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Formulation and evaluation of topical niosomal gel of baclofen

Mohamed A. El-Nabarawi[1], Ehab R. Bendas[1], Mohamed S. El-Ridy[2], Gehad A. Abdel-Jaleel[3] and Samar M. Nasr-Alla[1]*

[1]*Department of Pharmaceutics and Industrial Pharmacy, Faculty of Pharmacy, Cairo University, Cairo, Egypt*
[2]*Department of Pharmaceutical Technology, National Research Center, Dokki, Giza, Egypt*
[3]*Department of Pharmacology, National Research Center, Dokki, Giza, Egypt*

ABSTRACT

The aim of the present study was to formulate topical gel containing baclofen niosomes. Baclofen is a direct agonist at $GAPA_B$ receptors with an anti-inflammatory effect. Baclofen has narrow therapeutic index and many gastrointestinal side effects. The current market products of baclofen are intrathecal injection and oral tablets which cause many problems after their administration, Thus, niosomal formulations as carriers for the topical delivery of baclofen will prolong the contact time and increase the permeability through the skin so improve its, as anti-inflammatory. The baclofen niosomes were prepared by altering the ratios between various non-ionic surfactants (Span 60, 40), cholesterol and charge inducing agents using thin film hydration method, the prepared niosomal formulations were evaluated for their encapsulation efficiency and maximum entrapment efficiency attained a maximum value of 80.31%, and characterized by transmission electron microscopy, differential scanning calorimetry, particle size analysis, zeta potential analysis and in vitro release after 24 hours. The maximum cumulative release percentage was between 65.55%. Two gelling agents were used for the preparation of various baclofen gels and baclofen niosomal gels, in vitro permeation studies. Our results suggested that the niosomal delivery of baclofen in carbopol gel base acts as a suitable topical drug delivery system.

Key words: Baclofen, Niosomes, *Invitro*, Carbopol 934, Rat paw edema.

INTRODUCTION

Inflammation is a vascular reaction during which the net result is the delivery of fluid, dissolved substances and cells from the circulating blood into the interstitial tissue in an area of injury or necrosis[1]. Chemokines are the largest family of cytokines in human immunophysiology. These proteins are defined by four invariant cysteines and are categorized based on the sequence around the first two cysteines, which leads to two major and two minor Sub families. Chemokines function by activating specific G protein–coupled receptors, which results in, among other functions, the migration of inflammatory and non-inflammatory cells to the appropriate tissues or compartments within tissues[2]. chemokines which are considered inflammatory and are only produced by cells during infection or a pro- inflammatory stimulus. The role of inflammatory chemokines is to induce the migration of leukocytes to the injured or infected site. In addition, inflammatory chemokines activate the cells to mount an immune response and initiate wound healing[3]. such as IL-1, TNF-alpha, LPS, or viruses and actively participates in the inflammatory response attracting immune cells to the site of inflammation. Examples are: CXCL-8, CCL2, CCL3, CCL4, CCL5, CCL11, and CXCL10[4].

Baclofen interacts with two types of receptors, GABAA and GABAB[5]. The gamma amino butyric acid B (GABAB) receptor is a G protein-coupled receptor (GPCR) involved in synaptic transmission. So it interferes with the function of pro-inflammatory chemokine receptors[6].Therefore baclofen has inhibitory effects on chemokine induced chemotaxis. In vitro, baclofen reduces chemotaxis of human peripheral blood mononuclear cells towards

CCL2, CCL5, CXCL10, CXCL2 and CX3CL1 in a dose-dependent manner which are chemokine receptors: responsible for inflammation[6].

Recent data indicate it is also expressed that baclofen noticeably alleviated signs of inflammation as well as mobilization of neutrophils, monocytes and lymphocytes into the skin. This study demonstrates a new role of the GABAB receptor in inflammation, making it a potential new therapeutic target to treat inflammatory skin diseases[6]. Baclofen is a highly effective anti-inflammatory but it has a very narrow therapeutic index with inter individual variability in pharmacokinetics and pharmacodynamics, and short biological half-life. These factors necessitated niosomal formulation for baclofen. Also oral administration of baclofen often causes nausea, constipation, urinary frequency, insomnia, tinnitus, hypotension, drowsiness, dizziness, sedation, fatigue, weakness increase liver enzymes and increase of blood sugar. Topical application of drugs at the pathological sites offer potential advantages of delivering the drug directly to the site of action and thus producing high tissue concentrations of the drug with reduced side effects. Therefore, improved baclofen topical formulations with a high degree of skin permeation and prolonged maintenance in the target area at a therapeutic level could be a therapeutic success. Vesicular system ensure adequate penetration and more importantly localization of the drug within the skin[7].

EXPERIMENTAL SECTION

2.1. Materials: Baclofen was a gift sample from Masr Pharmaceutical Chemicals Company, Abuzaabal, Egypt. Sorbitan monostearate (Span 60), Merck Schuchardt OHG, Hohenbrunn,Germany. Sorbitan monopalmitate (Span 40), Cholesterol (CHOL) from Lanolin, minimum 99% (GC), Dihexadecyl hydrogen-Phosphate (Dicetyl Phosphate, free acid crystalline) (DCP),Fluka, Sigma-Aldrich Chemie GmbH, Steinheim, Germany. Octadecylamine (Stearylamine) (SA), minimum 97% (GC), Pluronic F127 (PF127), Sigma Chemical Co., St. Louis, USA, Carbopol 934, B.F., (Goodrich Chemical Company, Ohio, USA), Triethanolamine Nasr Company for Pharmaceutical Industries, Cairo, Egypt, Carragenan (Sigma Chemical Co., St. Louis, USA).All other chemicals were of analytical grade.

2.2. Preparation of baclofen niosomes: Baclofen niosomes were prepared by the thin film hydration method[8-13], using non-ionic surfactants, Span 60 and Span 40 which are safe and non-toxic. Cholesterol was used as an enhancer of niosomal membrane rigidity[14]. Two molar ratios between non-ionic surfactant and cholesterol were employed, 1:1[15] and 4:2[10]. DCP was used to prevent niosome aggregation and impart negative surface charge to the vesicles[16, 17]. SA was used as a positive charge inducer. Composition of baclofen niosomal formulations were illustrated in Table 1.

Table 1 : Baclofen niosomal formulations

Formulation	Baclofen (mg)	Span60 (mg)	Span40 (mg)	Cholesterol (mg)	DCP (mg)	SA (mg)	NIS:CH:CIA Molar ratio	Mean drug entrapped ±S.D.
F1	5	52.66	_____	47.34	____	____	1:1	60.21±1.86
F2	5	49.4	_____	44.3	6.3	____	1:1:0.1	15.31±1.91
F3	5	50.9	_____	45.8	____	3.19	1:1:0.1	48.65±2.67
F4	5	68.98	_____	31.01	____	____	4:2	80.31±2.02
F5	5	64.72	_____	29.10	6.17	____	4:2:1	25.42±2.66
F6	5	66.81	_____	30.04	____	3.14	4:2:1	58.02±1.71
F7	5	_____	51.00	48.99	____	____	1:1	43.54±2
F8	5	_____	47.71	45.80	6.47	____	1:1:0.1	4.37±0.72
F9	5	_____	49.34	47.35	____	3.30	1:1:0.1	31.46±1.42
F10	5	_____	67.57	32.42	____	____	4:2	48.65±1.04
F11	5	_____	63.22	30.34	6.43	____	4:2:1	15.21±0.42
F12	5	_____	65.35	31.36	____	3.27	4:2:1	37.60±0.503

2.3. Determination of entrapment efficiency: The amount of entrapped drug was estimated by subtracting the amount of free drug, measured at λmax 265.5 nm, collected from the separation and washing of the niosomal suspension, from the initial amount added at the start of the preparation[18] using table top refrigerated centrifuge, Unio 32R, Hanil Scientific Industrial Co., Korea.

The entrapment efficiency (%) was defined as: [18, 19]

$$\text{Entrapment efficiency\%} = \frac{\text{Amount of entrapped drug}}{\text{amount of total drug added}} \times 100$$

2.4. Characterization of baclofen niosomes

2.4.1. Transmission electron microscopy: Transmission electron micrographs of baclofen niosomal formulations were obtained using transmission electron microscope, Jeol, JEM-1230, Japan. The dye used was 1% phosphotungstic acid [20-22].

2.4.2. Particle size and zeta potential determinations: Vesicle properties, particle size diameter and zeta potential, were determined at room temperature by Zeta Potential/ Particle Sizer NICOMP_ 380 ZLS, PSS-NICOMP particle sizing systems, Santa Barbara, CA, equipped with a 5-mW laser. Niosomal formulations were diluted with phosphate buffered saline, pH 7.4, for Zeta potential and particle size determination, respectively.

2.4.3. Differential scanning calorimetry: Niosomal pellets were lyophilized. Differential scanning calorimetry (DSC) thermograms for individual components, Span 60, Span 40, Cholesterol, SA and DCP, as well as the drug powder, were investigated. A heating rate of 5˚C/min was employed over a temperature range (30–250) ˚C.

2.4.4. In-vitro release study: Eight baclofen niosomal formulations and their corresponding blanks were selected for this study, (F1, F2, F4, F5, F7, F8, F10 and F11).It should be noted that positively charged baclofen niosomes, F3, F6, F9 and F12 were omitted from this experiment due to reported aggregation and in-vivo toxicity [23, 24]. We just use stearyl amine to study the effect of positive charge on baclofen niosomes entrapment and characterizations. The *in-vitro* release of baclofen from different niosomal formulations were evaluated by the dialysis bag diffusion technique [25-27]. The baclofen niosomal suspension was placed in a cellulose acetate dialysis bag and sealed at both ends. The dialysis bag was immersed in the receptor compartment containing 25 ml of phosphate buffered saline (pH 5.5), which was stirred at 100 rpm and maintained at 37 ± 0.2°C. A two ml sample of the receiver medium was withdrawn at predetermined time intervals; 2, 4, 6, 8 and 24 h and replaced by equivalent volume of fresh medium to maintain constant volume. The samples were analyzed for drug content spectrophotometrically at 265.8 nm. The results are the mean values of the release experiments. Each result was the mean of three determinations. Percentage drug released was plotted as a function of time.

2.5. Physical stability study: The effect of storage of baclofen niosomes will be studied. Baclofen niosomal suspension formulations F1 and F4 which gave the highest release of drug from niosomal suspensions, were sealed in 10 ml glass vials after removal of free drug and stored in refrigerator at 4ºC for a period of 3 months. Samples from each niosomal formulation were withdrawn at definite time intervals. The retention of entrapped drug was measured after 1, 2 and 3 monthes of storage for the selected formulations. The initial entrapment efficiency was calculated for three monthes duration according to the following equation:

$$\text{Baclofen retained in niosomes\%} = \frac{\text{Entrapped drug (mg)}}{\text{Total drug added (mg)}} \times 100$$

2.6. Selection of baclofen niosomal formulations for second studies: Both F1 and F4 formulations showed high drug release (62.75%- 65.55%) over 24 hrs, also they represented the highest entrapment among the niosomal formulations (60.21% and 80.31%) respectively. Hofland [28] reported that increasing the alkyl chain length of surfactant cause reduction in toxicity, so increasing alkyl chain length of surfactant leads to formation of gel which is safer than liquid state, and as reported previously Span 60 has a longer saturated alkyl chain compared to Span 40.

2.7. Formulation of various gelling agents: Two gelling agents used for the preparation of gels which are Pluronic F-127, (30%) and Carbopol 934, (1%). Preparation of Plunoric matrix gel (30% w/w) was performed by the gradual sprinkling of PF127 in distilled water and then stirred with magnetic stirrer, Velp Scientifica, Italy at medium speed, Then the dispersion was stored in refrigerator (4°C) for 24 hours for bubbling air to be removed, and increasing orderliness of the cross link for the gel and became transparent. The gels were formed when the solutions are equilibrated at room temperature. The weighed amount of Carbopol 934 was sprinkled gradually in distilled water and then stirred with magnetic stirrer at medium speed. Stirring was continued until no lumps were observed and then triethanolamine was added for neutralization. The resulting gel was stored in refrigerator (4°C) for at least 24 hours until it was fully swollen and transparent.

2.8. Preparation of polymeric baclofen gels: The pure baclofen was incorporated in 30% Pluronic F-127, (F13) and 1% carbopol gel (F16). By using a glass rod we prepare smooth homogenous baclofen polymeric gels. Which were illustrated in Table 2.

2.9. Preparation of niosomal baclofen gels

The best formulation (F1 - Span 60: Cholesterol 1: 1) to prepare (F14 and F17) (F4 - Span 60 : Cholesterol 4 : 2) to prepare (F15 and F18) were selected for the preparation of gels. Niosomal dispersions were incorporated to the gel bases and stirred using glass rod to get 2% w/w of smooth homogenous baclofen niosomal gels. Which were illustrated in Table 2.

Table 2: Composition of different Baclofen niosomal gels formulations

Formulation	Drug	Non-ionic surfactant	NIS: Ch: CIA	Gelling agent
F13	Baclofen	_____	_____	**Pluronic F127 (30%)**
F14	Baclofen	Span 60	1:1:0	**Pluronic F127 (30%)**
F15	Baclofen	Span 60	4:2:0	**Pluronic F127 (30%)**
F16	Baclofen	_____	_____	**Carpobol 934 (1%)**
F17	Baclofen	Span 60	1:1:0	**Carpobol 934 (1%)**
F18	Baclofen	Span 60	4:2:0	**Carpobol 934 (1%)**

2.1.0. Evaluation of various baclofen gels

Clarity: It was determined by visual inspection under black and white background and it was graded as follows: turbid:, clear:, very clear (glassy)**.**

Homogeneity: It was determined by visual inspection for the appearance of gel.

Precipitation: It was determined by visual inspection for the presence of any aggregates.

***2.1.1. In-vitro* permeation studies of gels:** *In vitro* permeation studies were carried out to compare the permeation of polymeric gels (F13 and F16), niosomal gels (F14, F15, F17 and F18) by Franz diffusion cell using cellulose membrane as a semi permeable membrane. The gel formulation was placed between the lower (receptor) and the donor compartment. The diffusion Franz cell Vangard International Inc New Jersey, USA, was filled with phosphate buffer saline (pH 5.5) and maintained at 37±0.5◦C[29] and stirred continuously on a magnetic stirrer at 50 rpm throughout the experiment. At fixed time interval, samples were withdrawn at several time intervals and the drug percentage released analyzed for by U.V Spectrophotometer method at 265.8 nm. The volume of aliquot was replaced with the same volume of fresh buffer.

2.1.2. In-vivo study of gel formulations: The anti-inflammatory activity was carried out by carrageenan induced paw oedema method to compare the activity of the formulated niosomal, polymeric gels and marketed baclofen tablet. After gets ethical clearance male albino rats of wister strain (150-200 g) were used for this study.
The animals were divided into three groups having four animals in each group:

Group I control untreated, received carrageenan (as 1% conc) only.

Group II received blank niosomal gel containing Span60: CH (4:2) molar ratio which does not contain drug.

Group III received niosomal baclofen gel 1% of Span60: CH (1:1) molar ratio**, (F17).**

Group IV received niosomal baclofen gel 1% of Span60: CH (4:2) molar ratio, **(F18)**.

Group V received baclofen gel 1% (standard), **(F16)**.

Group VI received marketed baclofen 10mg oral tablet® (standard).
The rats were marketed on the left hind paw just beyond the tibiotarsal junction, then Hind footpad thickness (paw volume) was measured immediately before carrageenan injection and immediately after carrageenan injection and after 1, 2, 3, 4, 5, 6, 7, 8 and 24 hours [30] using plethysmometer**,** UGO Basile, model no. 21025 Comerio, Italy . Mean paw oedema was measured and the percentage inhibition of inflammation was calculated.

2.1.3. Ethical aspects

Animal experiments were conducted with the approval of the Research Ethics Committee of the National Research Centre, Egypt.

2.1.4. Statistical analysis

All data were presented as mean values±SD (mean±SD). Analysis of variance (ANOVA, one way) followed by LSD, using SPSS® software. Independent Student's t-test was also employed. Difference at $p<0.05$ was considered to be significant.

RESULTS AND DISCUSSION

3.1. Assessment of entrapment efficiency

The results of entrapment efficiency are illustrated in Table 3. F4 exhibited the highest entrapment efficiency 80.31%, and F8 exhibited the lowest entrapment efficiency 4.37%.

Table3: Entrapment efficiency of different Baclofen niosomal formulations

Formulation	Mean drug entrapped ±S.D.
F1	60.21±1.86
F2	15.31±1.91
F3	48.65±2.67
F4	80.31±2.02
F5	25.42±2.66
F6	58.02±1.71
F7	43.54±2
F8	4.37±0.72
F9	31.46±1.42
F10	48.65±1.04
F11	15.21±0.42
F12	37.60±0.503

Regarding encapsulation efficiency, it is observed that there is a significant effect of surface charge on entrapment efficiency at ($p<0.05$). The reduction in entrapment upon the addition of DCP could be due to the repulsive interactions between the negatively charged lipid and the carboxyl group present in baclofen molecules which influence drug incorporation within lipid bilayers [31]. The same by using positive charge inducer (SA) led to significant difference ($P<0.05$) in the percentage of drug. Although SA carries an opposite charge to that of carboxyl group in baclofen, its presence in place of DCP also decreased EE% of the drug. This could be explained by electrostatic induced chain tilt and the subsequent changes in the lateral packing of the bilayers by the effect of charge inducing agents [32]. Niosomal molar ratio affects an impact on entrapment efficiency at ($p<0.05$). The entrapment efficiency of charged and neutral baclofen niosomes prepared with the molar ratio (4:2:1) is higher than that of niosomes prepared with the other molar ratio (1:1:0.1) even after changing the type of non-ionic surfactant. Decreasing the amount of cholesterol content from 50%, in the molar ratio Span60: Cholesterol (1:1), to 33% in the molar ratio Span 60: Cholesterol (4:2), resulting in increasing the drug entrapment efficiency. This may be due to the following two conflicting factors [33, 34]:

1. With increasing cholesterol, the bilayer hydrophobicity and stability increased[35] and permeability[36] decreased which leads to efficiently trapping the hydrophopic drug into bilayers as vesicles formed.

2. In contrast, higher amounts of cholesterol may compete with the drug for packing space within the bilayer, hence excluding the drug as the amphiphiles assemble into vesicles.

Another study suggested that may be due the fact that increasing cholesterol beyond a certain concentration can disrupt the regular linear structure of the vescular membranes[34, 37]. Surfactant type affects entrapment efficiency. One way ANOVA at ($p<0.05$ level) reveals a significant increase in mean % entrapment of both neutral and charged baclofen niosomes at both molar ratios (1:1:0.1) and (4:2:1). This can be explained by the fact that Span 60 has higher phase transition temperature[15, 17] than Span 40. It has been reported[38].The higher the transition temperature of the surfactant. The higher the encapsulation efficiency may be correlated to the hydrophobicity of the alkyl chain (C18) compared to Span 40 (C16),53°C for Span 60 compared to 42°C for Span 40[39]. Also, this could be due to the surfactant chemical structure. All span types have the same head group and different alkyl chain of the sorbitan esters[21]. Increasing the alkyl chain length is leading to higher entrapment efficiency[40], Since Span 60 is having longer saturated alkyl chain (C18) compared to Span40 (C16), thus produces niosomes with higher entrapment efficiency.

3.2. Characterization of baclofen niosomes

Transmission electron microscopy

Transmission electron micrographs confirm the formation of vesicular structures of baclofen niosomes prepared using Span 60 (Figure 1) and Span 40 (Figure2). The vesicles are discrete and separate with uniform size.

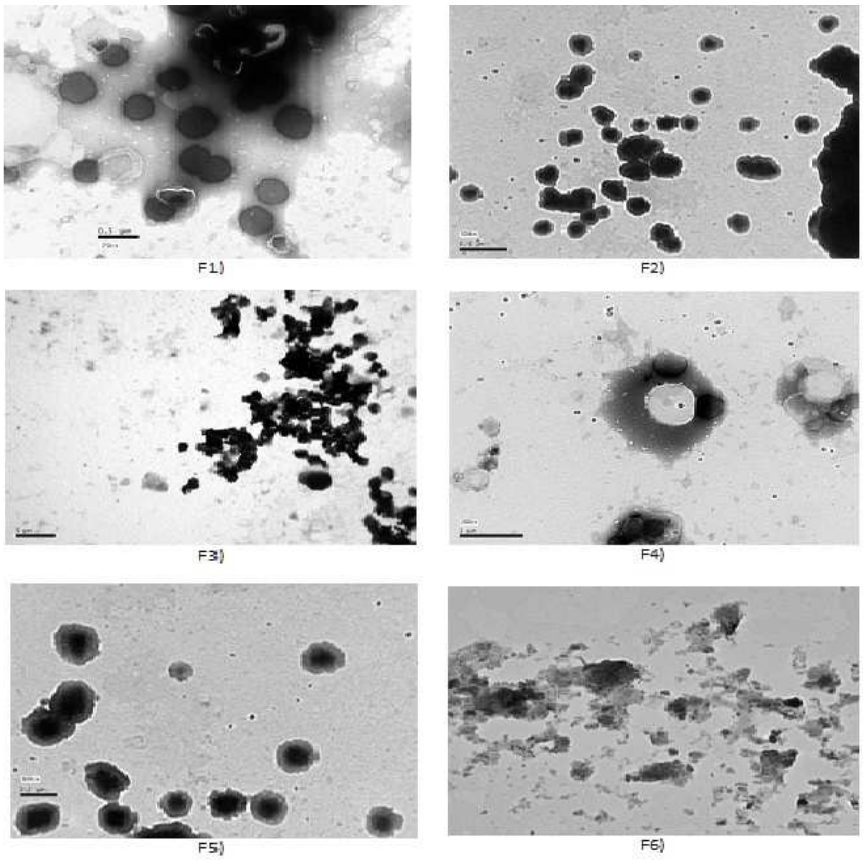

Figure (1): Electron micrograph of Baclofen niosomal suspension prepared using Span60

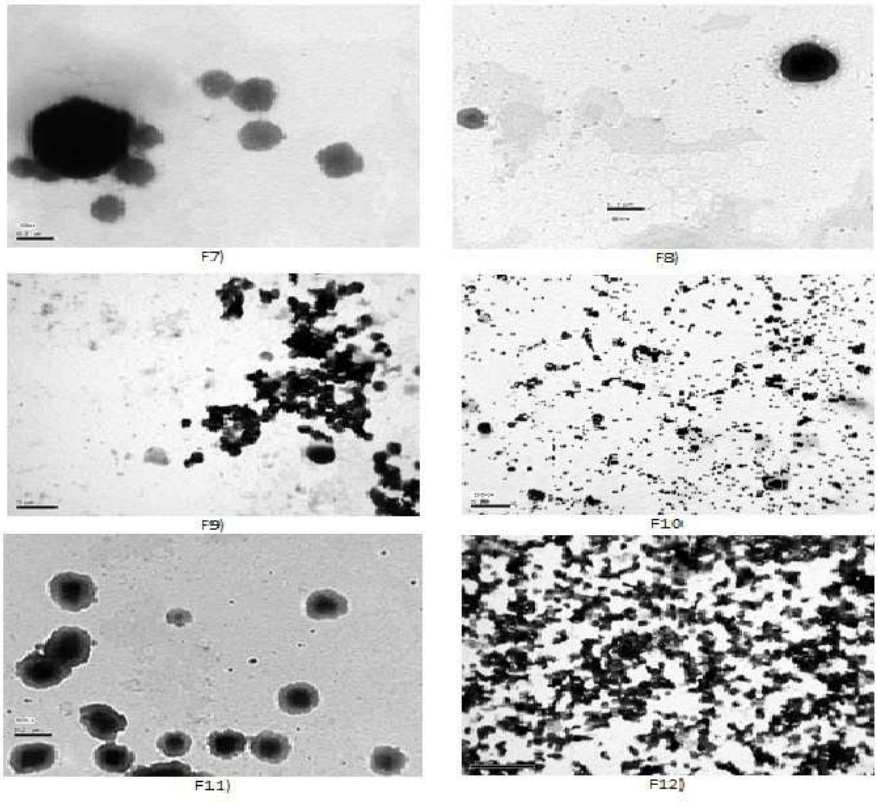

Figure (2): Electron micrograph of Baclofen niosomal suspension prepared using Span 40

Zeta potential determination

The values of zeta potential of niosomal vesicles are represented in Table 4. The zeta potential values of neutral and negatively charged niosomal formulations, prepared using Span 60 and Span40, have negative charge, although negatively charged niosomes have more negatively charges than neutral niosomes. Positively charged niosomes have positive charges of either molar ratio (1:1:0.1) or (4:2:1).

Table 4 reveals that the remarkable increase of zeta potential measurement in both positive charged and negative charged niosomes was observed with increased concentrations of stearyl amine and dicetylphosphate in the niosomal formulations. They noticed that neutral, negatively and positively charged Span 40 niosomes have higher zeta potenial than others prepared by using Span 60 at the equivalent molar ratios, because Zeta potential values of Span niosomal formulations increased with the hydrophilicity of the surfactants increased. This could be due to the fact that the surface free energy of the Span surfactants increases with increased HLB value[41] So that the larger HLP value, generally form high zeta potential value as Span 40 (HLB = 6.7) and Span 60 (HLB = 4.7) [34].

Table 4: Average zeta potential of the Baclofen niosomal formulations

Formulation	Surface charge	Avg. Zeta Potential (mv)
F1	neutral	**-0.14**
F2	negative	**-4.26**
F3	positive	**+4.93**
F4	neutral	**-0.8**
F5	negative	**-7.97**
F6	positive	**+10.99**
F7	neutral	**-0.38**
F8	negative	**-5.96**
F9	positive	**+8.88**
F10	neutral	**-2.59**
F11	negative	**-9.26**
F12	positive	**+12.33**

Particle size

Figure 3 reveals that the niosomal formulations prepared using Span 60 or Span 40, with or without charge inducing agent, molar ratios (1:1:0.1) (4:2:1) are in the nano-size range except the positively charged due to aggregation behavior [24].

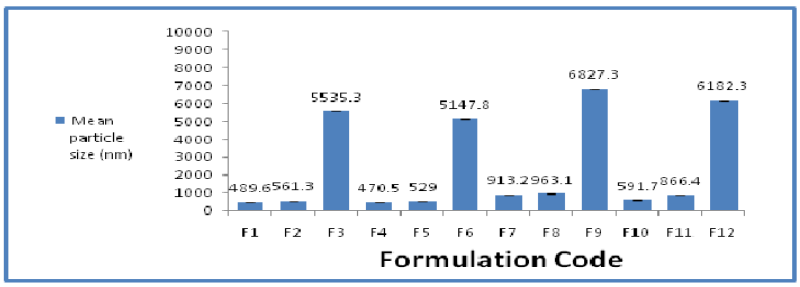

Figure (3): Distribution of particle size by bar diagram

Differential scanning calorimetry

Differential scanning calorimetry was carried out on the freeze-dried niosomal pellets of baclofen niosomes. DSC for the individual constituents of niosomes, Span60, Span40, DCP, SA, cholesterol and baclofen shows thermal peaks at 53.02˚C, 52.54˚C, ,74.75˚C 53.23˚C ,145.4˚C and 190.57 ˚C, respectively, corresponding to their melting temperatures as shown in figure4.

Figure 4 show that both transition temperature and transition energy of Span 60 peak of all niosomal formulations are less than that of pure Span 60. The effect of entrapped ethambutol hydrochloride on thermodynamic parameters of the niosomal vesicles (Figure 4) was evident where the niosomal formulations entrapping baclofen exhibit disappearance of its characteristic exothermic peak.

Figure 4 shows that the DSC thermograms of niosomal formulations prepared using Span 40 with or without charge inducing agent, 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.

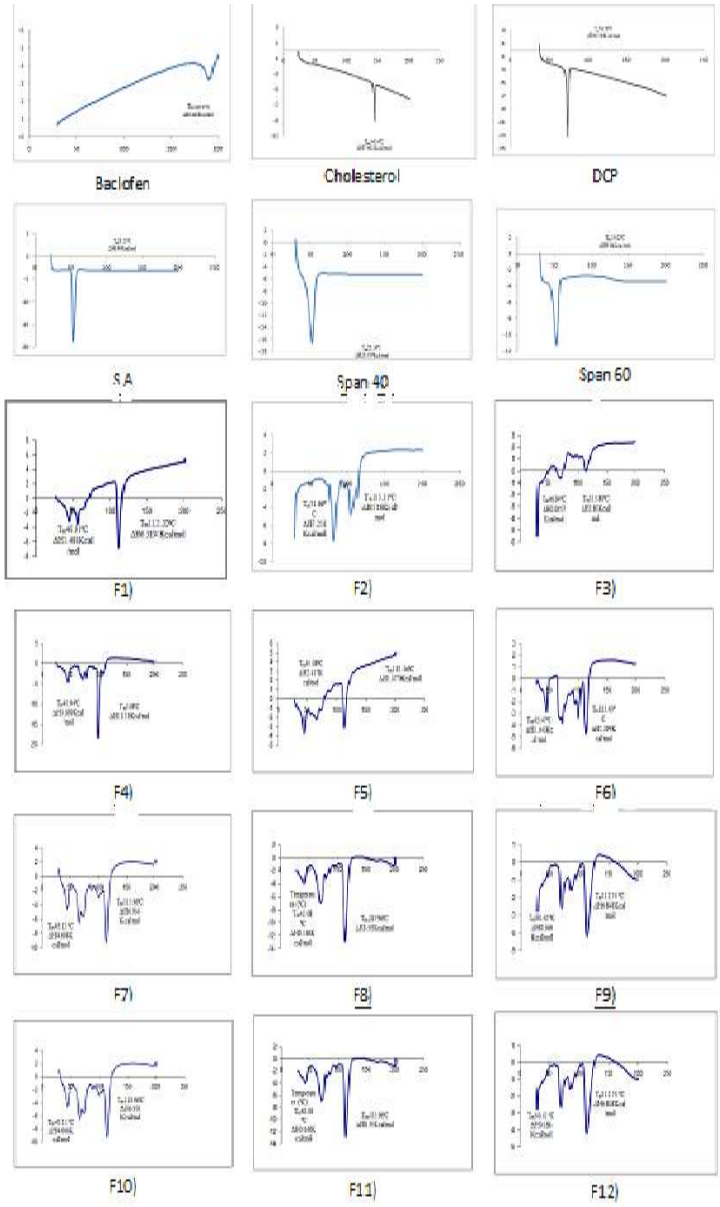

Figure (4): DSC thermogram of components and Baclofen niosomal formulations

3.3. In vitro release study: The experiment is done for neutral and negatively charged baclofen niosomes, F1, F2, F4, F5, F7, F8, F10 and F11. Positively charged niosomes were omitted from the experiment due to reported toxicity [23] , aggregation [24]. The obtained results (Figure 5) reveal that the release of baclofen from niosomes is biphasic, with an initial relative fast release phase followed by a slower one. this has been already reported for some liposomes[42, 43] and niosomes[44]. Analysis of all release profiles followed diffusion controlled mechanism with an initial relative fast release phase followed by a slower release one. This result was agreed with Mehta et al.[45], who have reported that diffusional release is observed for triton Niosomes; and Muzzalupo et al. [46] ,who had reported that diffusional release is observed for Pluronic L64 and P105 Niosomes. Similar results were obtained by Raslan et al. [47] and Coska et al[48]. The (%) of baclofen released from the niosomal formulations after 24 hours can be arranged in the following decreasing order: F4>F1>F5>F10>F2>F7>F11>F8 baclofen niosomes, viz., 65.55, 62.75, 59.18, 58.16, 55.79, 53.64, 51.876and 49.5%, respectively.

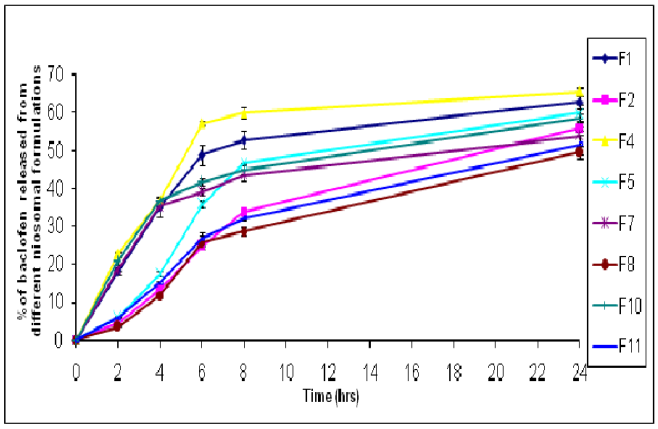

Figure (5): *In-vitro* release profile of neutral and negatively charged Baclofen niosomes in phosphate buffer saline (pH 5.5)

The obtained release results (Figure 5) showed that DCP cause retention in the release efficacy as it has ability to stabilize the structure of niosomal membrane and to render it less permeable Also were ascribed to that charged lipids serve to tighten the molecular packaging of the vesicle bilayers[49] , resulting in slow release from charged niosomes. The cholesterol content, viz., 30-50 mole % caused marked reduction in the efflux of the drug[50], which was in accordance with its membrane stabilizing activity. Cholesterol is known to abolish the gel to liquid phase transition of niosomal system resulting in niosomes that are less leaky increasing the cholesterol beyond a certain level (80:70) starts disrupting the bilayered structure leading to loss of drug entrapment levels in this case[50].

3.4 Physical stability study: After 30, 60, 90 days table 5 reveals the percentage of baclofen retained in the niosomal formulation from F1, were 57.29%, 56.98% and 56.35%. For F4 the percentage of baclofen retained in the niosomes were 78.26%, 78.11% and 77.94% and presented in table 6. It can be noted that there is no significant difference in entrapment efficiency for the both formulations upon the storage in refrigerator at 4ºC at ($P<0.05$).

Table5: Stability study of neutral niosomal formulation prepared using Span 60: cholesterol molar ratio (1:1) (F1)

Time in days	Formulation	Mean(%)drug entrapped ± S.D
Initial	F1	58.02±2.13
30	F1	57.29±1.46
60	F1	56.98±1.33
90	F1	56.35±1.1

Table6: Stability study of neutral niosomal formulation prepared using Span 60: cholesterol molar ratio (4:2) (F4)

Time in days	Formulation	Mean(%)drug entrapped ±S.D
Initial	F4	78.64±0.41
30	F4	78.26±0.07
60	F4	78.11±0.25
90	F4	77.99±0.04

3.5. Evaluation of various baclofen gels: of freshly prepared baclofen gels and baclofen niosomal gels in table7.

Table7: Physical properties of Baclofen gels and Baclofen niosomal gels prepared by different gelling agents

Formula	Gelling agent	%Conc of gelling agent	Appearance	Color	Homogeneity	Precipitation
F13	Pluronic F127	30%	Gel	Clear	Homogenous	No
F14		30%	Gel	turbid	Homogenous	No
F15		30%	Gel	turbid	Homogenous	No
F16	Carbopol 934	1%	Gel	Clear	Homogenous	No
F17		1%	Gel	turbid	Homogenous	No
F18		1%	Gel	turbid	Homogenous	No

***3.6. In-vitro* permeation studies of gels:** The permeation profiles of the polymeric and niosomal gels were shown in Fig. 5. The pluronic F127 gel and carbopol 934 containing pure drug (F13) and (F16) showed the cumulative percentage of drug permeation 87.74 % and 99.51 % in 24 hours respectively. (Figure 6) showed that F1 baclofen niosomes pellets in pluronic F127 (F14) and in carbopol 934 (F17) showed the cumulative permeation of drug 17.15% and 53% in 24 hours respectively. F4 baclofen niosomes pellets in pluronic F127 (F15) and in carbopol 934 (F18) showed the cumulative percentage of drug permeation24.17 % and 58.89 % in 24 hours respectively. The diffusion of baclofen from different gels prepared by pluronic F127 through cellulose membrane was slower than diffusion of baclofen from different gels prepared by carbopol 934 as it was before detected by Lagarce et al[51] that pluronic F127 gel in preparation of baclofen microsphere preparations plays a role in the reduction of release rates of baclofen from different formulations due to the pluronic F127 gel displayed both the highest viscosity at 37˚C and poloxamer gels, and especially pluronic F127 gels, have shown very interesting properties in controlling drug release[52-54]. The niosomals gel formulations showed controlled drug permeation due to the entrapment of drug in vesicles[34].

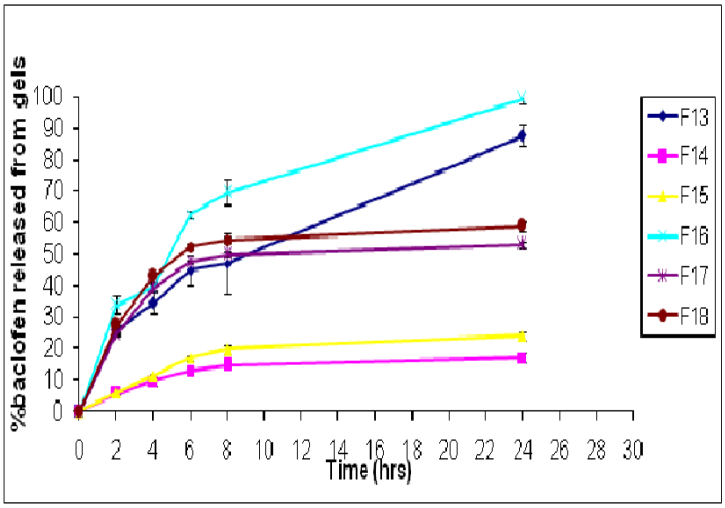

Figure (6): In-vitro permeation profile of Baclofen through artificial membrane from different polymeric gels and niosomal gels in phosphate buffer saline (pH 5.5)

3.7. In-vivo study of baclofen formulations: It is obvious that the application of control saline (group one) showed no anti-inflammatory effect, as well as the plain niosomes (group two), but other groups showed the anti-inflammatory effect on the paw edema. The percentage of reduction in paw oedema was gradually increased in the case of free drug gel (F16) (group five)gradually increased up to 5th hr and later it declined on 6th, 7th, 8th and 24th hr shown. Both niosomal formulations baclofen in Carpobol matrix gel (F17 and F18) lead to superior anti-inflammatory compared to the free baclofen in Carbopol matrix gel (F16) starting from sixth hour (lower edema rate% and higher inhibition rate%) at P< 0.05 after 24 hours suggesting higher drug retention. F18 showed the most anti-inflammatory efficacy compaired to the other niosomal formulation. F18 and marketed baclofen® tablets showed obviously lower edema volume% (22.57% versus18.40%) and higher edema inhibition % (84.83% versus 87.63%). There was insignificant difference in the volume of edema percentage F18 and marketed baclofen® tablets at P<0.05 after24 hours.

In-vivo study of baclofen formulations showed (F17 and F18) lead to superior anti-inflammatory compared to the free baclofen in Carbopol matrix gel (F16) starting from sixth hour till the end of the experiment This may be attributed to the creation of a reservoir effect for the drug in the skin due to deposition of other components of niosomes with the drug thereby increasing the drug retention capacity into the skin[55], and may be attributed to the fact that higher molar ratio of cholesterol can produce a condensing effect on niosomal membranes making them more rigid thus decreasing the rate of permeation across the skin and leading to a decreased permeability of the drug from the lipid bilayers resulting in lowering in its bioavailability[56].

CONCLUSION

Baclofen niosomal entrapment efficiency ranges from 4.37% to 80.31%. Baclofen niosomal formulations show mean particle diameters in the nano-range except the positively charged niosomes. Zeta potential is used in our study for measurement of the surface charge of the niosomes. Zeta potential values of negatively charged niosomal formulations have more negatively charges than neutral niosomes but positively charged niosomes have positive charges. The release of drug from the investigated niosomal formulations is biphasic, The comparative release data indicates that, by encapsulation of drug into niosomes, it is possible to sustain and control the release of the drug for a longer duration [19]; this in turn led to decreasing the dosage regimen of the drug, thus decreasing the drug toxicity; in addition to achieving a rapid action of the drug due to the fast initial release phase. The incorporation of free baclofen and two niosomal baclofen formulations into Carbopol 934 reflect faster permeation behavior than Pluronic F127. In-vivo examination reflects the insignificance in the anti-inflammatory behavior between baclofen niosomal gel (F18) and marketed baclofen® tablets at P>0.05 after24 hours, which is expected to minimize the side effects due to selective built up of drug concentrations at the site of action as topically applied niosomes can increase the residence time of drugs in the stratum corneum and epidermis, while reducing the systemic absorption of drug. They are through to improve the horny layer properties, both by reducing transepidermal water loss and by increasing smoothness via replenishing lost skin lipids.

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