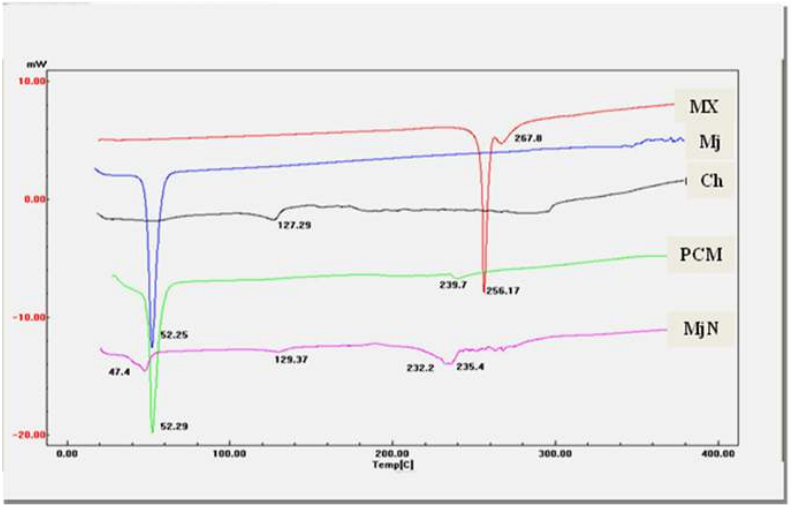

Fig. 3. DSC thermograms of MX (MX), Myrj 52 (Mj), cholesterol (CH), physical mixture of MX with Mrj 52 and cholesterol (PCM) and meloxicam niosomes prepared with Myrj 52 as a surfactant (MjN)

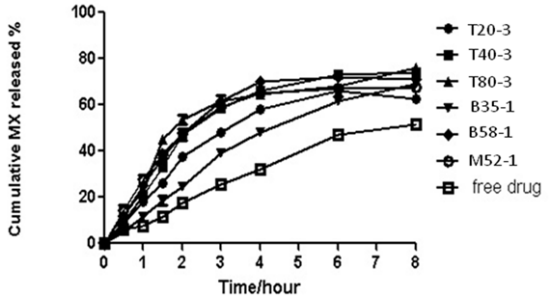

Fig. 4. In-vitro release of MX from different niosomal formulations and from free MX suspension

All the studied niosomal formulations showed highly significant increased release ($P < 0.01$) of MX compared to free MX suspension. The drug release from different niosomes was found to follow the Higuchi diffusion model indicating matrix-controlled diffusion of the released drug.

The selected niosomal formulations (T80-3, B58-1 and M52-1) were incorporated with different gel bases, namely sodium alginate, sodium carboxymethylcellulose (sodium CMC) and hydroxypropyl cellulose (HPC). The gel formulations were prepared with or without addition of glycerol (30%) and propylene glycol (10%). The composition of the different gel formulations is summarized in Table 3.

3.2.4.1 In vitro drug release from different niosomal gels

The release curves are shown in (Figs. 5-7). In each figure, (A) showing the release of MX from gels without glycerol and propylene glycol, while (B) showing the release of MX from gels containing glycerol 30% (w/w) and propylene glycol 10% (w/w).

The release pattern of MX could be ranked as: - sodium carboxymethyl cellulose gels > sodium alginate gels > hydroxypropyl cellulose gels. Addition of glycerol 30% (w/w) and propylene glycol 10% (w/w) markedly enhances the release of the drug, as they act as permeation enhancers.

Generally; niosomal gels showed lower release of MX than free MX gels. The entrapped MX molecules could leak out gradually from the vesicles into the surrounding gel. This finding is in agreement with Beata et al. [29] who found that the cholesterol present in the monolayer would limit1,2-dipalmitoyl-*sn*-glycero-3-phosphoethanolamine (DPPE) mobility [30] and favor an upright orientation of the DPPE hydrocarbon chains thus enhancing Van der Waals interactions. Consequently, it would contribute to a spontaneous mixing between the two membrane lipids and to an increased stability of the monolayer. Inclusion of cholesterol within the niosomal structure could markedly reduce the flux of (MX) during the release phase, which is in accordance with the membrane stabilizing activity of this lipid [29,31].

The release data of MX from different niosomal and free drug gels were analyzed and the results pointed to Higuchi diffusion pattern of drug release (data not shown).

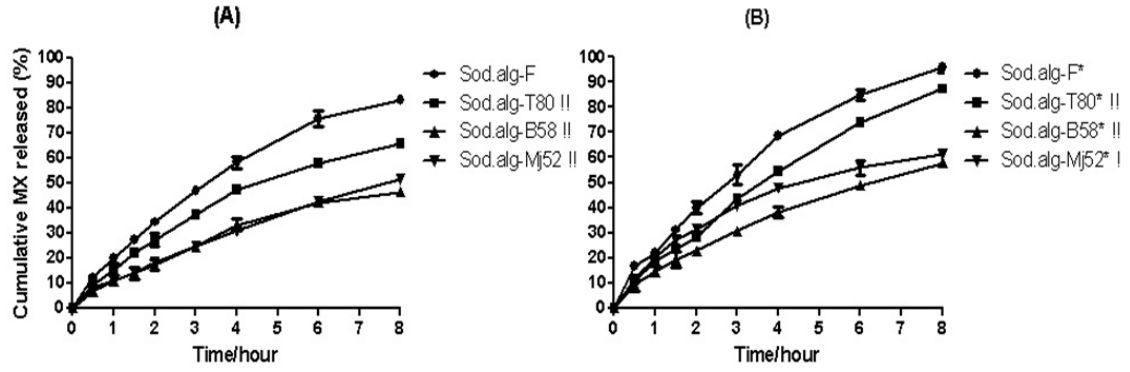

Fig. 5. Release of MX from different sodium alginate gels. (A) gels without glycerol and propylene glycol, (B) gels containing glycerol 30% (w/w) and propylene glycol 10% (w/w)

! significantly different compared to free drug gels, !! highly significantly different compared to free drug gels

Table 3. The composition of different gel formulations

Code	Polymer		MX 0.3% (w/w) (free or niosomal form code)	PG (% w/w)	Glycerol (% w/w)
	Type	Conc. (% w/w)			
Sod.alg-F	Sodium alginate	8	Free drug	---	---
Sod.alg-T80			T80-3	---	---
Sod.alg-B58			B58-1	---	---
Sod.alg-Mj52			M52-1	---	---
Sod.alg-F*			Free drug	10	30
Sod.alg-T80*			T80-3	10	30
Sod.alg-B58*			B58-1	10	30
Sod.alg-Mj52*			M52-1	10	30
SCMC-F	Sod.CMC	3	Free drug	---	---
SCMC-T80			T80-3	---	---
SCMC-B58			B58-1	---	---
SCMC-Mj52			M52-1	---	---
SCMC-F*			Free drug	10	30
SCMC-T80*			T80-3	10	30
SCMC-B58*			B58-1	10	30
SCMC-Mj52*			M52-1	10	30
HPC-F	HPC	15	Free drug	---	---
HPC-T80			T80-3	---	---
HPC-B58			B58-1	---	---
HPC-Mj-52			M52-1	---	---
HPC-F*			Free drug	10	30
HPC-T80*			T80-3	10	30
HPC-B58*			B58-1	10	30
HPC-Mj-52*			M52-1	10	30

***** *Gels containing PG and glycerol*

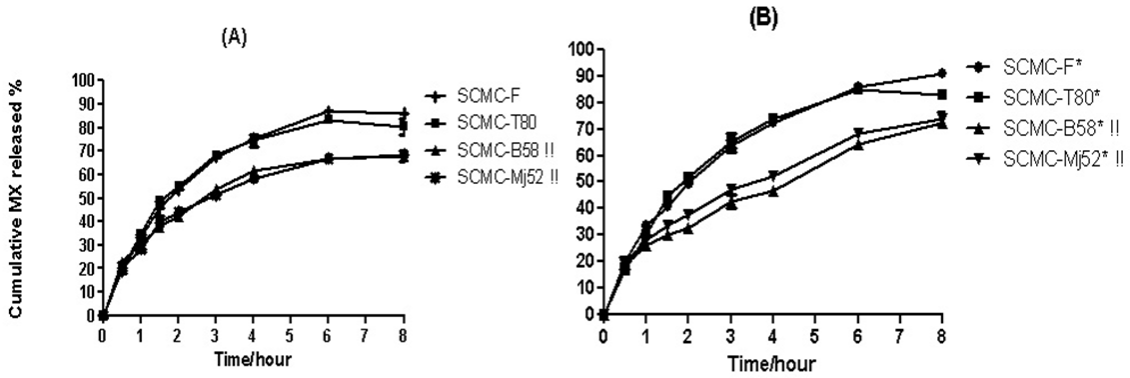

Fig. 6. Release of MX from different sodium carboxymethylcellulose gels. (A) gels without glycerol and propylene glycol, (B) gels containing glycerol 30% (w/w) and propylene glycol 10% (w/w)

! significantly different compared to free drug gels, !! highly significantly different compared to free drug gels

3.2.4.2 Viscosity and pH of selected niosomal gels

The viscosity and pH values of the MX gels selected for the skin permeation study are presented in Table 4. The pH values of all gel formulations ranged from 6.00 to 7.4. The MX gels could be ranked in the following order according to their viscosity: hydroxypropyl cellulose gels > sodium alginate gels > sodium carboxymethyl cellulose gels. Apparently the viscosity and pH were not affected by the type of niosmes incorporated in the gel.

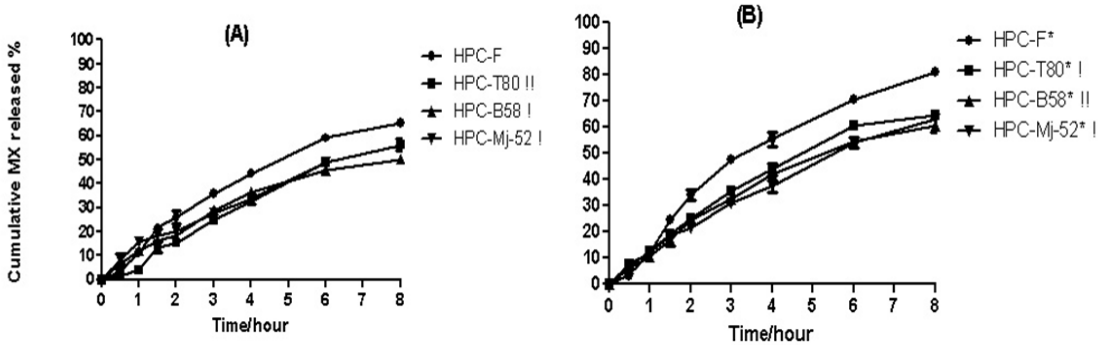

Fig. 7. Release of MX from different hydroxypropyl cellulose gels. (A) gels without glycerol and propylene glycol, (B) gels containing glycerol 30% (w/w) and propylene glycol 10% (w/w)

! significantly different compared to free drug gels, !! highly significantly different compared to free drug gels

Table 4. Viscosity and pH of selected MX niosomal and free drug gels

Polymer	Formulation	Viscosity (Pa.S)	pH
Sod.alg	Sod.alg-F*	85.75±3.1	6.2±0.4
8.0%	Sod.alg-T80*	81.25±2.7	6.4±0.4
	Sod.alg-B58*	81.11±3.5	6.0±0.6
	Sod.alg-Mj52*	82.49±4.1	6.2 ±0.1
Sod.CMC	SCMC-F*	44.15±2.5	6.9±0.3
3.0%	SCMC-T80*	43.05±1.7	7.0±0.1
	SCMC-B58*	43.77±1.5	6.9±0.4
	SCMC-Mj52*	44.92±1.2	6.6±0.3
HPC	HPC-F*	98.55±1.7	7.4±0.2
15.0%	HPC-T80*	96.22±1.3	7.1±0.4
	HPC-B58*	95.47±2.1	7.1±0.3
	HPC-Mj-52*	96.03±2.5	7.4±0.1

Mean±SD, n=3

3.2.5 In vitro skin permeation study

Only gels containing glycerol and propylene glycol were included in this study, since they showed higher drug release compared to those free from glycerol and propylene glycol.

Table 5 shows the flux (*J*ss) and permeability coefficient (*k*p) of different niosomal and free drug formulations. It can be seen that the niosomal gels showed higher skin permeation of MX through the excised rat skin as indicated by the increased flux and permeability coefficient values. The differences in permeation parameters values were significant ($P< 0.05$) or highly significant ($P< 0.001$) compared to those of the free drug gels.

Several mechanisms could explain the ability of niosomes to modulate drug transfer across skin. Interaction between skin and niosomes 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 [32,33]. 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 noisome vesicles to the surface of skin, demonstrated in a previous report [32], results in higher flux of the drug due to direct transfer of drug from vesicles to the skin.

3.2.5.1 In vivo anti-inflammatory activity of MX gels

Based on the in vitro release data, sodium CMC based gels were selected for further anti-inflammatory efficacy evaluation using the paw edema test.

Results are summarized in Table 6. Statistical analysis using student's *t*-test showed that the anti-inflammatory effect of niosomal gel formula (SCMC-Mj52*) was significantly higher ($P < 0.05$). The anti-inflammatory effect of the niosomal gel sustained for longer time than that of free MX gels as seen in Table 6. This could be due to superior skin penetration and deposition potential of niosomal gel formulation which definitely indicated depot forming properties of niosomal gel.

Table 5. The in vitro skin permeation parameters of MX from different niosomal and free drug gels

Polymer	Formulation	*J*ss (µg/cm^2/h)	*k*p (cm/h)×10^{-3}
Sod.alg 8.0%	Sod.alg-F*	44.75±23.1	29.537±0.004
	Sod.alg-T80*	65.61±11.2$^{!!}$	46.106±0.006$^{!!}$
	Sod.alg-B58*	63.10±12.0$^{!}$	43.822±0.008$^{!}$
	Sod.alg-Mj52*	65.53±11.6$^{!!}$	50.281±0.010$^{!!}$
	SCMC-F*	58.62±02.1	43.895±0.012
Sod.CMC 3.0%	SCMC-T80*	67.31±10.5$^{!}$	48.071±0.021$^{!}$
	SCMC-B58*	72.23±05.2$^{!!}$	53.881±0.014$^{!!}$
	SCMC-Mj52*	74.20±10.5$^{!!}$	54.558±0.002$^{!!}$
	HPC-F*	53.75±21.0	50.707±0.012
HPC 15.0%	HPC-T80*	72.60±16.2$^{!!}$	56.279±0.009$^{!!}$
	HPC-B58*	68.23±13.2$^{!}$	52.891±0.018$^{!}$
	HPC-Mj-52*	78.20±12.1$^{!!}$	60.153±0.012$^{!!}$

Mean±SD, n=3
! *significantly different compared to free drug gels*
!! *highly significantly different compared to free drug gels*

Table 6. Percent swelling and percent inhibition of edema by MX in sodium CMC niosomal gel and free drug gel

Time	Placebo		Free MX gel (SCMC-F*)		Niosomal gel (SCMC-Mj52*)	
(h)	%swelling	%inhibition	%swelling	%inhibition	%swelling	%inhibition
0	66.6±3.87	__	66.6±0.055	__	82.7±13.157	__
1	83.3±8.58	__	68.2±2.74	15.6±3.39	68.8±6.62	14.9±8.18
2	78.5±9.62	__	70.6±6.87	08.2±8.92	58.3±12.43	24.1±16.15
3	64.2±15.49	__	60.3±5.99	5.0±9.43	54.9±1.51	13.5±2.39
4	59.5±9.01	__	48.4±5.99	1.6±12.17	54.9±13.50	4.3±27.44
5	42.8±6.87	__	46.0±5.49	1.6±11.74	45.3±10.63	3.1±22.71
6	52.3±11.98	__	52.3±8.58	2.9±15.90	40.104.01	25.6±7.44
8	35.7±5.99	__	35.7±25.75	2.1±70.54	25.3±11.47	30.6±31.41

4. CONCLUSION

MX was successfully entrapped within the bilayer membrane of the niosomes prepared using different surfactants with high entrapment efficiencies (EE%) ranging from 88 to 99%. Niosomes formulated with Tween 80, Brij 58 and Myrj 52 showed higher release of MX (from niosomal suspensions) than those formulated with Tween 20, 40 and Brij 35. The in vitro release of MX from niosomal gels exhibited lower but sustained release of drug compared to that of free drug gels. Incorporation of glycerol and propylene glycol into the prepared gels resulted in increased in vitro drug release. Selected niosomal gel showed higher skin penetration compared to the free drug gel. Also, the anti-inflammatory effect of the selected niosomal gel was significantly higher than that of the corresponding free drug gel. The obtained results indicated that the prepared niosomal gel significantly enhanced the skin permeation of MX and that it might show good performance as trandermal delivery system of the drug.

CONSENT

It is not applicable.

DISCLAIMER

This manuscript was presented in the conference "5th International Conference and Exhibition on Pharmaceutics & Novel Drug Delivery Systems"

Available link is "http://www.omicsonline.org/2153-2435/2153-2435.S1.025-066.pdf " date16-18th March'2015-Crowne Plaza, Dubai, UAE, Formulation and evaluation of hydrogels containing miloxicam niosomes for topical delivery, Pharm Anal Acta, Volume 6, Issue 1

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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