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Research Article

Acyclovir Loaded Solid Lipid Nanoparticle Based Cream: A Novel Drug Delivery System

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ABSTRACT

Objective of the present investigation was enthused by the possibility to develop solid lipid nanoparticles (SLNs) of hydrophilic drug acyclovir. Also study vitro and vivo drug delivery. Methods: Drug loaded SLNs (ACV-SLNs) were prepared by high pressure homogenization of aqueous surfactant solutions containing the drug-loaded lipids in the melted or in the solid state with formula optimization study (Different lipid concentration, drug loaded, homogenization / stirring speed and compritol 888ATO: drug ratio). ACV - SLN incorporated in cream base. The pH was evaluated and rheological study. Drug release was evaluated and compared with simple cream- drug, ACV – SLN with compritol 888ATO and marketed cream. The potential of SLN as the carrier for dermal delivery was studied. Results: Particle size analysis of SLNs prove small, smooth, spherical shape particle ranged from 150 to 200 nm for unloaded and from 330 to 444 nm for ACV loaded particles. The EE% for optimal formula is 72% with suitable pH for skin application. Rheological behavior is shear thinning and thixotropic. Release study proved controlled drug release for SLNs especially in formula containing compritol88 ATO. Stability study emphasized an insignificant change in SLNs properties over 6 month. In-vivo study showed significantly higher accumulation of ACV in stratum corneum, dermal layer, and receptor compartment compared with blank skin. Conclusion: AVC-loaded SLNs might be beneficial in controlling drug release, stable and improving dermal delivery of antiviral agent(s).

Keywords: Solid lipid nanoparticles (SLNs), Acyclovir, Release study, vivo trails.

INTRODUCTION

Herpes simplex virus (HSV) infection has been a global health problem affecting around 21 million people every year. According to a recent survey in US population, approximately 50% to 80% of adults suffer from oral herpes infection while at least one in every five is infected with genital herpes[1]. HSV infection primarily affects the dermal or basal epidermal layer of the skin, mucous membranes, and occasionally esophagus and brain[2]. Among the various antiviral drugs used for the alleviation of HSV infection, acyclovir (ACV) is the most commonly prescribed treatment[3]. Nevertheless, all currently available ACV-based oral formulations suffer from disadvantages like poor bioavailability, multiple dosing regimens, systemic toxicity, and certain adverse drug reactions. To improve the efficacy of treatment, valacyclovir (prodrug of ACV), and famciclovir (prodrug of penciclovir) have also been tried but together with ACV, their effectiveness is constrained by tedious dosing regimens and limited accumulation at the site of infections (basal epidermis and dermis (E+D))[4,5]. Compared with oral administration, topical administration of ACV leads to tenfold higher concentration over the entire epidermis[6]. However, this concentration fails to produce desired therapeutic effect at the site of infection[7]. Although stratum corneum (SC) barrier can be overcome by chemical (penetration enhancers) and physical techniques (iontophoresis, electrophoresis, and sonophoresis)[8–12], yet these practices are inducing either irritation or damage, resulting in the disruption of skin barrier function. Hence, it is crucial to enhance the penetration of ACV (up to dermis), while maintaining the normal skin barrier function[13]. Solid Lipid Nanoparticles (SLNs) are solid, submicron sized particles carriers comprised of biodegradable lipids that are compatible with inflamed and damaged skin[14–16]. By virtue of their unique capability to load lipophilic as well as hydrophilic agents, SLNs stands attractive for use in dermal/topical delivery of various cosmetics and pharmaceutical agents[14,15] with enhanced penetration through skin[17,18]. However, their utility in achieving an efficient dermal delivery of antiviral agents has been seldom explored. The present study, therefore, examines the feasibility of using SLNs as a potential carrier for enhanced dermal/topical delivery of ACV. The present investigation was enthused by the possibility to develop solid lipid nanoparticles (SLNs) of hydrophilic drug acyclovir (ACV) and evaluate their potential as the carrier for dermal deliver.

MATERIALS AND METHODS

Materials

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ACV (> 99% pure by high-performance liquid chromatography (HPLC)), Compritol 888ATO (glyceryl behenate) were obtained as generous gift from Glaxo smith Kline Pharmaceutical Ltd, Egypt GSK Pharma, Precifac ATO (cetyl palmitate), stearyl alcohol, cetyl alcohol, isopropyl myristate, stearic acid, beeswax, spermaceti, Tween20, Tween80, Span60, and sorbitan mono oleate were obtained as a gift from Luna Company(Egypt). Diterol AL40 (Potassium olive oil hydrolyzed wheat protein and myristyl lactate) was obtained as gift from Nerol manufacturing and trading company (Egypt), and all other chemicals were analytical reagent grade.

Methods

Preparation of solid lipid nanoparticles

The lipid phase was melted and the drug was dispersed in the lipid melt to obtain a clear solution. The dispersion medium (i.e., distilled water with surfactants) was heated to the temperature of the lipid melt. The hot lipid phase was dispersed in a surfactant solution by high-pressure homogenization using IKA HPH (2000/4 SH5, Germany) at 26000 rpm for 5 mints followed by high-speed stirring using Ultra-Turrax T25 (IKA-Werke, Staufen, Germany) at 500 rpm for 10 mints then the cycle repeated twice. The dispersion was then subjected to. The obtained Nano dispersion was allowed to cool down to room temperature, forming lipid nanoparticles by recrystallization of the dispersed lipid[19]. Different lipids, including beeswax, spermaceti, Comperitol888, cetyl alcohol, stearyl alcohol, stearic acid, and cetyl palmitate, were tested. We used Tween 80, Tween 20, and Diterol AL40PF as our surfactants.

Preparation of oil/water cream

Both the lipid and aqueous phases of the cream were heated separately to 75°C. Hot water was then added to the lipid phase with stirring. The resulting emulsion cooled down to 40°C and the drug (Acyclovir) was added. Under constant stirring, the o/w cream cooled down to ambient temperature. For the SLNs containing cream, 50% of the SLNs dispersion containing ACV (with a double concentration of ACV) was mixed with half of the o/w cream. Table 1 shows the ingredients of the cream base.

Optimization of Process Variables

Lipid (type and amount)

SLN formulation trails included many lipids for example stearic acid and cetyl palmitate using tween 80 with the former and Diterol AL40PF with the later as surfactants. Choosing lipid type depends on particle size analysis, polydispersiability PDI and zeta potential measurement.

Drug loading

Three different ACV concentrations were used for optimization of drug loading 20, 60 and 100 mg of the total SLNs dispersion weight. SLNs evaluated according to particle size analysis, PDI, zeta potential measurement and entrapment efficiency and triple replicate.

Compritol 888 ATO

Recently, there has been great interest in the multiple roles those compritol 888 ATO plays in various pharmaceutical delivery systems. Different ratios of compritol 888 ATO versus ACV in 0.25:1, 0.5:1, 1:1 and 2:1 were studied for drug release. An automated temperature –controlled continuous flow through diffusion cells (six cells) was used to evaluate the amount of drug released from the development formulations.

Homogenization speed and stirring time

Homogenization at different speed as at 15000, 20000, 23000 and 26000 rpm for 5 mints each, also stirring at different time as 5, 10 and 15 mints were used as formula optimization variables. Assessment for optimal speed and time depends on particle size analysis, PDI, zeta potential measurement.

Thermal Analysis: Differential Scanning Calorimetric (DSC)

Pharmaceutical grade acyclovir, stearic acid and physical mixture powders were used for thermal analysis. The DSC curves were obtained using Shimadzu differential scanning calorimeter (DSC-50, Kyoto, Japan) over a temperature range of 10-300 ^{o}C with heating rate of 10^{o}C/min in an alumina crucible, under nitrogen atmosphere. An empty aluminum pan was used as reference standard.

pH measurement

The pH values of the prepared formulations were measured using a pre-standardized digital pH meter (Jenway 3510, Staffordshire, UK) at25 ^{o}C. One gram of each formulation was dispersed in 10 ml distilled water and the electrode was dipped into the dispersion until constant reading was obtained. The pH measurements were average of three replicates[20,21].

Rheological measurements

Rheology of both ACV-SLNs based cream and ACV simple cream was performed with a cone and plate rheometer (Anton Paar®GmbH, Ostfildern, Germany). Up and down portions of the flow curves were determined using parallel plate geometry (50 mm diameter), where, the gap between the two plates was 1 mm. About 0.5 g of the tested formulation was applied to the plate and left until the temperature of the plate reached 25 ± 1^{o}C. The measurements were made over range from 10, 15, 20, 25, 50, 75 up to 200 rpm with 20 s between each two successive the whole range of speeding setting speeds. The rheological behavior of each formulation was evaluated by plotting the shear stress versus the obtained shear rate values. The flow behavior was studied according to Farrow's equation[22] Log D = N Log S - Log ŋ Where, D is the shear rate (sec $^{-1}$), S is the shear stress (Pa), N is Farrow's constant and ŋ is the viscosity (Pa.s). N is the slope of the plot of log D against log S, which indicates the deviation from Newtonian flow. When N is less than one, it indicates dilatant flow (shear rate thickening). If N is greater than one, it indicates pseudo plastic or plastic flow (shear rate thinning). When the system showed thixotropic behavior, the hysteresis area (H.A.) between the upward and a downward curve was measured adopting the trapezoidal rule.

Particle Size Analysis

Optical microscopy

Slight volumes of the SLNs dispersion made were spread on a glass slide and examined for the particle structure for both unloaded and loaded vesicles using light microscopy (Olympus, Philippines). Photomicrographs were taken at a

magnification of CK X41 using digital camera fitted with microscope (Nikon cool-pix S220, Japan).

Size analysis and Zeta potential

The particle size and polydispersity index of SLNs was determined by using Zeta sizer by dynamic light scattering (Nano ZS, Malvern, Worcester-shire, UK). Six replicates were measured and values were measured as mean± standard deviation (SD).

Transmission electron microscopy

The morphological examination of ACV-loaded and unloaded SLNs was performed using transmission electron microscopy (TEM) HU-12A (Hitachi Ltd, Mito, Japan) at the Faculty of Agriculture Research Park. About 1 ml of SLNs formulations was diluted with 5 ml distilled water. One drop of nanoparticle dispersion (aqueous solution of SLNs) was placed on the copper grid and then negatively stained with one drop of 2% (weight/volume) aqueous solution of phosphotungstrate acid for contrast enhancement. The samples were allowed to dry before examination under the TEM.

Determination of entrapment efficiency

The entrapment efficiency of prepared SLNs was analyzed by indirect method. The prepared SLNs formulations was centrifuged at 25,000 rpm for 20 min in a refrigerated centrifuge (Sigma 3K30, rotor no. 12150, Sigma, UK) at a temperature below 10°C, obtained supernatants were appropriately diluted and ACV was analyzed spectrophotometrically (UV-1800Shimadzu, Kyoto, Japan) at λ_{max} 264 nm against blank that was prepared for slandered calibration curve. The entrapment efficiency was calculated as follows: EE (% w/w) = $[(W_a - W_s) / W+_a]$. 100 Where, W_a stands for the mass of ACV added to the formulation and W_s is the mass of ACV determined in the supernatant.

In Vitro Release Study

An automated, temperature-controlled continuous flow through Franz diffusion cells (six cells) with a diffusional area of 1.76 cm^2 was used to evaluate the amount of drug released from the developed formulations. The diffusion cells were thermoregulated with a water jacket at37□C. Cellulose nitrate membrane filters were soaked with phosphate buffer of pH 7.4 and mounted to diffusion cells. The receptor phase contained 7.1 mL of phosphate buffer and passed through the receptor chamber at a controlled rate using a multichannel peristaltic pump.400 mg of each sample of (SLNs- ACV loaded based cream and ACV simple cream) was applied to the donor compartment using a calibrated pipette, and the cells were covered to prevent evaporation of vehicle and ensure integrity of the formulations throughout the respective study period. Accurate samples (2 mL) were withdrawn at time intervals 0.25, 0.5, 1, 2, 3, 4, 6, 8, 10 and 12 hr. and analyzed by spectrophotometer. The previous method was repeated for up to 24 hr. release for both ACV- Compritol 888 ATO SLNs based cream with drug compritol ratio 1: 0.25 and marketed ACV cream.

In Vivo Study

Preparation of full thickness skin

Sprague–Dawley rats (8–10 weeks old and weighing 250–300 g) were obtained from the Central Animal Facility. Methodology was approved by faculty Ethics Committee (REC-FPSPI-6/39). Hairs from the dorsal surface were removed using an animal hair clipper (Sterling 2, Wahl, UK) following which full thickness skin was harvested. The subcutaneous tissue was removed surgically, and the dermis side was wiped with isopropyl alcohol to remove adhered fat. The skin was washed with phosphate-buffered saline (pH 7.4). All measurements were carried out in a single ventilated room (temperature, ~28–30°C; relative humidity, ~38–40%).

Permeation experiments

Full thickness skin was mounted between the two compartments of Franz diffusion cells with the stratum corneum side up and with effective area of (0.64 cm2). The receptor compartment (capacity 7.5 mL) was then filled with phosphate-buffered saline (pH 7.4) and maintained at 37±1°C with a constant stirring speed at 800 rpm using a magnetic stirrer. The dermal side of the skin which was placed in contact with the receptor compartment fluid was equilibrated for 30 min[26]. Then ACV-SLNs, cream, and ACV simple cream, all in a dose equivalent to 20 mg ACV were evenly spread on SC side of the skin in donor compartment and covered with Para film (American Can, USA). Aliquots (2 ml) were withdrawn from the receptor compartment with immediate replacement with fresh medium. The concentration of the drug in the withdrawn sample was determined using spectrophotometer at wave length 264. The excess formulation was carefully removed from the skin surface after 24 h and skin was washed three times with phosphate-buffered saline (pH 7.4) before drying gently with the cotton swab. The skin was then stored at -20°C until further analysis.

Quantification of drug in the skin

After completion of the permeation experiment, the excess formulation was removed from the skin surface. The skin was washed three times with phosphate-buffered saline (pH 7.4) and dried. SC was then separated from dermal tissues (epidermis and dermis (E+D)) using serial tape stripping. A 15±2 strips for rat and 23±3 strips for human cadaver skin were removed using adhesive tape (Scotch845 Book Tape, 3M). The procedure was standardized to avoid damage to the skin. These tape strips and the remaining skin portions (E+D) were collected in separated Eppendorf. ACVextraction from the various skin layers including tape strips was accomplished by adding 1 mL of 0.1 N NaOH to the Eppendorf, followed by high-speed vortexing for 30 s and then incubation at 70°C water bath for 30 min. To this alkaline extract, methanol was added to precipitate the proteins and then samples were centrifuged at 2,300×g for10 min. The supernatants were then analyzed for ACV content in each skin layer using spectrophotometer, by measuring the absorbance at λ_{max} 264 nm using phosphate buffer at pH 7.4 i.e. SC and dermal layer (E+D) and reported as percentage of applied dose/cm2 area. For determination of extraction efficiency of ACV from skin, blank skin samples were spiked with known concentration of ACV solution for 24 h and then extracted as described above.

RESULTS AND DISCUSSION

Table 1: Ingredients of cream base.

Ingredients	Percentage	Ingredients	Percentage
stearic acid	0.5	cetyl palmitate	0.41
cacao butter	0.5	isopropyl myristate	4.1
bees wax	0.5	span 60	0.615
cetyl alcohol	0.41	tween 80	1.43
steryl alcohol	0.41	tween 20	0.81
		distilled H2o	20

Table 2: Formulation of SLNs using stearic acid (each data repeated triple ±S.D).

S. No.	Stearic acid %	Particle size d.nm	PDI	Zeta-Potential mV
1.	6.50%	463.00±13.028	0.676 ± 0.166	- 37.20 ± 0.566
2.	5.00%	338.10 ± 9.134	0.577 ± 0.157	-31.90 ± 0.095
3.	2.50%	251.30 ± 18.305	0.542 ± 0.080	-35.00 ± 0.174
4.	1.25%	149.60 ± 10.347	0.542 ± 0.102	-28.80 ± 0.099

Table 3: Formulation of SLNs using cetyl palmitate (each data repeated triple ±S.D).

S. No.	cetyl palmitate %	Particle size d.nm	PDI	Zeta-Potential mV
1.	6.50%	518.30 ± 49.228	0.880 ± 0.110	- 22.90 ± 0.990
2.	5.00%	353.00 ± 51.270	0.533 ± 0.177	-17.60 ± 0.424
3.	2.50%	271.20 ± 59.409	0.680 ± 0.184	-36.70 ± 0.210
4.	1.25%	213.633 ± 73.459	0.488 ± 0.093	-27.60 ± 0.16

Table 4: Effect of ACV loading concentrations on particle size, PDI, zeta-potential and EE%.

loading %	Particle size d.nm	PDI	Zeta-Potential mV	EE%
40%	700.725 ± 74.764	0.451 ± 0.134	- 33.70 ± 0.354	87.602%
24%	542.175 ± 32.749	0.56 ± 0.196	-34.30 ± 0.149	80.373%
8%	343.333 ± 74.674	0.587 ± 0.153	-31.00 ± 0.141	72.00%

Table 5: Effect of different compritol 888 ATO ratio (each data repeated triple ± S.D).

ACV: compritol	Particle size d.nm	PDI	Zeta-Potential mV	EE%
1:2	797.666 ±10.499	0.714 ± 0.012	-31.90 ± 4.24	58.290%
1:1	608.033 ±118.495	0.596 ± 0.088	- 33.90 ± 3.82	58.460%
1:0.5	565.633 ± 51.972	0.624 ± 0.059	-32.10 ± 2.69	65.450%
1: 0.25	443.60 ± 40.989	0.534 ± 0.030	-43.40 ± 0.141	74.12%

Table 6: Effect of homogenization speed during SLNs formulation on particle size, PDI, zeta-potential. EE% (each data repeated triple ± S.D)

Technique used	Particle size d.nm	PDI	Zeta-Potential mV	EE%
15,000 rpm/5min	841.566 ±199.565	0.691 ± 0.123	-31.30 ± 0.137	53.240%
20,000 rpm/5min	650.233 ± 218.124	0.575 ± 0.119	- 33.20 ± 0.495	57.700%
23,000 rpm/5min	480.666 ± 70.571	0.646 ± 0.153	-31.90 ± 1.34	65.780%
26,000 rpm/5min	304.100 ± 51.298	0.647 ± 0.072	-33.20 ± 0.707	68.98%

Table 7: pH-value of different formulation (each data repeated triple ± S.D).

Formulation	pH-value
Simple Cream	4.976±0.077
Unloaded SLNs dispersion	4.99 ± 0.165
ACV Loaded SLNs dispersion	5.48 ± 0.02
ACV based cream	5.663 ± 0.280
ACV-SLNs based cream	5.616 ± 0.201

Formulation Optimization

Effect of lipids type and concentration

In order to optimize SLNs – ACV loaded, different formulation variables such as type and concentration of solid lipids also different type of surfactants were evaluated and the nanoparticle dispersions were characterized for Nano size, PDI, Zeta potential and ultra-microscope. Tables 2, 3 and figuers1, 2 showed formulations with different solid lipids percentage rang 6.5, 5, 2.5 and 1.25% with respect to aqueous phase and a fixed amount of hydrophilic surfactant. The most suitable formulation was selected for the next experiment. The beast formulation was made with different lipids including beeswax, spermaceti, cetyl alcohol, stearyl alcohol, stearic acid and cetyl palmitate. Tween 80 and Diterol AL40 were the surfactant used. Based on our data shown in previous tables, the formulations containing stearic acid and cetyl palmitate as lipids had the most stability, smallest size, and best appearance The measured zeta potentials for both SLNs indicated very good shelf life physical stability, and polydispersity index values were lower than 1, suggesting

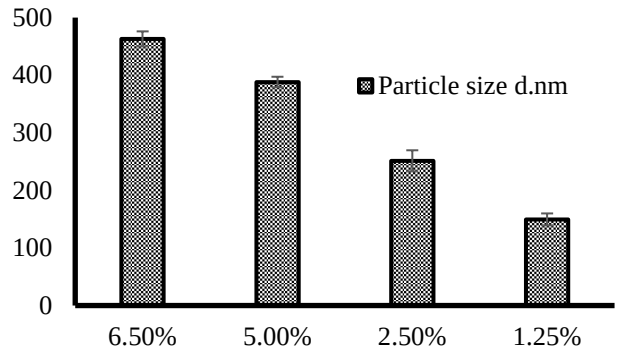


Figure 1: Correlation between lipid % (Steaeic acid) and particle size.

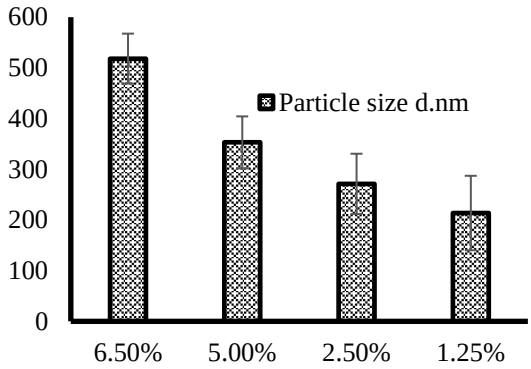


Figure 2: Correlation between lipid % (cetyl palmitate) and particle size.

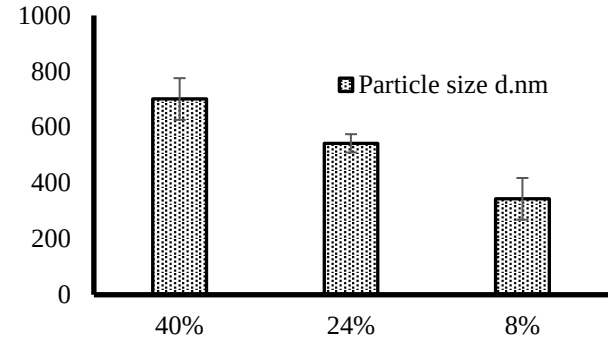


Figure 3: correlation between ACV % and particle size.

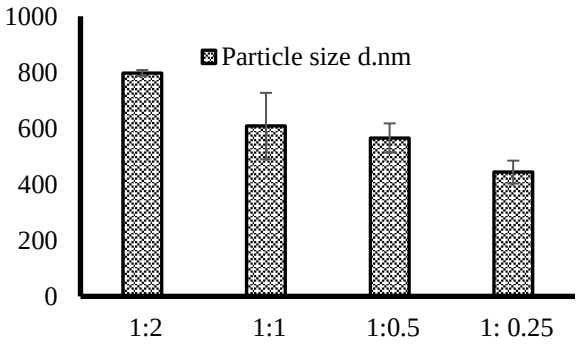


Figure 4: correlation between compritol 888 ATO: ACV ratio and particle size.

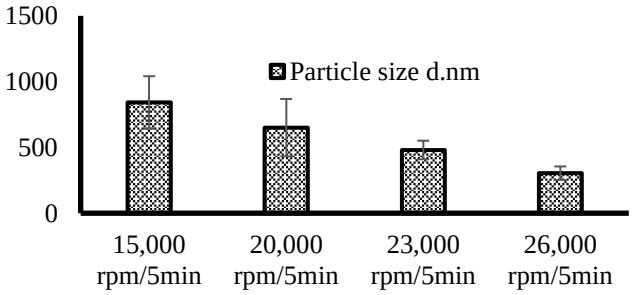


Figure 5: correlation between homogenization speed and particle size.

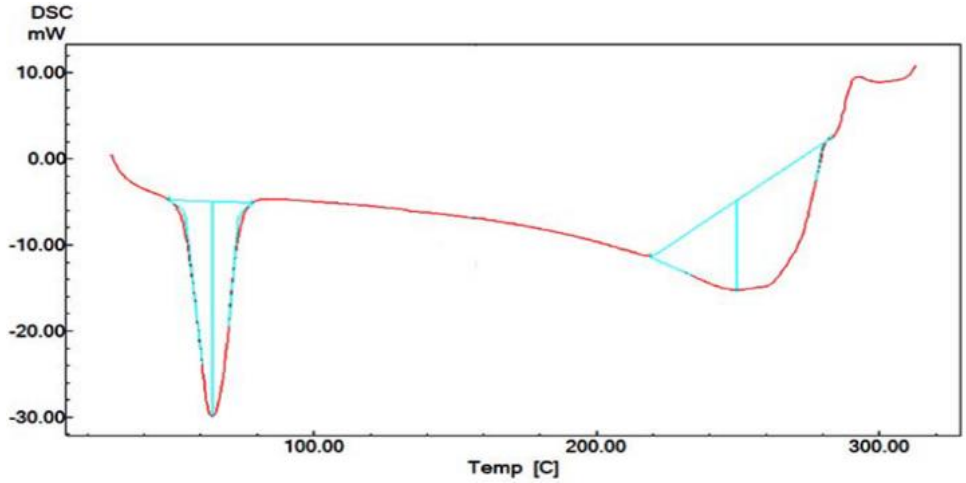
Table 8: Stability study: Malvern zeta sizer, TEM and EE%.

	Stability over 6 month					
	at 25 ºC			at 4 ºC		
	SLNs	ACV-SLNs	+compritol	SLNs	ACV-SLNs	+compritol
TEM size	109	327	380	106	351.5	363
Malvern zeta sizer	166.4	420.0	563.0	170.0	400.0	480.0
PDI	0.554	0.574	0.570	0.627	0.663	0.529
Zeta-potential	-26.9	-29	-27.9	-28.5	-25.5	-24.6
EE%	-	77.06%	79.42%	-	75.46%	65.35%

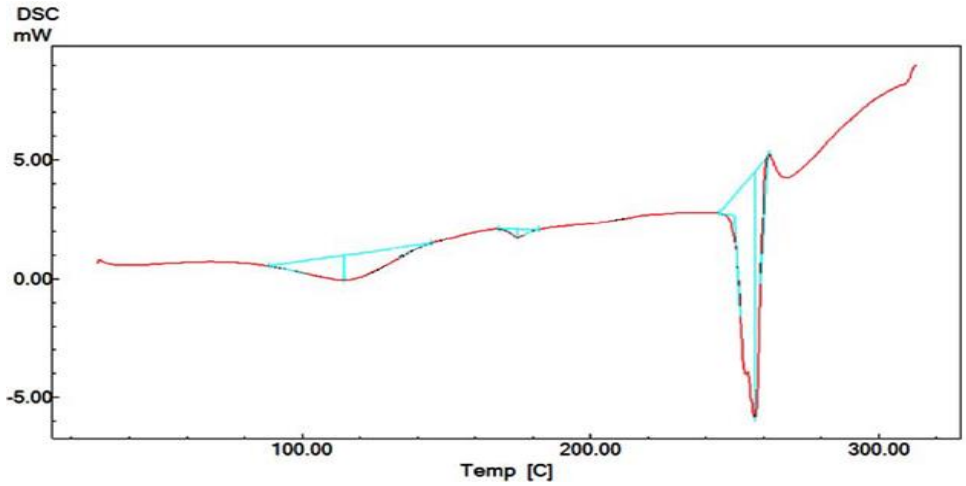
Table 9: percentage accumulation of ACV in different skin layers afterv24-hr permeation through rate skin.

	Stratum corneum	Epidermis and dermis	Receptor compartment
ACV solution	0.08 ± 0.03	0.23 ± 0.018	0.40 ± 0.014
ACV simple cream	0.16 ± 0.019	0.52 ± 0.116	1.04 ± 0.096
AVC-SLNs	0.20 ± 0.012	7.89 ± 1.71	1.18 ± 0.082

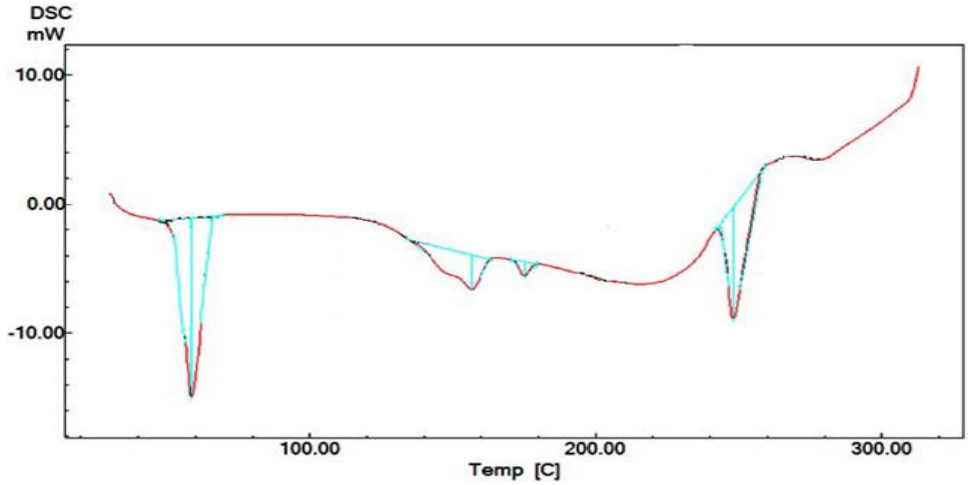
a relatively narrow size distribution. Data for all of the formulated SLNs were not shown. The hydrophilic–lipophilic balance value and the structure of surfactant have significant effects on formulation quality. Based on



Stearic Acid



ACV



Mixture ACV + Stearic Acid

Figure 6: DSC Thermogram of Stearic acid, ACV and physical mixture.

the results depicted in Table 2, SLNs with stearic acid was selected as the best formulation of 1.25% (size 149.666 ± 10.347nm, PDI 0.542±0.102 and zeta potential -28.8) with suitable Nano size, lower standard deviation and PDI and high zeta potential. The best formulation was incorporated into the cream base.

Effect of ACV loading

For optimization of drug loading i.e. 0.5%, 0.3% and 0.1% (w/w) with respect to aqueous and also represent 8%, 24% and 40% (w/w) respectively of lipid included in the formulations were prepared and evaluated for particle size, zeta potential and entrapment efficiency (Table 4, Figuer3). Based on the previous factors 8% w/w drug loaded was chosen as the best concentration to continue the experiment. Average particle size was 343.333±74.674 nm

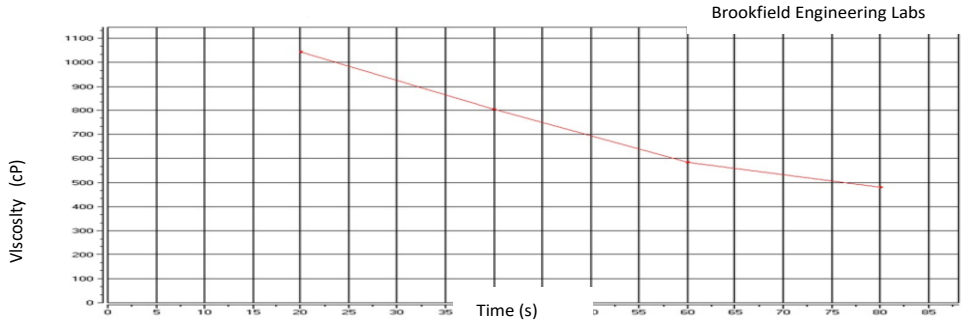
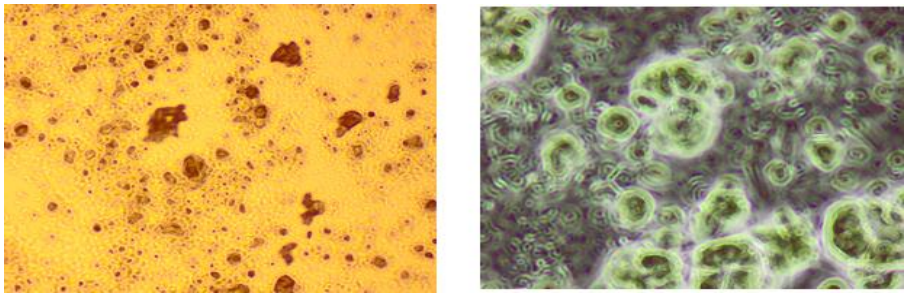
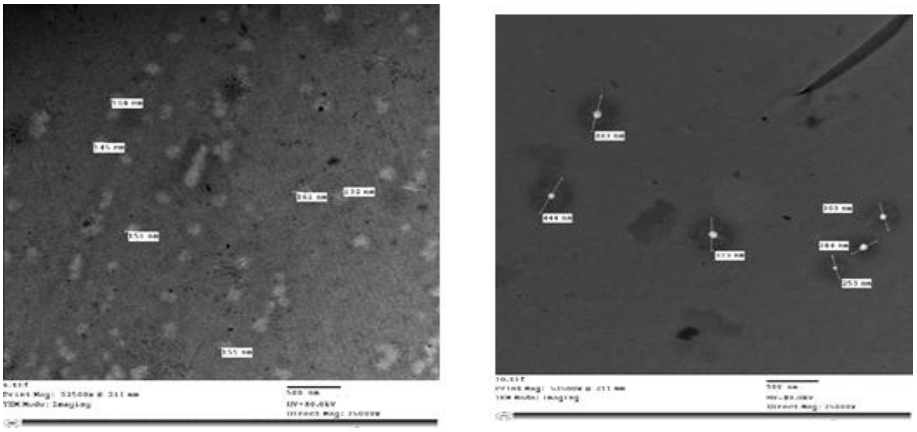


Figure 7: Rheological behavior of SLNs- ACV, based cream.



(a) (b)

Figure 8: Optical microscopy photograph of a-SLNs dispersion b- AVC-SLNs.



(a) (b)

Figure 9: TEM photograph of a-SLNs b- SLNs- AVC.

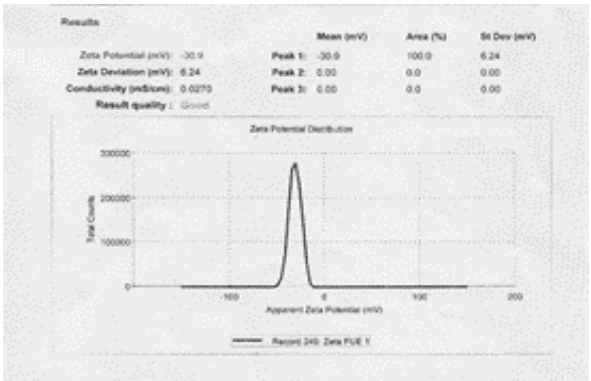


Figure 10: Zeta-potential for SLNs - AVC.

and PDI was 0.587± 0.153 with zeta potential -31.0 ±0.141and EE% 72%.

Effect of Compritol 888 ATO

Different researches have highlighted the feasibility of using compritol 888 ATO as a lubricant or coating agent for oral solid formulations. It has also been explored as a matrix forming agent for controlling drug release. To assess the effects of compritol 888 ATO concentration on drug release, different ratios of drug: compritol were prepared. Ratios of 1:0.25, 1:0.5, 1:1 and 1:2 were evaluated for particle Nano size, PDI, Zeta potential and encapsulated efficiency. SLNs size ranges were 443.6±40.989, 565.633±51.972, 608.033±118.495, 797.66±10.499 with PDI 0.534±0.030, 0.624±0.059, 0.596±0.088, 0.714±0.012 respectively. The potential were high range between -31.9±4.24 and -43.4±0.141 with EE% range from 58.29% to 74.12%. According to the previous results ratio of 1:0.25 was chosen for in-vitro release study. Table 5 and Figure 4 clarify the results.

Effect of homogenization speed and stirring time

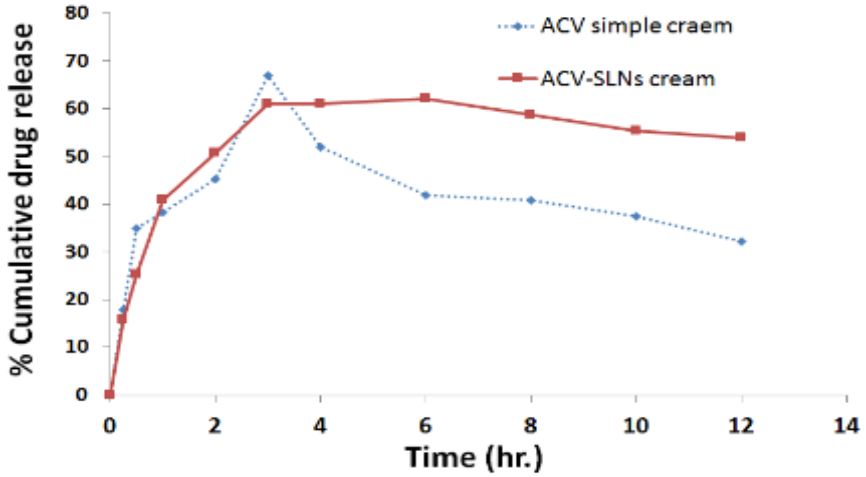


Figure 11: % cumulative release of ACV versus time for ACV simple cream and ACV – SLNs based cream.

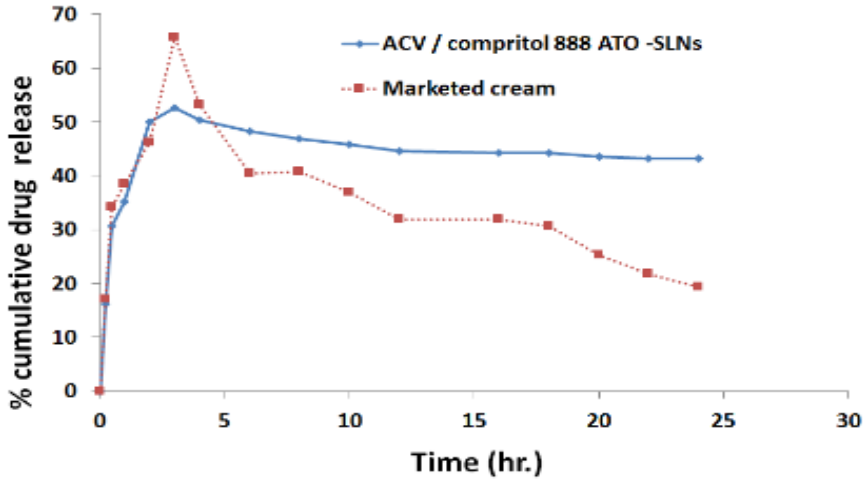


Figure 12: % cumulative release of ACV versus time for ACV / compritol 888 ATO – SLNs based and Marketed cream.

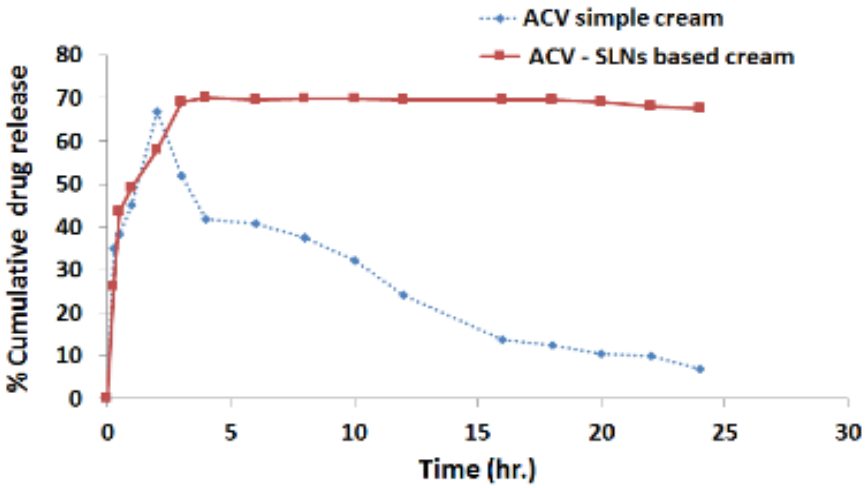


Figure 13: % cumulative release of ACV versus time for ACV simple cream and ACV – SLNs based cream using rate's skin.

Table 6 and Figure 5 summarize the effect of homogenization speed and stirring time on the particle characteristics of SLNs. Increases in homogenization speed (from 15,000 to 26,000 rpm) lead to a considerable decrease in the particle size (841.566±199.565 nm to 304.1±51.298 nm) and PDI range (0.691± 0.123 to 0.647± 0.072), zeta potential range (-31.3±0.137 to -33.2± 0.707) also entrapment efficiency range (53.2% to 68.98%). Further increase in stirring time from 5 to 20 min was accompanied with insignificant change in particle size

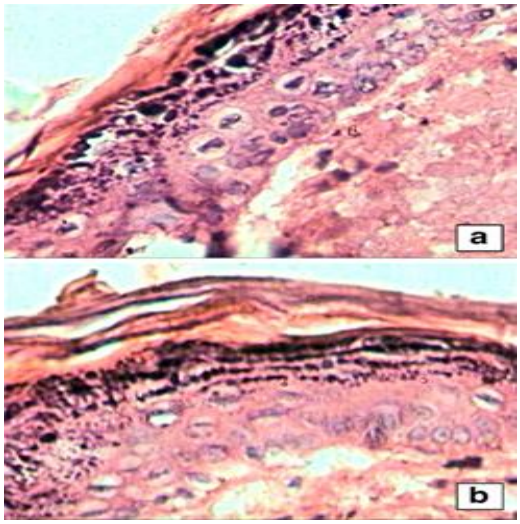


Figure 14: Histopathology of skin revealing the changes in the morphology of different layers of (a) blank skin (b) ACV SLNs-treated skin.

as well as entrapment efficiency. Notably, homogenization speed 26,000 for 5 min followed by stirring at 500 rpm for 5 min lead to the formation of particles with optimal size, PD, zeta potential and entrapment efficiency.

Thermal analysis

Figure 6 shows the differential scanning calorimetry (DSC) thermograms of stearic acid, pure AVC, and physical mixture of AVC/stearic acid. Pure ACV showed a single endothermic peak at 261.47°C and a depressed endothermic peak was also found at almost the same temperature from the curve of the physical mixture. Sharp endothermic peak at 68.8°C indicate melting point of stearic acid, In addition to this, no endothermic peak was found in the range of 200–300°C in the thermogram. The same previous peaks were also found at almost the same temperature from the curve of the physical mixture. This results prove neither Physical nor chemical interactions are exist between drug and lipid.

PH measurement

The pH measurements of 10% w/v aqueous dispersion of the selected NLC formulations are recorded in Table 7. The pH values were in the range from 4.99±0.165 to 5.616±0.201 indicating that the pH values of the prepared formulations lies within the acceptable range for topical preparations. This is explained by the fact that the pH value of the SC and the upper viable epidermis has been recorded to be 4.0–4.5 and 5.0–7.0, respectively, and the local pH value of the SC extracellular lipid matrix is 6[23].

Rheological Studies

The rheological behavior of the prepared SLNs formulations was studied to determine the appropriate lipid type and concentration that can form semisolid matrices in a single-step process. The rheological data namely Farrow's constant (N) and the hysteresis area (H.A.) for the selected SLNs formulations reveal that the semisolid SLNs formulations exhibited Farrow's constant (n) values larger than one, which indicates shear thinning characteristics with variable thixotropic (data are not shown). The yield values for the investigated semisolid SLNs formulations were greater than 20 Pa. Products with yield points below this value will flow readily by themselves. A lotion or creams with yield value above 20 Pa will flow more slowly. The combined shear thinning behavior and thixotropic are desirable characteristics for topical formulations, as they facilitate processing during manufacture and the flow from the container, and improve spreading on the skin. In addition, the applied film can gain viscosity instantaneously and thus resist running[24]. Figure 7 showed rheological behavior of SLNs-ACV based cream.

Characterization of Solid Lipid Nanoparticles

Optical microscopy

The morphology of all SLNs formulations was determined by optical microscope equipped with digital camera. The photomicrograph of optimal formula was shown in (Figure 8). These photomicrographs confirmed the formation of vesicular structures. The microscopic appearance of all formulations showed spherical vesicles for both loaded and unloaded SLNs. Unfortunately, information concerning microstructure of SLNs not be visualized by the low-magnification power of optical microscope, therefore transmission electron microscope was employed to elucidate morphology of Nano particles.

Transmission electron microscopy

TEM of SLN aqueous dispersion either unloaded or loaded with ACV showed homogenous small-sized spherical structures with smooth surface nanoparticles especially those entrapping ACV. Figure 9 shows photomicrographs clarify monolayer coating of surfactant surrounding solid lipids. The TEM studies confirmed the observed particle size of SLNs ranging between 100 nm and 175nm for drug unloaded SLNs, and between 206 nm and 444nm for ACV-SLNs. To study the connection between lipid content and the achieved particle size, different formulations with variable amount of stearic acid and a fixed amount of Tween 80 measured their particle size. Elevation of the lipid content of formulation increased the particle size of SLNs. Due to desired physicochemical specifications, SLNs based on stearic acid lipid content formula was selected for in- vivo study.

Zeta sizer analysis

Optimization of SLNs formulation and studying variation parameters were depend on Nano size, PDI and zeta potential. Average particle size of SLNs from Zeta sizer studies was found to be higher than the TEM. This is may be attributed to the difference in the principles underlying these techniques. Thus Zeta sizer allowed the observation of SLNs in a hydrated state that is closer to that of the SLNs in suspension. For TEM the sample was dried at 55°C. Thus, particle sizes of SLNs recorded via different techniques were found to be in the following order TEM<Zeta sizer, some reference is consistent with this result[25]. The zeta potential values were high and negatively charged as they ranged from -28.8 mV to - 43.4 mV ensure stability for longer periods of time (Figure 10). PDI < 1.0 indicating uniform particle size distribution.

Entrapment efficiency (EE %)

The mean EE% values of all the examined formulations are studied which ranged from 87.602% to 51.81%.

Concerning the effect of drug loading EE% was increased with increasing drug percentage i.e. (72%, 80.373 % and 87.602% for drug concentration 8%, 24% and 40% respectively). But according to the limitation of Nano size and PDI, EE of 71% for 8% drug loaded was suitable. The EE value was increased with increasing homogenization speed in order from lowest to highest speed. Also using different drug/compritol 888ATO ratios were affected EE% with maximum of 74.12%, but changing stirrer time insignificantly affected EE%.

In Vitro Release

The percentage cumulative release of acyclovir for the formulated creams was compared. ACV simple cream was compared with that SLNs-ACV based cream (Figuer11), and formula containing ACV/compritol 888ATO – SLNs based cream was compared with marketed cream (Figure12). The cumulative % of acyclovir released over a period of 12 hr. was plotted against time (figure 11) for the first comparison. Figure 12 showed the cumulative % of acyclovir released over 24 hr. periods for the second comparison. A third comparison using rat's skin was done between ACV simple cream and SLNs-ACV based cream formula for 24 hrs. period (Figure 13). All formulations showed gradual release of the drug over 12 h and 24 h of the release period followed with Plato (steady state) for SLNs- drug release. The mean values for the maximum SLNs – ACV concentrations were 70.153µg/ml in skin tissues, compared with 62.054 µg/ml concentration in cellulose membrane. The mean values for time to reach maximum drug concentration equal 3 hr. Furthermore, K elimination was 0.019 hr^{-1} and 0.022 hr^{-1} respectively. $T_{1/2}$ elimination was 33.76 hr. and 31.42 hr., AUC_{0-12} was 676.42 and 653.21 µg.hr/ml. MRT was 10.79 hr. and 11.2 hr. respectively. The volume of distribution was 0.998 and 0.968 liters, total clearance rate was o.312 and 0.356 ml/min.

Stability Studies

All of the SLNs exhibited good stability during storage. The physical stability of the SLNs during prolonged storage was determined by monitoring changes in zeta potential, particle size, drug content, and appearance. The optimal SLNs formulation prepared was studied for physical and chemical stability during the 6-month period at 4ºC and 25 ºC. No obvious color change, degradation, or phase separation was observed. The mean diameter of SLNs –ACV loaded and the entrapment efficiency measured were 327 nm and 77.06 %, respectively at 25 ºC (Table 8). Also ACV/compritol 888ATO – SLNs has a good stability after the storage period with Nano size and EE value of 380 nm and 79.42% respectively.

In Vivo Study

Permeation through and accumulation of ACV in various skin layers of rat skin after application of ACV-SLNs, commercial cream formulation of ACV and free ACV solution for 24 h was studied. Before starting this experiment, the extraction efficiency was measured by comparing the amount of ACV added and that obtained after extraction and it was found to be 91.5±0.5%. As observed in Table 9, the amounts (% applied dose/ cm2) of free ACV solution in SC, dermal layer (E+D) and receptor compartment (RC) in rat skin were found to be0.08±0.003, 0.23±0.018 and 0.4±0.014, respectively. As compared with free ACV solution and simple cream formulation, ACV-SLNs showed 34.30 and 15.17 times higher permeability in the dermal layer. In RC, both simple cream and free ACV solution showed statistically insignificant ($p>0.05$) drug concentration. Rat skin showed significantly higher accumulation of ACV in stratum corneum, dermal layer, and receptor compartment compared with blank skin (Figure 14).

CONCLUSION

The prepared SLNs exhibited optimum Nano size (ranging from 100 to 175 nm) for unloaded particles and (from 206 to 444 nm) for drug loaded. Formulations of SLNs containing cetyl palmitate or stearic acid as lipids achieved a high stability, small size and good appearance. DSC study indicating compatibility of stearic acid and ACV drug. SLNs loaded with ACV (a hydrophilic drug) were prepared by homogenization. The collected zeta potentials indicated very good physical stability during shelf-life, and polydispersity index values were lower than 1 (ranging between 0.6 and 0.3) indicating a relatively narrow size distributions. The optimized SLNs-ACV formula resulted in suitable high encapsulation of ACV (72%), and (343.44±74.674) mean nm diameter and desired pH. Slower drug release from SLNs based cream compared with simple cream. Using 0.25% compritol 888ATO in SLNs-ACV formula give Plato plot indicating sustained, steady state drug release. Kinetic parameters of SLNs drug – based cream through rat's skin gave better values i.e. 70.153µg/ml C_{max} , 0.019 hr^{-} K_{el} , 33.76 hr $T_{1/2}$ el, 676.42 AUC_{0-12} and 11.2 hr MRT. Rheological studies proved combined shear thinning behavior and thixotropic characters of SLNs-ACV based cream that are desirable characteristics for topical formulations. SLNs-ACV and SLNs-ACV with compritol 888ATO aqueous dispersions have good stability over 6 month period at 4ºC and 25 ºC. No change in Nano size, PDI, zeta potential and EE%. In vivo skin permeation studies showed that there was 34.30- and 15.17-fold accumulation of ACV- SLNs in dermal tissues of rat skin, respectively, as compared with ACV simple solution and commercial formulation of ACV. CLSM images revealed that dye-loaded SLNs penetrated and distributed throughout the rat's dermis and they preferentially followed entry via hair follicles. No cutaneous toxicity was exhibited by SLN formulation as confirmed by histological examination. The incorporation of prepared SLNs in some cream or gel formulation for potential application purpose is also desirable.

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