Original Article

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Cyproterone acetate-loaded solid lipid nanoparticles (SLNs) and nanostructured lipid carriers (NLCs): preparation and optimization

Parisa Ghasemiyeh[1], Amir Azadi[2], Saeed Daneshamouz[1], Soliman Mohammadi-Samani[1,3*]

[1]Department of Pharmaceutics, School of Pharmacy, Shiraz University of Medical Sciences, Shiraz, Iran.
[2]Pharmaceutical Sciences Research center, School of Pharmacy, Shiraz University of Medical Sciences, Shiraz, Iran.
[3]Center for Nanotechnology in Drug delivery, School of Pharmacy, Shiraz University of Medical Sciences, Shiraz, Iran.

Abstract

Due to limited water solubility of cyproterone acetate and also limited skin permeation; lipid base nanocarriers with different size ranges were considered for topical delivery of cyproterone acetate. Lipid nanoparticles were prepared by solvent diffusion evaporation technique. Different variables including; surfactant/lipid ratio, mixing rate and addition time of organic phase to aqueous phase were utilized in optimization process in order to fabricate nanoparticle at specified particle size ranges to target the particle to hair follicles. Statistical data showed that the model is significant (p-value of 0.0001) to prepare lipid based nanoparticle having specified size ranges. The results showed that there is interaction between different parameters and 3-D graphs indicated the optimum point of interactions between various parameters. Drug entrapment efficiency was 99.03% and loading capacity was 1.91%. Release studies showed that 50- 75% of drug will be releasable from the nanoparticles within the first 24 hours depend on the size range of the nanoparticles. The R-square value of 0.9839 indicated that there is a good relationship between experimental data and the fitted models suggested by Design-Expert software. Cyproterone acetate release from these lipid-based nanoparticles, was significantly slower than the permeation of the free drug from dialysis tubing, which confirm that this delivery system is capable to control the release rate of the cyproterone acetate. Drug release pattern from nanoparticles was best fitted to Higuchi model which is a suitable model for matrix based delivery systems.

Keywords: solid lipid nanoparticles (SLNs), nanostructured lipid carriers (NLCs), cyproterone acetate (CPA), size optimization, hair follicular targeting.

1. Introduction

Solid lipid nanoparticles (SLNs) are first generation of lipid based nanocarriers that are formed from solid lipids and they also stabilized by emulsifiers (1). Lipids that are used in SLNs preparation commonly are biocompatible, biodegradable and non-toxic. SLNs have size ranges between 40 to 1000 nm (2). There are different ways for the preparation of SLNs; such as hot or cold pressure homogenization (3), ultrasonic-solvent emulsification technique (4), solvent diffusion evaporation method (5), O/W micro-emulsion quenching technique (6), etc. Selection of appropriate preparation technique has profound effects on drug stability, loading efficiency, particle stability and particle size of the nanoparticles. Although loading efficiency and particle size of the

Corresponding Author: Soliman Mohammadi-Samani, Center for Nanotechnology in Drug delivery, School of Pharmacy, Shiraz University of Medical Sciences, Shiraz, Iran.
Email: smsamani@sums.ac.ir

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SLNs are important formulation variable but due to the possibility of drug expelling out of the SLNs this nanocarrier has its own limitations. To overcome this limitation Nanostructured lipid carriers (NLCs) were introduced.

NLCs are second generation of lipid nanocarriers that are designed from a mixture of solid lipids and liquid lipids (oils), the advantages of these nanoparticles from SLNs is the improvement of drug release properties and increment of drug loading efficiency (7).

The most important advantages of these lipid nanoparticles compared with others is the controlled and modulated drug release (8), high entrapment efficiency (9), better physical stability (10) and increasing the bioavailability of class 2 and class 4 drugs of Biopharmaceutical Classification systems (BCS) (11). SLNs also have some disadvantages such as drug expelling during storage (7) and initial burst release for some drugs (12).

SLNs and NLCs are popular nanocarriers for topical delivery of lipophilic drugs (13), these nanocarriers have the ability to target different skin organelles and limit the systemic availability of the drugs which are considered topical and local delivery purposes, yet these nanocarriers can increase skin penetration (14, 15); especially these nanocarriers could be used for follicular targeting of specified drugs intended for the therapeutic purpose of acne, hirsutism and alopecia (16). One of the advantages of lipid nanoparticles is the possibility of the prolonged drug release from the delivery system which can help to reduce the dose of the drug and also increase its interval of administration (17).

Previous researches has emphasis on the effects of size of the nanoparticles in skin penetration rate and follicular targeting (18), so in this study size optimization to target different skin organelles was considered. For this purpose Design-Expert software (version 7.1, Stat-Ease Inc., Minneapolis, USA) was used to reach the best model fitting from D-optimal design (19). Using this optimization technique could be recruited to design specific size range for targeting purposes and to limit extensive systemic absorption of drugs intended for topical delivery (20).

Cyproterone acetate (CPA) is an antiandrogen drug and antineoplastic agent and also has progestational effects, which is used for treatment of advanced prostate cancer in males (21, 22). CPA blocks the binding of dihydrotestosterone to the specific receptors in the prostatic carcinoma cell. CPA alone or in combination with ethinyl estradiol used in androgenic disorders in women such as acne and hirsutism (21). CPA is a lipophilic drug (log P=3.81) and its water solubility is 0.00152 mg/ml and it has low skin permeation (22). To increase topical delivery of CPA and also to target specific organelles such as hair follicles fabrication of the lipid based nanocarriers were considered in this project (23). In this regard to limit the various side effect of the systemic administration of CPA including adrenal suppression, decreased libido, galactorrhea, gynecomastia, etc. fabrication of CPA loaded SLN and NLC with appropriate size range were considered.

2. Materials and methods

2.1. Materials

Stearic acid and cholesterol and Brij 72 were obtained from Merck, Brij 35 and triolein were purchased from Sigma, cyproterone acetate was kindly gifted by Iran Hormon. Acetone, acetonitrile and ethanol were HPLC grade and were purchased from Merck domestic supplier in Iran. All other chemical were analytical grade and used with no further purification.

2.2. SLN Preparation

At First a mixture of surfactants containing Brij 35 and Brij 72 (1:1 ratio w/w, HLB equal 10.91) was dissolved in 20 ml of distilled water and was heated up to 70 °C using the water bath. After that stearic acid was dissolved in 10 ml of acetone, and organic phase was added to the aqueous phase under continuous stirring condition at predetermined rate of mixing at 70 °C. The stirring was continued till volume of the mixture of solvents reached to 10 ml and smell of the acetone was disappeared. Finally stirring was discontinued and promptly the mixture was cooled within the appropriate ice bath below to 5 °C and mixing the mixture was performed for 5 minutes, which help to solidify the lipid nanoparticles.

2.3. SLN Characterization

2.3.1. Particle Size and Size Distribution Analysis and Zeta Potential Measurement

After the nanoparticles preparation, size of the nanoparticles was measured freshly by Particle Size Analyzer (PSA; SHIMADZU SALD-2101), which was a laser diffraction particle size analyzer. Also, zeta potential of nanoparticles was measured by Zeta-Chek (Microtract, ZC007, Germany).

2.3.2. Optimization by D-optimal Design

For the purpose of formulation optimization, a D-optimal design was planned. Three independent variables were determined using the Design-Expert software (version 7.1, Stat-Ease Inc., Minneapolis, USA): These variables were Surfactant/lipid ratio (S/L), Mixing rate, Addition time of organic phase to aqueous phase. The ranges of each of these variables were also determined: S/L ranges were from 1 to 3, mixing rate ranges were from 400 to 1400 rpm and addition time ranges was from 15 to 150 seconds. Then the software was suggested 21 runs regarding to these independent variables (Table 1).

Table 1. Different runs which was suggested by Design-Expert.

Run	S/L ratio	Mixing rate	Addition time
1	3	1400	150
2	1	400	15
3	1.8	804	150
4	3	400	150
5	1	400	102
6	3	1400	15
7	3	400	150
8	3	1400	15
9	1	1050	15
10	1.8	1400	70
11	2.8	1307	83
12	2	900	83
13	3	1400	150
14	1	1400	150
15	1.8	786	67
16	1	1400	150
17	2.8	900	137
18	2.3	400	15
19	3	803	69
20	2.3	1025	15
21	1	1050	15

Each run was done two times and then the average of the two sizes was used for other statistical analysis. Also the span index of each sample was calculated using following equation:

$$Span\ index = \frac{D90 - D10}{D50} \quad \text{(Eq. 1)}$$

Which D_{90}, D_{50} and D_{10} are 90%, 50% and 10% under sized diameter of the particles population respectively.

2.4. NLC Preparation

The NLC also was prepared using the same method of SLN preparation. In this regard mixture of the lipids were dissolved in acetone as organic phase and mixture of the surfactants were dissolved in water as aqueous phase and after the heating the aqueous phase up to 70 °C organic phase was added to aqueous phase at the same conditions were applied to SLNs.

We targeted three different sizes; 100 nm, 300 nm and 600 nm by Design-Expert software and it was suggested three independent variables as seen in Table 2 for each targeted size according to our D-optimal model.

2.5. Drug Loading Assessment

CPA was added to the organic phase (containing lipids dissolved in acetone) and then CPA-loaded lipid nanoparticles were formed by solvent diffusion evaporation method. We added different percentages of CPA to evaluate the drug expulsion and loading capacity. Finally, we obtained the best formulation containing defined amount of CPA and a mixture of lipids in which drug expulsion was not occurred.

Entrapment efficiency (%EE) and loading capacity (%LC) were calculated by centrifugation

Table 2. Independent variables that were suggested for each targeted size.

Target size(nm)	S/L ratio	Mixing rate(rpm)	Addition time(sec)
100	1.87	1400	15
300	3	1029	150
600	3	403	150

technique (24), in this method we used centrifugal filter tubes (MWCO 10KDa, Amicon Ultra-4, Millipore Co., MA, USA). In this regard 5 ml of CPA-loaded nanoparticles were put in the upper chamber and centrifuged at 4000 rpm for 20 min (Eppendorf, centrifuge 5810 R), then filtrate was injected to HPLC Column C_{18} (KNAUER, Germany) to calculate the unloaded drug, each experiment was done in triplicate.

$$Loaded\ drug = Total\ drug - Unloaded\ drug \quad \text{(Eq. 2)}$$

$$\%\,Entrapment\ efficiency\,(\%EE) = \frac{Loaded\ drug}{Total\ drug} \times 100 \quad \text{(Eq. 3)}$$

$$\%\,Loading\ capacity\,(\%LC) = \frac{Loaded\ drug}{Loaded\ drug + Total\ lipid} \times 100 \quad \text{(Eq. 4)}$$

2.6. Drug Release

In this regard a dialysis membrane (Dialysis tubing cellulose membrane, D9652-100FT, Sigma-Aldrich) was used to confirm the ability of CPA to pass through this membrane (membrane treatment: removal of glycerol included as a humectant was accomplished by washing the tubing in running water for 3-4 hours. Removal of sulfur compounds was accomplished by treating the tubing with a 0.3% (w/v) solution of sodium sulfide at 80 °C for 1 minute. Then washed with hot water (60 °C) for 2 minutes, followed by acidification with a 0.2% (v/v) solution of sulfuric acid, then rinsed with hot water to remove the acid), for this purpose 10 ml of 80 µg/ml CPA solution in phosphate buffer saline (PBS)+25% ethanol was packed within dialysis membrane and put in the release medium (PBS+25% ethanol) 37 °C and stirring rate of 100 rpm, sampling was done after 0 min, 15 min, 30 min, 1h, 2 h, 4 h, 8 h and 24 h. Then samples were injected into the HPLC (KNAUER, Germany) and the pattern of CPA release was analyzed by a developed method of analysis. Also, drug release study from lipid nanoparticles was performed for each targeted size (100, 300 and 600 nm) in triplicate. 10 ml of CPA-loaded lipid nanoparticles were delivered in dialysis membrane and put in 200 ml of release medium (75% Phosphate buffer saline+25% ethanol) at 37 °C and stirring rate of 100 rpm, sampling was done at 0 min, 15 min, 30 min, 1h, 2 h, 4 h, 8 h and 24 h. The samples were injected into the HPLC (KNAUER, Germany) and the pattern of CPA release from lipid nanoparticles were analyzed completely.

2.7. Morphological Assessment

Atomic force microscopy of CPA-loaded lipid nanoparticles was utilized to confirm the size range and also elucidate the morphological characteristics of the nanoparticles. In this regard at first one drop of sample was fixed in a glass slide and then atomic force microscopy (AFM-JPK, NanoWizard® II) was done with a probe to detect nanoparticles shapes and sizes.

3. Results

3.1. Particle Size and Size Distribution Analysis and Zeta Potential Measurement

A typical graph of SLNs and NLCs particle size distribution obtained via laser diffraction technique which was shown in Figure 1. According to this figure sharp shape of the peak

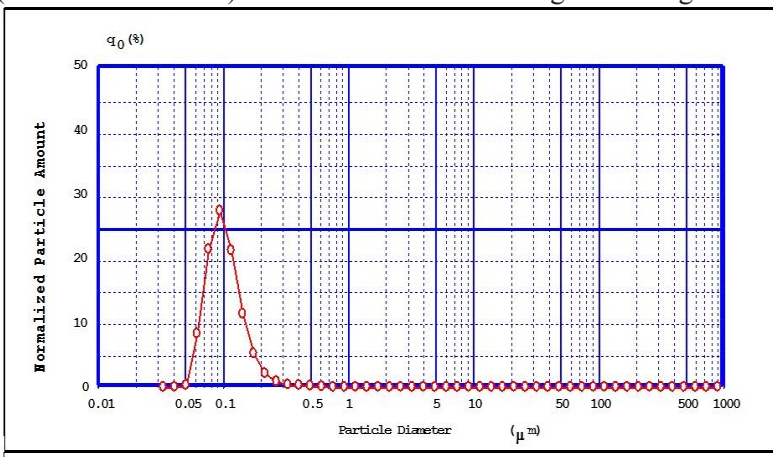

Figure 1. A typical graph of particle size distribution of nanoparticles with average 100 nm mean diameter.

indicated the low polydispersity index (PDI) between nanoparticles. Mean volume size of this sample was 103 nm and median diameter was 98 nm. Also, Span index of 0.89 was calculated using equation 1. Zeta potential of these nanoparticles was -35 mv. The negative zeta potential was also compatible with other reports of lipid nanoparticles zeta potential (25-27). The presence of negative zeta potential can guarantee electrostatic repulsion between nanoparticles and so the stability of the formulation can be achieved.

3.2. Optimization

According to Table 1, 21 experiments were designed to assess the effects of 3 different compositional and technical variables including surfactant/ lipid ratios, mixing rates and lipid phase addition times to aqueous phase. The results of the interaction levels between different variables mentioned above were presented in Table 3. According to different strategies which was considered in these experiments it is possible to fabricate particles with average size between 100- 600 nm indicating that by controlling the appropriate compositional or technical variables tuning the size for special purpose is feasible. The extent of the effect of each different variable on mean particle size of the particles and also possible interaction between different variables and optimum point of interactions was depicted in Figure 2.

Three dimensional model graphs in this figure revealed the relationship between different independent variables and response variable (particle size). By these graphs an optimum condition for each desired size would be achievable based on precise determination of the effect of each of three independent variables for targeting the desired size. There is some bending in these graphs which shows the interactions between the independent variables. By these graphs a tendency between each variable and particle size would be considered and the "optimum surface" in which the smallest size can be achieved would be predictable. Statistical assessment of the effect of different variables on size was presented in Table 4.

Table 3. Responses (Size and span index) of 21 runs which was suggested by Design-Expert.

Run	S/L ratio	Mixing rate	Addition time	Size(nm)	Span index
1	3	1400	150	104	0.925
2	1	400	15	1075	1.323
3	1.8	804	150	398	1.112
4	3	400	150	570	0.787
5	1	400	102	750.5	1.687
6	3	1400	15	82.5	0.815
7	3	400	150	547	0.675
8	3	1400	15	106.5	1.018
9	1	1050	15	512.5	1.079
10	1.8	1400	70	83.5	0.835
11	2.8	1307	83	101.5	0.877
12	2	900	83	368.5	1.039
13	3	1400	150	104.5	0.935
14	1	1400	150	420	1.041
15	1.8	786	67	369.5	1.027
16	1	1400	150	389	0.971
17	2.8	900	137	358.5	0.937
18	2.3	400	15	655.5	1.238
19	3	803	70	360.5	0.950
20	2.3	1025	15	352.5	0.932
21	1	1050	15	595.5	1.199

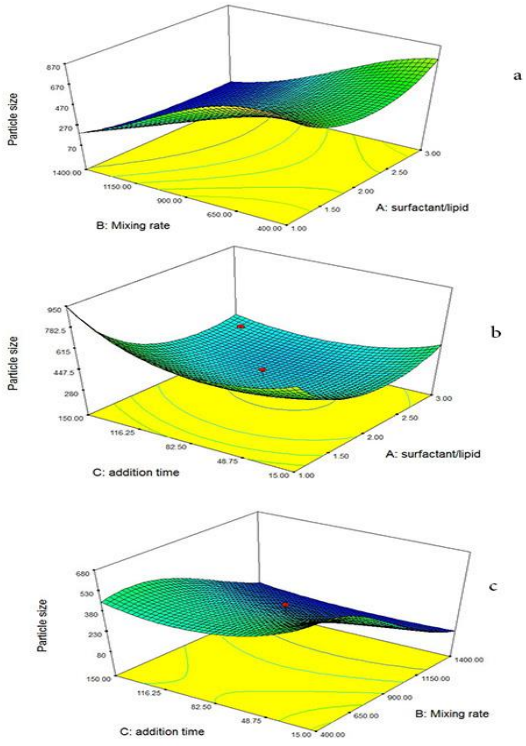

Figure 2. Binary effect of different varibles on mean particle size of the nanoparticles (A:S/L ratio, B:mixing rate, C:addition time).

Low CPA loading and also presence of drug expulsion during the storage of manufactured nanoparticles revealed that SLN based lipidic carrier were not adequate delivery system for CPA. In this regard we aimed to change the structure of the nanocarrier to nano structure lipid carriers (NLC). Therefore cholesterol and triolein were added to steraric base of the lipid carrier. Compositions of each formulation of NLC and the effects of cholesterol and triolein amounts on average size of NLC were shown in Table 5. Further assessment of the standard deviation of the size also revealed that

Table 4. Analysis of Variance (ANOVA) for Particle Size (ANOVA for Response Surface Quadratic Model).

Source of variations	Sum of squares	Degree of freedom	Mean square	F value	Prob.> F
Model	2.38	9	0.26	74.59	< 0.0001
A - (S/L ratio)	0.2	1	0.2	57.57	< 0.0001
B - Mixing rate	1.14	1	1.14	321.74	< 0.0001
C - Addition time	2.33E-04	1	2.33E-04	0.066	0.8026
AB	0.056	1	0.056	15.67	0.0022
AC	0.022	1	0.022	6.25	0.0295
BC	0.046	1	0.046	13.10	0.004
A^2	0.08	1	0.08	22.67	0.0006
B^2	0.12	1	0.12	33.61	0.0001
C^2	0.047	1	0.047	13.20	0.0039
Residual	0.039	11	3.54E-03		
Lack of Fit	0.03	6	5.00E-03	2.78	0.1408
Pure Error	8.99E-03	5	1.80E-03		
Core total	2.42	20			

Table 5. NLC Optimization based on the addition of different amounts of cholestrol and triolein.

Target size=100nm					
Sample No.	**%Cholesterol**	**%Triolein**	**%Stearic acid**	**Particle size(nm)**	**Span index**
1 (model)	0	0	100	106	0.97
2 (lipid=fixed)	0	0	100	108	1.03
3	30	0	70	110	1.15
4	20	10	70	80	0.74
5	15	15	70	62	0.72
6	**10**	**20**	**70**	**109**	**1.08**
7	0	30	70	511	0.88
Target size=300nm					
Sample No.	**%Cholesterol**	**%Triolein**	**%Stearic acid**	**Particle size(nm)**	**Span index**
1 (model)	0	0	100	329	0.78
2 (lipid=fixed)	0	0	100	377	2.45
3	30	0	70	265	1.47
4	20	10	70	112	1.11
5	15	15	70	110	1.10
6	**10**	**20**	**70**	**308**	**1.28**
7	0	30	70	448	0.93
Target size=600nm					
Sample No.	**%Cholesterol**	**%Triolein**	**%Stearic acid**	**Particle size(nm)**	**Span index**
1 (model)	0	0	100	643	1.08
2 (lipid=fixed)	0	0	100	2117	1.46
3	30	0	70	642	1.12
4	20	10	70	566	1.08
5	15	15	70	584	0.87
6	**10**	**20**	**70**	**609**	**0.94**
7	0	30	70	548	0.97

incorporation of the cholesterol and triolein could reduce span index of the particles and more uniform nanoparticles were achieved. In this regard mean number base average size were same as volume based mean average size of the nanoparticle. Therefore in the next sections of the experiments only NLC based lipidic nanocarriers were considered.

3.3. Drug Loading and Drug Expulsion

At first CPA was used in 10 % by weight of total lipids, in this cases drug expulsion was observed a few hours after manufacturing process, for overcoming the drug expulsion problem we cosidered two differnt approaches; at first the ratio of drug/lipid was reduced gradually, and on the other hand liquid lipids (oils) such as triolein or oleic acid were added to prepare NLC instead of SLN to minimize crystalization of the drug within the lipidic matrix (28, 29).

Finally the optimum formulation with 2% cyproterone acetate and a lipid mixture of 70% stearic acid, 20% triolein and 10% cholesterol was obtained in which drug expulsion was not ocurred and CPA was completely dissolved in formulation, with this optimium formulation, also desired size was achived. We used this optimum formulation for three different sizes (100, 300 and 600 nm) and entrapment efficiency(%EE) and loading capacity(%LC) were determined in

Table 6. Drug loading results of NLC formulation with different average size range.

Target size	Sample	% EE	Mean	Std Dev	%CV	% LC	Mean	Std Dev	%CV
	1	98.00				1.91			
100 nm	2	98.60	98.68	0.7344	0.744	1.92	1.92	0.0138	0.719
	3	99.46				1.93			
	1	99.07				1.93			
300 nm	2	98.76	99.19	0.512	0.516	1.92	1.93	0.0096	0.499
	3	99.76				1.94			
	1	99.27				1.93			
600 nm	2	99.03	99.22	0.1654	0.167	1.92	1.93	0.0036	0.188
	3	99.35				1.93			

triplicate for each sample (Table 6).

3.4. Drug Release

The release profiles of CPA from NLCs with different size ranges were depicted in Fig. 3. Results confirm that CPA release from lipid nanoparticles is completely dependent on the size of the nanoparticles and the percentage of surfactants, also our data revealed that the percentage of surfactants is dominant factor. With small size lipid nanoparticles, faster release rate was obtained, which is in agreement by diffusion theory, with small particles the release distance for passing by drug molecule is shorter and then faster release rate is expected.

AFM results showed that lipid nanoparticles were spherical and also the average particle size of the particles have good agreement with the results of laser diffraction technique. The results of AFM graphs were shown in Figure 4.

3.5. Atomic Force Microscopy (AFM)

AFM results showed that lipid nanoparticles were spherical and also the average particle size of the particles have good agreement with the results of laser diffraction technique. The results of AFM graphs were shown in Figure 4.

4. Discussion

During this project various technical and compositional variables effect on mean particles size and size distribution of the SLNs and NLCs were considered. Due to the direct effect of particles size on disposition of these nanoparticles, tuning of the size using technical or compositional variables would be a good option for formulator to tailor specific size for particular purpose such as targeting the nanocarriers to skin organelles such as hair follicles to extend the effect of drugs and also in order to keep higher concentrations of active pharmaceutical in site of action. During this project by using such variables we target three

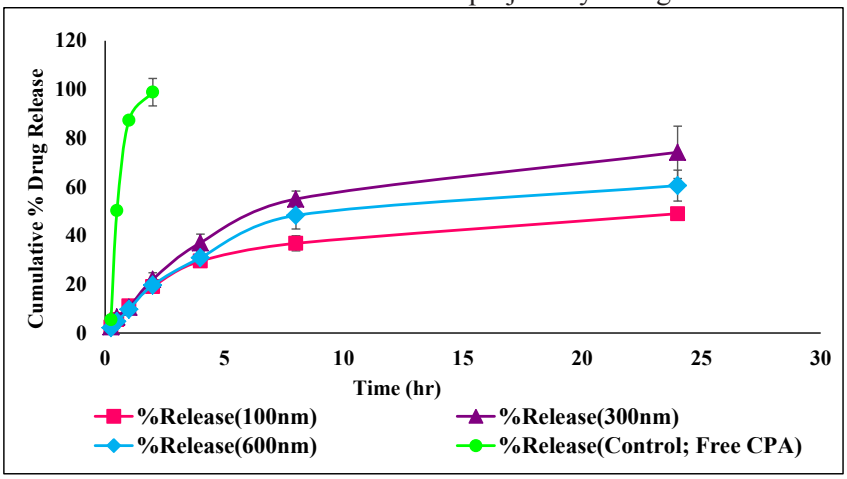

Figure 3. The release pattern of CPA from different nanoparticles having average size 100, 300, 600 nm.

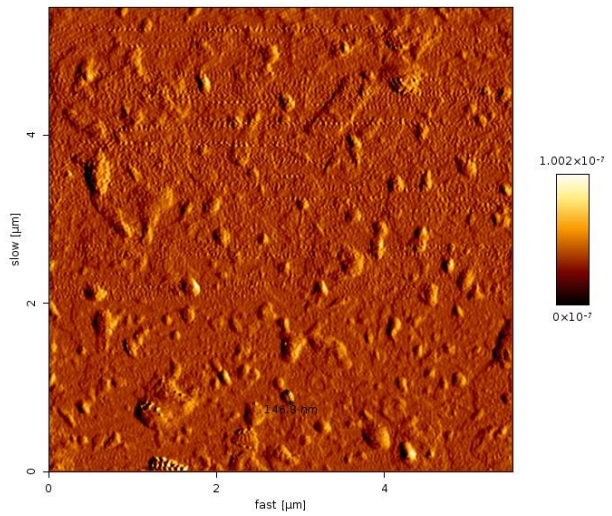

Figure 4. Atomic force microscopy (AFM) graph of lipid nanoparticles.

different sizes in nanometer range from 100- 600 nm. In this regard a statistical design method was considered to evaluate the extent of the effect of each factor independent from the others and also interaction of these factors was assessed.

4.1. D-optimal Design

As mentioned earlier, three independent variables; S/L ratio (A), mixing rate (B) and addition time (C) were selected for D-optimal Design and response variable was consider as the size of the nanoparticles. According to the output of the Design-Expert software (version 7.1, Stat-Ease Inc., Minneapolis, USA) final equation in terms of coded factors was proposed as:

Log10 (Particle size) = +2.48 -0.14 * A -0.35 * B -4.863E-003 * C -0.088 * A * B -0.053 * A * C +0.080 * B*C +0.18 * A^2 -0.21 * B^2 +0.13 * C^2 (Eq. 5)

This equation revealed that by increasing the S/L ratio (A), particle size could be reduce also by increasing the mixing rate (B) and addition time (C), same results would be obtained.

The suggested model was "quadratic" with the F value of 74.59 and p-value of 0.0001 (p-value<0.05 and F value of 74.59 implies that the model is significant), also lack of fit was not significant. The high R-square (0.9839), adjusted R-square (0.9707) and predicted R-square (0.9241) indicated that there is a good relationship between experimental data and the fitted models. Adequate precision, which measures signal to noise ratio, was 25.707 (a ratio greater than 4 is desirable) then confirming that this model has statistically significant signal to noise values.

4.2. Analysis of variance (ANOVA) for Response Surface Quadratic Model

According to the Table 4, among the three independent variables; S/L ratio (A) and mixing rate (B) were significant factors but the addition time (C) was not significant, but assessment of the binary combined effects of these variables show significant differences, which shows that there is interaction between S/L and mixing rate with addition time this finding emphases that although addition time alone, was not significant factor but it could not be omitted in optimization process because of its interaction with other factors.

4.3. NLC Preparation

As mentioned, for NLC preparation, three different sizes was targeted by Design-Expert software. In all of these samples which were shown in Table 5, total lipid was kept in fixed condition (150mg), but we used different percentages of stearic acid, cholesterol and triolein as lipid phase, then particle size and span index of each sample were assessed. Based on the results significant effect of triolein on particle size was seen, first by incrasing the percentage of triolein, particle size was decreased but above an optimum level its

effect was reversed and particle size was increased. So with this trend the optimum percentage of different lipids to obtain desired size was achived (70% stearic acid, 20% triolein and 10% cholesterol).

Due to the limited solubility of the CPA in lipidic matrices in high ratios of CPA/lipids considerable expulsion was observed. Incorporation of the liquid lipid in formulations enhanced CPA solubility in lipidic matrices and further evaluations showed that maximum allowable CPA in these formulation with no expulsion is 2%.

Entrapment efficiency of CPA in NLCs, was very high as seen in Table 6 and these data are completely compatible with previos studies on NLCs (25, 30, 31).

Using of high percentage of surfactants, facilitate the drug disolution and increased the release rate. CPA release from all of these lipidic nanoparticles, was significantly slower than the permeation of the free drug in the same dissolusion media, which confirm that this delivery system are capable to control the release rate of the CPA. Drug release pattern from nanoparticles was best fitted to Higuchi model which is a suitable model for matrix based delivery sytems. Higuchi model of drug release was also reported in previos studies on lipid based nanoparticles delivery systems (32-34).

5. Conclusion

Solid lipid nanoparticles (SLNs) and nanostructured lipid carriers (NLCs) are lipid nanocarriers which are used to deliver both hydrophilic and lipophilic drug. Although potent drugs such as CPA would be considered as effective drug in various skin disorders but due to the limited water solubility of these compound, skin permeation of such therapeutic moeities is limited and topical application of conventional formulation does not have therapeutic value (22). On the other hand novel delivery system such as SLNs and NLCs have the advatage of targeting ability and also would be appropriate means to overcome limited solubility of such valuable therapeutic drugs. In this regard using such delivery systems enable us to formulate new delivery system to ovecome previous limitation of valuable potent drugs such CPA still limit the systemic adverse effects of such clinicaly important drugs. As mentioned earlier because of the important effect of nanoparticles size on skin permeation and follicular targeting, in this study size optimization and formulation optimization was done by Design-Expert software to obtain the desired particle size and optimum formulation. Also, nanoparticles characterizations such as drug loading and drug release was done for different particle sizes to distinguish the effect of size on these parameters and predict the optimum size for best clinical responses.

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Conflict of Interest

None declared.

6. References

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