Available online at *www.ujpronline.com*
Universal Journal of Pharmaceutical Research
An International Peer Reviewed Journal
ISSN: 2831-5235 (Print); 2456-8058 (Electronic)
Copyright©2016; The Author(s): This is an open-access article distributed under the terms of the CC BY-NC 4.0 which permits unrestricted use, distribution, and reproduction in any medium for non-commercial use provided the original author and source are credited

RESEARCH ARTICLE

INVESTIGATION OF PRONIOSOMES GEL AS A PROMISING CARRIER FOR TRANSDERMAL DELIVERY OF GLIMEPIRIDE

Elsaied Hamada Elsaied, Hamdy Mohamed Dawaba, Elsherbini Ahmed Ibrahim, Mohsen Ibrahim Afouna
Department of Pharmaceutics, Faculty of Pharmacy (Boys), Al-Azhar University, Cairo, Egypt.

Article Info:

Article History:
Received: 6 October 2016
Reviewed: 10 November 2016
Accepted: 8 December 2016
Published: 15 January 2017

Cite this article:

Elsaied EH, Dawaba HM, Ibrahim EA, Afouna MI. Investigation of proniosomes gel as a promising carrier for transdermal delivery of Glimepiride. Universal Journal of Pharmaceutical Research 2016; 1(2): 1-10.
http://doi.org/10.22270/ujpr.v1i2.R1

***Address for Correspondence:**

Hamdy Mohamed Dawaba, Department of Pharmaceutics, Faculty of Pharmacy (Boys), Al-Azhar University, Cairo, Egypt; Tel: +201022950831.
E-mail: *hamdydawabah@azhar.edu.eg*

Abstract

Objectives: The aim of the study was to develop a proniosomal carrier system that is capable of efficiently delivering entrapped glimepiride over an extended period of time for the treatment of type 2 diabetes.
Methods: Proniosomal gels were developed based on Span 60 with and without cholesterol. The entrapment efficiency of drug inside niosomes developed from hydration of the proniosomes gel was also characterized. The *in vitro* release and skin permeation of glimepiride from various proniosomes gel formulations were investigated. The stability studies were performed at 4°C and at room temperature.
Results: The maximum entrapment efficiency was obtained when the cholesterol concentration was 10% of total lipid (90.02%). *In vitro* release through mixed cellulose ester membrane showed sustained release of drug from proniosomes gels. *In vitro* drug permeation across rabbit skin revealed improved drug permeation and higher transdermal flux with proniosomes gels compared to hydro-alcoholic gel of drug. Also, good physical stability was also achieved with proniosomes gels. Kinetics of *in vitro* skin permeation showed diffusion model of drug release from formulations.
Conclusion: The study proved that the concentration of cholesterol had great influence on the properties of proniosomes gels. Hence, proniosomes preparation containing 10% cholesterol can significantly increase trans-epidermal flux and prolong the release of glimepiride.
Keywords: Cholesterol, glimepiride, proniosomes gel, Span 60, sustained release, transdermal drug delivery.

INTRODUCTION

The transdermal mode offers several distinct advantages; the skin presents a relatively large and readily accessible surface area for absorption, Further benefits of transdermal drug delivery (TDD) include the potential for sustained release, the ease of termination, if need arises (e.g. systemic toxicity) with less pain sensation[1] and avoiding the first pass hepatic metabolism[2]. The self-assembly of non-ionic amphiphiles in aqueous media results in closed bilayer structures called niosomal vesicles or simply niosomes[3]. They are analogous to liposomes (phospholipids vesicles) and can encapsulate both hydrophilic and lipophilic solutes, hence, serve as drug carriers[4]. Among all routes of administration, the enhanced transdermal delivery of niosome encapsulated drugs was seriously considered. TDD with niosomes appears promising for hydrophobic and amphiphilic drug molecules and would require that the dose to be applied in high concentration and within niosomes prepared from low phase transition surfactant mixtures[5]. Moreover, for transdermal application, niosomal vesicles should be included into polymeric matrix like methylcellulose gels. However, the use of polymeric matrix in the formulation may affect drug penetration and niosomes integrity[6]. This problem in addition to the physical instability of the niosomal dispersions can be circumvented by the formulation of alcoholic solutions of the nonionic surfactant or of liquid crystalline compacts called proniosomes. Both systems are of great stability due to very little water content. Previously, proniosomes have been introduced as a stable form alternative to the liquid formulations of niosomes, offering advantages in terms of formulation stability and shipping expenses[7]. They are transformed into niosomes immediately upon hydration; hence, they are called proniosomes[8-10]. The great advantages offered by proniosomes are their ease of use and their hydration is much easier than the time consuming

shaking process required to hydrate surfactants in the conventional dry film method[11]. Furthermore, unacceptable solvents are avoided in proniosomes formulations. The systems may be directly formulated into transdermal patches and doesn't require the dispersion of vesicles into polymeric matrix[12].

Glimepiride (GMD) is a 3rd generation sulfonylurea drug used for treatment of type 2 diabetes. Clinical studies have proven GMD to be safe and effective in reducing fasting and postprandial glucose levels, as well as glycosylated hemoglobin concentrations, with dosages of 1–8 mg/day. GMD has been associated with severe and sometimes fatal hypoglycemia and gastric disturbances like nausea, vomiting, heartburn, anorexia, hemolytic anemia due to the transient high blood concentration that occurs after oral administration and increased appetite after oral therapy[13]. GMD has a relatively short elimination half-life (5 h), thereby requiring twice or thrice daily dosing in patients. Since these drugs are usually intended to be taken for a long period, patient compliance is also very important[14]. To circumvent these drawbacks, GMD was entrapped in vesicular carrier system to improve therapeutic efficacy of GMD via transdermal route. The purpose of this study to develop GMD proniosomes carrier systems using the common, non-irritant, safe and available non-ionic surfactants Span 60 with and without cholesterol and to investigate the possibility of using proniosomes gels for transdermal delivery of GMD.

MATERIALS AND METHODS

GMD was a kind gift from Medical Union Pharmaceuticals, Abu Sultan, Ismailia, Egypt; Sorbitan monostearate (Span 60), Cholesterol (> 99%), Sodium azide and Hydroxy Propyl Methyl Cellulose (HPMC; MW 86,000 Da, viscosity of 2% solution 4000 cP) were procured from Sigma-Aldrich, St. Louis, MO, USA. All other chemicals and solvents were of analytic grade and obtained from El-Nasr Company for pharmaceutical chemicals, Cairo, Egypt.

Spectrophotometric scanning of GMD

Standard solution of GMD was prepared by transferring 10 mg of glimepiride into a 100 ml volumetric flask and the volume was made up to 100 ml using phosphate buffer containing 10% methanol (pH 7.4) as a solvent to get the concentration of 100µg/ml. Fresh aliquot (3 ml) from the standard solution was pipette out and subjected to spectrophotometric scanning using Shimadzu spectrophotometer (model UV-1800 PC, Kyoto, Japan). The scanning range was from 200-400 nm using phosphate buffer containing 10% methanol (pH 7.4) as blank.

Preparation of Proniosomes gels

Proniosomes gels were prepared by the coacervation phase separation method previously reported[15]. In glass vials accurately weighed amounts of Span 60 were mixed with the calculated amount of cholesterol. The cholesterol was added as 0%, 10%, 30% and 50% of total lipids. Absolute ethanol (about 400 mg) was added to the surfactant or surfactant/cholesterol mixtures. Precisely, surfactants: alcohol (1:1) then vials were tightly sealed and warmed in water bath (55-60°C) for 5-10 min while shaking until complete dissolution of cholesterol. To each of the formed transparent solutions, about 0.16 ml hot distilled water (55-60°C) was added while warming in the water bath for 3-5 min till a clear or translucent solution was produced. The mixtures were allowed to cool down at room temperature and observed for the formation of white creamy proniosomal gel (Table 1). The obtained formulations were kept in the same closed glass vials in dark for further characterization[15].

Drug loading into proniosomes gels

GMD was added as (5, 10, 12, 15, 17 and 20 mg) to the nonionic surfactant/cholesterol mixture (400mg) and dissolved by the aid of absolute ethanol while warming at 50-60°C in water bath. It was noted that the addition of the drug didn't show turbidity or precipitated crystals in preparations.

Hydration step and formation of niosomes

About 7 ml of phosphate buffer (pH 7.4) were added into each vial followed by heating for 10 minutes at a temperature 60±3°C in a water bath while vortexing. The final volume was adjusted to 10 ml by the same buffer[15].

Preparation of 50% alcoholic HPMC gels containing GMD

The weighted amount of HPMC-4000 cp was dispersed in distilled water containing 50% ethyl alcohol and stirring for sufficient time (about 30 min) was allowed for complete hydration and gel formation, then the weighted amount of GMD was added and dispersed thoroughly. The concentration of HPMC-4000 cp was 2% and the final concentration of GMD was adjusted to be 15 mg/gm.

Characterization of proniosomes GEL BASES

Total drug content

One ml of aqueous dispersion of niosomes was disrupted through addition to 50 ml methyl alcohol followed by shaking for 15 min.

Table 1: Formulation design of proniosomes gels.

Formula Code	Formula Composition (mg)				Physical Appearance
	GMD	Span 60	Cholesterol	Ethyl alcohol	
P6	15	400	0	400	White creamy gel
P6A	15	360	40	400	White creamy gel
P6B	15	280	120	400	White creamy gel
P6C	15	200	200	400	White creamy gel

GMD = Glimepiride

The solution was diluted to 100 ml with methyl alcohol[16]. Aliquots were withdrawn and the absorbance was recorded at 228 nm.

Particle size analysis

The mean size of niosomal vesicles was analyzed by dynamic light scattering technique using Zetasizer Nano ZS (Malvern Instruments Ltd., Worcestershire, UK). Before measurement, samples were dispersed in distilled water followed by sonication for 5 min using Qsonica Ultrasonic Homogenizer Q125 (Qsonica, LLC. Newtown, CT, USA). Triplicate measurements were carried out at 25±0.5°C[17].

Microscopic examination

Light microscopy

A thin layer of proniosomes gel was spread on a glass slide. After placing the cover slip, the sample was examined for the gel structure and the presence of insoluble drug crystals using ordinary light microscope with magnification powers (40 X). Photomicrographs were taken for either the proniosomes gel or niosomes developed after adding a drop of water through the side of the cover slip while the slide still under microscope[12].

Transmission electron microscopy (TEM)

Transmission electron microscopy (JEOL-JEM-2100, Tokyo, Japan) was used to investigate the morphology of niosomes. A drop of the diluted dispersion was applied to a carbon-coated 300 mesh copper grid and left for 1min to allow some of the niosomes to adhere to the carbon substrate. The remaining dispersion was removed by absorbing the drop with the corner of a piece of filter paper and the sample was air dried[18].

Fourier transform-infrared spectroscopy (FT-IR) studies

The FT-IR spectral analysis was conducted to verify the possible chemical intermolecular interactions between GMD, Span 60 and cholesterol. The samples were scanned using FT-IR spectrophotometer (Nicolet 200 FT-IR, USA) in the spectral region between 4000 and 400 cm^{-1}. The smoothing of the spectra and the baseline correlation procedures were applied. The detector was purged with dry nitrogen gas to reduce moisture and to increase the signal level.

Differential scanning calorimetry (DSC)

The thermotropic properties of the samples were explored to assess the degree of crystallinity and the presence of possible interactions between GMD, Span 60 and cholesterol. DSC analysis was performed with a DSC Q2000 V24.4 Build 116. The calorimeter was calibrated for temperature and heat flow accuracy using the melting of pure indium (mp156.6°C and ΔH of 25.45 J gm^{-1}). The temperature range was from 0 to 300°C with a heating rate of 10°C/min. The gas used was nitrogen with a purging rate of 50 ml/min. The weight of each sample was 5-9 mg.

Determination of the entrapment efficiency of GMD in niosomes

Freeze-thawing/centrifugation method was used to determine the entrapment efficiency of GMD in niosomes as follows: 1 ml samples of niosomes dispersion were frozen for 24h at -20°C in Eppendorf tubes. The samples were removed from the freezer and were let to thaw at room temperature, then centrifuged at 20000 rpm for 60 min at 4°C. Two times washings with phosphate buffer (pH 7.4) were done for complete removal of drug adsorbed on the surface of niosomal vesicles. The supernatant was separated each time from niosomal pellets and prepared for the assay[15]. Each result was the mean of three determinations (±SD). The drug content was determined spectrophotometrically at 228 nm against with drug free systems as blank in order to avoid interference of lipids. The % of GMD entrapped was calculated by subtracting amount of un-entrapped drug from the total drug incorporated[19].

$$\%\ \text{Encapsulation efficiency} = \frac{\text{amount of drug encapsulated}}{\text{total amount of drug}} \times 100$$

Assessment of GMD release rates from proniosomes across mixed cellulose ester (MCE) membrane

The release of GMD from different proniosomes gels were determined using modified Franz cell fabricated locally with 6.6 cm^2 of diffusion area. MCE membrane (0.45μm pore size), previously soaked in phosphate buffer (pH 7.4), was mounted between the donor and receptor compartments of the diffusion cells. The proniosomes gels were placed on MCE membrane in the donor chamber whilst phosphate buffer containing 10% methanol (pH 7.4) was used as a receiver medium in the receptor chamber. The temperature was kept at 32±0.5°C and the stirring rate was 100 rpm in Kottermann shaker (D3165 Hangisen, Germany). About two milliliter samples were withdrawn at specified time intervals (0.5, 1, 2, 3, 4, 6, and 8 h) and replaced with equal volumes of fresh receiver medium to keep the volume constant during the experiment. Samples were analyzed spectrophotometrically at 228 nm against samples collected from diffusion of drug free systems as blank.

Assessment of GMD release rates from niosomes

The niosomal pellets prepared from proniosomes gels of Span 60 were separated by centrifugation and washed twice as described above. The pellets were resuspended in 50 ml of phosphate buffer (pH 7.4). The resultant suspension was placed in a stoppered glass flask in a shaking water bath at 37°C. One ml samples were withdrawn at the following intervals; 0, 1, 2, 3, 4, 6 and 8 h after incubation. Samples were centrifuged as described above and supernatants were assayed at 228 nm. The percentage of drug release was plotted as a function of time[15].

***Ex vivo* skin permeation study**

Abdominal skin of male rabbits (2-2.5 kg) was used in the study. Hair was removed from the abdominal skin with the aid of an electric animal clipper and shaver. Care was taken not to damage the skin surface. Rabbits were sacrificed and the abdominal skin of the rabbit was separated. Sodium azide (0.02%) is applied to the skin as preservative and the skin was stored at -20°C and used within three days for the permeation study. The permeation experiments were run by using the same diffusion cells previously described in the release through MCE membrane. However, skin membranes were mounted, with the stratum corneum side towards the donor (drug loaded system) and the dermal side facing and in contact with the receptor compartment which contained phosphate buffer containing 10%

methanol (pH 7.4) maintained at 32±0.5°C[20] and the stirring rate was 100 rpm in Kottermann shaker. Proniosomes gels and alcoholic HPMC gels were applied to the stratum corneum side. 2 ml aliquots were sampled at 0.5, 1, 2, 3, 4, 6, 8, 12 and 24 h after starting the experiment from the receptor medium and replaced with equal volume of the fresh receiver medium. Samples were analyzed spectrophotometrically at 228 nm. Each experiment was carried out in triplicate. All skin permeation parameters such as steady state transdermal flux (SSTF), permeability coefficient (PC), maximum diffusion (D_{max}) and diffusion coefficient (D) of GMD across rabbit skin were estimated for different formulations. Permeability parameters of GMD were constructed by plotting the cumulative amount of the drug permeated per unit area (Q) as a function of time. The steady state flux (J_{SS}) was calculated from the slope. The permeability coefficient (P_C) was calculated by dividing the flux by the initial drug load (C_o).The diffusion coefficient (D) was calculated by plotting the cumulative amount of drug permeated versus square root of time($\sqrt{t}$) according to Equation[13].

$$Q=2C_o\,(D_t/\pi\,)^{½} \qquad \text{(Higuchi model)}$$

Assessment of physical stability for proniosomes gels

Proniosomes gels stability and aggregation of niosomes dispersions as a function of temperature were determined as the change in total drug content and entrapment efficiency after storage. The proniosomal gels and niosomes dispersions were stored in glass vials at room temperature or kept in refrigerator (4°C) for 3 months[18].

Statistical analysis

The data were reported as mean±SD (n=3) and statistical analysis of the data were carried out using one-way ANOVA at a level of significant of $p<0.05$.

RESULTS AND DISCUSSION

Spectrophotometric scanning of GMD

Glimepiride was analyzed in the whole study using UV spectrophotometric method. The UV spectrum of glimepiride in phosphate buffer containing 10% methanol (pH 7.4) showed absorption maxima at 228 nm. The obtained results came in accordance with other researchers' investigations[21-24].

Formation of proniosomes gels

A sol phase of Proniosomes gels were formed when distilled water (about 30-40% w/w of total lipids) is added at 60°C as drops to the nonionic surfactant dissolved into hot absolute ethanol while vortexing. Since the solvent (absolute ethanol) amount is small and the formation of micelles is not possible into this solvent, it is expected that the addition of small amount of water favors the self-assembly of the surfactant into w/o microemulsion sol phase, where the aqueous droplets bound by surfactant interfacial films which are dispersed in the continuous solvent phase[25]. Cooling the sol phase results in a decrease in the solubility of Span 60 and cholesterol in the solvent and consequently, lower solvent-cholesterol affinities due to the limited solvent system present.

Table 2: Characterization parameters of proniosomes gels.

Formula Code	Characterization Parameters*			
	Drug Content (mg/ml)±SD	Vesicle Size		Entrapment Efficiency %
		Before sonication (µm)±SD	After sonication (nm)±SD	
P6	1.49±0.42	4.23±1.08	293.2±2.38	89.07±0.21
P6A	1.46±0.51	5.11±2.19	298.5±2.12	90.02±0.23
P6B	1.52±0.09	5.86±2.56	310.2±3.21	87.56±0.30
P6C	1.48±0.17	7.26±3.48	636.8±4.57	86.88±0.70

*Each parameter has been investigated in triplicate. (n=*3*)

The formed gel structure proposed to be amphiphilic system comprising double layers of oriented molecules placed head to head and tail to tail with water present as droplets or sheets of water molecules between the hydrophilic residues of the surfactant layers[12]. Span 60 produced white creamy gel in the presence or absence of cholesterol as it has high transition temperature (Tc=53°C) and is solid at room temperature, so it acts as gelator by itself. The formation of proniosomes gels is a thermo-reversible process as an increase in temperature results in the solvation of the gelator into the solvent and returns again to the gel structure upon cooling[15].

Hydration of proniosomes gels

Niosomes are formed only when the proniosomes gels comes into contact with an aqueous environment such as moisture found on the skin or mucosal surfaces. The contact of proniosomes gels with water leads to swelling of the lamellar liquid crystal bilayers and vesicles due to the interaction of the hydrophilic head groups of the surfactant with water molecules[12]. The surfactant tubules serve as conduits for water penetration into the gel network and the gel fragments[26]. The complete hydration of proniosomes gels was found to take long time ranging from 1h to 2h at room temperature with continuous stirring or vortexing. So, warming the gel with excess water above the transition temperature of Span 60 (>60°C) accelerates the transformation to niosomal structures within 5-10 min only.

Drug loading in proniosomes gels

Total 15 mg of GMD was found to be completely dissolved into proniosomes gels. This is perhaps due to high solubility of the drug in surfactant/cholesterol/ethanol mixture. Microscopic examination showed no drug crystals precipitated.

Drug content

The actual amount of GMD added for all practical purposes into the vesicular dispersions was 1.5 mg/ml and the drug content of the developed formulations was not found to be significantly different (p<0.001) from the added amount (Table 2).

Vesicle size investigation

The mean size of niosomal vesicles was analyzed by dynamic light scattering technique using Zetasizer Nano ZS (Malvern Instruments Ltd., Worcestershire, UK). This technique analyzes the fluctuations in light scattering due to the Brownian motion of vesicles. Before measurement, samples were dispersed in distilled water to obtain a suitable scattering intensity at 90°C with respect to the incident beam[17]. To promote the development of fine dispersions, sonication for 5 minutes was performed. Preliminary studies confirmed that sonication of the vesicles is necessary to achieve fine dispersions[17]. However, an inverse correlation was observed between the sonication time and the EE% of the drug. Therefore, a good compromise was achieved with a sonication time of 5 min[17]. The obtained results were represented in Table 2. The mean vesicle size of the dispersion ranged from 4.23 µm (P6 formulation) to 7.26 µm (P6C formulation) before sonication and ranged between 293.2 nm (P6 formulation) to 636.8 nm (P6C formulation) after sonication. Cholesterol had positive influence on the developed vesicles and is thought to be responsible for the higher diameters. Proportional correlations were observed between the cholesterol content and the mean vesicle size.

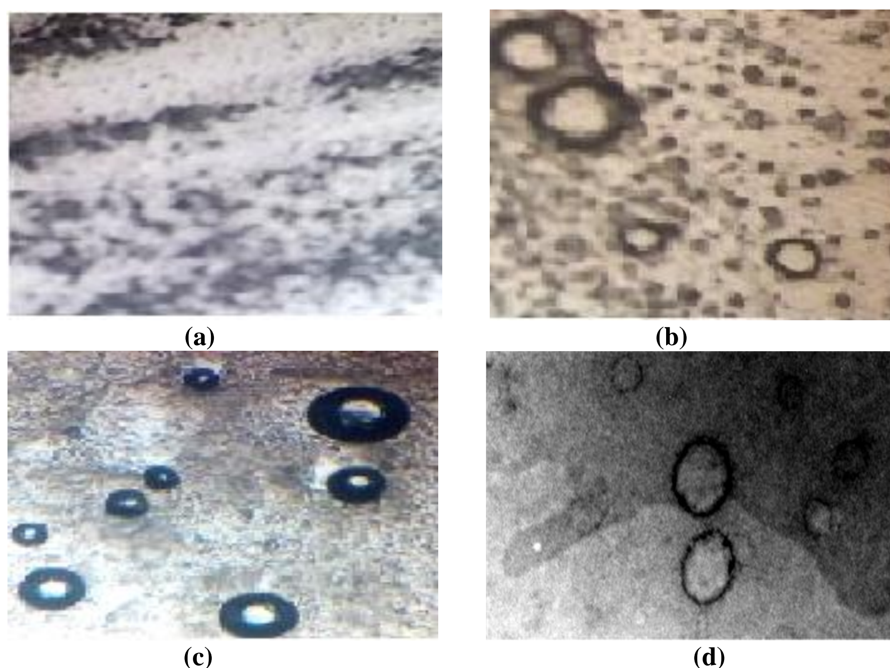

Figure 1: Photographs of: (a) Proniosamal gel under optical microscope (b) and (c)Niosomal vesicles under optical microscope (d) TEM micrographs of niosomalvesicles at 60000x magnification power.

Microscopic examination

Gel structure

The gel structures were formed from floccules of small tubular and vesculating particles which have creamy opaque appearance and also no drug precipitates (Figure 1a). The units of the gel are often bound together by van der Waals forces so as to form crystalline regions throughout the entire system. The dominant coherent gel phase is built up by surfactant/cholesterol lamellae with water phase mainly bound interlamellarly to the hydrophilic head groups of surfactant/ cholesterol lamellae. This resulted in an interconnected network of a mixture of lamellar liquid crystals resembling palisades or tubular aggregates and vesculating lamellae[12].

Niosomes dispersions:

Niosomes developed under ordinary microscope gave rise to randomly scattered spherical structures comprising multilamellar and multivesicular vesicles (Figure 1b and c). The examined niosomes appeared as small unilamellar, spherical nano-vesicles under TEM analysis (Figure 1d). The explanation for this observation is as follows: due to limited solvent presents, proniosomes formed were mixture of lamellar liquid crystals stacked together (best described as compact niosomes). Addition of water causes swelling of bilayers as well as vesicles due to interaction of water with polar groups of the surfactant. Upon addition of small amount of water, the bilayers tend to form spherical structures randomly giving rise to multilamellar, multivesicular structures (Figure 1b and c). When proniosomes are shaken with excess amount of water, complete hydration takes place leading to the formation of niosomes (Figure 1d). Large vesicles were formed after hydration of proniosomes without agitation as energy applied during agitation results in the breakage of large multilamellar vesicles into smaller unilamellar vesicles[12].

FT-IR studies

The FT-IR spectra of GMD, Span 60, cholesterol and physical mixture were displayed in Figure 2. The GMD showed strong absorption peak at 1159 cm^{-1}

corresponding to the sulphonamide group (S=O) and peaks at 1696 cm^{-1} corresponding to carbonyl group (C=O). The broad band's at 3374 cm^{-1} and 3377 cm^{-1} could be assigned to O-H stretching vibrations of Span 60 and cholesterol, respectively. The FT-IR spectrum of the physical mixture revealed that the characteristic bands of GMD did not disappear or exhibit major shifts. Furthermore, no new bands were formed. These findings pointed out the lack of considerable intermolecular interactions between GMD, Span 60 and cholesterol.

Differential scanning calorimetry (DSC)

DSC is a fast and reliable method to screen drug-excipients interactions as indicated by appearance of a new peak(s), change in the peak shape and its onset, peak temperature/ melting point and relative peak area or enthalpy. Figure 3 depicts various DSC thermograms obtained during the study. Pure GMD showed a sharp endothermic peak at 205.72°C. Thermogram of Span 60 exhibits an endothermic peak with onset at 44.62°C and maximum occurrence at 52.24°C. Cholesterol is reported to show an endothermic peak at 148-150°C. It is evident that the original peaks of GMD disappear from the thermogram of proniosomal gel (containing Span 60, cholesterol and GMD or Span 60 and GMD). These observations confirm incorporation of GMD into proniosomal gel proving complete entrapping of drug into the vesicles.

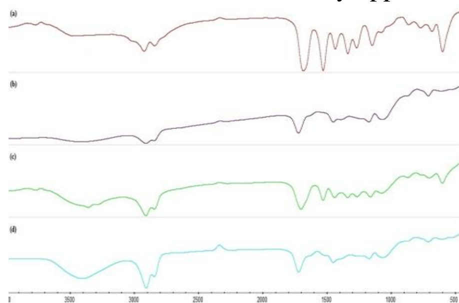

Figure 2: FT-IR spectra of (a) GMD, (b) Span 60, (c) Span 60 and GMD and (d)Span 60, GMD and Cholesterol.

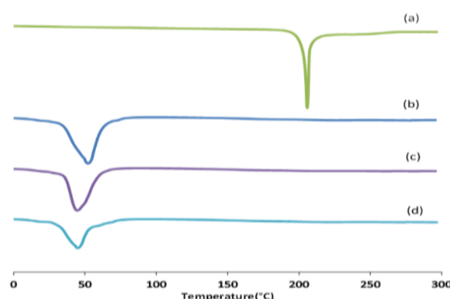

Figure 3: DSC thermogram of (a) GMD, (b) Span 60, (c) Proniosomes gel of Span 60 and GMD and (d) proniosomes gel of Span 60, GMD and Cholesterol.

Entrapment efficiency of GMD in niosomes

Freeze-thawing/Centrifugation method was used to determine the entrapment efficiency of GMD in niosomes. This method was selected as the statistical analysis showed that freezing the prepared niosomes for 24 h at−20°C followed by centrifugation resulted in a significant increase in the entrapment efficiency (p<0.05) compared to that of the non-frozen exhaustively dialysis method[15]. In freezing, drug and vesicles are concentrated; particles are closely packed in contact with each other resulting in fusion of niosomal vesicles. Therefore, large aggregates which include the drug are formed which after shaking, niosomal vesicles are formed entrapping efficiently the drug[15].

Factors affecting entrapment efficiency of glimepiride

Effect of cholesterol

Cholesterol is one of the common and essential additives in niosomes dispersions in the present study. Incorporation of cholesterol was known to influence vesicle stability and permeability[27]. The effect of cholesterol on GMD entrapment was varied according to its concentration. A significant increase (p<0.05) in the entrapment efficiency of GMD was obtained when 10% of cholesterol was incorporated into niosomes prepared from Span 60 (P6A) followed by a decrease in entrapment efficiency of the drug upon further increase in cholesterol content (Table 2).

Reason revealed for this type of behavior is cholesterol molecules accommodate itself as ‘‘vesicular cement’’ in the molecular cavities formed when surfactant monomers are assembled into bilayers to form niosomal membranes and this space filling action results in the increased rigidity and the improved entrapment efficiency. On further increase of cholesterol beyond certain concentration it competes with the drug for the space within the bilayers, hence excluding the drug and can disrupt the regular linear structure of vesicular membranes[28].

***In vitro* release studies**

Figure 4 showed the percentage GMD released from proniosomes gels over 8 h. From the obtained results it was clear that formulation P6 showed the lowest release rates among all proniosomal preparations. Addition of 10% cholesterol (formulation P6A) gave a higher release rate than cholesterol free formulation (formulation P6). Increasing cholesterol content up to 50% was accompanied by gradual decrease in the release rate, however, remained significantly higher than that of cholesterol free formulation (p<0.05). This could be due to the fact that the addition of cholesterol appeared to disrupt the ordered array of the hydrocarbon chains in the gel phase[15]. Below the transition temperature, addition of cholesterol made the membrane less ordered, while above the transition temperature made the membrane more ordered[29,30]. At 37°C, formulation P6 (0% cholesterol) gave rise to highly ordered gel phase than those containing cholesterol; consequently, they produced lower release amounts of GMD. The decreased release rates upon increasing cholesterol content into proniosomes is due to cholesterol is largely hydrophobic and has one polar group (the hydroxyl group), making it amphipathic. This gives cholesterol the opportunity of forming van der Waals interactions with lipid tails, and so, decreases their mobility which makes bilayer membranes more rigid, hydrophobic and less permeable to drugs[15].

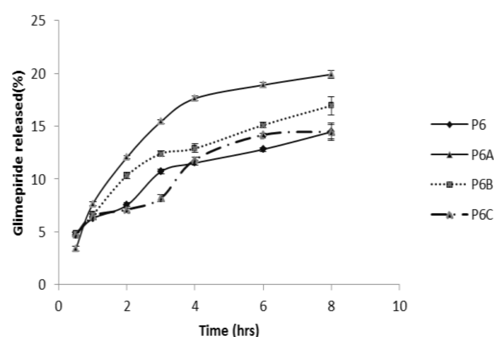

Figure 4: Effects of cholesterol on the *in-vitro* release of GMD from proniosomes gels.

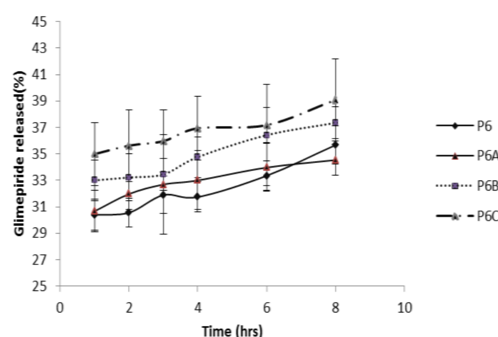

Figure 5: *In vitro* release of GMD from niosomes after incubation in phosphate buffer pH 7.4.

Moreover, it is well known that by increasing the gelator content into gel formulations, the cross-linking of tubular and vesculating structures of the gel becomes more condensed and the consistency of the gel increases and as a result the formed proniosomal gels become less leaky[15].

Kinetic analysis of the release data of GMD proniosomal systems

The kinetic analysis of GMD release data from different Proniosomes gels were tested according to zero, first order kinetic and diffusion controlled model. The results indicate proniosomal systems fitted better with Higuchi diffusion model.

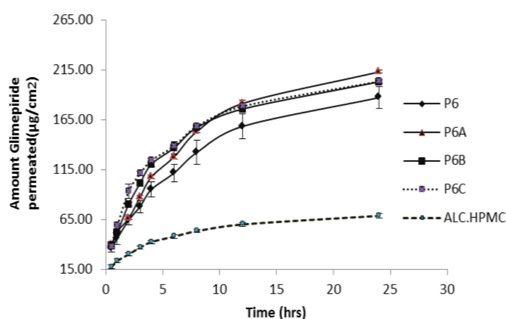

Figure 6: Effects of cholesterol content on GMD permeability across rabbit skin.

Glimepiride release rates from niosomes

The release profiles of GMD from niosomes of different cholesterol contents is an apparently biphasic release process. Rapid drug leakage was observed during the initial phase where about 30–35% of the entrapped drug was released from various formulations in the first hour. However, during the following 8 h, a slow release occurred in which only further 3% to5% of GMD was lost from different niosomal preparations (Figure 5). This could be explained on the basis that the drug is mainly incorporated between the fatty acid chains in the lipid bilayers of niosomal vesicles. This leads to rapid ionization and release upon dispersing niosomes in increased buffer (pH 7.4) volumes until reaching equilibrium[31]. Figure 5 showed that formulation P6A displayed the lowest extent of drug release after 8h. The percent GMD released from formulations P6A and P6C after 8 h was 34.54% and 39.08%, respectively. The increase in release rates of GMD from formulation P6Cwas statistically significant (p<0.05) compared to P6 and P6A formulations. This result attributed to the cholesterol concentration.10% cholesterol produced an optimum hydrophobicity that decreased the formation of the transient hydrophilic holes, responsible for drug release through liposomal layers[32]. On the other hand, further increase in cholesterol amounts into niosomes formulations could increase the release of GMD[28]. Another explanation is that increasing cholesterol beyond a certain concentration can disrupt the regular linear structure of the vesicular membrane and increase the drug release[27].

***Ex vivo* skin permeation studies**

After 24 h about 213.19, 187.37, 203.18, 203.58 µg/cm^2 were permeated across rabbit skin from P6A, P6, P6B, and P6C proniosomal formulations respectively as showed in Figure 8. Addition of 10% cholesterol (P6A) significantly enhanced GMD permeability from proniosomes gel (p<0.05). The steady state transdermal fluxes (SSTF) of GMD from P6A (7.339 µg/cm^2.hr) higher than SSTF of GMD from P6, P6B and P6C (6.356, 6.560 and 6.306 µg/cm^2.hr respectively) and five times more than alcoholic HPMC (Table 3). Statistical analysis showed a significant difference between SSTF of P6A and other formulations. The reduced permeation of GMD from proniosomal gels of Span 60 is primarily attributed to its high transition temperatures which made them in a highly ordered gel state at the permeation temperature (32°C)[20].

Table 3: Permeability parameters of GMD released across rabbit skin.

Formula Code	Permeability Parameters			
	D_{max} (µg)	JSS (µg/cm^2.hr)	PC (cm/hr)	(D)
Alc. HPMC	454.41	2.031	3.692×10^{-4}	4.121×10^{-6}
P6	1236.63	6.356	1.155×10^{-3}	3.836×10^{-5}
P6A	1407.02	7.339	1.334×10^{-3}	5.131×10^{-5}
P6B	1341.01	6.560	1.238×10^{-3}	4.334×10^{-5}
P6C	1343.59	6.306	1.146×10^{-3}	4.060×10^{-6}

D_{max}: maximum amount of drug permeated, JSS: steady state flux, PC: permeability coefficient and D: diffusion coefficient

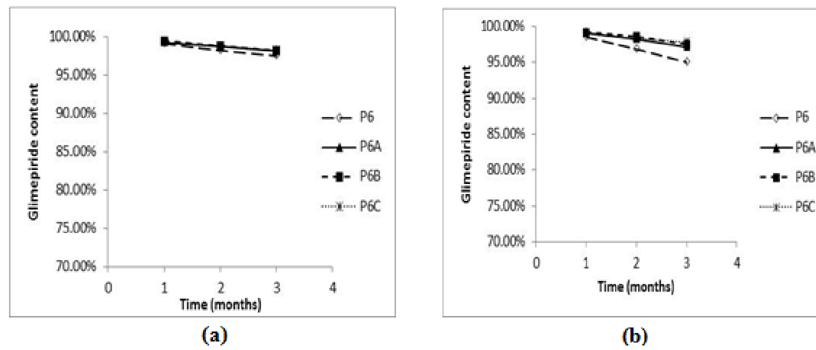

Figure 7: GMD content in proniosomes gels after storage for 3 months (a) at 4°C (b) at 25°C.

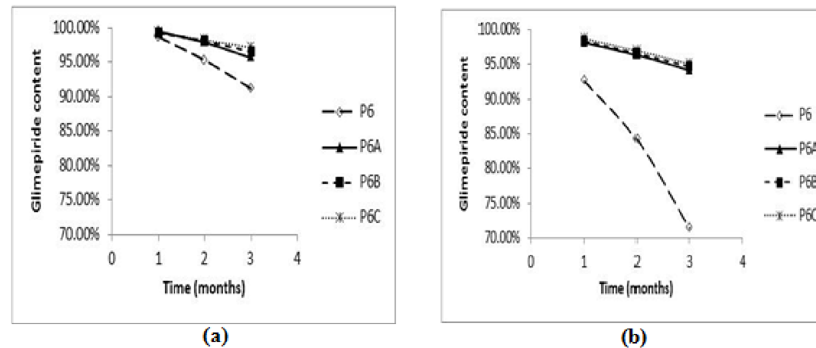

Figure 8: GMD content in proniosomes dispersions after storage for 3 months (a) at 4°C (b) at 25°C.

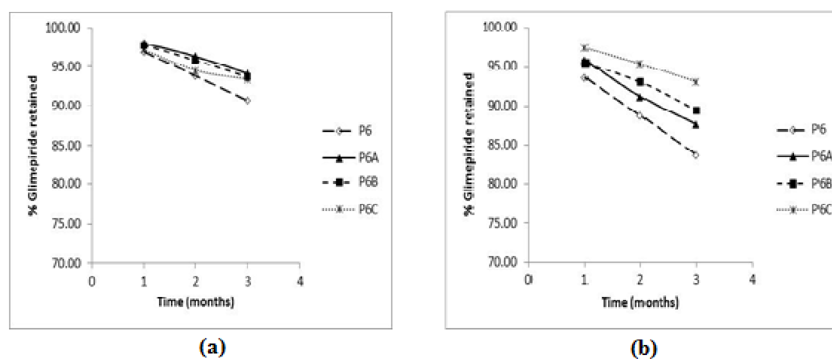

Figure 9: % GMD retained in proniosomes gels after storage for 3 months (a) at 4°C (b) at 25°C.

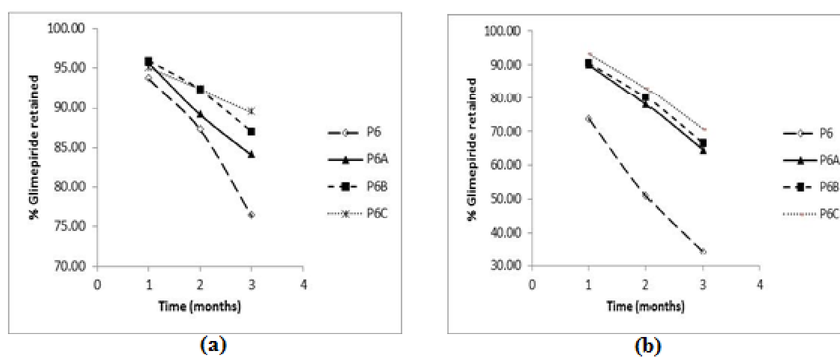

Figure 10: % GMD retained in proniosomes dispersions after storage for 3 months (a) at 4°C (b) at 25°C.

Kinetic analysis of the permeation data of GMD Proniosomal systems

The kinetic analysis of the permeation data of GMD from different proniosomal systems were tested according to zero, first order kinetic and diffusion controlled model. The results clearly revealed that all proniosomes gels fitted better with the diffusion controlled model.

Physical stability

The results of stability studies were compiled in Figure 7- Figure 10. The effects of storage temperature on entrapment efficiency were expressed as % of GMD retained entrapped according to the following equation.

$$\%\ \text{Glimepiride retained} = \frac{\text{Entrapped glimepiride after storage}}{\text{Entrapped glimepiride before storage}}\ X\ 100$$

Generally, drug leakiness from niosomal dispersions upon storage in refrigerator was significantly low; while at room temperature there was an appreciable drug loss and decreased in entrapment efficiency. Hence the niosomes dispersions needs to be refrigerated for use as is the case with all other vesicular systems[18]. Proniosomes gels were more stable than niosomal vesicles under refrigerated and room temperature condition. This suggests that proniosomes offered a more stable system that could minimize the problems associated with conventionally prepared niosomes like degradation by hydrolysis, or oxidation, sedimentation, aggregation and fusion during storage. P6A (1.86% loss in drug content and 5.89% decrease in entrapment efficiency) were more stable than other investigated formulations at 3 months. For better understanding, we must consider the role of cholesterol in the stability issue. Cholesterol is a waxy steroid metabolite found in the cell membranes. Cholesterol is added usually to the non-ionic surfactants to give rigidity and orientational order to the niosomal bilayer[33]. Cholesterol enables the formation of vesicles, reduces aggregation and provides greater stability. Cholesterol is also known to abolish gel to liquid phase transition of niosomal systems resulting in niosomes that are less leaky[33]. Cholesterol content also plays an important role in the stability of niosomes. Incorporation of cholesterol at 10% caused a decreased leakage and permeability of niosomal formulations. Cholesterol produced an optimum hydrophobicity that decreased the formation of the transient hydrophilic holes as well as decreased the membrane fluidity that is responsible for drug leakage[15]. Also, it was reported that increasing cholesterol beyond a certain concentration can disrupt the regular linear structure of the vesicular membrane and increase the drug leakage[27].

CONCLUSIONS

Concluding the above mentioned results; Proniosomes gels could enhance the solubility of certain poorly soluble drugs but to a maximum limit after which any increase in the drug concentration may lead to drug precipitation. The EE% of GMD (as a model for poor soluble drugs) into niosomes prepared by the proniosomal method was a function of cholesterol content. Cholesterol content affected the drug release and permeation properties of proniosomal system. All proniosomal gels fitted to diffusion model when using MCE membranes and when using rabbit skin for release and permeation studies, respectively. It is clear that niosomal formulations containing 10% cholesterol are the most stable among other tested formulations.

AUTHOR'S CONTRIBUTION

Elsaied EH: writing original draft, conceptualization, methodology, investigation. **Dawaba HM:** Writing, review, and editing, supervision, resources. **Ibrahim EA:** writing, review, and editing. **Afouna MI:** writing, review, and editing, project administration. All authors read and approved the manuscript.

DATA AVAILABILITY

The data and material are available from the corresponding author on reasonable request.

ACKNOWLEDGEMENTS

The authors extend their thanks and appreciation to the Al-Azhar University, Cairo, Egypt to provide necessary facilities for this work.

CONFLICT OF INTEREST

None to declare.

REFERENCES

1. Keleb E, Sharma RK, Mosa EB, Aljahwi A-AZ. Transdermal drug delivery system-design and evaluation. Inte J Advances Pharm Sci 2010;1(3):201-11. *https://doi.org/10.5138/ijaps.2010.0976.1055.01026*
2. Kalluri H, Banga AK. Transdermal delivery of proteins. AAPS Pharmscitech 2011;12(1):431-41. *https://doi.org/10.1208/s12249-011-9601-6*
3. Karim KM, Mandal AS, Biswas N, Guha A, Chatterjee S, Behera M, Kuotsu K. Niosome: a future of targeted drug delivery systems. J Advanced Pharmaceutical Techn Res 2010; 1(4):374. *https://doi.org/10.4103%2F0110-5558.76435*
4. Sankhyan A, Pawar P. Recent Trends in Niosome as Vesicular Drug Delivery System. J Applied Pharmaceutical Sci 2012; 02(6):20-32. *https://doi.org/10.7324/JAPS.2012.2625*
5. Kaur H, Dhiman S, Arora S. Niosomes: A Novel Drug Delivery System. Int J Pharmaceutical Sci Rev Res 2012; 15(1):113-20. *https://doi.org/10.20959/wjpps20177-9607*
6. Reddy PD, Swarnalatha D. Recent advances in novel drug delivery systems. Int J PharmTech Res 2010; 2(3):2025-7.
7. Hu C, Rhodes DG. Erratum to 'proniosomes: a novel drug carrier preparation. Int J Pharm 1998; 185: 23–35.
8. Gupta A, Prajapati SK, Balamurugan M, Singh M, Bhatia D. Design and development of a proniosomal transdermal drug delivery system for captopril. Tropical J Pharm Res 2007; 6(2):687-93.*https://doi.org/10.4314/tjpr.v6i2.14647*
9. Fang JY, Yu SY, Wu PC, Huang YB, Tsai YH. *In vitro* skin permeation of estradiol from various proniosome formulations. Int J Pharmaceutics 2001; 215(1):91-99. *https://doi.org/10.1016/S0378-5173(00)00669-4*
10. Varshosaz J, Pardakhty A, Mohsen S, Baharanchi H. Sorbitan monopalmitate-based proniosomes for trans-dermal delivery of chlorpheniramine maleate. Drug Deliv 2005;12(2):75-82. *https://doi.org/10.1080/10717540490446044*
11. Singh S, Trivedi S, Jain S. Design and development of proniosome based transdermal delivery of ondansetron hydrochloride. Int J Pharm Biol Res 2012; 3(5):191-201. *https://doi.org/10.22159/ijap.2019v11i2.30614*
12. Vora B, Khopade AJ, Jain N. Proniosome based transdermal delivery of levonorgestrel for effective contraception. J Controlled Rel 1998; 54(2):149-65. *https://doi.org/10.1016/S0168-3659(97)00100-4*
13. Ahmed OA, Afouna MI, El-Say KM, Abdel-Naim AB, Khedr A, Banjar ZM. Optimization of self-nanoemulsifying systems for the enhancement of *in vivo* hypoglycemic efficacy of glimepiride transdermal patches. Expert opinion on drug delivery 2014; 11(7):1005-13. *https://doi.org/10.1517/17425247.2014.906402*
14. Ahmed TA, Khalid M, Aljaeid BM, Fahmy UA, Abd-Allah FI. Transdermal glimepiride delivery system based on optimized ethosomal nano-vesicles: Preparation,

characterization, *in vitro*, *ex vivo* and clinical evaluation. Int J Pharmaceutics 2016; 500(1):245-54. https://doi.org/10.1016/j.ijpharm.2016.01.017

15. Mokhtar M, Sammour OA, Hammad MA, Megrab NA. Effect of some formulation parameters on flurbiprofen encapsulation and release rates of niosomes prepared from proniosomes. Int J Pharmaceutics 2008; 361(1):104-11. https://doi.org/10.1016/j.ijpharm.2008.05.031
16. Radha G, Rani TS, Sarvani B. A review on proniosomal drug delivery system for targeted drug action. J Basic Clin Pharm 2013; 4(2):42-8. https://doi.org/10.4103%2F0976-0105.113609
17. Tayel SA, El-Nabarawi MA, Tadros MI, Abd-Elsalam WH. Duodenum-triggered delivery of pravastatin sodium via enteric surface-coated nanovesicular spanlastic dispersions: Development, characterization and pharmacokinetic assessments. Int J Pharm 2015; 10: 483(1):77-88. https://doi.org/10.1016/j.ijpharm.2015.02.012
18. Ammar H, Ghorab M, El-Nahhas S, Higazy I. Proniosomes as a carrier system for transdermal delivery of tenoxicam. Int J Pharmaceutics 2011; 405(1):142-52. https://doi.org/10.1016/j.ijpharm.2010.11.003
19. Alsarra IA, Bosela AA, Ahmed SM, Mahrous G. Proniosomes as a drug carrier for transdermal delivery of ketorolac. European J Pharmaceutics Biopharmaceutics. 2005; 59(3):485-90. https://doi.org/10.1016/j.ejpb.2004.09.006
20. Zidan AS, Mokhtar M. Multivariate optimization of formulation variables influencing flurbiprofen proniosomes characteristics. J Pharm Sci 2011; 100(6):2212-21. https://doi.org/10.1002/jps.22453
21. Induri M, Bhagavan RM, Rajendra PY, Kumar P. Development and validation of a spectrophotometric method for quantification and dissolution studies of glimepiride in tablets. E-J Chemistry 2012; 9(2):993-8.
22. Bhargavi S, Suryasagar G, Sowmya DK, Ashok K, Nama S. UV Spectrophotometric method for determination of glimepiride in pharmaceutical dosage forms. Int J Pharm Sci Rev Res 2013; 21(2):131-3.
23. Venkatesh B, Indira S, Srinivas P. Formulation and evaluation of glimepiride oral capsules. Int J Pharm Sci Invention 2014; 3(10):1-13.
24. Sambasivarao A. Formulation and evaluation of immediate release tablets of glimepiride by using solid dispersion technique using Gelucires. World J Pharm Pharmaceutical Sci 2016; 5(5):1659-78.
25. Murdan S, Gregoriadis G, Florence AT. Sorbitan monostearate/polysorbate 20 organogels containing niosomes: a delivery vehicle for antigens? European J Pharmaceutical Sci 1999; 8(3):177-85. https://doi.org/10.1016/S0928-0987(99)00014-7
26. Sudaxshiina M, Van Den BB, Gregory G, Alexander T. Water in sorbitan monostearate organogels (water in oil gels). J Pharm Sci 1999; 88(6):615-9. https://doi.org/10.1021/js980343j
27. El-Samaligy M, Afifi N, Mahmoud E. Increasing bioavailability of silymarin using a buccal liposomal delivery system: preparation and experimental design investigation. Int J Pharm 2006; 308(1):140-8. https://doi.org/10.1016/j.ijpharm.2005.11.006
28. El-Laithy HM, Shoukry O, Mahran LG. Novel sugar esters proniosomes for transdermal delivery of vinpocetine: preclinical and clinical studies. European J Pharmaceutics Biopharmac 2011; 77(1):43-55. https://doi.org/10.1016/j.ejpb.2010.10.011
29. Arunothayanun P, Bernard M-S, Craig D, Uchegbu I, Florence A. The effect of processing variables on the physical characteristics of non-ionic surfactant vesicles (niosomes) formed from a hexadecyl diglycerol ether. Int J Pharmaceutics 2000; 201(1):7-14. https://doi.org/10.1016/S0378-5173(00)00362-8
30. Kaur IP, Rana C, Singh M, Bhushan S, Singh H, Kakkar S. Development and evaluation of novel surfactant-based elastic vesicular system for ocular delivery of fluconazole. J Ocular Pharmacol Therapeutics 2012; 28(5):484-96. https://doi.org/10.1089/jop.2011.0176
31. Wissing S, Kayser O, Müller R. Solid lipid nanoparticles for parenteral drug delivery. Advanced Drug Delivery Rev 2004; 56(9):1257-72. https://doi.org/10.1016/j.addr.2003.12.002
32. Cócera M, Lopez O, Coderch L, Parra J, De la Maza A. Permeability investigations of phospholipid liposomes by adding cholesterol. Colloids and Surfaces A: Physicochemical and engineering aspects 2003; 221(1):9-17. https://doi.org/10.1016/S0927-7757(03)00104-3
33. Nasir A, Harikumar S, Amanpreet K. Niosomes: An excellent tool for drug delivery. Int J Res Pharmacy Chem 2012; 2(2):479-87.