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APPLICATION OF FACTORIAL DESIGN TO FORMULATE AND OPTIMIZE LOSARTAN POTASSIUM- LOADED SOLID LIPID NANOPARTICLES

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ABSTRACT
Losartan Potassium (LP) belongs to class III in biopharmaceutics classification system with antihypertensive activity. The aim of this study is to design Losartan Potassium loaded Solid lipid nanoparticles (LP-SLNs) and to optimize on their physio-chemical properties including particle size (PS), entrapment efficiency (%EE), zeta potential (ZP) and in-vitro release of drug. Losartan Potassium loaded solid lipid nanoparticles (LP-SLNs) were prepared by means of a w/o/w double emulsion solvent evaporation technique employing stearic acid alone or mixture of stearic acid and Geleol™ as a lipid matrix. A (2^3) non-randomized full factorial design was adopted to optimize LP-SLNs physicochemical properties. Three independent parameters were selected in this design including the lipid type, the concentration of polyvinyl alcohol (%w/v) and the amount of lipid (mg). The impacts of these parameters on particle size (PS), entrapment efficiency (%EE), zeta potential (ZP) and *in-vitro* release of drug were inspected. The designed preparations of LP-SLNs possessed a spherical shape with a particle size in the range of 294 nm to 671 nm and a negative zeta potential range from -18.04 to -8.02 mV. The % entrapment efficiency of LP-SLNs formulations were found to be in the range of 20.18 to 32.19. Fourier Transformation Infrared Spectroscopy (FTIR) revealed no possible interactions between LP and other additives while differential scanning calorimetric (DSC) manifested transformation of LP from crystalline to amorphous state in SLNs formulation. Stability studies of the chosen freeze dried LP-SLNs formulations showed a high stability during a period of three months.

KEYWORDS: Solid Lipid Nanoparticles, Losartan Potassium, factorial design, stearic acid, Geleol™

INTRODUCTION
Nanotechnology deals with the formation and development of small structures of nanometer scale size, with potential applications in biomedical and pharmaceutical fields. Pharmaceutical applications include nanomaterial-based drug delivery systems (DDSs) with its great potential to provide targeted and controlled drug delivery.[1,2]

Pharmaceutical Nanotechnology has played a vital role to overcome several drawbacks of macro-scale conventional dosage forms (e.g tablet and capsules). This is attributed to its different chemical, physical, and/or biological properties. This classical macro-scale dosage forms suffered from low and variable bioavailability, poor patient compliance, and numerous adverse effects which were corrected using pharmaceutical nanotechnology.[3,4] Nanosystems which commonly used in nanomedicine are polymeric nanoparticles[5], liposomes[6], metallic nanoparticles[7], dendrimers[8], etc.

Solid lipid nanoparticles (SLNs) are one of these nanosystems that were developed in 1990. SLNs are defined as a type of colloidal nano-particles which composed of lipids being solid at room temperatures.[9] The structure of SLNs include a hydrophobic solid core with a coating layer of phospholipids and the drug is either dissolved or dispersed inside the core according to drug type.[10-12]

The SLNs formulated from biocompatible and biodegradable materials are able to encapsulate both hydrophilic and hydrophobic drugs which make it a viable tool for controlled and targeted drug delivery.[10,13] SLNs showed desirable characteristics of biocompatibility, biodegradability, storage stability, limited toxicity, high surface area, extended drug release, drug protection from degradation, superior cellular uptake and improvement of drug solubility, permeability and bioavailability.[13,14]

Losartan potassium (LP) is an orally active, non-peptide angiotensin II receptor antagonist used for treatment of hypertension and heart failure control.[15] It acts as a specific AT1 receptor blocker resulting in a reduction in the pressor impact of angiotensin II.[16] It is clearly

obvious that LP has a pronounced action in comparison to angiotensin converting enzyme inhibitors and peptide receptor antagonists due to its improved tolerability, selectivity and specificity.[17]

LP is well absorbed from gastrointestinal tract after oral administration. Nevertheless, LP undergoes extensive first pass metabolism in liver with highly variable and poor oral bioavailability (approximately 25%–35%).[18] Due to its short elimination half-life (1.5–2 hours) and narrow therapeutic index, LP exhibits weak therapeutic efficacy.[18,19]

LP is freely soluble in water, soluble in isopropyl alcohol and slightly soluble in acetonitrile with a partition coefficient (octanol/water) equal 4.01.[20] Owing to its high solubility and low permeability, LP is considered as a class III drug in the biopharmaceutics classification system (BCS).[21]

It was reported that SLNs were excessively used for enhancing the bioavailability of drugs belonging to BCS Class-III by improving their permeability nature and avoiding hepatic first pass metabolism for examples SLNs encapsulating isoniazid[22], acyclovir[23] and zidovudine.[24]

LP is a good candidate for delivery by means of SLNs as this would enhance lymphatic uptake, avoid first pass metabolism in liver and thus improve bioavailability, reduce the dose necessity and minimize the side effects.[25,26] Moreover, prolonged drug release from LP loaded SLNs would reduce frequency of dosing and maintain drug level in plasma within the therapeutic window.

The first aim of this study is to design LP-loaded SLNs for treatment of hypertension. The second objective was to optimize on the physio-chemical properties of LP-loaded SLNs by studying the effect of different formulation parameters, such as the lipid type, lipid amount, and PVA concentration (%w/v) in the external phase.

MATERIALS AND METHODS

Materials

LP was obtained as a gift sample from Epico Pharmaceutical Industries, Cairo, Egypt. Stearic acid purchased from El-Nasr Pharmaceutical Chemical Co., Egypt. Geleol™ Mono and Diglyceride (glyceryl monostearate 40-55%) was received as a free sample from Gattefosse sas, France. The surfactants used are polyvinyl alcohol (PVA, M.wt 32,000-50,000 with a degree of hydrolysis equal 87-89%) (Sigma –Aldrich Chemical Co., USA) and Tween 80 (El-Nasr Pharmaceutical Chemical Co., Egypt). Methanol, dichloromethane (DCM), phosphate buffer saline (PBS) and acetonitrile (HPLC) were obtained from Sigma – Aldrich Chemical Co., USA.

Experimental design

In the current research, a (2^3) non-randomized full factorial design was applied for optimization of LP-loaded SLNs (Table 1). Three formulation parameters (A, B & C) with two levels for each parameter were studied; (A) lipid type (stearic acid alone and mixture of stearic acid and Geleol™ [3:1]), (B) lipid amount (300 mg and 400 mg) and (C) the concentration of PVA (0.5 and 1% w/v). The responses were Particle size (PS), Encapsulation efficiency (%EE), Zeta potential (ZP), and in-vitro release. Table 2 shows all possible formulations in this design.

Preparation of LP-SLNs

LP-loaded SLNs were formulated using w/o/w double emulsion solvent evaporation technique. The selected amount of lipid (stearic acid or mixture of stearic acid and Geleol™) was dissolved in 5 ml organic solvent mixture composed of Methanol: DCM (3: 2). Inner aqueous phase was prepared by dissolving Twenty five milligrams of LP in 1 ml aqueous solution containing 0. 25% v/v Tween 80. Both aqueous and organic phases were mixed and sonicated by means of ultrasonic homogenizer(Cole-Parmer Instrument Co., 4710 series, United States) supplied with 3.2mm probe at 70 W for 1 minute to form w/o emulsion. This primary emulsion (w/o) was gradually poured into 75 ml aqueous PVA solution at room temperature under high speed homogenizer (Virtis 23, The Virtis Company, Inc., Gardiner, USA) and then continued stirring for 2 hours to allow evaporation of organic solvent and formation of SLNs.[24]

In-Vitro Characterization

Particle surface morphology

Surface morphology of LP-loaded SLNs was characterized by means of transmission electron microscopy (JOEL, JEM- 100SX Electron Microscope, Japan). A sample of nanodispersion was loaded into a formvar film-coated grid and then subjected to air-drying to form a dry film.

Particle Size (PS) and polydispersity index (PDI)

The particle size and PDI of LP-SLNs were measured using a ZetaPALS Particle Size Analyzer with Software Ver. 5.23 (Brookhaven Instruments Corporation, USA). A sample of SLN dispersions was vortexed and then diluted (1:10 ratio) with double distilled water before measurement. All the measurements were conducted in triplicates and the mean was recorded.

Zeta Potential (ZP)

The surface charge of LP-SLNs was determined using a Zeta Potential Analyzer Ver. 5.59 (Brookhaven Instruments Corporation, USA). The system was kept at 25 °C. All the measurements were performed in triplicates and the mean was recorded.

Entrapment Efficiency (EE)

Entrapment efficiency was measured using an indirect method.[24,27] SLNs were separated via ultracentrifugation (Sigma 3-30KS, Sigma Laborzentrifugen GmbH., Germany) at 25,000 rpm for 30 minutes. The concentration of free LP in the collected supernatant was measured by a reported high-pressure liquid chromatography (HPLC) technique with some changes.[28] The HPLC system composes of Waters™ 486 Tunable Absorbance Detector, Waters™ 600 Controller and Waters™ 717 plus Autosampler (Water Corporation, USA). The mobile phase of potassium dihydrogen phosphate (0.025 M, pH 6.0): acetonitrile (65:35% v/v) was used at a flow rate of 1.5 ml/min with UV detection at 230 nm. The amount of LP entrapped in SLNs was calculated according to equation 1. All measurements were conducted in triplicates and the mean was recorded as the percentage of LP entrapment efficiency.

$$\% \text{ Entrapment efficiency} = \frac{total\ drug - free\ drug\ (supernatent)}{total\ drug} x100 \quad \text{(Equation 1)}$$

In vitro Release Study

An aliquot of LP-free drug solution in water and LP loaded SLNs (equivalent to 1 mg of LP) were placed into a 4 cm dialysis sac (cellulose tubing, M.wt. cut-off 12.000-14.000 Daltons, USA) sealed tightly at both the ends. The sac was soaked overnight in distilled water to ensure wetting and swelling of membrane. The dialysis bag containing the tested samples was dipped into 50 mL of PBS (pH 7.4). The release medium was stirred on a magnetic stirrer (Daihan, MSH-20A, Korea) at a speed of 150 rpm and kept at 37 ± 2°C. At different time intervals, samples (1ml) were taken from the release medium and replaced with 1 ml of fresh PBS.[27] These withdrawn samples were analyzed by the adopted HPLC assay.

Differential scanning calorimetric (DSC)

A DSC was an important tool to evaluate the physical state of drug before and after processing. The thermograms of LP, stearic acid, Geleol™, a physical mixture of equal amount of LP, stearic acid and Geleol™ (1:1:1) and LP-loaded SLNs (F5 and F8) were obtained using differential scanning calorimeter (DSC-60 Plus, Shimadzu Corporation, Japan). Data was analyzed by means of TA-60WS software. Approximately, 5 mg of each sample was heated in aluminum pans. The thermograms of each sample were obtained at a heating rate of 10°C/min, covering a heating range of 28-300 °C. Nitrogen used as an effluent gas.

Fourier Transformation Infrared Spectroscopy (FTIR)

Infrared spectra (IR) of LP, stearic acid, Geleol™, a physical mixture of equal amount of LP, stearic acid and Geleol™ (1:1:1) and LP-SLNs (F5 and F8) were obtained by means of a Bruker Tensor 27 FT-IR spectrometer (Bruker Optics GmbH, Ettlingen, Germany) supplied with DLATGS (Deuterated L-alanine-doped Triglycine Sulfate) detector. The samples and KBr were mixed thoroughly and then compressed into discs (KBr discs). These KBr discs were subjected to scanning from 4,000 to 250 cm−1.

Stability study

F5 and F8 were lyophilized in a Laboratory freeze dryer VaCo 5 (Zirbus, GmbH, Germany) by means of sucrose solution (2% w/v) as a cryoprotectant. Stability of freeze dried formulations (F5&F8) was performed under two temperatures (4°C and 25°C) for a period of one, two and three months. The mean ZP, %EE and PS were measured directly after lyophilization and after each month.[24,27]

Statistical analysis

Minitab statistical software (Minitab 17) was used to achieve statistical manipulation of the predetermined factors. The regression equations were obtained and the significance of each factor and interaction on the chosen responses were reported. The coefficient values of the selected factors were derived for optimization. Stability results were analyzed by means of one-way ANOVA (analysis of variance) with Tukey's post hoc test. Factors and interactions would be considered statistically significant if the p-value was < 0.05.

RESULTS AND DISCUSSION

Morphology of LP-SLNs

The surface morphology and shape of LP- SLNs formula (F5) were observed by transmission electron microscope (TEM) (Figure 1). The TEM image showed LP- SLNs with a spherical shape and smooth surface. LP- SLNs revealed acceptable PDI with no evidence of aggregation. Size measurements in accordance with TEM are comparable to results obtained by particle size analyzer.

Particle size and PDI

The average PDI and PS values for the tested preparations are summarized in Table 3. The size of the examined SLN formulations was in the range of 294 nm to 671 nm, while PDI values were in the range of 0.082 to 0.345.

It is reported that PDI range of 0.3 to 0.7 is acceptable. All PDI values for the tested formulations were below 0.35, indicating uniform size distribution and good homogeneity.[29]

The deduced linear regression equation for particle size (PS) was as follows:

Particle Size = 464.42 - 2.68 A + 56.63 B - 121.85 C + 1.13 AB - 12.66 BC - 2.56 AC - 14.43 ABC (Equation 2).

Two factors (B &C) were statistically significant (p-values< 0.05) whereas, factor A (lipid type) showed insignificant effect (p-value=0.571). All the interactions

exhibit significant effects except D and F (p-value equal 0.811 and 0.589 respectively). The effects of the selected factors on PS were graphically demonstrated using contour plots (Figure 2). According to the provided regression equation, the concentration of PVA (factor C) was the most effective factor on PS. The second and the third important parameters were factor B (lipid amount) and ABC, respectively. From these findings, F5 was selected as the optimum formula regarding PS (PS=294nm). F5 formula was prepared with a high concentration of PVA (1%w/v), low amount of lipid (300mg) and stearic acid as a lipid matrix.

The PS was decreased with increase in the concentration of PVA. This was attributed to the stabilization effect of surfactant molecules (PVA) during emulsification process. PVA forms a thick protective coat around particles which hinders their aggregation, leading to formation of nanoparticles with a small size. Also PVA imparts sufficient viscosity to the external aqueous phase which resists premature emulsion coalescence and particles aggregation.[24,30,31]

The PS was significally increased with increase in the lipid amount. This may be attributed to the disability of PVA solution to achieve stabilization for the emulsion at higher amount of lipid, leading to formation of particle aggregates (large size).[24] Moreover, at higher amount of lipid, viscosity of the organic phase was increased leading to decrease in the homogenization shearing capacity which is not enough for size reduction.[31,32]

Zeta potential

ZP is defined as the electric charge on the surface of particle which makes a repulsive barrier around particles and prevents particles coalescence. This repulsive effect is important to enhance emulsion stability.[33] The average ZP values for the tested preparations are demonstrated in Table 3. All the preparations exhibited a negative ZP in the range of -18.04 mv (F3) to -8.02mv (F6). These negative charges were attributed to the lipids chemical nature. Ionization of theses lipids (stearic acid and Geleol™) might be responsible for these negative charges.[24,34] Theoretically, these founded levels of ZP aren't enough to stabilize the SLN dispersion. Fortunately, the tested formulations showed a good stability which can be explained by the presence of PVA molecules that coat the particles and prevent their aggregation.[24,34]

The deduced linear regression equation for zeta potential (ZP) was as follows:

Zeta Potential = 12.596 + 0.047 A + 1.593 B - 2.983 C - 0.045 AB - 0.215 BC + 0.178 AC + 0.481 ABC (Equation 3).

All factors showed significant effects (p-values< 0.05) on ZP except factor A (p-value=0.814). All interactions exhibit insignificant impacts (p-values >0.05) except ABC (p-values< 0.05). The effects of the selected parameters on ZP were graphically demonstrated using contour plots (Figure 3). The most efficacious factor was the concentration of PVA (C) with a negative coefficient value followed by positive B (400mg lipid) and positive ABC, respectively. F3 with 0.5 %w/v PVA, 400 mg lipid and stearic acid as a lipid matrix was selected as the optimum formula regarding ZP (ZP= -18.04 mv).

It was found that ZP was decreased with increase in concentration of PVA. This was explained by coating of SLNs with PVA molecules, thereby shielding the charge on the surface of particles result in low ZP values.[31] While increasing lipid amount resulted in a significant raise in ZP. This can be attributed to the cumulation of the ionized negative charge of the lipid owing to increasing its abundance. This finding agrees with that reported by El-Gizawy et al.[27] and M. Nabi-Meibodi et al.[34]

Entrapment efficiency

Quantitative analysis of LP using HPLC method showed an accurate, precise, reproducible and reliable method of detection. This technique revealed a linear relationship between drug concentrations and peak areas (AUC) in the range of 2-50 µg/ml. The mean linear regression equation for LP calibration curves was Y= 10838(±102) X – 36097(±337) with a regression coefficient (R2) of 0.9999. Table 4 and table5 summarize the validation parameters of HPLC assay.

The mean %EE values for the tested SLNs preparations are reported in Table 3. The % EE of the examined nanodipersion ranged from 20.18 ± 0.89 (F1) to 32.19 ± 1.23 (F8).

The linear regression equation for %EE was as follows:

%EE = 26.341 + 1.585 A + 0.940 B + 3.076 C - 0.121 AB - 0.155 BC + 0.121 AC + 0.405 ABC (Equation 4).

All factors exhibited significant effects (p-values< 0.05) on % EE whereas, all interactions showed insignificant effects (p-values >0.05). Contour plots showing the impact of selected factors on % EE were shown in Figure 4. The most influential factor was the concentration of PVA (C) with a positive coefficient value followed by positive A (mixture of stearic acid and Geleol™) and positive B (400mg lipid), respectively.F8 with 1% PVA, 400 mg lipid and mixture of stearic acid and Geleol™ as a lipid matrix was chosen as the optimum formula regarding entrapment efficiency (%EE= 32.19 ± 1.23).

%EE was significally increased with increase in the PVA concentration. This may be explained by the increased viscosity of the external aqueous phase and the effective coating of particles by the polymer (PVA).[35] These aforementioned reasons are responsible for prevention of LP leaching in external aqueous phase which helps to enhance the EE of the drug. This finding agrees with that reported by Haggag et al.[31]

Increasing lipid amount exhibited a significant raise in %EE. This may be due to the availability of more lipid molecules (i.e. more space) to entrap more drug leading to enhancement of %EE. This finding is close to findings reported by Singh et al.[24]

It was found that using a binary mixture of lipids (stearic acid + Geleol™) makes a significant enhancement of %EE compared to using a single lipid matrix (stearic acid). This can be explained on the fact that the degree of lipid crystallization has a strong impact on the entrapment efficiency. Stearic acid usually forms highly crystalline lipid matrix with an ideal crystal lattice. This crystalline state expels the drug from nanoparticle core to its outer surface and then to the external aqueous phase, leading to decrease in %EE.[36] While blending Geleol™ (glyceryl monostearate 44-50%) with stearic acid (binary lipid mixture) resulted in a decrease in the degree of crystallinity of lipid phase and formation of imperfect crystal lattice with more space to encapsulate the drug. Analogous findings were reported by Ebrahimi et al.[37] and Rawat et al.[38]

In-vitro release

The release profiles of LP-SLNs and free drug solution in water are demonstrated in Figure 5. The kinetic orders, the correlation coefficient (R2) and the release rate constants (K) are reported in Table 6. The release rate constants (K) were selected as an index for release behavior. The average K (mg.hr -1/2) for the tested formulations ranged from 5.1855 ± 0.0827 (F4) to 6.7147 ± 0.0376 (F5). The correlation coefficient (R2) clearly showed that the best model depict LP release from LP-SLNs were the Higuchi model for all tested formulation (F1 to F8).

The stepwise linear regression equation for K was as follows:

K (rate constant) = 5.8784 - 0.3704 A - 0.2319 B + 0.3143 C - 0.0637 AB - 0.0393 BC - 0.0735 AC - 0.0910 ABC (Equation 5).

All factors and interactions displayed significant effects (p-values< 0.05) on K. Contour plots elucidating the effect of different factors on k were shown in Figure 6. Factor A (lipid type) was the most effective factor on K in accordance with the above regression equation. In the second and the third rank, factor C (PVA concentration) and factor B (lipid amount) were represented, respectively. It is clear that there is a good correlation between LP-SLNs size and their release behavior represented by K.[27] F4 exhibited the largest size and the slowest release (the lowest K), while F5 revealed the smallest size and the fastest release (the highest K).

Increasing PVA concentration results in a significant increase in K value whereas, decreasing lipid amount leads to a significant increase in the value of K. This is due to the fact that any parameter decreases mean SLNs size, such as high PVA concentration and low lipid amount (-ve level), is expected to increase specific surface area of SLNs and thus increase the rate of drug release from it.[27,31]

According to our results, it is obvious that SLNs fabricated from binary lipid mixture (stearic acid + Geleol™) have lower K values (slower release rate) than those fabricated from single lipid matrix (stearic acid). This may be attributed to the disruption of polymorphism and deformation of crystal lattice of stearic acid by Geleol™ lipid. These mentioned reasons result in more accommodation of drug in the binary mixture of lipid with depressed expulsion of the drug to the outer surface of SLNs and thus decrease drug leaking and control and prolong drug release (lower K values). Findings reported by Shah et al.[39] and Rawat et al.[38] support our findings.

Optimization of LP-SLNs physicochemical properties was conducted regarding to their PS, %EE, ZP and in-vitro drug release. The optimization design led to formulation of solid lipid nanoparticles with the targeted physicochemical properties. Formulation F5 was one of these optimized formulations which showed the smallest particle size (294 nm), relatively low PDI (0.294) and the highest release rate. Formulation F8 showed the highest %EE (32.19 ± 1.23), good PDI (0.306), slow drug release rate and acceptable particle size (368 nm) (within the targeted range). So F5 and F8 were chosen for further DSC, FTIR and stability study.

Differential scanning calorimetry (DSC)

The DSC thermograms of losartan potassium, stearic acid, Geleol™, physical mixture and LP-SLNs (F5&F8) are presented in Figure 7. The thermogram of LP showed a single sharp endothermic peak at 269.39°C corresponding to melting temperature of the drug.[21] This thermal behavior is close to data published by Amer et al.[40] Stearic acid showed a sharp melting endotherm at 58.22 °C whereas, Geleol™ showed endothermic peak at 64.51°C corresponding to their melting points. Moreover, physical mixture thermogram still shows the characteristics endotherm of the LP at 269.92 °C which reflects the crystalline state of the drug. By contrast, the thermogram of LP-SLNs F5and LP-SLNs F8 showed absence of endothermic peak of LP. This could suggest loss of LP crystallinity and the transformation to the amorphous form in SLNs formulation.[23,40]

Fourier Transformation Infrared Spectroscopy (FTIR)

The infrared study was used to investigate any possible interactions between drugs, lipids and other ingredients. Figure 8 shows the FTIR spectra of LP, stearic acid, Geleol™, physical mixture and LP-SLNs (F5&F8). The FTIR spectrum of LP (Fig. 8 a) showed a broad absorption band at 3,411cm−1 (tetrazole ring), a sharp peak at 1,463cm−1 due to imidazole ring and C=N stretch gives a peak at 1,417cm−1.[21,40] Physical mixture (Fig. 8 d) has shown all peaks of LP and the two lipids. LP-SLNs F5 and LP-SLNs F8 (Fig. 8 e and f) retained

main peaks of LP with decreased intensity that may be attributed to encapsulation of LP in SLNs and dilution effect of other excipients.[27] No additional peaks were recorded, suggesting absence of any interaction between LP and other components.

Stability study

The mean PS, %EE, and ZP of LP-SLNs F5 and F8 after storage at refrigerator temperature (4°C) and room temperature (25°C) for 3 months were shown in Table 7. According to the stability results, insignificant changes (P>0.05) regarding the values of PS, %EE, and ZP were noticed which indicated the good stability of LP-SLNs after freeze drying.

Table 1: Formulation variables of LP-SLNs Preparations.

Formulation Variables	Levels	
	Low	High
A= Lipid type	Stearic acid	Mixture of stearic acid and Geleol™ (3:1)
B= Lipid amount (mg)	300	400
C= Concentration of PVA (%w/v)	0.5	1
Code	-	+
Tested responses Constraints Y1= PS(nm) targeted (200-400nm) Y2= ZP(mv) targeted (more than -5 mv) Y3= %EE maximize Y4= K (mg.hr -1/2) minimize		

Table 2: (2^3) Full factorial design of LP-SLNs.

Formula	Codes			Lipid type	Lipid amount (mg)	Concentration of PVA (% w/v)
	A	B	C			
F1	_	_	_	Stearic acid	300	0.5
F2	+	_	_	Mixture of Stearic acid and Geleol (3:1)	300	0.5
F3	_	+	_	Stearic acid	400	0.5
F4	+	+	_	Mixture of Stearic acid and Geleol (3:1)	400	0.5
F5	_	_	+	Stearic acid	300	1
F6	+	_	+	Mixture of Stearic acid and Geleol (3:1)	300	1
F7	_	+	+	Stearic acid	400	1
F8	+	+	+	Mixture of Stearic acid and Geleol (3:1)	400	1

Table 3: *In-vitro* characteristics of LP-SLNs.

Formula	PS (nm)	ZP(mv)	PDI	%EE
F1	529 ± 11	-13.37 ± 0.95	0.345 ± 0.009	20.18 ± 0.89
F2	501 ± 26	-14.16 ± 0.71	0.341 ± 0.015	24.16 ± 1.03
F3	644 ± 22.8	-18.04 ± 1.37	0.273 ± 0.007	23.42 ± 0.77
F4	671 ± 16.5	-16.73 ± 1.33	0.325 ± 0.024	25.29 ± 0.69
F5	294 ± 34	-8.4 ± 0.44	0.294 ± 0.006	27.21 ± 1.31
F6	307 ± 17.2	-8.02 ± 0.56	0.082 ± 0.019	30.05 ± 0.91
F7	401 ± 31	-10.33 ± 0.51	0.310 ± 0.015	28.21 ± 1.05
F8	368 ± 8	-11.65 ±.1.24	0.306 ± 0.013	32.19 ± 1.23

Data are means ± S.D (n=3)

Table 4: Intraday validation parameters of losatan poatssium (n=3).

Nominal value (µg/ml)	Recovered concentration (µg/ml)	SD (µg/ml)	% RSD	%Recovery
2	1.950235	0.012385	0.635051	97.5117
5	5.088353	0.038362	0.753923	101.7671
10	10.002729	0.135713	1.356762	100.0273
20	20.058780	0.098011	0.488619	100.2939
30	29.880891	0.027718	0.092761	99.6029
40	39.602183	0.638325	1.611842	99.0054
50	50.353230	0.196620	0.390482	100.7065

Table 5: Inter-day validation parameters of losatan poatssium (n=3).

Nominal value (µg/ml)	Recovered concentration (µg/ml)	SD (µg/ml)	% RSD	%Recovery
2	2.071877	0.027623	1.333245	103.5938
5	5.160085	0.009682	0.187631	103.2016
10	9.995479	0.051699	0.517224	99.9547
20	19.687396	0.324613	1.648837	98.4369
30	29.884849	0.192205	0.643151	99.6161
40	40.113950	0.068057	0.169658	100.2848
50	50.081472	0.587609	1.173306	100.1629

Table 6: Release kinetics for LP-SLNs formulations.

Formula	Kinetic order	Correlation Coefficient (R^2)	Rate constant (K)
F1	Higuchi	0.9687 ± 0.003	6.0882 ±0.0081
F2	Higuchi	0.9718 ±0.0002	5.4787 ±0.098
F3	Higuchi	0.9861 ± 0.001	5.637 ±0.056
F4	Higuchi	0.9819 ± 0.01	5.1855±0.0827
F5	Higuchi	0.9559 ± 0.005	6.7147 ±0.0376
F6	Higuchi	0.9679 ±0.0007	6.1641±0.0219
F7	Higuchi	0.9702 ± 0.008	6.5589 ±0.0337
F8	Higuchi	0.9722 ± 0.004	5.3799 ±0.0514

Data are means ± SD (n=3).
Unit of K for Higuchi is (mg.hr $^{-1/2}$)

Table 7: Stability results of LP-SLNs (F5 and F8) for a period of 90 days.

F5						
Time (day)	PS(nm)		ZP(mv)		%EE	
	At 4°c	At 25°c	At 4°c	At 25°c	At 4°c	At 25°c
0	296.6±2.9	294.2±3.7	-8.26±2.3	-8.4 ±0.9	26.88±1.5	27.21 ±2.4
30	304±3.8	299.6±5.7	-8.05±1.9	-8.14±0.83	24.98±2.5	26.48 ±3.1
60	309±1.3	307.8±4.6	-8.46±1.1	-8.78±0.77	23.22±3.2	25.55 ±3.9
90	305.6±10.9	311.1±2.9	-8.56±1.7	-7.85±0.72	23.99±1.78	23.33 ±4.4
F8						
0	370.4±3.5	368.9±4.2	-11.9±1.8	-11.5±1.3	32.32±3.3	32.19 ±2.3
30	375.9±4.6	372.6±5.5	-10.8±1.6	-11.9±1.8	31.12±2.3	30.99 ±1.8
60	379.5±6.3	377.5±7.6	-10.3±1.2	-10.59±2	30.78±2.85	29.79 ±5.3
90	384.6±7.9	381.5±9.4	-10.9±0.9	-10.1±1.6	28.97±6.32	30.55±1.8

Values are expressed as means ±SD (n=3).

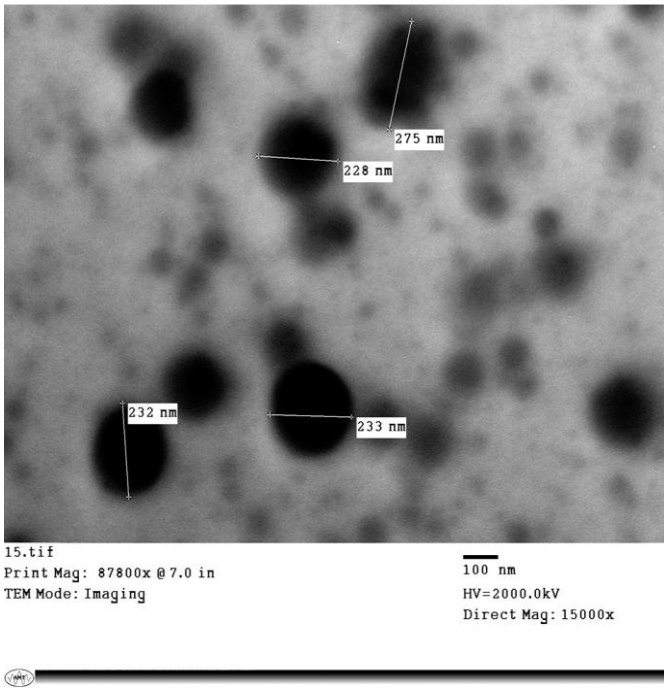

Figure 1: TEM image of LP-SLNs (F5).

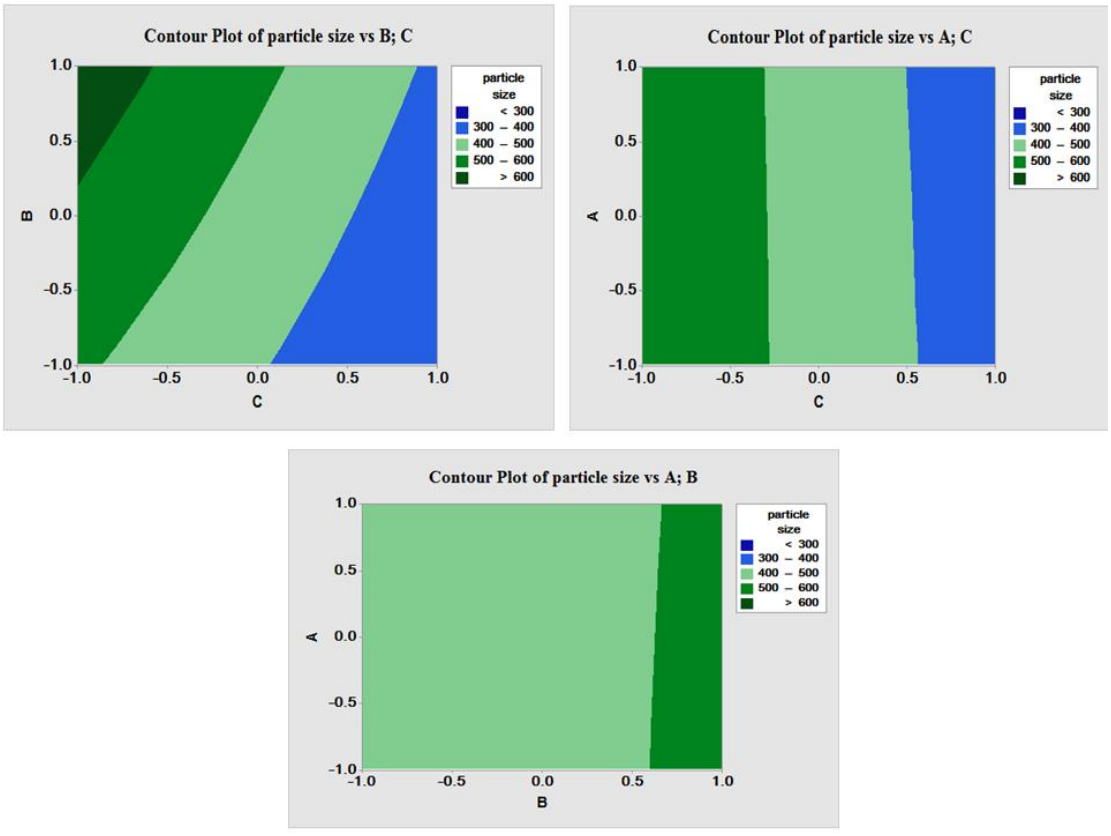

Figure 2: Contour plots describe the effect of independent variables (A, B & C) on PS response.

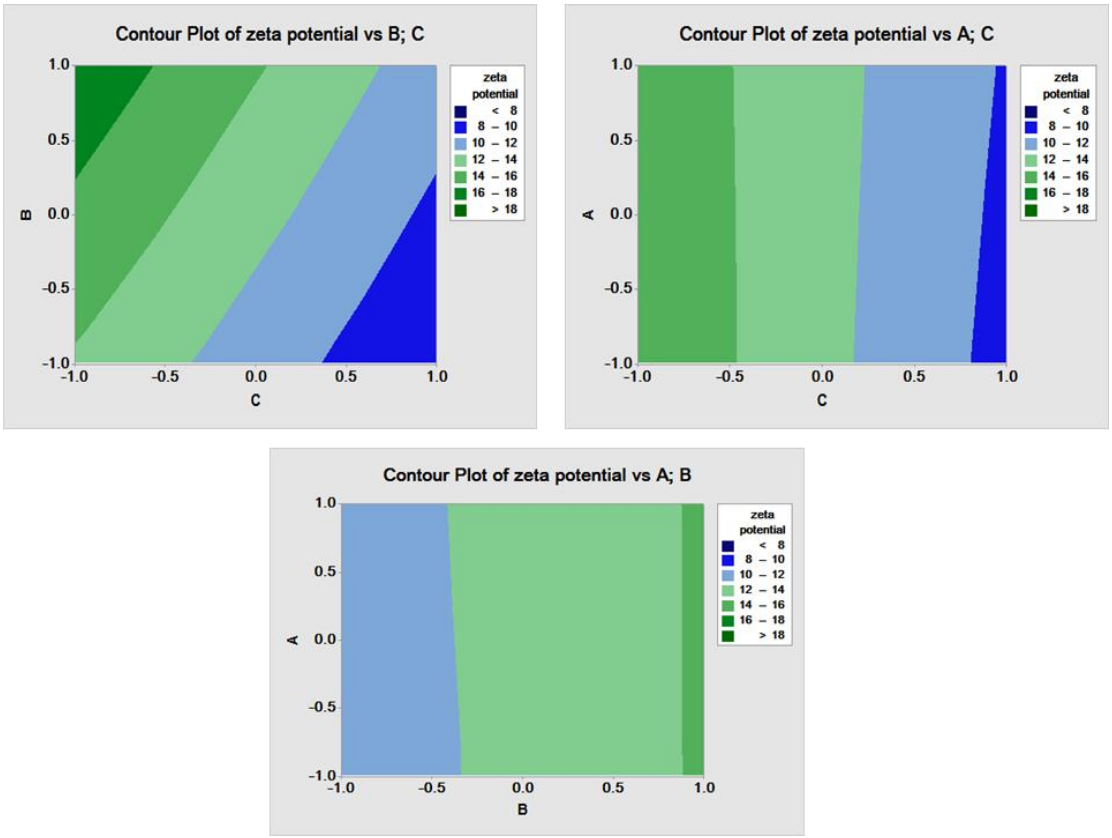

Figure 3: Contour plots show the impact of independent variables (A, B & C) on ZP response.

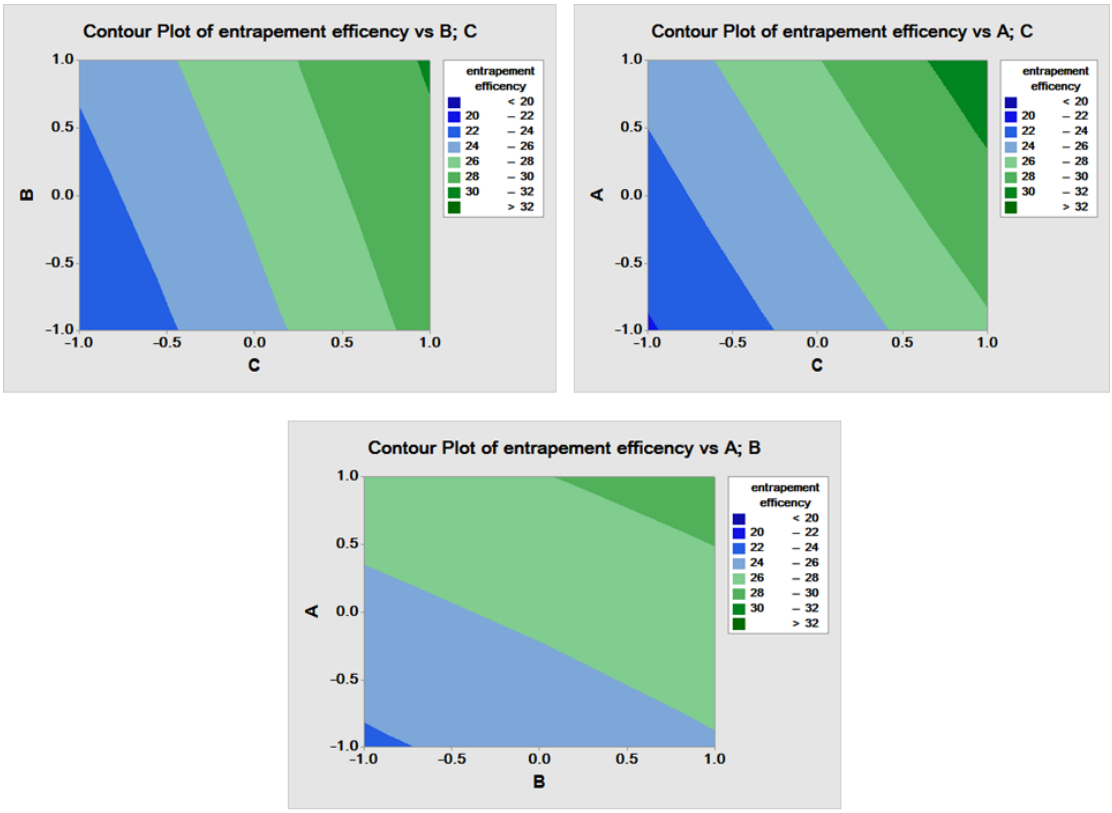

Figure 4: Contour plots describe the effect of independent variables (A, B & C) on %EE response.

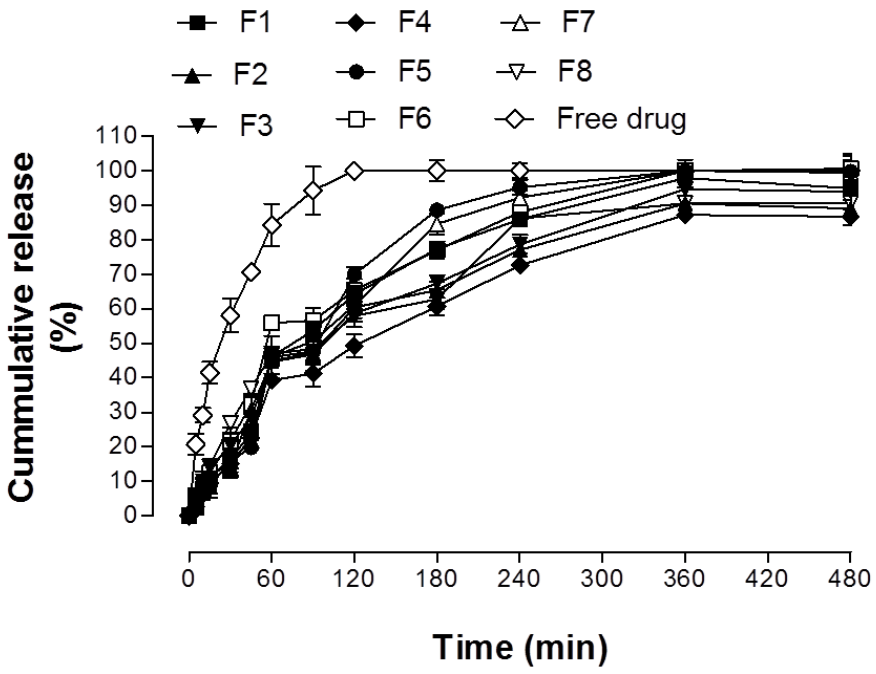

Figure 5: ***In-vitro*** **release profiles of LP free drug solution in water and LP-SLNs formulations.**

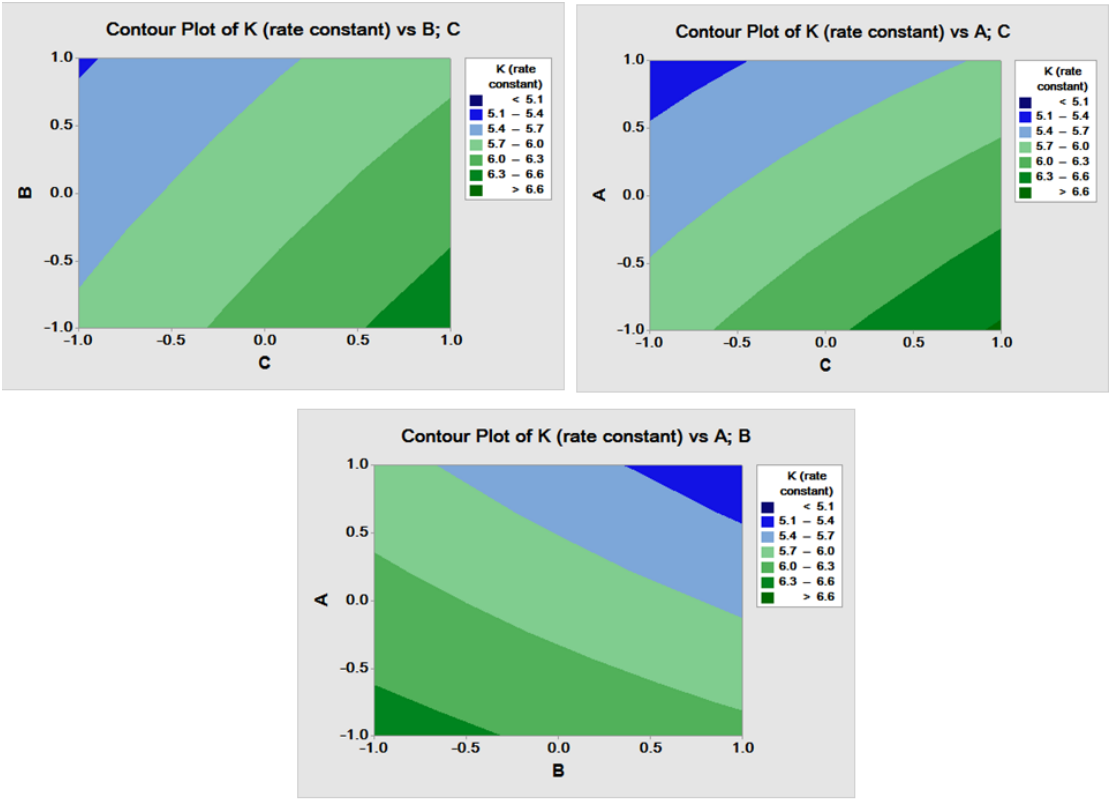

Figure 6: Contour plots describe the effect of independent variables (A, B & C) on K value.

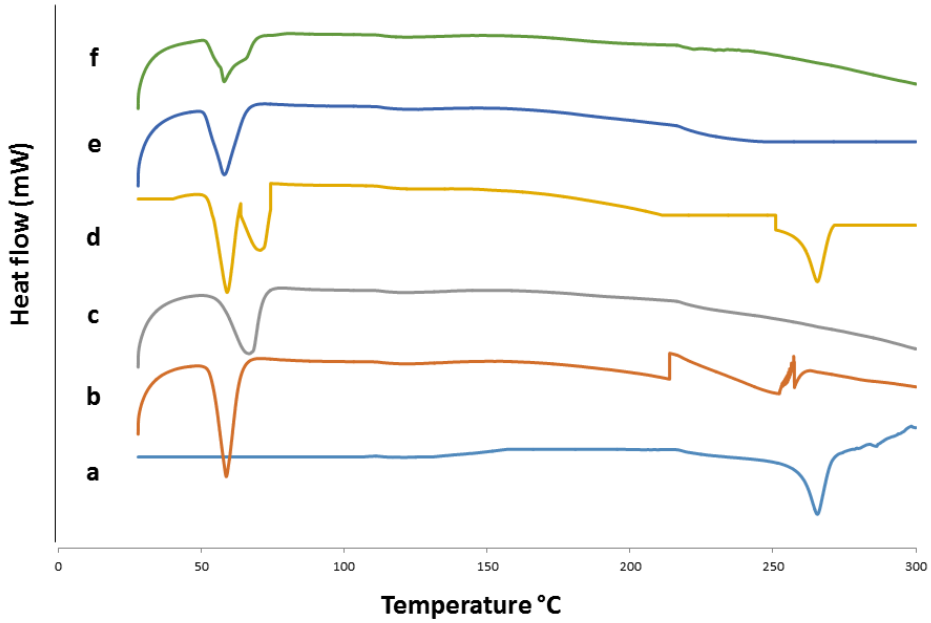

Figure 7: **The DSC thermograms of (a) LP, (b) stearic acid, (c) Geleol™, (d) physical mixture, (e) LP-SLNs F5 and (f) LP-SLNs F8.**

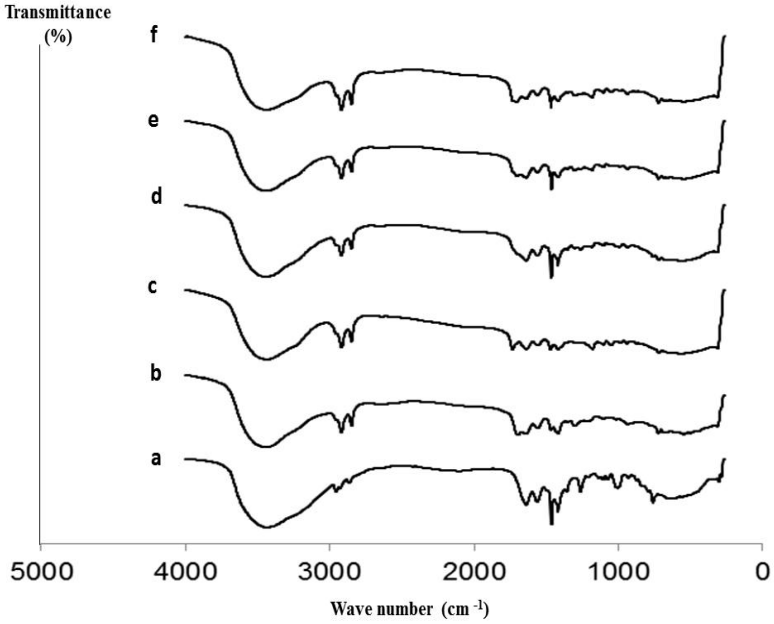

Figure 8: FTIR of (a) LP, (b) Stearic Acid, (c) Geleol™, (d) physical mixture, (e) LP-SLNs F5 and (f) LP-SLNs F8.

CONCLUSION

The LP-SLNs were prepared by w/o/w double emulsion solvent evaporation technique employing different formulation parameters of (lipid amount, lipid type and %w/v PVA concentrations). The optimization of LP-SLNs utilizing a full factorial design (2^3) was succeeded. The resulted SLNs have spherical shape with a relatively low PDI. LP-SLNs showed good physicochemical characteristics concerning mainly PS, %EE, ZP and *in-vitro* release. The compatibility of preparation components with LP was obvious from FTIR studies. DSC showed that LP lost its crystallinity and transformed to the amorphous state in LP-SLNs formulations. Stability studies of the chosen freeze dried

LP-SLNs formulations showed a high stability during a period of three months.

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