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RESEARCH ARTICLE

Development of Betamethasone Dipropionate-loaded Nanostructured Lipid Carriers for Topical and Transdermal Delivery

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ARTICLE HISTORY

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Abstract: ***Introduction*:** Betamethasone dipropionate is a highly effective corticosteroid anti-inflammatory. However, the main drawback of its topical use is the limited skin penetration into deeper skin layers. Also, its systemic use has shown many side effects.

***Objective*:** The goal of this research was to formulate betamethasone dipropionate in nanostructured lipid carriers (NLC) formulae that contain oleic acid to aid its penetration to deeper skin layers and to aid absorption to local regions upon topical application.

***Methods*:** NLC formulae were prepared by high shear homogenization then sonication. Formulae were characterized for their particle size, size distribution, electric potential, occlusion factor, entrapment efficiency, drug loading, transmission electron microscopy, *in vitro* drug release, and *ex vivo* skin penetration. Compatibility of ingredients with drug was tested using differential scanning calorimetry. Formulae were shown to have appropriate characteristics. NLC formulae were superior to traditional topical formulation in drug release.

***Results*:** Upon testing *ex vivo* skin penetration, betamethasone dipropionate prepared in NLC formulae was shown to penetrate more efficiently into skin layers than when formulated as a traditional cream. NLC formulation that contained higher percentage of oleic acid showed higher penetration and higher amount of drug to pass through skin.

***Conclusion*:** In general, NLC with lower oleic acid percentage was shown to deliver betamethasone dipropionate more efficiently into deeper skin layers while that of a higher oleic acid percentage was shown to deliver the drug more efficiently into deeper skin layers and through the skin, transdermally.

Keywords: Absorption, betamethasone dipropionate, NLC, oleic acid, skin penetration, transdermal.

1. INTRODUCTION

Nanoparticles-based drug delivery systems have gained an increased attention in the last decades. This took place because nanoparticles have proven to obtain numerous useful characteristics such as controlled release of the drug molecule [1] and penetration enhancement through semipermeable membranes and skin [2]. Lipid-based nanoparticulate drug delivery systems are receiving growing attention. These systems, such as nanoemulsions, liposomes, niosomes, ethosomes, virosomes, ufasomes, vesosomes have the advantages of biocompatibility, well tolerability, reduced toxicity and increased bioavailability of poorly water soluble drugs [3, 4].

Amongst the most important lipid-based nanoparticulate drug delivery systems, Nanostructured Lipid Carriers (NLC) arouse as beneficial and highly effective tools for imparting useful properties for drug delivery such as controlled drug release, drug targeting, increased drug stability, high drug payload, enhanced lymphatic transport and increased penetration of stratum corneum [5].

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NLC were stated to be advantageous over Solid Lipid Nanoparticles (SLN) in exhibiting improved properties for drug loading, modulation of the delivery profile and stable drug loading during storage [6, 7].

Betamethasone dipropionate, being a corticosteroid, has been widely used as soothing anti-inflammatory or immunosuppressant for topical as well as systemic use. It can be used in the treatment of rheumatoid arthritis, osteoarthritis, and ankylosing spondylitis. Topical application of corticosteroids, including betamethasone dipropionate, often produces inhibition of skin conditions in which inflammation is a major feature, such as eczema, seborrhoeic dermatitis, and some forms of psoriasis [8]. The main advantage of betamethasone over other corticosteroids is the lack of retention of sodium and water [8].

The objective of this work was to develop, characterize and evaluate NLC formulae; optimize the conditions and formulae compositions for production of the optimum formulae; and loading optimum formulae with betamethasone dipropionate for the delivery of this drug to/through skin.

2. MATERIALS AND METHODS

2.1. Materials

Glycerylmonostearate (GMS), oleic acid (OLA), tween 80 (T80), glycerol, sodium monohydrogen phosphate and potassium hydroxide were purchased from Al-Gomhoria Company for chemicals, Egypt. Medical Union Pharmaceuticals company (MUP) generously supplied cremophor RH 40 and betamethasone dipropionate as gifts. Sodium tauroglucocholate was purchased from Thomas Baker Chemical Industries Pvt. Ltd., Mumbai, India.

Phospholipon 90G® was purchased from Spectrum Chemicals and Laboratory Products, New Brunswick, NJ, USA. Span 20 was purchased from Loba Chemie Pvt. Ltd., Mumbai, India. Potassium dihydrogen ortho phosphate was purchased from Piochem for laboratory chemicals, Egypt. Methanol (HPLC grade) was purchased from Fisher Chemical, Fairlawn, NJ, USA. Dialysis Tubing cellulose membrane (molecular weight cut-off 12,000 g/mole) was purchased from Sigma-Aldrich Chemical Company, St. Louis, USA. All other chemicals used were of analytical grade and were used without any further processing or purification.

2.2. Methods

2.2.1. Compatibility Screening of Selected Lipids

Test for compatibility of liquid lipids with solid lipids was performed following procedure reported by Doktorovová *et al*., with slight modification [9]. The lipid matrix components were mixed together in the selected final ratios and then were heated up to 70°C to melt the solid lipid with liquid lipid. After complete melting, shaking was done to assure complete mixing of the components together. Lipid matrices were made into pellets and left for solidification. Pellets of lipid mixtures were checked 1 h, 1 day, 7 days and 30 days (day 0, day 1, day 7, and day 30, respectively) after solidification. Mixtures separating into more than one phase were excluded; mixtures forming one phase without any separation during this whole period of testing were selected for formulating NLC.

2.2.2. Determination of Melting Range of Selected Lipid Mixtures

The solid lipid or the lipid mixtures under test were molten at 70°C. The molten lipid was shaken thoroughly to mix the components completely and then left to solidify. The solid lipid mixture was inserted into a capillary tube by dipping the capillary tube in the solid lipid mixture. Determination of melting point of the lipid matrix was then done using Stuart apparatus, model SMP10. The temperature at which lipid started to melt was recorded.

2.2.3. Formulation of Drug-free NLC

Nanostructured lipid carriers were formulated using combination of the two techniques named high shear homogenizer and ultrasonication [10-13].

Aqueous phase, containing surface active agent(s) and glycerol as viscosity modifier, was heated up to 70°C. Oily phase was heated up to the same temperature separately. The two hot phases were then mixed together just before homogeniza-

Table 1. Composition of the investigated NLC formulations (25g).

Formula	GMS (mg)	OLA (mg)	T 80 (mg)	Cr (mg)	STG (mg)
GO1 F1	675	75	1500	-	-
GO1 F2	675	75	750	-	-
GO1 F3	675	75	-	1500	-
GO1 F4	675	75	-	750	-
GO1 F5	675	75	-	-	1500
GO1 F6	675	75	-	-	750
GO1 F7	675	75	750	750	-
GO1 F8	675	75	375	375	-
GO1 F9	675	75	500	500	500
GO1 F10	675	75	250	250	250
GO3 F1	525	225	1500	-	-
GO3 F2	525	225	750	-	-
GO3 F3	525	225	-	1500	-
GO3 F4	525	225	-	750	-
GO3 F5	525	225	-	-	1500
GO3 F6	525	225	-	-	750
GO3 F7	525	225	750	750	-
GO3 F8	525	225	375	375	-
GO3 F9	525	225	500	500	500
GO3 F10	525	225	250	250	250

tion. The hot mixture afterwards was rapidly transferred to high shear homogenizer (Ultra-Turrax Homogenizer, model Ultra- Taurax® T-25, IKA, Jahnke & Kunkel GmbH, Staufen, Germany) and was set for homogenization for 5 minutes. After that the formed emulsion was then set for ultrasonication (Ultra sonicator cleaner, model SK 3210 HP, Human Lab. instruments Co., Korea). Formulae compositions and amount of each component are listed in Table **1**.

2.2.4. Determination of Particle Size and Particle Size Distribution

Average particle size of the prepared SLN and NLC formulae was measured using dynamic laser light scattering apparatus at ambient temperature (mastersizer 2000 *vs*. 5.54 hydro 2000 S, Malvern instruments Ltd., Malvern, and Worcs, UK).

Two main concepts are used frequently to determine the distribution of particles in the population according to particle size. These two concepts are span and Polydispersity Index (PI).

Span is calculated according to the following formula:

$$\text{Span} = (d_{0.9} - d_{0.1})/d_{0.5}$$

where, $d_{0.9}$, $d_{0.5}$ and $d_{0.1}$ are the particle diameters below which 90%, 50% and 10% of the particles are situated, respectively.

On the other hand Polydispersity index (PI), which is an important measure for the homogeneity of particles population, was also calculated. This was done using the following formula:

$$PI = (\sigma/z)^2$$

where, σ is the standard deviation, and z is the mean particle size.

2.2.5. In vitro Occlusion Test

The test utilized for determination of *in vitro* occlusion of the formulae was originally adapted by de Vringer [14] and was reported afterwards [15].

Beakers (100 ml) were filled with 50 ml of water and then covered carefully with filter paper followed by sealing. A fixed amount of the formulae (5 g) were applied evenly on the top of the filter paper surface. Beakers were then stored in an insulated hot air oven at 32°C and relative humidity of 50-55%. Test took place for 48 hours. The amount of water was weighed at predetermined time intervals. Time intervals of 6h, 12h, 24h and 48h were chosen. At the end of the experiment, a visible film formed of the lipids of the formulae could be seen and removed intact by the aid of spatula. A beaker covered by a filter paper with no formula applied to it was used as a control. The loss of water occurred due to the flux of water vapor outside the beaker through the filter paper surface. As a result, water losses from formulae-applied beakers were compared to the water losses from the control beakers. Occlusion factor of the formulae was calculated using the following equation:

$$\text{Occlusion Factor (OF)} = [(W_{LR}-W_{LS})/ W_{LR}] \times 100$$

where, W_{LR} is the water loss from control reference and W_{LS} is the water loss from formulae-applied beakers. Experiment was performed in triplicate for statistical validation of results.

2.2.6. Determination of Drug Solubility in Lipid Matrices

Solubility of drug in excipients of formulae is an important parameter to take care of. It mainly controls the amount of drug to be added in the formula and the entrapment efficiency. This was reported in many publications [9, 16, 17].

For determination of solubility of the drug in the different lipid matrices the shake-flask method introduced by Higuchi and Connors [18] was followed. This method was shown to be the most reliable and widely used solubility measurement method. This method is used for determining thermodynamic solubility [19]. Using shake-flask method for determination of thermodynamic solubility of Active Pharmaceutical Principle (API) in the lipid matrix was reported by Shen and Zhong [16]. This method was followed with simple modification.

An excess amount of betamethasone dipropionate was introduced to the lipid mixture in a capped vial. Vials, afterwards, were placed in a shaking water bath which was adjusted to a temperature slightly above the melting point of the lipid matrices. Vials were stirred in the water-bath at 60 rpm for 48 hrs. A vortex mixer was used to facilitate the solubilization frequently. After standing for 48 h and reaching equilibrium, lipid matrices saturated with the drug were carefully decanted from the vials leaving remnants of undissolved betamethasone dipropionate at the bottom of the vials. The amount of drug in each lipid matrix was then determined using UV spectrophotometry.

2.2.7. Formulation of Betamethasone Dipropionate-loaded NLC

The same techniques used for preparing drug-free NLC were applied for the formulation of NLC loaded with betamethasone dipropionate. However, a simple change in the procedure was done. Instead of using blank lipid mixture for preparing the drug-free formulae, the lipid used to formulate the drug-loaded formulae was saturated first with the drug. In other words, lipid mixtures in study were separately saturated with the drug and then made into pellets. The betamethasone dipropionate-saturated lipid pellets were used to formulate the drug-loaded formulae according to the protocol mentioned for the preparation of drug-free NLC.

2.2.8. Determination of Zeta Potential

The electrica potential or zeta potential of the prepared particles was determined using Phase Analysis Light Scattering (PALS) technique. An adequate volume (1 ml) of the sample was loaded into the cuvette without any dilution. Cuvette was then inserted into the instrument (Zetasizer Nano ZS, Malvern Instruments Ltd., Malvern, UK). A multi-frequency measurement was performed to determine the mean electric potential. Measurement was done at the Nanotechnology Unit, National Research Center, Al Dokki, Guiza, Egypt.

2.2.9. Determination of Entrapment Efficiency

The used method for separating free and entrapped drug in this research was through utilization of centrifuge for its simplicity [9]. A volume of 5 ml of each drug-loaded sample was diluted to 15 ml with distilled water and then was centrifuged at Relative Centrifugal Force (RCF) = 2215 g for four cycles each spent 45min. to separate the lipid and aqueous phase. The supernatant was then analyzed by UV-VIS spectroscopy at 259 nm. The amount of free drug was determined and the percent entrapment efficiency (EE %) of the drug was given by the formula:

$$EE\% = ((W_a - W_s) / W_a) \times 100$$

where, EE% is the percent entrapment efficiency, W_a is the amount of drug loaded in formula, and W_s is the amount of the drug determined in the supernatant.

2.2.10. Determination of Percent Drug Loading

A volume of 5 ml of each drug-loaded sample was diluted to 15 ml with distilled water and then was centrifuged at 3000 rpm for four cycles each spent 45 min. to separate the lipid and aqueous phase. The supernatant was then analyzed by UV-VIS spectroscopy at 259 nm and the weight of lipid particles was also determined.

The percent drug loading (DL %) was given by the formula:

$$DL\% = ((W_a - W_s) / Wt_{Ptc}) \times 100$$

where, DL % is the percent drug loading, W_a is the amount of drug loaded in formula, W_s is the amount of the drug determined in the supernatant, and W_{tPtc} is the weight of lipid nanoparticles.

2.2.11. Differential Scanning Calorimetry (DSC)

Accurately weighed samples [1-6 mg] were placed in 40-µl aluminum pans and then crimped inside them upon closure of the pans using special piston. Sample-containing pan was then heated up at a rate of 10°C/min. under constant purging of nitrogen gas at a rate equivalent to 30 ml/min. The thermal range used was between 25°C and 250°C. Only Glycerylmonostearate (GMS) was heated only to 80°C. A reference of an empty aluminum pan having the same dimensions and quality was used for comparing differences in energy absorption or release by the sample.

2.2.12. Transmission Electron Microscopy (TEM)

Solid lipid nanoparticles and nanostructured lipid carriers loaded with betamethasone dipropionate were placed on copper grid and then stained with phophotungstic acid (2% W/W). Copper grid containing the stained sample was left to dry at room temperature. After that, grid was fixed to the holder and placed properly inside the transmission electron microscope for viewing the sample. Measurement was operated at the electron microscope center, National Research Center, Al Dokki, Guiza, Egypt).

2.2.13. Determination of In-vitro Release Profile

2.2.13.1. Dissolution Medium

Betamethasone dipropionate is practically water insoluble [20], and its solubility in different aqueous solutions was used as a criterion for selecting the media utilized in the dissolution test.

Dissolution medium was chosen to consist of phosphate buffer, pH 5.5, containing tween 80 (1% W/W) for enhancing betamethasone dipropionate solubility conserving sink conditions [21].

2.2.13.2. Dissolution Testing Process

The release of betamethasone dipropionate from NLC was done using the procedure reported by other research teams for determining the *in vitro* release profile of dihydroartemisinin and the poorly water soluble drug, bicalutamide, from nanostructured lipid carriers [17, 22].

In this method, well-closed glass vessels containing 100 ml of the dissolution media were warmed up in a shaking water bath. The temperature of the water bath was adjusted to be 32 ± 0.5°C. The speed of shaking of the water bath was adjusted to be about 100 rpm. An accurately measured amount of the formula was placed into dialysis bag and then the dialysis bag was tightly closed at both ends and placed in the glass vessel. Samples of the dissolution media were withdrawn from each vessel at predetermined time point using pipette. The volume of the sample withdrawn was 5 ml. The volume lost from each vial was replaced

by another fresh dissolution liquid warmed up to the same temperature in the same water bath and having the same volume taken.

Samples withdrawn were then analyzed using spectrophotometric method previously mentioned above.

2.2.14. Determination of Ex vivo Skin Penetration

The *ex vivo* skin penetration study was done following method reported for tenoxicam determination with slight modifications [23].

All animal-related procedures of the experiment were performed under the approved institutional protocols and after ethical committee approval. This approval holds the number of 20156A1, faculty of pharmacy, Suez Canal University, Egypt.

Dorsal hair of male westar rat was carefully removed by razor without causing any injury to the skin surface. Animals were sacrificed and then skin was carefully excised. Subcutaneous fat was then removed. Skin was mounted in fresh distilled water heated up to 37°C one hour before beginning of the experiment.

Skin membrane was then mounted in Franz cells with outer side facing the donor compartment and the inner one facing the receptor one. Receptor compartment was filled with 1% W/W of sodium lauryl sulfate [24]. Appropriate amounts of the formulae were then applied to the donor compartment. Donor compartment was then covered by parafilm® to prevent dehydration of the formula during the whole period of experiment.

Franz cells were then mounted in a modified water bath adjusted at 32°C ± 0.5 with magnetic stirrer beneath to stir the solution of the receptor compartment. At predetermined times; the content of the receptor compartment was evacuated and set for analysis. Fresh, preheated solution was used to replenish the taken liquid and maintain sink conditions.

Samples withdrawn were then analyzed using spectrophotometric method previously mentioned while formulae loaded in the donor compartment were carefully extracted and analyzed for drug content. The amount of drug delivered to skin but not delivered transdermally, *i.e*. located in skin, was calculated using the following formula:

$$D_{SL} = D_{OL} - (D_R + D_D)$$

Where, D_{SL} is the amount of skin-located drug, D_{OL} is the amount of drug originally loaded to donor compartment, D_R is the amount of the drug determined in the receiver compartment at the end of experiment, and D_D is the amount of the drug determined in the donor compartment at the end of experiment.

2.2.15. Statistical Analysis

Two way ANOVA testing of the data was done using GraphPad Prism®, GraphPad software Inc, Ver. 6.05. Statistical analysis was done on data obtained after all tests; excluding the qualitative ones such as compatibility screening, DSC, and TEM.

3. RESULTS AND DISCUSSION

3.1. Compatibility Screening of Selected Lipids

Since lipid matrix of NLC is composed of mixture of solid and liquid lipids, clarity or homogeneity of the lipid matrix is an important issue. If the lipid matrix is not clear in liquid state, while hot, and homogenous while solid, it is excluded from formulating the required NLC. In other words, only clear and homogenous lipid matrices are selected for formulating NLC [9]. Glycerylmonostearate (GMS) was chosen as the solid lipid. Oleic acid (OLA) was chosen as the liquid lipid since it was proven to have penetration enhancing properties through skin [25].

It was shown that OLA and GMS formed a clear and homogenous lipid matrix for formulation of NLC through the entire range of concentrations used in this study. Lipid pellets prepared from Mixtures of OLA and GMS remained homogenous (Fig. **1**) through the whole period of investigation; 30 days.

3.2. Melting Range of Selected Lipid Mixtures

Since, an important condition in formulation of NLC is that lipid nanoparticles should remain solid at human body temperature to function properly [26], determination of melting point for suggested lipid mixtures is important.

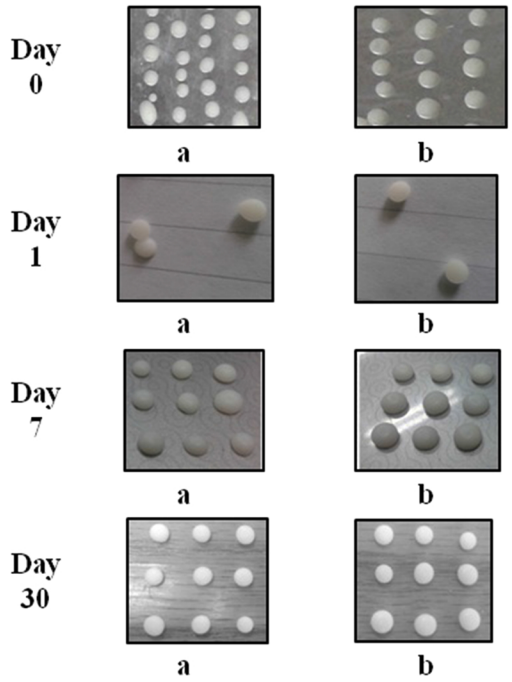

Fig. (1). Lipid pellets prepared from GMS and OLA mixture in the ratio of 90:10 (**a**) and 70:30 (**b**) at day 0 (I), day 1 (II), day 7 (III) and day 30 (IV) after preparation.

The addition of OLA to GMS has resulted in lowering melting point of the produced lipid mixture. Lipid mixture having 10% W/W OLA melted at 51.7°C, while the mixture having 30% OLA was recorded to melt at lower temperature, 46°C. This suggested that addition of liquid lipid to solid one resulted in decreasing melting point. The higher the ratio of the liquid lipid in the lipid mixture, the lower is the melting point of the resulting mixture. The effect of increasing the ratio of liquid lipid to the solid lipid in the lipid matrix on the melting point of the matrix might be attributed to a decrease in their crystallinity in comparison to their bulk counterparts [12].

It is also worth noting that the two bulk lipid mixtures had melting points higher than that of the body temperature which indicate the suitability of the two mixtures to formulate NLC.

3.3. Effect of Different Surface Active Agents on Physicochemical Properties of Prepared NLC

Three surface active agents were selected for formulation of NLC. These surfactants were tween 80 (T80), Cremophor RH 40 (Cr) and Sodium Tauroglycocholate (STG). Blends of these surfactants were also utilized. A blend of equal amount of T80 and Cr was used. Another blend of equal amounts of the three surfactants was used.

It is worth noting that T80 and Cr are nonionic surfactants that have HLB value of 15. They were used in this study to investigate the effect of geometry and size of the surface active agent on its emulsification behavior and capability. On the other hand, STG is one of the naturally occurring bile salts. Being one of the bile salts, STG is considered to be anionic surface active agent that has steroidal nucleus.

It was shown from Table **2**, that STG was incapable of producing NLC of adequate size. All formulae prepared using STG had mean particle size higher than 1000 nm (out of the nano range). This finding was also applicable for formulae prepared using the surfactant blend containing STG as one of its components. This agreed to the results obtained for preparing formulae using sodium deoxycholate, a structurally related bile salt, as surfactant [27]. These results might be justified on the basis of the process of emulsification. It is well known that, regarding to the emulsification process, the situation is highly dynamic. Mass transport is generally affected by many factors including molecular weight, charge and dimensions of the moving molecules. Sodium tauroglycocholate, having a molecular weight of 594.74 daltons (Da), is considered to be a large molecule. Thus, poor emulsification capabilities of STG might be attributed to the large molecule of STG which occupy large volume and thus low kinetics for adsorption on the new surfaces produced during particle size reduction. Also, the steroidal nucleus of the surface active agent, being bulky, is suggested to play an important role in reducing the kinetics of the molecule thus reducing it capability to cover the new surfaces leading to low system stabilization resulting finally in larger particles [28, 29].

It was shown that using mixture of T80 and Cr in equal ratios has resulted in NLC formulae with best particle size and particle size distribution. Formulae prepared using this mixture were shown to have span value less than 1 and PI one less than 0.1, *i.e*. they were forming a monodispersed population.

Table 2. Particles size and particle size distribution of NLC formulations.

Formula	d_{mean} (nm)	Span	PI
GO1 F1	189.7 ± 15.7	4.702359	2.4569
GO1 F2	270.7 ± 16.6	6.173967	4.2353
GO1 F3	193.0 ± 2.6	0.870216	0.0841
GO1 F4	224.7 ± 15.3	3.764179	1.5743
GO1 F5	1778.7 ± 125.8	1.996102	0.4427
GO1 F6	1373.7 ± 27.58	3.599287	1.4394
GO1 F7	169.0 ± 1.0	0.773256	0.0667
GO1 F8	189.7 ± 4.2	0.853242	0.0809
GO1 F9	539.3 ± 13.25	2.139094	0.5084
GO1 F10	1176 ± 331.3	1.493992	0.2480
GO3 F1	174.7 ± 0.6	0.840226	0.0784
GO3 F2	353.3 ± 38.5	8.166004	7.4093
GO3 F3	197. 7 ± 8.3	0.863857	0.0829
GO3 F4	227.0 ± 2.6	5.669255	3.5712
GO3 F5	1874.3 ± 52.8	3.098829	1.0670
GO3 F6	2971.3 ± 89.6	2.146696	0.5120
GO3 F7	190.3 ± 3.8	0.877759	0.0856
GO3 F8	212.3 ± 0.6	0.913433	0.0927
GO3 F9	568.3 ± 29.2	2.542578	0.7183
GO3 F10	1138.7 ± 24.6	2.253889	0.5644

The use of surfactants in the concentration of 6% W/W has resulted in lower particle size of the prepared formulae in comparison to the ones prepared using 3% W/W. This could be explained on the basis of fact stating that adsorption dynamics of surface active agent to the newly formed surfaces of lipid are concentration-dependant [28].

Concerning occlusion of the particles, Fig. (**2**) illustrated that formulae prepared using T80/Cr mixture in equal weights had the highest occlusion factors reflecting the power of this mixture to produce smallest particle sizes amongst sole surfactants and/or other surface active agents. Formulae prepared using such a surfactant blend in total concentration of 6% W/W showed occlusion factor higher than 65% after 48h.

This might be attributed to the fact that occlusion of particles is negatively correlated to their particles size, *i.e*. adhesive forces between a flat surface and particles increase as the mean sizes of the particles decrease [30].

Another finding was that the occlusion factor of formulae prepared using lipid matrix richer in oleic acid showed slightly higher occlusion factor, provided that they had comparable particle sizes. This could also be justified on the basis that lipids having lower melting point and crystallinity, in

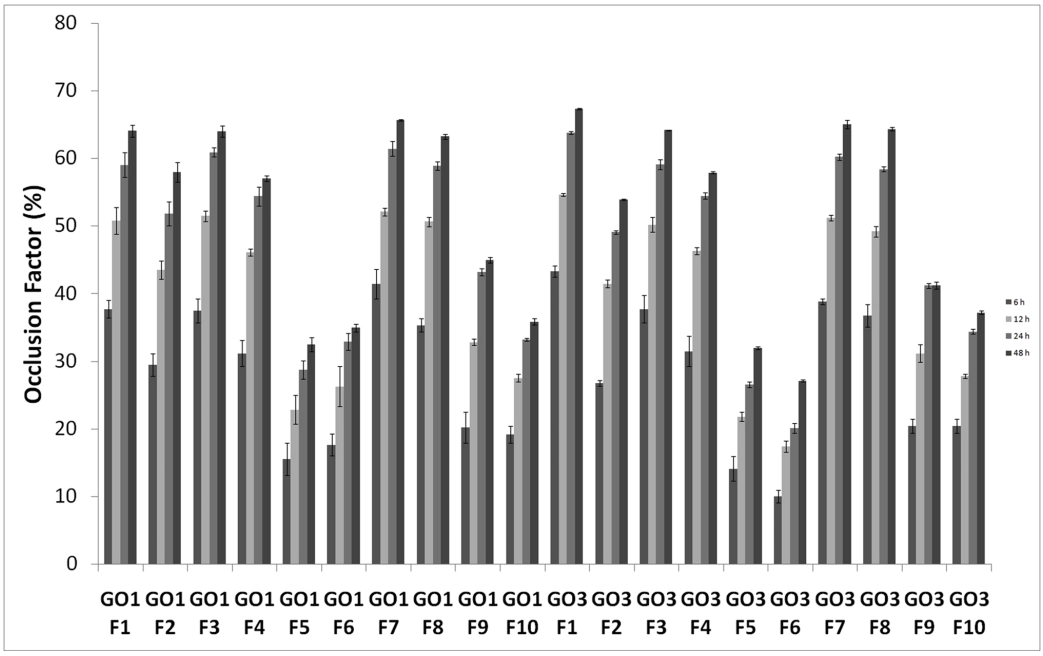

Fig. (2). Occlusion factor of formulae.

this study the higher-OL-proportion lipid, were reported to have higher occlusion factors [30].

3.4. Optimization of the Formulae

The use of mixture of T80 and Cr was shown to result in NLC formulae having a good particles size and uniform distribution. Moreover, formulae prepared using such a blend showed occlusion factor of more than 65% after 48 h which is considered to be satisfactory for topically applied formulae. However, the blend was composed of two hydrophilic surface active agents. This blend was suggested to be improved by incorporating a hydrophobic surfactant. The hydrophobic agent would be embedded mostly in the lipid part in the contrary to the hydrophilic one. This was suggested to increase the emulsification capability of the surfactants blend leading to smaller particle size [31]. The commonly used lipophilic surface active agents include span 20 and lecithin. In this study span 20, lecithin and equal mixture of them were utilized. The compositions of the optimized formulae are tabulated in Table **3**.

It was shown, as listed in Table **4**, that using lecithin as sole lipophilic surface active agent together with the blend of T80 and Cr has resulted in formulae of 180 nm. This particle size has moved down bit a little upon using span instead of lecithin. The use of equal mixture of the two lipophilic surface active agents, in addition to the blend of the two hydrophilic ones, has achieved the best possible result. Formulae prepared by the quadrant blend have shown particles size in the range of 160 - 175 nm. This is suggested to be the result of using different molecules of different geometry and molecular weight leading to close packing of the different surfactant molecules on the surface of the hot lipid droplets, thus, forming a perfect film of the surfactant at the aqueous/lipoidal interface and hence stabilizing the formed emulsion resulting ultimately in small particle sizes of the final formula [28, 31].

Occlusion factors of the formulae were also satisfactory. They all passed the boundary of 65% after 48 h. Not surprisingly, formulae prepared using the quadrant mixture Formulae GO1 LS and GO3 LS were chosen for loading betamethasone dipropionate because they showed the best particle size and size distribution. Fig. (**3**). Showed that both GO1 LS and GO3 LS were monodisperse of surface active agents, being the smallest in size, showed the highest occlusion factor at all times. This was clearly illustrated by Fig. (**4**).

3.5. Drug Solubility in Lipid Matrices

The solubility of the drug in the lipid matrix is an important parameter to consider. This parameter affects many actions of the SLN or NLC. For

Table 3. Composition of the NLC formulations for optimization (25g).

Formula	GMS (mg)	OLA (mg)	T80 (mg)	Cr (mg)	Span 20 (mg)	Phospholipon NG90 (mg)
GO3 L	525	225	625	625	0%	250
GO1 L	675	75	625	625	0%	250
GO3 S	525	225	625	625	250	0 %
GO1 S	675	75	625	625	250	0 %
GO3 LS	525	225	625	625	125	125
GO1 LS	675	75	625	625	125	125

Table 4. Particles size and particle size distribution of optimized NLC formulations.

Formula	d_{mean} (nm)	Span	PI
GO1 L	180.7 ± 6.0	0.84296	0.07895
GO3 L	186.0 ± 2.7	0.79965	0.07105
GO1 S	187.0 ± 6. 6	0.77931	0.06748
GO3 S	170.0 ± 1.4	0.7936	0.06998
GO1 LS	175.7 ± 27.1	0.63495	0.04480
GO3 LS	159.3 ± 0.6	0.68660	0.05238

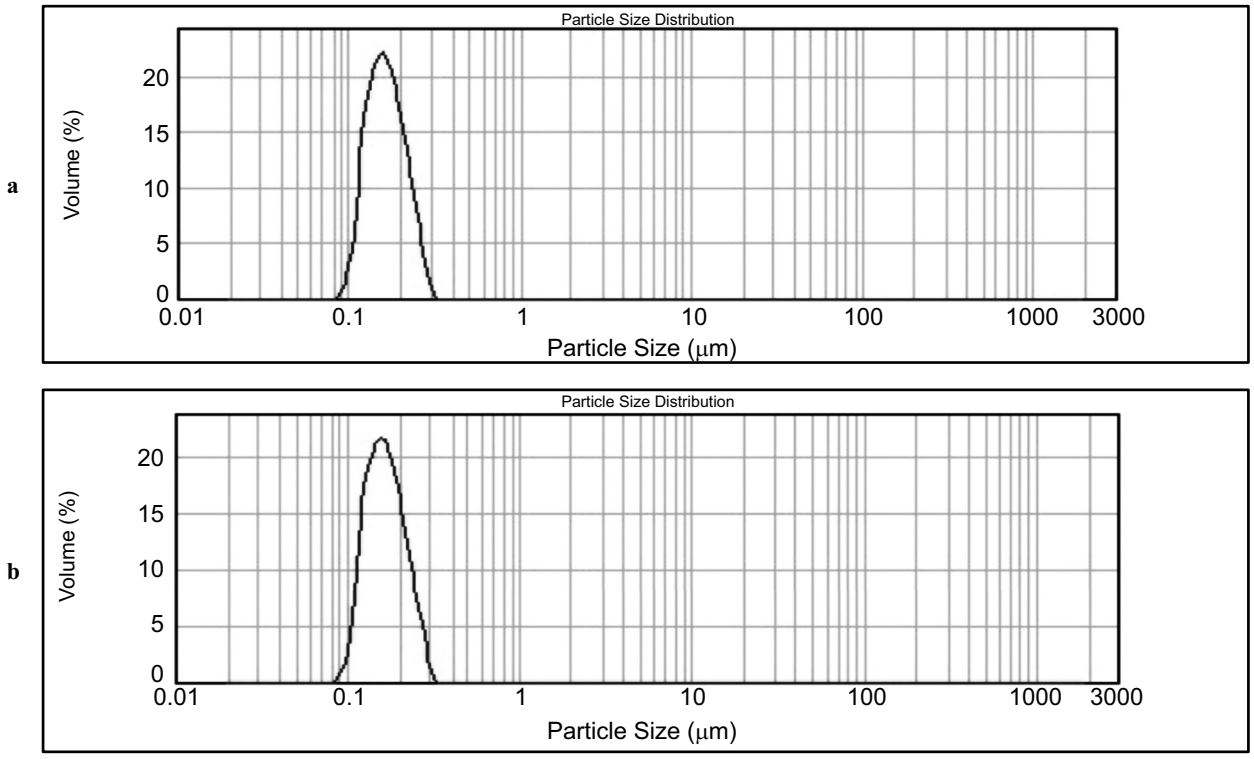

Fig. (3). Particle size distribution curves of formulae **a**) GO3 LS and **b**) GO1 LS.

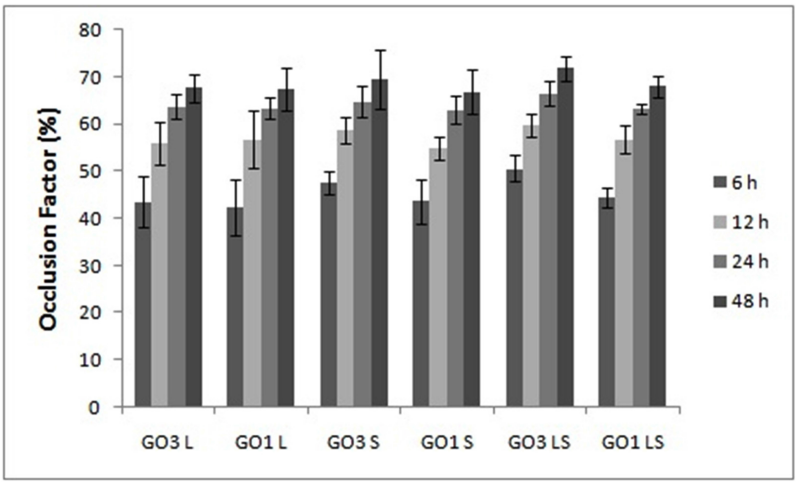

Fig. (4). Occlusion factors of optimized formulae.

example, solubility of drug in the lipid matrix was reported to affect entrapment efficiency (also called encapsulation efficiency) and percent drug loading of this particular drug in the finally prepared NLC [34].

Drug exhibited different solubility in matrices prepared using 10% W/W and 30% W/W oleic acid. Increasing oleic acid percentage in the lipid matrix has lead to an increased solubility of betamethasone dipropionate in the matrix. Solubility of drug in 30% oleic acid lipid matrix was shown to be 3.61 ± 0.05%, while the solubility of drug in the 10% oleic acid lipid matrix was shown to be of lower value, which is 2.69 ± 0.02%. this suggested that increasing the amount of liquid lipid, oleic acid, in the lipid matrix has resulted in an increase in the drug solubility. This might be suggested to take place due to imperfections made in the lattice of the lipid upon addition of liquid to the solid one. These imperfections created gaps that aided inclusion of the drug molecules easily in the lipid [12, 32].

3.6. Physicochemical Characteristics of Betamethasone Dipropionate-loaded NLC

Particle size, size distribution, zeta potential and occlusion factors at different time intervals (6 h, 12 h, 24 h, and 48 h) are listed in Table **5**. It was shown that loading the lipids with the drug has resulted in a significant increase in the average size of the nanoparticles. This was clearly illustrated by comparing particle sizes of NLC prepared using lipid matrices loaded and/or unloaded with the drug. This agreed to results reported by many researchers [32-34]. Parameters measuring particle size distribution, Span and PI, were also increased in value. However, the increased Span and PI value did not exceed the limit of monodispersion recordings. This means that NLC populations were also monodisperse despite showing broader distribution as compared to drug-free formulations. Zeta potential values were measured to be exceeding 20 mV. This indicates high stability of the prepared formulations [35]. Occlusion factors of the betamethasone dipropionate-loaded formulations were decreased as compared to that of the drug-free formulae. This was suggested to be attributed to the increase in particles size rather than the direct effect of betamethasone dipropionate on the occlusion of particles [30].

3.7. Entrapment Efficiency and Percent Drug Loading of Nanoparticles

Both formulae were shown to have entrapment efficiency exceeding 83% which is regarded to be very high percent. Entrapment efficiencies of formulae BL GO3 and BL GO1 were measured to be 89.98 ± 3.48% and 83.83 ± 3.98%, respectively. Formulae BL GO3 and BL GO1 showed percent drug loading of 3.25 ± 0.1255% and 2.25 ± 0.1097%, respectively.

It is clearly obvious that both drug loading percentage and entrapment efficiency values increased as the percentage of the liquid lipid in the lipid matrix increased. This could be attributed to the increased solubility of the drug in lipid matrix

Table 5. Particle size, span, PI, and occlusion factor of betamethasone dipropionate-loaded NLC formulations.

Formula	d_{mean} (nm)	Span	PI	Zeta Potential	Occlusion Factor			
					6 h	12 h	24 h	48 h
BL GO3	208. 7 ± 3.8	0.8387	0.0782	25.4 ± 8.1	35. 7 ± 1.4	47.6 ± 2.2	56. 7 ± 2.2	62.4 ± 0.5
BL GO1	200.0 ± 6.1	0.8549	0.0812	25.3 ± 6.0	38.8 ± 1.7	49.1 ± 7.8	58.1 ± 3.4	63.3 ± 0.6

as the lipid matrix contains higher proportion of liquid lipid [12, 33].

3.8. Differential Scanning Calorimetry (DSC)

Structural changes of materials are accompanied by heat exchanges, *e.g*., uptake of heat during melting or emission of heat during crystallization. DSC is designed to measure these heat exchanges during controlled temperature programs and allows drawing conclusions on the structural properties of a sample [34].

Figs. (**5** and **6**) illustrate the thermograms of pure drug, pure lipid matrix, physical mixture of lipid matrix and drug, drug-free NLC and drug-loaded NLC prepared using lipid matrices containing 30% W/W and 10% W/W oleic acid, respectively. It was clearly shown by these thermograms that 1) nanoparticles started to melt at lower melting point as compared to the bulk solid. This could be attributed to the increased surface area of NLC in comparison to bulk solid. 2) The sharp peak of pure betamethasone dipropionate, shown to be at 178°C in section (a) of the figures, has disappeared in the thermogram of drug-loaded NLC. This peak corresponds to the process of melting of betamethasone dipropionate. The disappearance of this peak demonstrates clearly that drug was completely soluble in the lipid matrix of the NLC formulations [33].

3.9. Transmission Electron Microscopy (TEM)

Transmission electron micrographs of NLC belonging to formulae BL GO3 and BL GO1 are shown in Figs. (**7** and **8**), respectively. It was illustrated by these micrographs that particles showed round, homogenous shading indicating spherical and homogenous particles.

The particle size determined by TEM was shown to be less than that determined by dynamic laser light scattering technique. This is suggested to happen because the latter technique measures the hydrodynamic diameter of particles rather than the actual one. Hydrodynamic diameter is the diameter of the particles in addition to the liquid layer formed around it as it moves [35].

3.10. *In vitro* Release Profile

The study of betamethasone dipropionate release profiles from different nanoparticles, negative controls and reference standard was performed. Two negative controls were utilized in this study; the first was the drug suspension in distilled water; while the second was drug suspended in a lipid-free mixture of all other aqueous phase components.

Release study showed that drug suspension in water exhibited the least release. Moreover, the drug suspension in water was also incomplete. This might be attributed to the low drug solubility in water [21]. Drug suspension in surfactants was shown to be fast in the beginning following plateau. The drug-surfactants suspension release was also incomplete but more than that of the drug suspension in water. This emphasized that the main reason standing behind the incomplete drug release form suspension was the drug solubility [21]. The maximum value of release profile of Diprosone® cream was shown to be 50% approximately, after 24 hours.

Release of betamethasone dipropionate from NLC was shown to be more effective than negative controls and the reference standard. It was higher both in magnitude and rate as shown by Fig. (**9**). This suggested that the solubility of drug could be considered as an important factor governing its release from the formula. Thus, the release of drug from lipid-free formula was higher than the drug suspension in distilled water. Also,

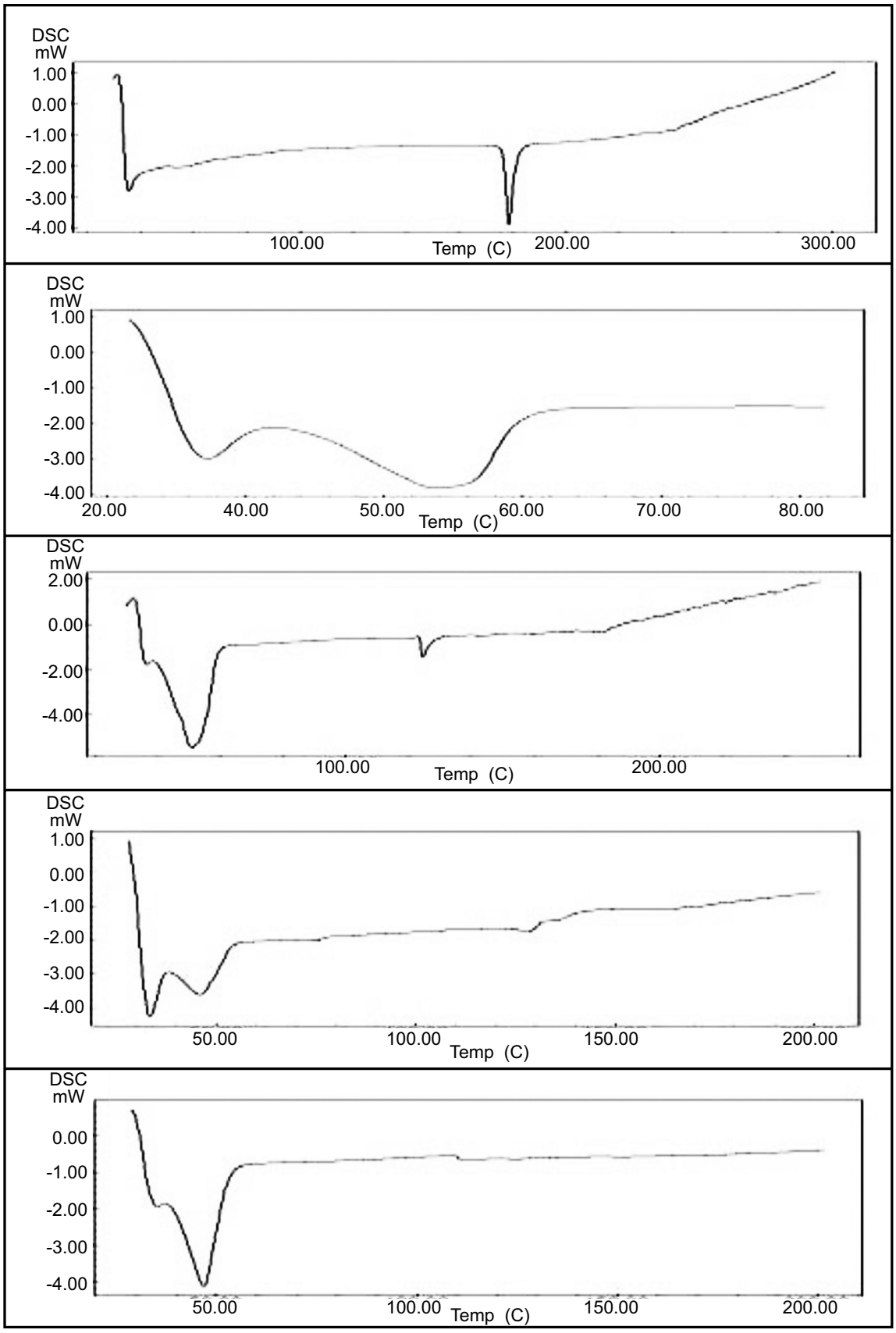

Fig. (5). DSC diagrams of **a**) Pure betamethasone dipropionate **b**) Pure lipid matrix containing 30% W/W of oleic acid **c**) Physical mixture of bulk lipid matrix and drug **d**) NLC of formula GO3 LS and **e**) NLC of formula BL GO3.

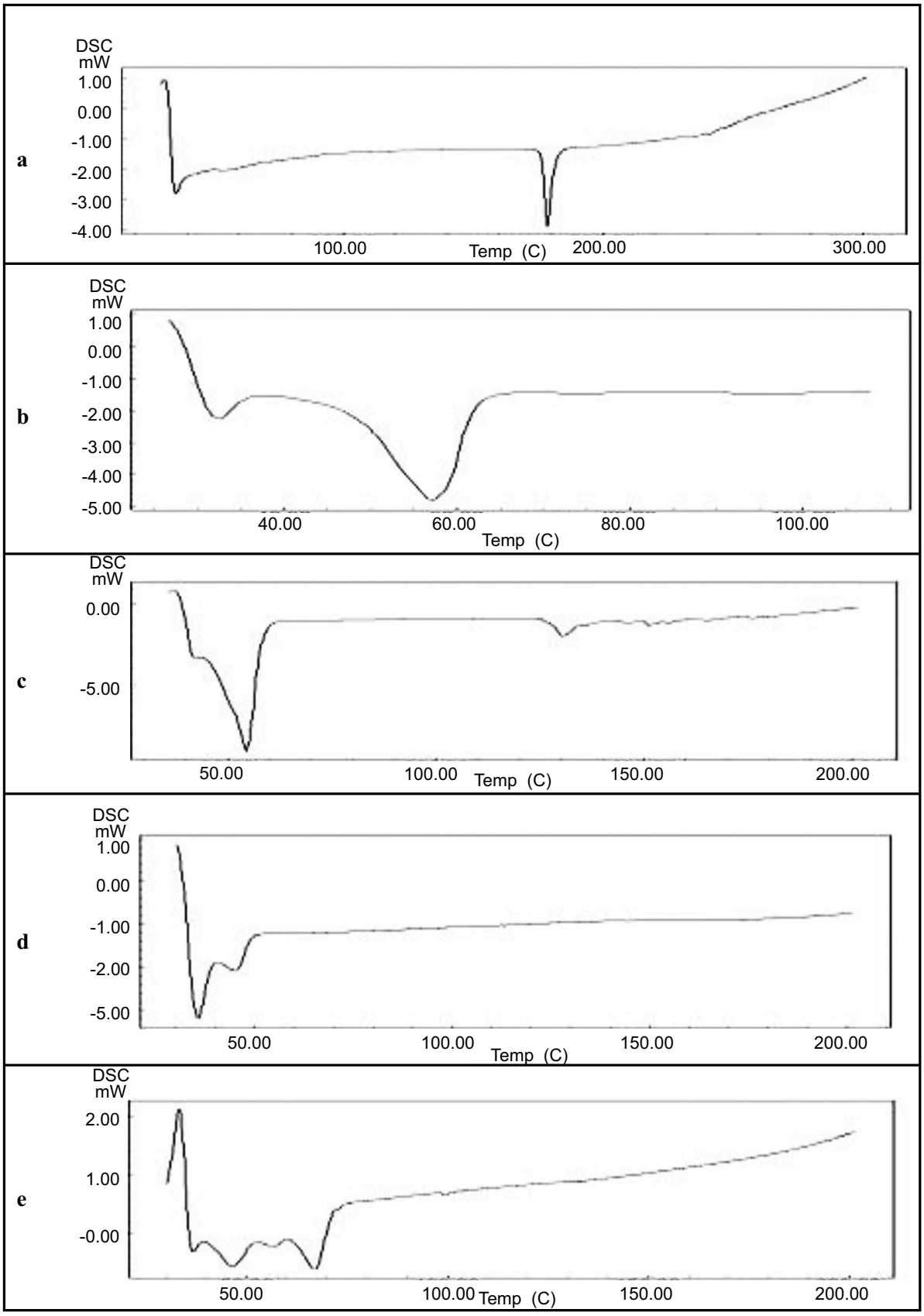

Fig. (6). DSC diagrams of **a**) Pure betamethasone dipropionate **b**) Pure lipid matrix containing 10% W/W of oleic acid **c**) Physical mixture of bulk lipid matrix and drug **d**) NLC of formula GO1 LS and **e**) NLC of formula BL GO1.

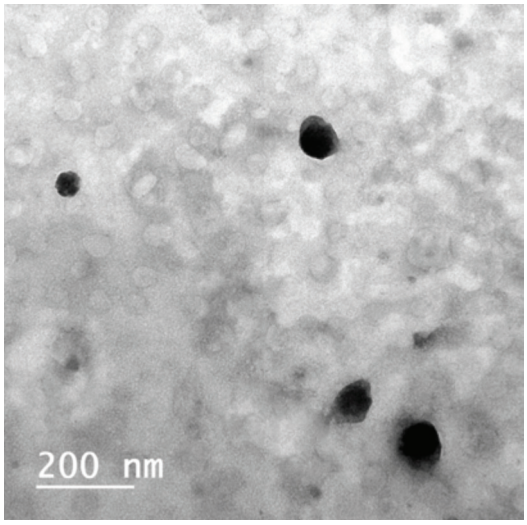

Fig. (7). Transmission Electron Micrograph (TEM) of BL GO3 Nanoparticles.

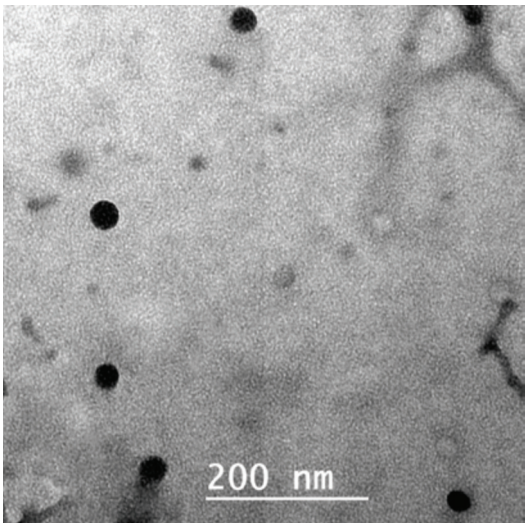

Fig. (8). Transmission Electron Micrograph (TEM) of BL GO1 Nanoparticles.

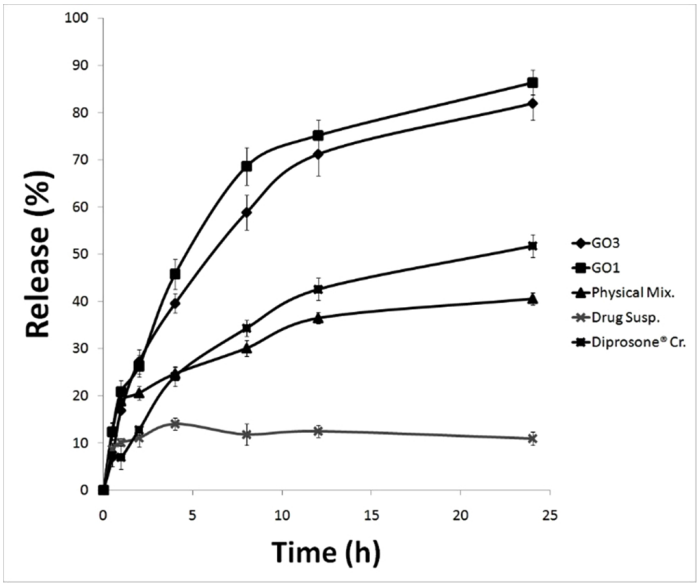

Fig. (9). Releases profile of negative controls, Diprosone® cream and betamethasone dipropionate-loaded NLC formulations.

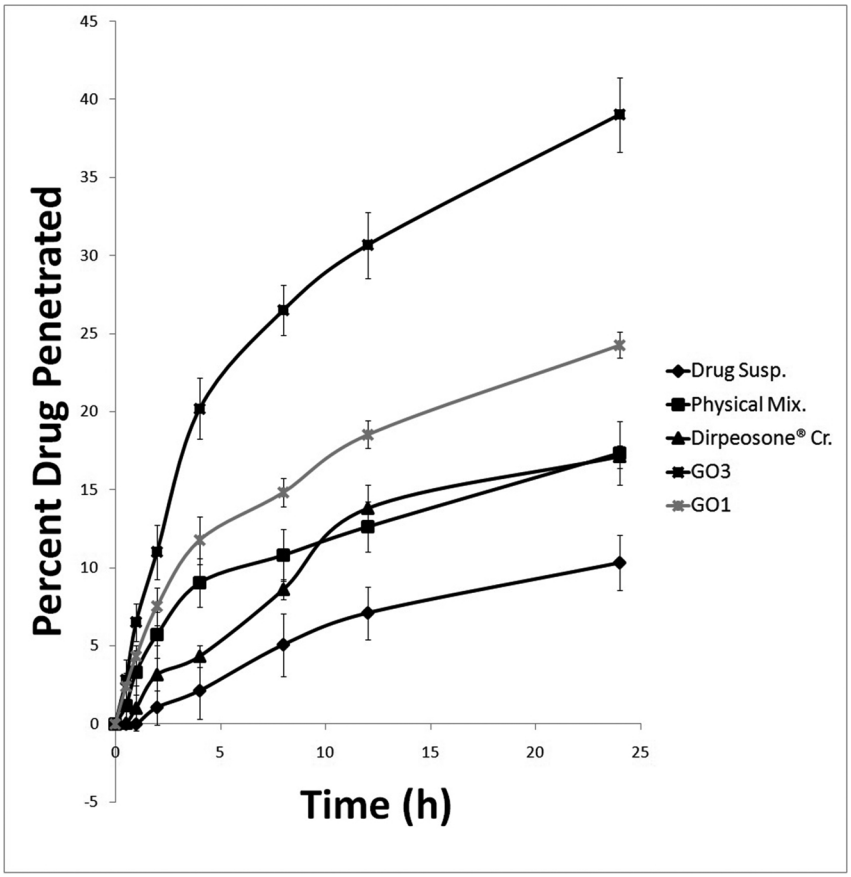

Fig. (10). *Ex vivo* skin penetration profile of negative controls, diprosone® cream and betamethasone dipropionate-loaded NLC formulations.

release of betamethasone dipropionate form both NLC formulae was higher than the cream. This might be attributed to the higher surface area of drug carrier in case of NLC as compared to the traditional formula, as well as, the higher drug solubility in case of NLC formulae due to incorporation of the drug in nanoparticles formula [12, 33].

3.11. *Ex vivo* Skin Penetration of Betamethasone Dipropionate

Since, as stated earlier, skin has been evidenced to be the largest organ in the body, the delivery of drugs to/through skin has become an important issue that has many advantages over the conventional routes of administration.

The *ex vivo* skin penetration study of betamethasone dipropionate from two NLC formulations, negative controls and reference standard was performed. The liquid lipid utilized in preparing the two NLC formulations, oleic acid, has been extensively identified as penetration enhancer [36]. The percent concentrations of liquid lipid in NLC formulae were chosen to be 10% and 30% W/W in order to investigate the effect of incorporating the liquid penetration enhancer in the carrier on skin penetration of the drug.

Fig. (**10**) showed that addition of surfactants in the formula has resulted in increasing the amount of betamethasone dipropionate penetrating the skin. This was clearly illustrated by comparing amounts of drug that penetrated the skin from drug suspension and lipid-free formula. However, addition of surfactants was not the only factor that aided the penetration of drug into skin. It was shown that amount of drug that penetrated skin from NLC formulation containing 10 % W/W oleic acid was significantly higher than that of drug suspension, lipid-free formula and/or reference standard formula in comparison. Another interesting finding was that the amount of betamethasone dipropionate that penetrated skin from NLC containing 30% W/W oleic acid was way higher than that of formula containing 10% W/W of oleic acid. These findings suggested that formulation of betame-

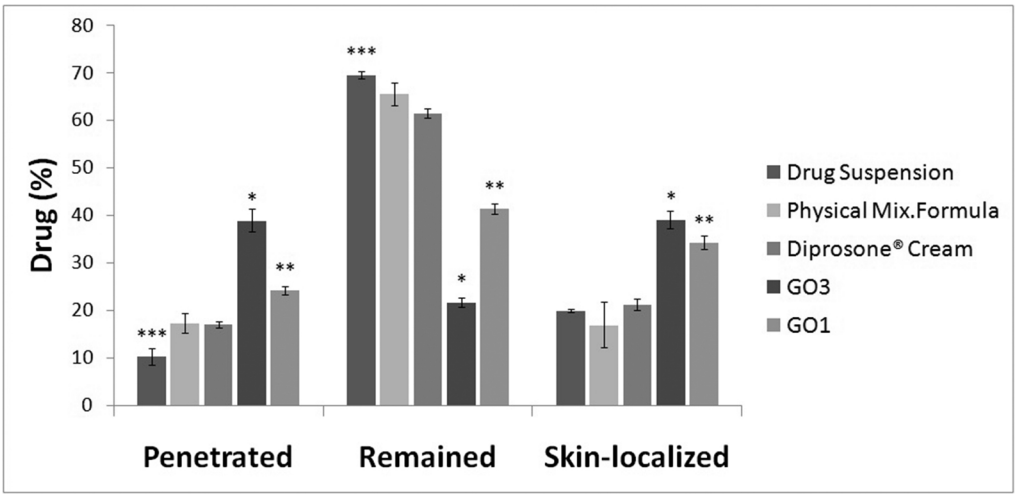

Fig. (11). Percent of drug penetrated through skin, remained at skin surface and skin-localized of all prepared formulae, drug suspension, lipid-free formula and diprosone® cream.

thasone dipropionate in NLC formulae has resulted in increased skin penetration. In addition, incorporation of OLA, this acted as chemical penetration enhancer, has aided deeper and more effective penetration of the drug. This was illustrated by comparing the amount of drug that penetrated skin from formulae containing higher and lower percentage of OLA. Comparing amount of drug that penetrated skin from NLC containing 30% W/V and 10% W/V OLA has shown that the former was way higher. This suggested the efficacy of OLA to aid the penetration of betamethasone dipropionate when formulated as NLC. This was also shown in previous studies [36].

It was illustrated by Fig. (**11**) that amount of drug localized in skin was 39.22% and 34.30% for NLC formulae prepared using lipid matrices containing 30% and 10% W/V OLA, respectively. This showed that increasing the percent of OLA in the lipid matrix has resulted in increasing the capability of the dosage form to aid drug penetration to/through skin by aiding the penetration of drug through stratum corneum.

This suggested that using the drug nanocarrier that contained higher amount of the penetration enhancer, 30% OLA, didn't affect much the amount of drug localized in skin as compared to that containing 10% W/V OLA. Instead, it affected enormously the amount of drug that passed through skin. This suggested that NLC containing 30% W/V OLA could be used for transdermal absorption of the drug, while, formula containing only 10% W/V OLA in the lipid matrix could be recommended for localizing higher amount of betamethasone dipropionate in the skin layers as compared to traditional cream.

CONCLUSION

Preparation of betamethasone dipropionate-loaded NLC containing the penetration enhancer oleic acid as liquid lipid was done. The technique used was through hot homogenization followed by sonication. The factor that had an important role in the determination of physicochemical properties was the surface active agent choice. Nanoparticles with best physicochemical properties were prepared using tween 80, cremophor RH 40, span 20 and Phospholipon 90 G ® as surfactants blend. Sodium tauroglycocholate failed to produce nanoparticles of the used lipid blend. Differential scanning calorimetry revealed that drug was soluble in the lipid matrix. TEM illustrated that particles were homogenous and spherical. Drug loading, entrapment efficiency, release pattern of the prepared NLC were adequate. The use of OLA has proven to increase the penetration of drug to deeper skin layers. As the proportion of OLA in the lipid matrix increased, the amount of betamethasone dipropionate delivered to deeper skin layers were also increase. Thus, the delivered amount of betamethasone dipropionate to deeper skin layers could be manipulated through controlling proportion of OLA in the lipid matrix.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

All the animal-related procedures were approved by the ethical committee of Suez Canal University, Egypt. (Approval No. 20156A1).

HUMAN AND ANIMAL RIGHTS

No humans were used in this study. All the reported experiments on animals in the study were in accordance with the Egyptian laboratory animals care and use guidelines.

CONSENT FOR PUBLICATION

Not applicable.

CONFLICT OF INTEREST

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