BETAMETHASONE DIPROPIONATE GEL FOR TREATMENT OF LOCALIZED PLAQUE PSORIASIS

SANAA EL-GIZAWAY1*, MAHA FADEL2, BASMA MOURAD3, FATMA EL-ZAHRAA ABD ELNABY3

1Department of Pharmaceutical Technology, Faculty of Pharmacy, Tanta University, Egypt, 2Department of Medical Laser Application, National Institute of Laser Enhanced Sciences, Cairo University, Egypt, 3Department of Dermatology, Andrology and Venereology Diseases, Faculty of Medicine, Tanta University, Egypt

Email: selgizawy@hotmail.com

Received: 20 Mar 2017 Revised and Accepted: 30 Jun 2017

INTRODUCTION

Psoriasis is a chronic inflammatory skin disease with increased epidermal proliferation related to dysregulation of immune system-estimated to affect around 2-3% of the world population [1-3]. This disease has different types: psoriasis vulgaris, guttate psoriasis, erythrodermic psoriasis, pustular psoriasis and nail psoriasis. The first type is the most common form of psoriasis, which is characterised by red, scaly and raised plaques. Classic psoriasis vulgaris mainly infects specific areas such as elbows, knees and the scalp. It can also remain localized or become generalized over time and the plaques may differ in size [1, 4]. Treatment of psoriasis depends on many factors such as the extent of the disease, its influence on patient's life, and the life perception of patient’s illness [4].

Different types of treatment can be used such as ultraviolet B (UVB), psoralen plus ultraviolet A (PUVA), methotrexate (MTX), cyclosporine, vitamin D3 analogues, topical retinoids and topical corticosteroids such as betamethasone dipropionate (BD). BD is a highly potent glucocorticoid receptor agonist which possesses immunosuppressive, anti-inflammatory and anti-proliferative effects. BD has been used in topical therapy for the treatment of mild to moderate psoriasis [1, 2, 5-8]. Targeting of topically applied drugs is becoming a major centre of interest for many pharmaceutical groups working in dermatology to improve drug penetration into different skin layers. Thus; several vesicular systems were used in the treatment of psoriasis such as methotrexate (MTX) loaded liposomes hydrogel®, MTX loaded transfersomes®, MTX loaded niosomes® and corticosteroid nano capsule suspension [9-13]. Transfersomes have been utilized for dermal and transdermal drug delivery, also have several advantages over other nano-systems such as: biocompatibility, biodegradability and transportation of therapeutic agents through narrow constriction without any significant loss [14]. The high deformability of transfersomes gives better penetration of intact vesicles [14]. Therefore; the aim of this work is to design and evaluate betamethasone dipropionate (BD) loaded transfersomes as a topical formulation for the treatment of localized plaque psoriasis.

MATERIALS AND METHODS

Materials

Betamethasone dipropionate (BD) was a kind of a gift from N and R Bio industries INC, China. Soya Phosphatidylcholine (SPC) and sodium deoxycholate (SDC>98%) were purchased from Flukabiochemika company, Saint Gallen, Switzerland. Absolute ethyl alcohol and chloroform were purchased from Fischer scientific company, London, UK. HEPES buffer (4-(2-hydroxyl)-1-piperazinethanesulfonic acid): 1 Molar was purchased from Sigma Aldrish Company, St Louis, MO. Tween 80, methyl paraben and propyl paraben were purchased from Acros Organics company, Geel, Belgium. Carboxy methyl cellulose sodium salt (CMC) was purchased from Fluka biochemika company, Saint Gallen, Switzerland. Soya Phosphatidylcholine (SPC) and sodium deoxycholate (SDC>98%) were purchased from Flukabiochemika company, Saint Gallen, Switzerland. Absolute ethyl alcohol and chloroform were purchased from Fischer scientific company, London, UK. HEPES buffer (4-(2-hydroxyl)-1-piperazinethanesulfonic acid): 1 Molar was purchased from Sigma Aldrish Company, St Louis, MO. Tween 80, methyl paraben and propyl paraben were purchased from Acros Organics company, Geel, Belgium. Carboxy methyl cellulose sodium salt (CMC) was purchased from Fluka biochemika company, Saint Gallen, Switzerland.

Design and preparation of BD loaded transfersomal (BD-T) formulations

Full factorial design

A 22 full factorial design was applied in this study to optimize entrapment efficiency, particle size, zeta potential, polydispersity index and in vitro drug release for the prepared BD-T formulations (table 1). Three factors were selected as independent variables: the drug content (A) at two levels (25 mg and 50 mg), the type of surfactant (B) at two levels (sodium deoxycholate and tween 80) and the amount of surfactants (C) at two levels (5 mg and 7.5 mg). The experimental trials were performed at all 8 possible combinations. Minitab® release 17 software was used for the generation and evaluation of the statistical experimental design.
Preparation of betamethasone dipropionate loaded transfersomal formulations (BD-T)

Different formulations of BD loaded transfersomes containing 50 mg SPC were prepared by conventional thin lipid film hydration technique using rotary evaporator (Helidolph, Germany) according to formulation composition reported in table (2). The amounts of lipid, surfactant (sodium deoxycholate or tween 80) and drug (BD) were dissolved in 6 ml chloroform and placed in a rotary evaporator for 2 h at a temperature of 42 °C, at a rotation speed of 80-83 rpm under vacuum. The organic solvent was totally evaporated and the formed lipid film was subsequently hydrated by adding 15 ml of the freshly prepared 0.01 M HEPES buffered saline (PH 5.5) and rotated at a speed of 120-122 rpm for 30 min at 40-42 °C [15, 16].

Characterization of BD loaded transfersomal (BD-T) formulations

Determination of entrapment efficiency percentage

The prepared BD-T formulations (F1-F8) were centrifuged using cooling centrifuge (Helidolph, Germany) at a speed of 40,000 rpm and a temperature of 4 °C for one hour. The transfersomal residue was separated from the supernatant containing the free drug (FD). The residue was washed twice with 15 ml 0.01M HEPES buffered saline and kept in 4 °C for further characterization studies (PS, PI, ZP, TEM, and in vitro drug release).

Table 1: Coded units of 2³ full factorial design for betamethasone dipropionate loaded transfersomal (BD-T) formulations

<table>
<thead>
<tr>
<th>Variables</th>
<th>Levels</th>
<th>High</th>
</tr>
</thead>
<tbody>
<tr>
<td>A (Drug content)</td>
<td>25 mg BD</td>
<td>50 mg BD</td>
</tr>
<tr>
<td>B (Type of surfactant)</td>
<td>Sodium deoxycholate</td>
<td>Tween 80</td>
</tr>
<tr>
<td>C (Amount of surfactant)</td>
<td>5 mg</td>
<td>7.5 mg</td>
</tr>
<tr>
<td>Coded values</td>
<td>-1</td>
<td>+1</td>
</tr>
</tbody>
</table>

In vitro drug release study

A volume of 0.5 ml of each transfersomal formulations was placed in a dialysis bag of 2 cm length (Dialysis tubing-visking: regenerated cellulose, size 5 inf Dia 24/32~19 mm: 30 M, Medicell. UK). Both ends were tied and the dialysis bag was suspended in 15 ml Eth-HS solution of PH= 5.5 and maintained at 37±0.5 °C. The system was stirred magnetically at 200 rpm. At predetermined time intervals; 2 ml aliquots of the release medium was sampled and replaced with 2 ml fresh Eth-HS solution. The samples were suitably diluted with Eth-HS solution and the drug concentration was measured spectrophotometrically at λmax of 238 nm [20]. Drug concentration was determined using the previously prepared standard curve of BD in Eth-HS solution and all experiments were conducted in triplicates.

Preparation of BD-T gel

Organoleptic properties

The organoleptic properties of the prepared BD-T gel were tested such as color, odor, texture and transparency.

Rheological study

A sample of one gram was tested in order to identify the flow behaviour of the gel (Cone over the plate, V type, Brookfield viscometer, UK). The shear stress in dyne/cm² and the viscosity in centipose were determined at a different shear rate (2, 6, 10, 20, 24, 60, 100 and 120 sec⁻¹) using spindle number 52.

In vitro BD release

The in vitro drug release from BD-T gel was studied using a USP dissolution apparatus (Electro lab model TDT-08L, India) with 8 cells each containing 250 ml vessel. One gram of BD-T gel was placed in a glass watch of 5 cm diameter and covered with a dialysis membrane (size 5 inf Dia 24/32~19 mm: 30 M, Medicell UK). Each glass watch was placed in dissolution vessels containing 100 ml of the dissolution medium (Eth-HS solution) at 32±0.5 °C (skin temperature). The dissolution medium was stirred with a paddle at constant rate of 100 rpm [11, 21]. Samples of 2 ml were withdrawn from the dissolution medium at specified time intervals and were suitably diluted with Eth-HS solution before measuring the concentration of BD released. The drug concentration was measured spectrophotometrically at λmax of 238 nm using the previously constructed calibration curve.

Stability study

The stability of BD loaded transfersomes was evaluated after storage for 6 mo at 4 °C and 25 °C by studying the change in the organoleptic characters, drug content (chemical stability) and in vitro drug release (physical stability) [19, 22-29].
The drug content was determined by dissolving a weight of 0.5 gm of the freshly prepared BD-T gel in 50 ml Eth-HS solution. Two ml samples were filtered and analyzed spectrophotometrically at \( \lambda_{\text{max}} \) of 238 nm after suitable dilution with Eth-HS solution.

Clinical study

Twenty patients with plaque psoriasis, with an age range of 24 to 60 y were enrolled in this study. The experimental protocol was approved by an ethical committee at Faculty of Pharmacy, Tanta University. The patients were enrolled from the outpatient dermatology clinics, Tanta University hospital. Patients were subdivided into two equal groups.

Group 1: for treatment with BD-T gel and Group 2: for treatment with marketed product. For each patient; one side was treated with the tested product and the other side was treated with placebo gel. The exclusion criteria were pregnancy, lactation and prohibition from topical corticosteroids. Patients were given treatment three times daily.

Physician global assessment (PGA) score of improvement was calculated at base line and after 2 mo of treatment. The PGA scores were 0 (clear), 1 (minimal), 2 (moderate), 3 (severe) and 5 (very severe) [30-35].

Kinetic analysis

The release data were analyzed mathematically according to zero order, first order and Higuchi models [equations 2 to 4] using StatistXL for MS Excel software. The release rate constants (K) and order, first order and Higuchi models [equations 2 to 4] were estimated.

Where:

\[ K_0 \] is the zero-order rate constant (concentration/time).
\[ t \] is the time (h).
\[ C_0 \] is the initial concentration of the drug.

\[ C_t = \text{amount of drug released in time } t. \]

EE% = 77.4±2.28 A+4.87 B+8.45 C+1.19 AB-2.82 AC+1.37 BC+0.914 ABC Equation (6).

The regression equation (7) representing the effect of independent variables on the particle size showed that the most effective factor was the drug content (A) in its high level. The second effective factor was B (the type of surfactant) in its high level and the third effective factor was the interaction AC in its low level indicating that high EE% was achieved by the use of A (high drug content) and C (low surfactant content). All the factors showed a significant effect on EE%.

K is the first order constant.

\[ Qt \] is the amount of drug release in time \( t \).

\[ KH \] is the Higuchi dissolution constant.

Table 2: In vitro characteristics of betamethasone dipropionate loaded transfersomal (BD-T) formulations

<table>
<thead>
<tr>
<th>Batch code</th>
<th>Formulation composition</th>
<th>In vitro characteristics</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Drug content (mg)</td>
<td>Surfactant type</td>
</tr>
<tr>
<td></td>
<td>(mg)</td>
<td>SDC</td>
</tr>
<tr>
<td>F1</td>
<td>25</td>
<td>Tween 80</td>
</tr>
<tr>
<td>F2</td>
<td>50</td>
<td>Tween 80</td>
</tr>
<tr>
<td>F3</td>
<td>25</td>
<td>Tween 80</td>
</tr>
<tr>
<td>F4</td>
<td>25</td>
<td>SDC</td>
</tr>
<tr>
<td>F6</td>
<td>50</td>
<td>SDC</td>
</tr>
<tr>
<td>F7</td>
<td>25</td>
<td>Tween 80</td>
</tr>
<tr>
<td>F8</td>
<td>50</td>
<td>Tween 80</td>
</tr>
</tbody>
</table>

Each *rmulat *nc *ntains [50]mgSPC(s *yaph *spatidylyh *line),SDCiss *duimde *xych *late., EEis the entrapment efficiency (%), PSis the particle size (nm). Plis the physicochemical and ZPis the zeta potential (mV), dataaremean±SD, n=3.

Entrapment efficiency percentage

The entrapment efficiency of different transfersomal formulations are reported in the table (2). From the table, the transfersomes prepared from low content of surfactant showed the highest EE irrespective to the type of surfactant. The regression equation representing the effect of independent variables on the EE % was equation (6). The equation showed that the most effective factor on EE % was the surfactant content (c) in its low level. The second effective factor was B in its high level (tween 80). The third one was the interaction AC in its low level indicating that high EE % was achieved by the use of A (high drug content) and C (low surfactant content). All the factors showed a significant effect on EE %.

EE % = 77.4±2.28 A+4.87 B+8.45 C+1.19 AB-2.82 AC+1.37 BC+0.914 ABC Equation (6).

Where:

EE % is the entrapment efficiency percentage.

Particle size (PS), polydispersity index (PI) and zeta potential (ZP) measurements

The regression equation (7) representing the effect of independent variables on the particle size showed that the most effective factor was the drug content (A) in its high level. The second effective factor was B (the type of surfactant) in its high level and the third effective factor was the interaction ABC in its negative level. All the factors showed a significant effect on PS.

Where:
PS is the particle size.

The regression equation (8) representing the effect of independent variables on the PI showed that the most effective factor was B in its high level (Tween 80), the second effective factor was C (surfactant content) in its high level and the third effective factor was A in its high level (high drug content). All the factors showed significant effects (p<0.05) on PI except the interaction ABC.

\[
PI = 0.510 - 0.0288 A - 0.0935 B - 0.0358 C + 0.0125 AB + 0.0133 AC + 0.0245 BC - 0.00900 ABC
\text{Equation (8)}
\]

Where:
PI is the poly dispersity index.

The regression equation (9) representing the effect of independent variables on the ZP showed that the most effective factor was the interaction AC in its high level; +A (high drug content) and +C (high surfactant content). The second effective factor was the interaction ABC in its low level and the third one was +C (high surfactant content). All the factors showed significant effects (p<0.05) on ZP except the factor A (drug content).

\[
ZP = 14.8 + 0.094 A - 0.394 B + 1.38 C + 1.38 AB + 2.36 AC - 0.856 BC - 2.03 ABC
\text{Equation (9)}
\]

Where:
ZP is the zeta-potential.

Transmission electron microscopy (TEM)
The TEM photographs for the eight tested formulations showed great variations in shape with nano size of the transfersomal vesicles. Formulations F1 to F4 showed typical spherical shape, unilamellar structure and smooth surface vesicles and formulations F5 to F8 showed high deformation in the shape of the formed vesicles.

Photographs of F4 and F8 represent the formulations F4 (a) and F8 (b) as shown in fig. 1.

In vitro drug release
The drug release profiles from the eight BD-T formulations are represented in fig. 2. The Percentages of BD released from BD-T formulations after 0.75 h were ranged from 2.37±0.03 % to 9.50±0.50 %.

The results of the kinetic analysis of the release data are reported in the table (3). All formulations showed best fitting to zero order kinetics (K0). From the table, F4 was the formulation that showed the highest release rate. The regression equation representing the effect of independent variables on the release rate constant (K0) was equation (10). From the equation, the most effective factor on increasing K0 was An in its high level (high drug content). The second effective factor was the interaction BC in its low level resulted from the interaction of the factor –C with the factor+B. The third one was B in its high level (Tween 80). All the factors showed significant effects (p<0.05) on K0 except the interaction AB and the interaction ABC.

\[
K_0 = 2.61 + 1.18 A + 0.170 B - 0.0936 C - 0.00669 AB + 0.0507 AC - 0.293 BC - 0.0073 ABC
\text{Equation (10)}
\]

Where:
K0 is zero order release constant.

A graphical representation of contour plots for the effects of the most effective factors on EE, particle size and release rate constant is showed in fig. 3 (a, b and c).

In vitro characterization of BD-T gel
The prepared BD-T gel was elegant, soft, odorless, colourless and having a transparent appearance. The gel exhibited a pseudoplastic flow with a thixotropic behaviour which was a desirable character in pharmaceutical gels. The rheological parameters for the prepared BD-T gel were calculated according to equations (11) and (12) of the power law and listed in the table (4) [36, 37]. The drug release profile was best fitted to Higuchi diffusion model with release rate constant Ka= 11.0±0.01 mg/hr-1 and T50 of 5.15±0.1 h.

\[
\eta = K\cdot \gamma \cdot \left(n-1\right)
\text{Equation (11)}
\]

Where:
\(\eta\) is the viscosity in cp.

K is the consistency index.
\(\gamma\) is the shear rate in sec-1.

n is the flow behaviour index.

\[
\log \eta = \log K + \log \gamma \cdot \left(n-1\right) \text{ Equation (12)}
\]

Fig. 1: Transmission electron microscope (TEM) photographs of betamethasone dipropionate loaded transfersomal formulations F4 (a) and F8 (b), (magnification x 7500)
Fig. 2: *In vitro* drug release profile from betamethasone dipropionate loaded transfersomal formulations, Data are mean±SD, n=3 for each formulation

Table 3: Drug release kinetic parameters for BD-T formulations

<table>
<thead>
<tr>
<th>Formulation No.</th>
<th>$K_0$</th>
<th>$T_{50}$ (hr)</th>
</tr>
</thead>
<tbody>
<tr>
<td>F1</td>
<td>1.097±0.015</td>
<td>11.34±0.056</td>
</tr>
<tr>
<td>F2</td>
<td>3.375±0.025</td>
<td>7.407±0.055</td>
</tr>
<tr>
<td>F3</td>
<td>2.035±0.029</td>
<td>6.093±0.006</td>
</tr>
<tr>
<td>F4</td>
<td>4.290±0.026</td>
<td>5.827±0.038</td>
</tr>
<tr>
<td>F5</td>
<td>1.393±0.011</td>
<td>8.973±0.068</td>
</tr>
<tr>
<td>F6</td>
<td>3.877±0.012</td>
<td>6.450±0.017</td>
</tr>
<tr>
<td>F7</td>
<td>1.162±0.029</td>
<td>10.607±0.060</td>
</tr>
<tr>
<td>F8</td>
<td>3.623±0.038</td>
<td>6.9±0.0700</td>
</tr>
</tbody>
</table>

Data are mean±SD, n=3 $K_0$: release rate constant presented as mg. h$^{-1}$

Fig. 3: Contour plots for the effects of the most effective factors on entrapment efficiency% (a), particle size (b) and release rate constant (c), n=3 for all formulations
Table 4: Rheological parameters of the prepared pseudoplastic BD-T gel

<table>
<thead>
<tr>
<th>System</th>
<th>R²</th>
<th>n</th>
<th>K</th>
</tr>
</thead>
<tbody>
<tr>
<td>BD-T gel</td>
<td>0.9447±0.0053</td>
<td>0.6553±0.0004</td>
<td>6696.28±11.581</td>
</tr>
</tbody>
</table>

BD-T gel betamethasone dipropionate loaded transfersomal gel, R² is the regression coefficient, n is the flow index, K is the consistency index, sample size =3, data are mean±SD

Stability of BD-T gel

All organoleptic characters including shape, consistency, color and odor; didn’t changed over six month’s storage at 4 °C and 25 °C. Drug release from the gel during 6 mo was used as an indication for physical stability. There was the insignificant difference between drug released from gel during 6 mo (p=0.144) either at 4 °C or at 25 °C (table 5). The release kinetics was best fitted to Higuchi diffusion model. From the table, there were no significant changes in the drug content during six months storage at 4 °C and 25 °C indicating the drug chemical stability.

Clinical evaluation of BD-T gel

The relation between clinical efficacy and patient’s age, sex and duration of illness in both groups were statistically evaluated, and the results showed insignificant effect (p>0.05) of patient’s age on the clinical efficacy of both BD-T gel and marketed cream. An overall comparison between the clinical efficacies of both groups was showed in the table (6),which showed that there was a statistically significant difference in the clinical efficacy between groups treated with BD-T gel and Diprosone® cream (p=0.016). The comparison between the two groups regarding safety and tolerability was represented in fig. 4. The results indicated that BD-T was more safe and tolerable compared with the reference product (p=0.00). From table (5), 30% of the patients treated with BD-T gel showed clear score (0), 60% showed minimal score (1) and 10% showed mild score (2). The patients treated with BD cream showed that 10% was a clear score (0), 10% was minimal (1) and 50% was mild (2), and 30% showed moderate score (3). The patient’s response to the treatment in both groups wasn’t affected significantly with the difference in age (p=0.093), p>0.05, gender =1 and duration of illness p=0.233. There was statistically difference between score before treatment and score after treatment. The clinical results for Group (1) and Group (2) are showed in fig. 5.

Table 5: Drug release kinetic parameters and drug content for betamethasone dipropionate loaded transfersomal gel during storage for six months

<table>
<thead>
<tr>
<th>Storage conditions</th>
<th>Storage times (months)</th>
<th>Drug content</th>
<th>Kdiff (mg/hr1/2)</th>
<th>T50 (hr)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fresh</td>
<td>97.60±1.01</td>
<td>11.02±0.100</td>
<td>5.145±0.096</td>
<td></td>
</tr>
<tr>
<td>4 °C</td>
<td>98.50±0.50</td>
<td>11.02±0.017</td>
<td>5.151±0.016</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>97.83±0.49</td>
<td>10.92±0.079</td>
<td>5.243±0.076</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>97.73±0.68</td>
<td>11.02±0.010</td>
<td>5.151±0.009</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>97.73±0.31</td>
<td>11.02±0.030</td>
<td>5.146±0.028</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>96.63±0.85</td>
<td>10.96±0.031</td>
<td>5.205±0.030</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>97.60±1.01</td>
<td>10.98±0.015</td>
<td>5.186±0.014</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>95.00±0.03</td>
<td>11.02±0.015</td>
<td>5.151±0.015</td>
<td></td>
</tr>
</tbody>
</table>

Data are mean±SD, n=3

Table 6: Comparison between the two studied groups according to Physician global assessment (PGA) score

<table>
<thead>
<tr>
<th>Score before treatment</th>
<th>%</th>
<th>%</th>
<th>χ²</th>
<th>MC p</th>
<th>χ²</th>
<th>Me p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clear</td>
<td>0.0</td>
<td>0.0</td>
<td>1.116</td>
<td>0.835</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Minimal</td>
<td>0.0</td>
<td>0.0</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mild</td>
<td>0.0</td>
<td>0.0</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Moderate</td>
<td>50.0</td>
<td>50.0</td>
<td>1.050</td>
<td>0.712</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Severe</td>
<td>30.0</td>
<td>30.0</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Very Severe</td>
<td>20.0</td>
<td>20.0</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Score for Control</th>
<th>%</th>
<th>%</th>
<th>χ²</th>
<th>MC p</th>
<th>χ²</th>
<th>Me p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clear</td>
<td>0.0</td>
<td>0.0</td>
<td>1.050</td>
<td>0.712</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Minimal</td>
<td>0.0</td>
<td>0.0</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mild</td>
<td>0.0</td>
<td>0.0</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Moderate</td>
<td>50.0</td>
<td>40.0</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Severe</td>
<td>30.0</td>
<td>50.0</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Very Severe</td>
<td>20.0</td>
<td>10.0</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Score after treatment</th>
<th>%</th>
<th>%</th>
<th>χ²</th>
<th>Me p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clear</td>
<td>30.0</td>
<td>10.0</td>
<td>9.424</td>
<td>0.016</td>
</tr>
<tr>
<td>Minimal</td>
<td>60.0</td>
<td>10.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mild</td>
<td>10.0</td>
<td>50.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Moderate</td>
<td>0.0</td>
<td>30.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Severe</td>
<td>0.0</td>
<td>0.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Very Severe</td>
<td>0.0</td>
<td>0.0</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

χ²: Chi square test, MC: Monte Carlo for Chi square test, *: Statistically significant at p ≤ 0.05
DISCUSSION

The use of thin film hydration method in the present work may be advantageous in improving EE compared with vortexing method [22]. All the tested BD-T formulations showed a relatively high EE% ranged from 69.1%±0.80 to 90.1%±0.76 (table 2). This increase in EE% may be attributed to the formation of a thin film with a large surface area which facilitates the complete hydration of vesicles [22].

Concerning EE%, the regression equation (6) showed that the formulation F4 was the optimized one due to the low surfactant content (-C), tween 80 (+B) and high drug content (+A). These results agreed with that reported by El-Zaafrany et al. [22]. The authors found that increasing surfactant content from 2% to 5% (W/W) increased the EE% non-significantly but further increase in its content showed a decrease in EE%.

The same finding was reported by Jain et al. [19], where they showed that EE% decreased with an increase in surfactant concentration. The authors explained these results by the possible coexistence of mixed micelles and vesicles at higher surfactant concentration, and consequently lower drug entrapment efficiency. The second effective factor was B (type of surfactant) in its high level; vesicles containing tween 80 have higher EE than vesicles...
containing SDC. To explain these findings we considered HLB (hydrophilic/lipophilic balance) of the two surfactants [22]. The HLB values for tween 80 and SDC are 15 and 16.7, respectively [22]. Based on these HLB values, affinity for lipids was expected to be higher in the case of using tween 80 rather than SDC and incorporation of a lipophilic drug was easier in the non-ionic surfactant compared with the anionic surfactant. This result reflected the relationship between the hydrophobicity of the drug and that of the surfactant as reported by El-Zafraany [22]. Another explanation for the higher EE showed by tween 80 may be due to its high solubilizing effect. The last effective factor was the interaction-ABC in its low level where the use of low hydrophobicity that results in smaller vesicles. The third effective factor was the interaction-ABC where; the use of high drug content (+A) with the low surfactant content (-C) lead to a significant increase in EE%.

Concerning PS, The regression equation (7) showed that the formulation F4 was the optimized one due to the use of high drug content (+A), tween 80 (+B) and low surfactant content (-C). The particle size of the prepared transfersomes decreased significantly (p<0.05) by increasing drug content from 25 mg to 50 mg. This may be due to the partitioning of the lipophilic drug into the coating layer resulting in an increase of its rigidity. Consequently, this enhanced the stability of vesicles with the smaller size. Also, from the regression equation (7); the second effective factor was B in its high level (tween 80). This result was explained by the solubilizing effect and the HLB value of tween 80 [38-40]. Generally, the use of edge activators with lower HLB resulted in vesicles with the smaller size and this was the case of F4 compared with SDC [22]. This relationship observed between vesicle size and surfactant HLB was attributed to the decrease in surface energy obtained with increasing hydrophobicity that results in smaller vesicles. The third effective factor was the interaction ABC in its low level where the use of low surfactant content (-C) with high drug content (+A) and tween 80 (+B) significantly reduced particle size.

The polydispersity index (PI) gives an important indication concerning sample homogeneity, as a value below 0.7 reflect relatively homogenous nanoparticles, with a minimum predisposition to deformation in the shape of the formed vesicles as showed in (fig. 1). This deformation may be explained by the formation of mixed micelles in combination with the transfersomal vesicles [22].

The in vitro release of all formulations were best fitted to zero order kinetics as indicated by the highest correlation coefficients. Concerning in vitro drug release, the regression equation (10) showed that the formulation F4 is the optimized one due to the use of high drug content (+A), low surfactant content (-C) and tween 80 (+B). The most effective factor was the high drug content (+A). This may be due to the significantly decreased PS and consequently the increased surface area of the nanovesicles resulting in faster drug release. Also, high drug content was found to significantly increase EE% and drug loading leading to an increased drug partitioning from the nanovesicles to the release medium. The second effective factor was the interaction-BC with tween 80 (+B) and low surfactant content (-C). It was reported that at high surfactant concentration, the drug release was low due to formation of rigid micelles [45]. These findings were agreed with Shaji et al. [48] and Gupta et al. [29], who reported that drug release was related to EE and drug loading. In the present study the optimum surfactant concentration for drug release was found to be achieved by using 5 mg tween 80. This finding is also agreed with Gupta et al. [29] and Kayani et al. [49], who found that the use of tween 80 as a non-ionic surfactant significantly increased (p<0.05) drug release from the transfersomal suspensions compared with SDC as an ionic surfactant. The possible explanation of the enhancing effect of tween 80 on drug release rate was the large volume of hydrophilic head group consisting of several polyethylene chains that impede penetration into tails of lipid bilayer of the transfersomes and increased the deformability of the vesicles and consequently, showed highest drug release [50].

The optimized BD-T formulation was F4 concerning EE, particle size and in vitro drug release. This formulation was selected to be prepared as a pharmaceutical gel using 5% Na CMC to increase the consistency and consequently the contact time on the application site. The prepared BD-T gel exhibited pseudoplastic flow with a thixotropic behavior. The prepared BD-T gel exhibited pseudoplastic flow with a thixotropic behavior as indicated by the highest correlation coefficients.

Zeta potential (ZP) was measured via the electrophoretic mobility of the particles in an electric field [43]. ZP is one of the factors that determine the physical stability of nanosystems [44-46]. The regression equation (9) showed that the formulation F6 was the optimized one due to the use of high drug content (+A), high surfactant content (+C) and SDC (-B). The most effective variable on ZP was the interaction AC in its high level. This result indicated the synergistic interaction between high drug content (+A) and high surfactant content (+C). This result agreed with that reported by Basha et al. [46], who found that the increase in surfactant concentration (sodium cholate, sodium deoxycholate, tween 80) increases the ZP value of the nanovesicles. In addition, Gonzalez-Mira et al. [47], reported that the higher concentration of the loaded drug (flurbiprofen) leads to higher ZP. The second effective factor significantly increased ZP value was the interaction-ABC indicating the significant effect of using of +A, +C and -B (SDC). This was in agreement with that reported by Basha et al. [46], who found that nanovesicles prepared with SC and SDC showed more negative ZP values compared with tween 80 with its non-ionic nature. Despite of the regression equation showed that F6 is the optimized it is not necessarily to be the most physically stable one. This is because ZP indicates only the electrical hindrance. Transfersomes prepared with tween 80 showed electrical and steric hindrance [42]. This steric hindrance prevented the aggregation and stabilized the system physically.

TEM showed that the use of high surfactant contents resulted in high deformation in the shape of the formed vesicles as showed in (fig. 1). This deformation may be explained by the formation of mixed micelles in combination with the transfersomal vesicles [22].
formulation. The transfersomal formulation was prepared by the thin film hydration method which was reported to be more advantageous in improving the stability of the prepared transfersomal formulations compared with other methods such as vortexing method [22, 45]. Treated patients suffering from localised plaque psoriasis showed insignificant difference regarding age, sex and duration of illness for both groups. The PGA score was used to assess the patient at baseline and after two months of treatment. Group 1 treated with BD-T gel showed significantly higher clinical effects, safety and tolerability compared with Group 2 treated with BD cream. [45, 53].

CONCLUSION

The use of BD-loaded transfersomes was effective and promising for carrying the drug into deeper layers of the skin. The transfersomal gel was an effective vehicle for applying BD topically in psoriatic lesions. The BD-loaded transfersomal system was more effective, tolerable and safe for treatment of psoriasis compared with the marketed product.

CONFLICT OF INTERESTS

The authors report no conflict of interest.

AUTHORS’ CONTRIBUTION


REFERENCES


How to cite this article