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Original Article

NIOSOME-ENCAPSULATED CLOMIPRAMINE FOR TRANSDERMAL CONTROLLED DELIVERY

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ABSTRACT

Objective: To decrease the extensive first-pass metabolism of clomipramine (CLM) in the liver by developing and optimizing niosomal transdermal formulation of CLM in order to improve its bioavailability.

Methods: Niosomes were prepared according to 4×2^2 factorial design where surfactant type was set at four levels (Tween 20, Tween 60, Span 20 and Span 60), surfactant to cholesterol (CH) ratio at two levels (1:1, and 1:0.5) and charge inducing agent (dicetyl phosphate) (DP) at two levels (present and absent). Entrapment efficiency (%EE) and release efficiency percentage after 6 h ($\%RE_6$) was chosen as dependent variables. CLM loaded niosomes were prepared by employing a thin film hydration technique using different non-ionic surfactants (Tween 20, Tween 60, Span 20 or Span 60), in the presence of CH and a negative charge inducer (DP) in different molar ratios. The prepared niosomes were characterized for entrapment efficiency, shape, size, zeta-potential and *in vitro* drug release.

Results: The studies demonstrated successful preparation of CLM niosomes. Niosomes showed percentage entrapment efficiency (%EE) of 72.54 ± 0.37% for optimized formula (F5) which composed of (1:1:0.1 molar ratio of Tween 60, CH and DP) and 25 mg of CLM. The selected formula F5 (1:1:0.1) was incorporated in gel base of HPMC-K15M (4%) and evaluated through *in vitro* release. Skin irritancy test performed on albino rats, showed no sign of irritation.

Conclusion: Transdermal delivery of niosomal CLM formulations is expected to improve drug bioavailability, while preferably avoiding undesired effects and improving patient compliance.

Keywords: Clomipramine hydrochloride, Antidepressant, Niosomes, Niosomal gel, HPMC, Transdermal.

INTRODUCTION

The delivery of drugs into and through the skin is recognized as an effective means of therapy for local dermatologic and systemic diseases. In recent years, transdermal delivery of drugs for systemic and local effect has gained considerable attention, because it offers many advantages over conventional administration such as avoidance of first pass metabolism and elimination of gastrointestinal irritation resulting in the improvement of patient compliance [1]. Gel base formulations make the drug molecules more easily to be removed from the system than cream and ointment ones [2]. Transdermal delivery is the best suited for drugs, which display high toxicity and/or narrow therapeutic windows [3]. The proposed advantage of transdermal delivery is the possibility to attain sustained and constant drug levels [4]. However, the major barrier of the skin is the stratum corneum (SC), the top layer of the epidermis. Low molecular weight (≤500 Da), lipophilicity, and effectiveness at low dosage are the ideal characteristics of the drugs for transdermal delivery. However, many drugs do not possess ideal physicochemical properties. Thus, manipulation of the drug or vehicle to enhance diffusion through skin becomes necessary [5].

Niosomes are such bilayer system containing nonionic surfactants and CH. They have longer shelf life, stability and ability to deliver the drug at the target site in a controlled or sustained manner which enhances bioavailability. Niosomes offer several advantages over liposomes such as higher chemical stability, intrinsic skin penetration enhancing properties and lower costs [6]. In addition, niosomes are versatile carrier systems that can be administered through various routes including intramuscular route [7], intravenous injection [8], peroral delivery [9], ocular delivery [10], pulmonary delivery [11] and transdermal delivery [12-13]. Particular efforts have been aimed at using niosomes as effective dermal and transdermal drug delivery systems [13-15].

Clinical depression is a disturbance of mood that is distinguishable from the usual mood fluctuations of everyday life. Suicide is a significant risk and up to half of all patients with depression may attempt suicide during their lifetime. Risk factors for developing depression include female gender and a positive family history[16]. CLM is a tricyclic antidepressant with actions and uses similar to those of amitriptyline. It has antimuscarinic properties and is also a potent serotonin reuptake inhibitor. In addition, it is one of the more sedating tricyclics. CLM has been estimated to have a plasma elimination half-life of about 21 hours. CLM is readily absorbed from the gastrointestinal tract, but due to extensive hepatic first-pass metabolism to the active metabolite, desmethylclomipramine, less than 50% of a dose reach the systemic circulation [16]. Based on the aforementioned reasons, the purpose of the current study was to prepare CLM encapsulated niosomes in order to be used as transdermal carriers for depression treatment. The influence of different processing and formulation variables such as surfactant structure (Tween 20, Tween 60, Span 20, or Span 60), CH content and presence of negatively charged DP on CLM entrapment efficiency was demonstrated. Also, particle size, zeta-potential, Differential Scanning Calorimetry analysis (DSC) and *in vitro* drug release were evaluated. The selected CLM niosomal dispersion was incorporated in gel base of HPMC-K15M (4%) and evaluated through *in vitro* release.

MATERIALS AND METHODS

Materials

CLM was kindly donated by Sigma Pharmaceutical Industries, Egypt-S. A. E. CH and DP were purchased from Sigma-Aldrich, USA. Polyoxyethylene sorbitan monolautate (Tween 20) was purchased from Nice Chemicals pvt. Ltd., Kerala, India. Polyoxyethylene 20 sorbitan monostearate (Tween 60) was purchased from Alpha Chemika, Mumbai, India. Sorbitan monostearate (Span 60) and Sorbitan monolaurate (Span 20) were purchased from Oxford Laboratory, Mumbai, India. Phosphotungestic acid hydrate was purchased from Sigma-Aldrich, Buchs, Japan. Hydroxypropyl methylcellulose (HPMC-K15M) was purchased from Tama, Tokyo, Japan. Methanol, chloroform, sodium hydroxide, potassium dihydrogen phosphate and disodium hydrogen phosphate were

purchased from Adwic, El-Nasr Chemical Co., Cairo, Egypt. Spectra/ Por dialysis membrane, 12,000–14,000 molecular weight cutoff was purchased from Spectrum Laboratories Inc., USA.

Preparation of CLM Niosomes

The composition of the tested niosomal formulae are reported in Table 1. Niosomes containing CLM were prepared by thin film hydration technique. [17-18]. Briefly, 25 mg CLM was weighed accurately and dissolved in 10 ml methanol. In a clean, dry, long necked quick fit round-bottom flask, a mixture containing non ionic surfactant, CH with or without DP, in different molar ratios, were completely dissolved in 10 ml chloroform. The drug solution was added to the flask getting a mixture of chloroform / methanol mixture (1:1, v/v). The organic solvents were slowly evaporated and completely removed at 58-60 ˚C under reduced pressure, using a rotary evaporator (Eyela OSB-2000, Japan) at 150 rpm such that a thin dry film of the components was formed on the inner wall of the rotating flask. The dried thin lipid film was then hydrated with 10 ml of phosphate buffer (PB, pH 7.4), by rotating the flask in a water bath using a rotavapor under normal pressure in order to ensure complete hydration of the film. The niosomes was allowed to equilibrate at room temperature, and the niosomal suspension was kept in the refrigerator (4 ˚C) to mature over night [19-20].

CLM niosomes were prepared according to 4 × 2^2 factorial experimental design to investigate the influence of formulation variables on the entrapment efficiency of the formulations and release profile of the drug. In this design, surfactant type (X1), surfactant to CH ratio (X2) and charge inducing agent (DP) (X3) were selected as independent variables, whereas entrapment efficiency (%EE) (Y1) and release efficiency percentage after 6 h (RE_6%) were chosen as dependent variables. The levels of the chosen independent variables were illustrated in Table 2.

Determination of CLM Entrapment Efficiency %EE

The proportion of encapsulated CLM was obtained by cooling centrifugation (Megafuge 1.0/1.0R, Kendro Laboratory Products (Fussex, UK)) of a known aliquot (1 ml) of the prepared niosomal suspension at 15,000 rpm for 1 h at 4 ˚C. The niosomes were separated from the supernatant and the amount of the entrapped drug was determined by lysis of the niosomal pellet with methanol, covered with parafilm to prevent evaporation and sonication to obtain a clear solution [21]. The concentration of the drug was determined spectrophotometrically (Shimadzu, model UV-1650 PC, Kyoto, Japan) by measuring the U. V. absorbance at λ_{max} = 252 nm. The entrapment efficiency is defined as follows [22]:

CLM %EE = (amount of CLM entrapped) / total amount of CLM) x 100

Table 1: CLM niosomal formulae composition (molar ratio)

Formula	Tween 20	Tween 60	Span 20	Span 60	CH	DP
F1	1	-	-	-	1	0.1
F2	1	-	-	-	1	-
F3	1	-	-	-	0.5	0.1
F4	1	-	-	-	0.5	-
F5	-	1	-	-	1	0.1
F6	-	1	-	-	1	-
F7	-	1	-	-	0.5	0.1
F8	-	1	-	-	0.5	-
F9	-	-	1	-	1	0.1
F10	-	-	1	-	1	-
F11	-	-	1	-	0.5	0.1
F12	-	-	1	-	0.5	-
F13	-	-	-	1	1	0.1
F14	-	-	-	1	1	-
F15	-	-	-	1	0.5	0.1
F16	-	-	-	1	0.5	-

Table 2: Levels of independent variables

Factors (independent variables)	Levels of variables
X1: surfactant type	Tween 20 - Tween 60 - Span 20 - Span 60
X2: surfactant to CH ratio	1:1 - 1:0.5
X3: charge inducing agent (DP)	Present – absent

Photomicroscopy and Transmission Electron Microscopy (TEM)

The formation of niosomal vesicles as well as their morphological aspects was evaluated by using photo and transmission electron microscopy.

Various niosomal formulations were examined microscopically at magnification of 40× with a binocular microscope (Leica-Queen 550IW, Germany) equipped with the camera to study their size [23-24]. A drop of niosome suspension placed on glass slide, examined and photographed for morphological evaluation.

The morphology of hydrated niosome dispersions was also examined by Transmission Electron Microscope microscope (model JEM-1230, Jeol, Tokyo, Japan) at 70 kV. A drop of dispersion was stratified onto a carbon coated copper grid and left to adhere on the carbon substrate for about 1 min. The dispersion in excess was removed by a piece of filter paper. A drop of 2% phosphotungstic acid solution was stratified and, again, the solution in excess was removed by a tip of filter paper. The sample was air-dried and photographed under TEM [13].

Particle size distribution and zeta-potential determinations

CLM niosomal suspension (100 µl) was diluted to 10 ml with distilled water, then subjected at room temperature to photon correlation spectroscopy and laser Doppler anemometry (Zetasizer ZEN 3600 Nano ZS (Red badge) Malvern Instr., UK) for measurement of the particle size [25]; the potential of CLM niosomes was also measured and evaluated for surface charge.

Differential Scanning Calorimetry (DSC)

DSC experiments were performed using a differential scanning calorimeter (DSC 60, Shimadzu, Kyoto, Japan) calibrated with indium. Differential scanning calorimetry (DSC) thermograms for individual components, CLM, Tween 20, Tween 60, Span 20, Span 60, CH and DP, as well as drug-loaded niosomes, were investigated. Samples (2-4 mg) were placed in flat-bottomed aluminum pan. The analysis was performed under nitrogen atmosphere. A heating rate of 10 °C /min was employed over a temperature range (30–250) °C for Span 60 niosomes and (-100–250) °C for Tween 20, Tween 60 and Span 20 niosomes.

In vitro release of CLM from Niosomes through artificial membrane

The *in vitro* release of CLM from the prepared niosomes was determined, in order to evaluate the effect of various factors used in the preparation of CLM niosomes, using the membrane diffusion technique [26-27]. After separation of the prepared niosomes by centrifugation, an accurately measured quantity of CLM niosomal dispersion formulations, equivalent to 25 mg of CLM was resuspended in 0.3 ml phosphate buffer (pH 7.4) and transferred to a glass cylinder having the length of 10 cm and diameter of 2.5 cm. This cylinder was fitted at its lower end with presoaked cellulose membrane (Spectra/Por dialysis membrane 12,000–14,000 M wt cutoff). The glass cylinder was attached to the shaft of the dissolution apparatus and then suspended in the dissolution flask of USP dissolution apparatus (NE4-COP Dissolution Testing Station, Copley Scientific, Nottingham, UK) containing 100 ml of phosphate buffer (pH 5.5) at 32 ± 0.5°C [10]. The glass cylinder was allowed to rotate at a constant speed (50 rpm). At predetermined time intervals (0.5, 1, 1.5, 2, 3, 4, 5 and 6 hr), 5 ml aliquots of the release medium were withdrawn for analysis and replaced with equal volume of fresh phosphate buffer (pH 5.5) solution to maintain a constant volume [28]. The drug content was determined spectro photometrically at λ_{max} 252 nm. The results were the mean values of three runs.

Release efficiency percentage after 6 h (RE_6%) was considered as a basis for comparison of the dissolution and was calculated based on the following equation:

$$\text{Release Efficiency (RE \%)} = {}_0\int^t y.\,dt \,/\, y_{100}\, t^{*}\, 100$$

Release efficiency is defined as the area under the release curve up to a certain time, t, and expressed as a percentage of the area of the rectangle described by 100% release in the same time.

Where the integral is the area under the curve up to release time t and y_{100} is for 100% dissolved drug. For each formula, values of (RE_6 %) were calculated [29]. The mechanism of CLM release from niosomal formulations was determined using the following mathematical models: zero-order kinetics, diffusion Higuchi kinetics and the Korsmeyer-Peppas models. Linearity was detected by linear regression analysis.

The coefficient of determination (R^2) was determined in each case. The large value of (R^2) indicated a superiority of the dissolution profile fitting to mathematical equations.

Incorporation of CLM niosomes in hydroxyl propyl methyl cellulose K-15M gel base

CLM niosomal formula F5 was incorporated in hydroxypropyl methyl cellulose K-15M (HPMC-K15M) base (4% w/w) and was represented by G1, where the weighed amount of HPMC was dissolved gradually in distilled water by the aid of magnetic stirrer at medium speed. Stirring was continued until the formation of gel base then left overnight for equilibration. Niosomal gel formulations were prepared by mixing the niosomal dispersion with the gel base.

In vitro release studies of CLM niosomal gels through artificial membrane

Accurately measured amounts of CLM niosomal gel formulations, equivalent to 25 mg of CLM were placed in a glass cylinder having the length of 10 cm and diameter of 2.5 cm. The same procedures were followed as previously mentioned. The percent CLM released as a function of time is presented as the arithmetic means of three measurements (± SD).

Statistical data analysis

Analysis of the factorial design was performed using Social Package for Statistical Study software (SPSS 17®, SPSS Inc., Chicago, USA).

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 [30]. Skin irritation studies were carried out on male albino healthy adult rats weighing (150–200 g). The animals were kept under standard laboratory conditions, with temperature of 25 ± 1 °C and relative humidity of 45-60%, with free access to water and food. Rats were divided into three groups (each group, n = 3):

1. Group A: No application (control).

2. Group B: Niosomal gel without CLM (placebo gel).

3. Group C: CLM niosomal gel (G1).

The dorsal surface of the rats was cleaned and hair was removed by shaving. CLM niosomal gel (G1) and the unloaded niosomal formula dispersed in HPMC-K15M gel (positive control) were placed over the skin with the help of the surgical adhesive tap and they were removed after 48 h [3,31] and the skin was observed for any visible change such as erythema for the next five days. Evaluation was carried out using Draize scale [32] on the base as follows:

0: No erythema development; 1: very slight erythema; 2: well defined erythema; 3: moderate to severe erythema; 4: severe erythema.

The animal experiments were conducted in full compliance with local, national, ethical and regulatory principles for animal care and were approved by the Cairo University Animal Care Committee (S. No. PI 1142).

RESULTS AND DISCUSSION

The low cost, greater stability and resultant ease of storage of nonionic surfactant vesicles [33] have lead to the exploitation of these vesicles as alternatives to phospholipid vesicles for the enhancement of dermal and/or transdermal bioavailability of drugs and substances.

Entrapment Efficiency %EE

The entrapment efficiency is the most important parameter from pharmaceutical viewpoint in niosomal formulations. A high percentage of entrapment would mean less time and effort involved in removal of unentrapped material [34]. The entrapment efficiencies of all niosomal formulations are reported in Table 3. In order to attain high CLM encapsulation efficiency, several factors, including the type of surfactant, the ratio of CH added and presence of DP were evaluated and optimized. CLM entrapment was influenced by the affinity of the drug for the niosome material, the thickness of the niosome bilayers, the drug solubility in water, and the compatibility between the drug and niosome material [35].

Factors affecting entrapment efficiency of CLM

A full 4×2^2 factorial design was applied to evaluate the effect of surfactant type (X1), surfactant to CH ratio (X2) and charge inducing agent (DP) (X3) on the entrapment efficiency (%EE). Analysis of factorial design demonstrated that the surfactant type had a significant effect on the entrapment efficiency ($p < 0.05$) as shown in Fig. 1. The entrapment efficiency of different surfactants could be ranked as follows: Tween 60 > Span 60 > Tween 20 > Span 20.

The entrapment efficiencies for niosomes prepared using Tween 60 were superior to those prepared using Tween 20. This shows that the vesicles obtained from stearyl (C18) chain surfactants (Tween 60) produce higher entrapment efficiencies than surfactants with lauryl (C12) chain (Tween 20), as the length of alkyl chain is a crucial factor of permeability. Thus, long chain surfactants produce high entrapment [36]. Additionally, the alkyl chain length influences the hydrophilic-lipophilic balance (HLB) value of the surfactant [37]. The lower the HLB of the surfactant the higher will be the drug entrapment efficiency and stability as in the case of niosomes prepared using Tween 60 (HLB = 14.9), whilst Tween 20 with a high HLB of 16.7, showed low entrapment efficiency. Our results are dissimilar to the results reported by ***Ruckmani K. and Sankar V., 2010*** which indicate that the higher the HLB of the surfactant, the higher will be the entrapment efficiency [38].

It is clear that CLM niosomes prepared utilizing Span 60 revealed higher entrapment efficiencies than those prepared using Span 20.

The higher entrapment may be due to the solid nature, hydrophobicity and high phase transition temperature of the Span 60 [39]. Spans 60 has the highest transition temperature (Tc = 53 ℃) amongst all spans (16 ℃ for Span 20, 42 ℃ for Span 40 and -12 ℃ for Sp 80) [40]. The span having the higher phase transition temperature provides the higher entrapment for the drug and vice versa [36, 39]. These findings are in agreement with previous researchers' work, where the increase in the alkyl chain length span 60 (C18) > Span 20 (C12) led to increase in the encapsulation efficiency (%EE) [41-42]. Additionally, the lower the HLB of the surfactant the higher will be the drug entrapment efficiency and stability [43-44]. In the present study, niosomes prepared using Span 60, HLB = 4.7, exhibited higher (%EE) compared to Span 20 with a higher HLB value, namely; 8.6.

Many non-ionic surfactants form vesicles when CH is included in the bilayer to the level of 30-50 mole% [45]. CH imparts rigidity to vesicles, which is very important under severe stress conditions [46]. CH improves the fluidity of the bilayer membrane and improves the stability of the bilayer membrane in the presence of biological fluids such as blood/plasma [47]. Depending on the data obtained from our factorial design, surfactant to CH ratio had a significant effect on the entrapment efficiency ($p < 0.05$). Higher entrapment was found at surfactant / CH molar ratio of 1:1. The effect of CH molar ratios on entrapment efficiency is demonstrated in Table 3. High entrapment was found in Tween 20 and Tween 60 formulation at CH /surfactant molar ratio of 1:1. The interaction of CH with Span 60 in the bilayer of niosomes is due to hydrogen bonding [46].

Inclusion of CH increases the viscosity of the formulation indicating more rigidity of the bilayer membrane. Moreover, drug partitioning will occur more easily in highly ordered systems of surfactant and CH. The ability of the lamellar surfactant phase to accommodate drug, depends upon the structure of the surfactant phase [48]. However entrapment efficiency increased with decreased CH content in Span 20 and Span 60 formulations. These results are in a good agreement with that reported by ***Muzzalupo et al., 2005***, where the best encapsulation efficiency was obtained when niosomes were made up of surfactant and CH at the molar ratio of 2:1 [49].

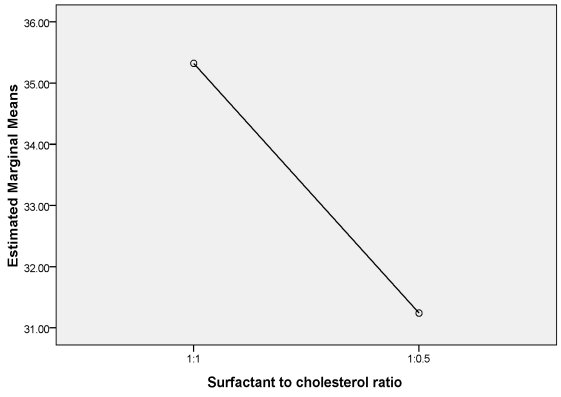
Table 3: % Entrapment efficiency %EE, Particle size, Zeta potential and RE_6% of CLM niosomal formulations

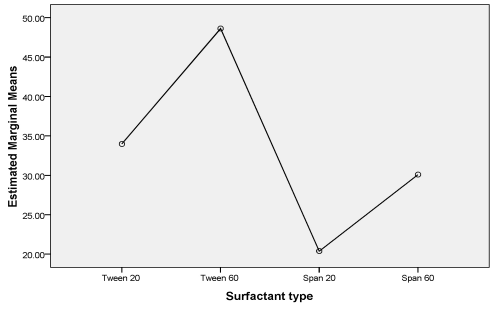
Niosomal Formulation	%Entrapment efficiency (%EE ±S. D.)	Particle size (nm) (mean ± S. D.)	Zeta potential (mV) (mean ± S. D.)	%RE_6[a] ± SD
F1	36.26 ± 1.87	434.40 ± 1.82	-41.70	54.97 ± 1.05
F2	35.17 ± 1.54	299.80 ± 0.30	-17.80	62.83 ± 1.97
F3	29.59 ± 1.37	220.20 ± 1.81	-38.40	72.95 ± 2.45
F4	34.95 ± 0.79	141.80 ± 1.49	-14.00	77.70 ± 1.17
F5	72.54 ± 0.37	825.00 ± 0.31	-32.80	29.72 ± 0.06
F6	46.03 ± 1.71	615.10 ± 1.56	-9.27	24.73 ± 0.75
F7	32.50 ±1.48	538.90 ± 0.93	-41.50	39.57 ± 1.26
F8	43.34 ± 1.40	488.70 ± 0.33	-26.60	50.43 ± 1.45
F9	4.30 ± 0.16	240.60 ± 0.81	-64.50	ND
F10	25.53 ± 1.61	197.10 ± 0.27	-46.80	ND
F11	9.69 ± 0.45	193.50 ± 0.68	-54.10	ND
F12	42.1 ± 2.38	180.40 ± 1.42	-37.00	ND
F13	26.92 ± 1.82	879.80 ± 0.55	-37.10	9.17 ± 1.37
F14	35.71 ± 1.90	261.10 ± 0.17	-35.80	14.86 ± 1.34
F15	29.07 ± 1.57	548.00 ± 0.76	-69.10	15.21 ± 1.37
F16	42.21 ± 2.81	255.00 ± 0.82	-44.40	16.52 ± 1.29

[a] %RE_6: Release Efficiency percentage after 6 h.

It is obvious from Fig.1 that DP had a great effect on the CLM entrapment ($p < 0.05$). In case of niosomes composed of Span 20 (F9–F12) or Span 60 (F13–F16), incorporation of DP was found to decrease the encapsulation efficiency of CLM. These results come in accordance with those reported for the incorporation of charge inducing agents into acetazolamide liposomes [50].

However, contrary to previous results, In case of niosomes composed of Tween 20 (F1–F4) or Tween 60 (F5–F8), the incorporation of DP was found to increase the entrapment efficiency of CLM at equal molarity of these non-ionic surfactants and CH.





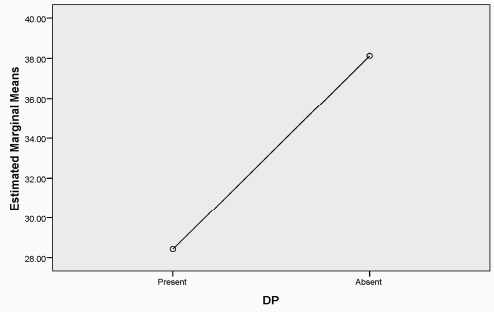


Fig. 1: Effect of surfactant type, surfactant to CH ratio and DP on the Entrapment Efficiency %EE

This increase could be explained by the fact that CH in the presence of DP was more efficiently able to stabilize the structure of the niosomal membrane in a molar ratio of 1:1 (non-ionic surfactant: CH) [33]. These results show that inclusion of DP alters the entrapment; but that it also depends upon the alkyl side-chain of the surfactant and CH molar ratio.

Photomicroscopy and Transmission Electron Microscopy (TEM)

The photomicrographs (x40) of CLM niosomes prepared by reverse phase evaporation technique is shown in Fig. 2.

The spherical morphology of the niosomal vesicles could be observed.

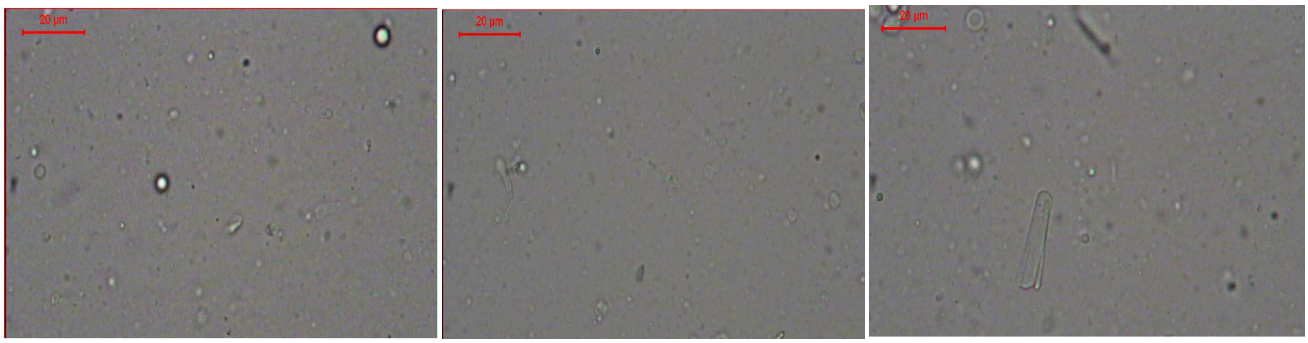
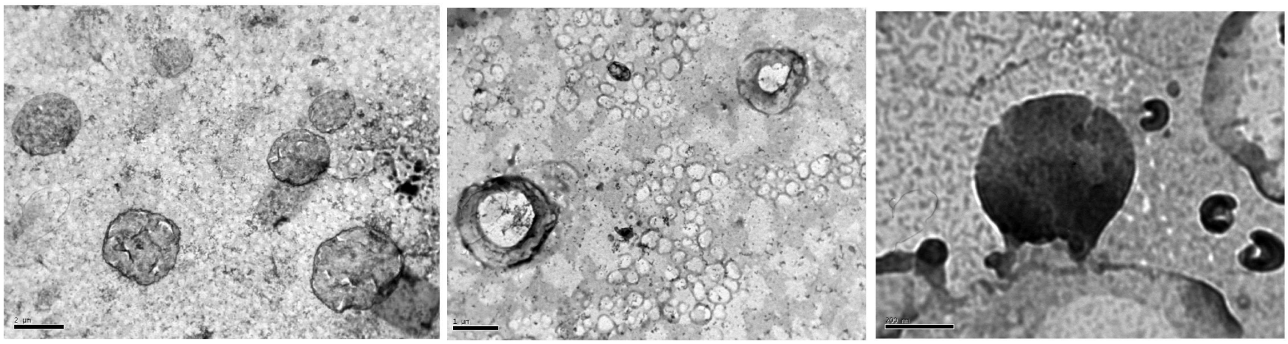


Fig. 2: Photomicrographs of CLM-loaded niosomes, magnification power 40×

Negative stain transmission electron micrographs of CLM niosomes F5 composed of Tween 60, CH and DP in a 1:1:0.1 molar ratio and F14 composed of Span 60 and CH in a 1:1 molar ratio are shown in Fig. 3 A, B and C respectively.

It is demonstrated that the vesicles are well identified and present in a nearly perfect sphere-like shape having a large internal aqueous space relative to the sphere diameter [51].



F5 (A) **F5 (B)** **F14 (C)**

Fig. 3: Negative stain transmission- electron micrographs of CLM **-loaded niosomes F5 (A) [Mag. 8000x]; F5 (B) [Mag. 815000x]; F14 (C) [Mag. 120000x]**

Particle size distribution and zeta-potential determinations

Results of particle size analysis showed that the niosomal formulations prepared using Tween 20, Tween 60, Span 20 or Span 60, with or without charge inducing agent, at both molar ratios are in the nano-size range. Niosomal formulations composed of Tween 60 (F5–F8) are larger in size than niosomes prepared using Tween 20 (F1–F4). Tween 60 has longer alkyl chain compared to Tween 20 and it was reported that surfactants with longer alkyl chains generally give larger vesicles [52]. This would account for the higher entrapment efficiencies obtained with Tween 60 niosomes. The same observation was recorded when particle size of niosomal formulations composed of Span 60 were compared to particle size of niosomal formulations composed of Span 20 prepared. Results of particle size analysis showed that, as the concentration of CH increases, the particle size of different formulations also increases, which was may be due to the formation of rigid bilayer structure [53] as illustrated in Table 3.

When zeta potential was studied to understand the surface charges of the vesicles it was noticed that the zeta potential values of neutral and negatively charged niosomal formulations, prepared using Tween 20, Tween 60, Span 20 or Span 60, of either molar ratio, falls in the zone in which the colloidal system dispersion is stable (more negative than -30 mV). The zeta potential of the niosoml formulations prepared using DP were more negatively charges than neutral niosoml formulations, the values of zeta potential of niosomal vesicles are represented in Table 3.

Differential Scanning Calorimetry (DSC)

DSC for the individual constituents of niosomes, Tween 20, Tween 60, Span 20, Span 60, CH, DP and CLM shows thermal peaks at 45.08 ˚C, 25.13 ˚C, 15.41 ˚C, 56.34 ˚C, 149.00 ˚C, 48.35 ˚C and 194.04 ˚C, respectively, corresponding to their melting temperatures as shown in Fig. 4.

Drug loaded niosomal formulations showed broad transitions which are characteristic for lipid mixtures containing CH, signifying good interaction of all components forming the bilayers of niosomes [54]. Fig. 4 A, B, C and D shows that the DSC thermograms of niosomal formulations prepared using either Tween 20, Tween 60, Span 20 or Span 60 with or without DP, using either molar ratio, reveal the effect of niosomal formulation on the individual constituents of niosomes as well as the effect of entrapped drug by changing the thermodynamic parameters of the thermal peaks. Fig. 4 (D) shows that both transition temperature and transition energy of Span 60 peak of niosomal formulation (F16) is less than that of pure Span 60.

A DSC thermogram of loaded niosomes exhibit disappearance of characteristic exothermic peak of CLM. The absence of the melting endotherm of CLM and shifting and/or broadening of the endotherms of surfactant bilayers of niosomes suggests possible interaction of CLM with bilayer components and can account for the enhanced entrapment of CLM into these formulations [51,55].

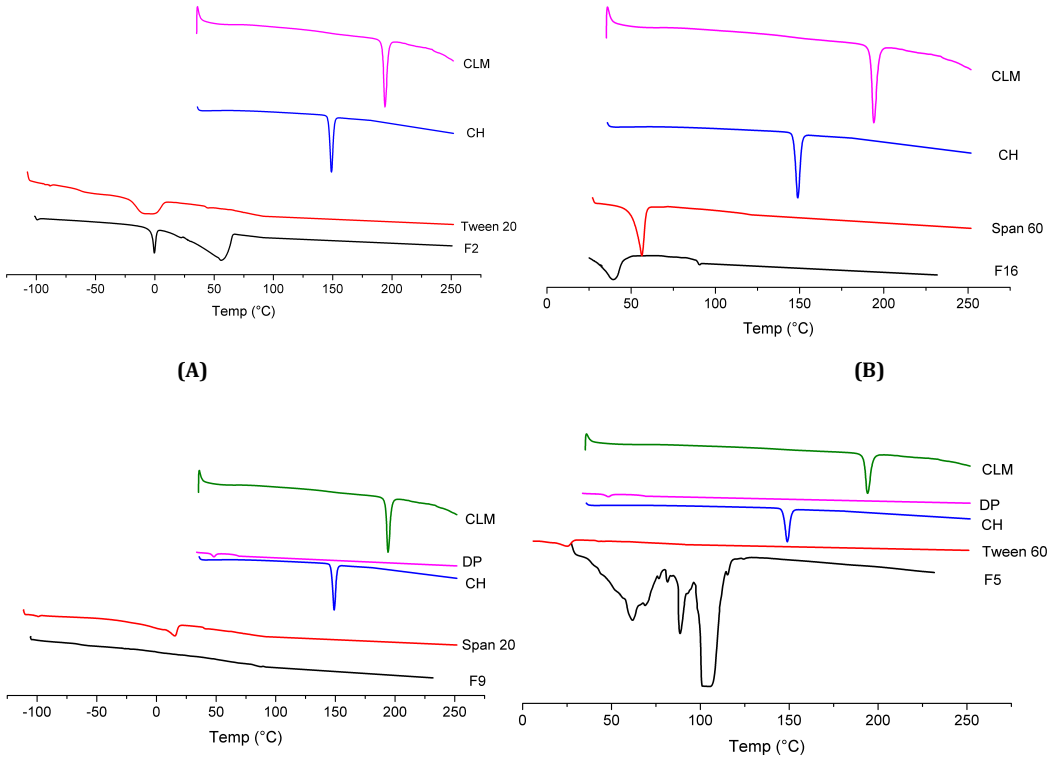


Fig. 4: Differential Scanning Calorimetry (DSC) measurements of niosome components and CLM loaded niosomal formulae (A) F2; (B) F16; (C) F9; (D) F5

In vitro Release of CLM from Niosomes

The release profile of CLM from niosomal formulations prepared using Tween 20, Tween 60 or Span 60 is graphically illustrated in Figs. 5, 6 and 7, respectively. The release efficiency percentage after 6 h (%RE_6) of the prepared niosomal vesicles are shown in Table 3. From the results, it is obvious that niosomal CLM formulations showed slower release rate than CLM solution.

Analysis of factorial design demonstrated that the surfactant type had a significant effect on the release efficiency percentage after 6 h ($p < 0.05$) as shown in Fig. 8. The release efficiency of different surfactants could be ranked as follow: Tween 20 > Tween 60 > Span 60. This can be explained by the fact that niosomes exhibit an alkyl chain length-dependent release and the higher the chain length, the lower the release rate [45].

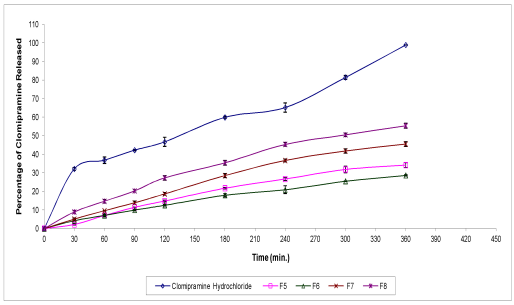


Fig. 6: Percentage release profile of CLM from niosomal formulae (F5–F8)

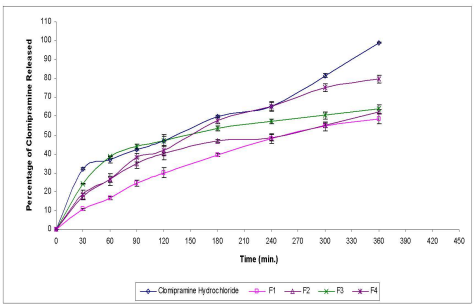


Fig. 5: Percentage release profile of CLM from niosomal formulae (F1–F4)

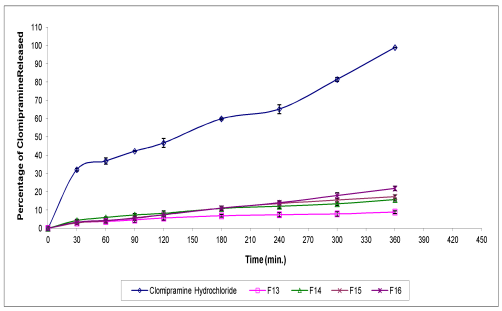


Fig. 7: Percentage release profile of CLM from niosomal formulae (F13–F16)

By inspection of the data, it could be concluded that niosomal formulations of Tween 20, Tween 60 or Span 60 with CH in a molar ratio 1:1 (non-ionic surfactant: CH) (F1, F2, F5, F6, F13, F14) showed slower release rate of the drug when compared to niosomes composed of (non-ionic surfactant: CH) in a molar ratio 1:0.5. Increasing CH markedly reduces the efflux of the drug. Inclusion of CH fills the pores in vesicular bilayers and abolishes the gel-liquid phase transition of liposomal and niosomal systems resulting in niosomes that are less leaky.

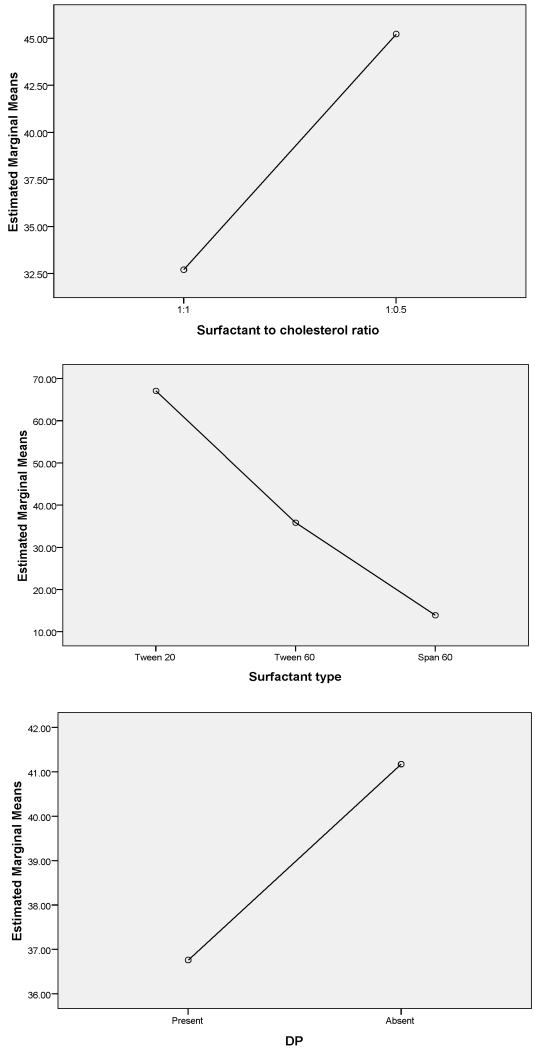


Fig. 8: Effect of surfactant type, surfactant to CH ratio and DP on the %Release efficiency

This confirms that CH in the formulation acts as a membrane stabilizing agent that helps to sustain drug release [38]. ***Cocera et al., 2003*** reported that CH produced an optimum hydrophobicity which decreased the formation of the transient hydrophilic holes, by decreasing membrane fluidity, responsible for drug release through liposomal layers [56]. It is obvious from Fig. 8 that DP had a great effect on CLM release ($p < 0.05$). By comparing the release data of CLM niosomes containing DP with that of DP free-niosomes, it is clear that the release is retarded in presence of DP. This comes in accordance with the effect of DP in stabilizing the niosomal membrane structure, rendering it less permeable [57].

Linear regression analysis for the release data of CLM from niosomes (F1–F16) is tabulated in Table 4. The data showed that the CLM is released following diffusion controlled mechanism for formulae (F1 and F5), zero release kinetics (for formulae F4, F6, F7, F13, F15 and F16) and Korsmeyer-Peppas for F2, F3, F8 and F14. The *n* value from the Korsmeyer-Peppas model for CLM niosomal formulations was > 0.54 indicating anomalous diffusion mechanism with erosion, except for F3, F13 and F14 between 0.23 and 0.54 which confirms the Fickian type diffusion [58].

In vitro release studies of CLM niosomal gels through artificial membrane

The release profile of CLM from the prepared niosomal gel in Sorensen's phosphate buffer of pH 5.5 is graphically represented in Fig. 9. It was evident that the incorporation of the drug in niosomes resulted in delayed release which was further delayed in case of niosomal gel due to formation of an additional diffusion barrier to drug release [59]. In case of the prepared niosomal gels a noticeable and gradual control of release behavior of CLM could be observed during the whole release run (19.00%) of CLM was released from G1 after 6 h.

Skin irritancy test

No obvious erythema, oedema or inflammation was observed on rats' skin after one week of application of the selected formulation. Thus it could be concluded that neither the surfactant used nor the CLM used in the niosomal formulation as well as the other excipients were non-irritant to the skin.

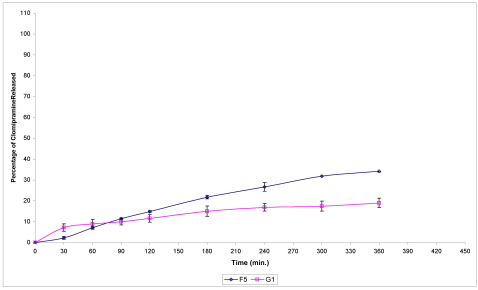


Fig. 9: *In-vitro* release profile of CLM from niosomal gel in Sorensen's phosphate buffer of pH 5.5

Table 4: Kinetic analysis of release data of CLM through artificial membrane

Niosomal Formulation	Zero order R^2	Higuchi diffusion R^2	Korsemeyer- Peppas R^2	Release Order	*n*
F1	0.989	0.994	0.993	Diffusion	0.759
F2	0.942	0.987	0.998	Peppas	0.613
F3	0.869	0.941	0.955	Peppas	0.485
F4	0.987	0.985	0.982	Zero order	0.641
F5	0.993	0.997	0.985	Diffusion	1.393
F6	0.999	0.986	0.998	Zero order	0.820
F7	0.999	0.974	0.996	Zero order	0.929
F8	0.991	0.989	0.994	Peppas	0.789
F13	0.996	0.978	0.978	Zero order	0.439
F14	0.994	0.988	0.999	Peppas	0.471
F15	0.993	0.954	0.969	Zero order	0.647
F16	0.983	0.932	0.944	Zero order	0.549

R^2= coefficient of determination.

CONCLUSION

From the presented study, it is clear that CLM niosomes were successfully prepared by the thin film hydration technique. Surfactant structure, CH content and the presence of negative charge inducer (DP) altered the entrapment efficiency %EE and release rate from CLM niosomes. Niosomal formulations characterization using TEM showed the spherical shape of the prepared niosomes. DSC studies gave evidence of possible interaction of CLM with niosomal components. Incorporation of drug in niosomes resulted in delayed release which was further delayed in case of niosomal gel. CLM niosomal gel showed no irritation to rat skin. Accordingly, transdermal delivery of niosomal CLM formulations is expected to improve drug bioavailability, while preferably avoiding undesired effects and improving patient compliance.

CONFLICT OF INTERESTS

Declared None

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