

Formulation and Characterization of Hydrophilic Drug Tramadol Hydrochloride Nanoparticles in Transdermal Drug Delivery System

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ABSTRACT

Objective: The aim of this study was to prepare gel containing Tramadol Hydrochloride Solid Lipid Nanoparticles (TRHC-SLNs) for Transdermal Drug Delivery System (TDDS).

Significance: Preparation of gel formulation containing solid lipid nanoparticles encapsulating Tramadol Hydrochloride was studied in order to use it as a topical analgesic agent in post-op and Cancer pain management without its addiction problems and short half-life.

Methods: The SLN formulations containing Tramadol Hydrochloride were prepared using glycerol monostearate (GMS) as the lipid matrix and soybean lecithin and tween 80 as the surfactant by Double Emulsification-Solvent Evaporation technique. The nanoparticles were optimized through a fractional factorial design. DPs were prepared by lyophilization technique. The morphology of the particles was examined using transmission electron microscopy. The *in vitro* drug release profiles were evaluated.

Results: The particle size, PDI, zeta potential, entrapment efficiency and drug loading capacity of the optimized SLNs were 197 ± 57.25 nm, 0.21 ± 0.013 , -19.8 ± 1.04 mV, $89.4 \pm 2.38\%$, $9.49 \pm 0.14\%$ respectively. TEM images revealed de-agglomerated particles. *In vitro* release studies showed sustained release of Tramadol and the release kinetics were best fitted to the first order kinetic model.

Conclusion: The results found here indicated that TRHC-SLNs could be successfully prepared and stabilized through freeze drying.

Keywords: Tramadol hydrochloride; Transdermal Drug Delivery System (TDDS); Solid Lipid Nanoparticles (SLN); Double emulsification-solvent evaporation

INTRODUCTION

Tramadol hydrochloride is a synthetic analgesic of aminocyclohexanols, which operates in both opioid and non-opioid ways, mostly used in post-ops and further to control the cancer pain. Its central mechanism is through the agonistic features of serotonergic and dopaminergic receptors and further prevention of their reuptake, and the opioid mechanism is through the μ receptors [1]. Shortly after oral consumption, its active metabolite (O-desmethyl Tramadol) (M1) which is a strong μ agonist, responsible for Tramadol addiction, is produced through First Pass Metabolism (FPM) in the liver [2]. Tramadol is 10 times stronger than Morphine also with fewer adverse effects profile, which makes it a good analgesic candidate.

Although it has less adverse effects than other opioids, its short half-life (4-6 hrs) and its opioid mechanism are count as disadvantages. It is best if its delivery system is modified in order to slow the metabolism to prevent the addiction and also in a control-released manner so that the patient compliance raise [3]. In this case Transdermal Drug Delivery System (TDDS) seems like a reasonable way to use Tramadol as a topical analgesic. This system is rather a new and non-invasive way that gives us a controlled release profile, which can lead to a controlled blood concentration of the drugs and further lessen the number of times that the drugs are consumed [4]. Although it is non-invasive and affordable, it can be further modified. One of the modifications is the use of Solid Lipid Nanoparticles (SLNs) in order to increase the system qualifications such as drug penetration.

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SLNs are introduced in 1990 as a replacement for emulsions, liposomes and polymeric nanoparticles, because of their tolerance in different conditions and their compatibility with human body [5]. This system is containing of spherical particles with 10-1000 nm in diameter. These particles have a lipophilic matrix-like core which is stabilized with the help of surfactants [6]. SLNs hold many benefits such as, the ability of interaction with both lipophilic and hydrophilic drugs, good bioavailability, protection from the chemical analysis of the drugs, controlling the drug delivery system, and the ability to be scaled-up [7]. SLNs can be used as carriers for Tramadol hydrochloride in TDDS in order to increase the penetration and the system output and further decrease the adverse effects.

In this study Tramadol-loaded SLNs were prepared using the double emulsification - solvent evaporation technique ($W_1/O/W_2$) and a Central composite experimental design was employed in order to produce optimized formulations in terms of different physicochemical characteristics.

MATERIALS AND METHODS

Materials

Glyceryl monostearate (GMS) was purchased from Sigma™ (St. Louis, MO, USA), Soybean lecithin; polyethylene glycol 400, tween 80 and sucrose were supplied by Samchun™ (Seoul, Korea). Sodium chloride, potassium chloride, disodium hydrogen phosphate, potassium dihydrogen phosphate and HPLC grade solvents including methanol, acetonitrile and acetic acid were obtained from Merck™ (Darmstadt, Germany). Tramadol Hydrochloride was provided from Zhengzhu Debo™ (China). Analytical grade water was provided using Milipore® water purification system. All other ingredients were of pharmaceutical grade and were used as received.

Methods

Preparation of tramadol hydrochloride-loaded SLNs: Tramadol hydrochloride-loaded SLNs were prepared by a double emulsion-solvent evaporation technique (DESE) [5]. Briefly, GMS and SL in various portions were dissolved in 5 ml dichloromethane (oil phase). Inner aqueous phase (w1) was prepared by dissolving portion of Tramadol hydrochloride in 2 mL of deionized water. The first aqueous (w1) phase was emulsified into the oil phase (containing lipids) using a high-shear homogenizer at 20000 rpm for 10 min. The w1/o emulsion was added using a syringe pump at a rate of 2 mL/min into 25 mL of aqueous Tween 80 solution under high-shear homogenization (20,000 rpm) for 15 minutes. After that, the organic solvent in the double emulsion was evaporated by a rotary evaporator to produce SLNs.

The nanoparticles were sedimented by centrifugation at 20,000 rpm using Beckman-Coulter Optima™ XPN-100 ultracentrifuge for 30 min at 25°C, and washed twice with double deionized water. The retrieved nanoparticles were re-suspended in 1 mL of 10% (w/v) sucrose and frozen at -20°C before finally being lyophilized at -40°C for 48 h (Table 1).

Quantification of tramadol by reversed phase HPLC: Tramadol HCl was quantified by previously reported reversed phase high

performance liquid chromatography (HPLC) method using Shimadzu LC-20AD HPLC system (Kyoto, Japan) equipped with SPD-10A VP UV-Visible detector and Shimadzu ODS C18 column (250 mm × 4.6 mm). The mobile phase was a mixture of water and acetonitrile (10:90) [8]. The pump flow was kept constant at 0.5 mL min⁻¹. Tramadol HCl was detected by its absorbance at 270 nm and the peaks were automatically integrated using lab solution® software. The method represented a good linearity between 1 and 100 µg mL⁻¹ with a mean correlation coefficient of 0.9991. The limit of detection (LOD) and limit of quantification (LOQ) was 0.11 µg mL⁻¹ and 0.9 µg mL⁻¹, determined by signal to noise ratio. Deionized water was used as the solvent for preparation of working standards of Tramadol HCl.

Characterization of nanoparticles: The mean particle size (Z-average) and polydispersity index (PDI) of nanoparticles and DPI formulations were determined by dynamic light scattering (DLS) using a Malvern® zetasizer-nanosizer (Malvern® instruments, United Kingdom). Zeta potential of nanoparticles was evaluated by the same instrument using electrophoretic mobility of nanoparticles using Smoluchowski's equation. All measurements were performed at ambient temperature of 25°C. The percentage of entrapment efficiency (EE%) and drug loading (DL%) were indirectly calculated by determination of the drug content in the clear supernatant which was obtained after centrifugation of freshly prepared colloidal nanoparticles. Analysis of the free drug content in the supernatant was performed by HPLC. Then after, EE% and DL% were calculated by reduction of un-entrapped drugs from total drug contents using following equations [9]:

Entrapment efficiency (%) = [(Mass of initial drug - mass of free drug)/mass of initial drug] × 100% (Equation 1)

Loading efficiency (%) = [(Mass of initial drug - mass of free drug)/weight of nanoparticles] × 100% (Equation 2)

Experimental design studies: The Central-composite design experiments were carried out in 13 sets with different values of independent variables. According to the fractional factorial experimental design, the amounts of tween 80 were kept constant at 1%, whereas, the amount of lipid to soy lecithin ratio and the drug were varied. Different experimental runs with various compositions as F₁ to F₁₃ were designed as shown in Table 2. The particle size and PDI of the prepared formulations were evaluated using Malvern™ Zetasizer - Nanosizer. All the measurements were performed in triplicate. All results were analyzed statistically using design-expert® Software (V.7.0.0, Stat-Ease, Inc. Minneapolis, USA). Formulations were experimentally prepared as designed by the software and the significance of interaction between variables was statistically evaluated using one-way analysis of variance (ANOVA).

In model fitting analysis, three of the responses, namely particle size (Y₁) and pDI (Y₂) of all formulations were treated by the software and for each response, best fitting mathematical model

Table 1: Ranges and constrains of variables used in experimental design.

Factors	Levels used	
Independent variables	1	-1
A=GMS (mg)	58.5	10
B=Drug (mg)	34.14	5.86

Table 2: Central composite experimental design (n=13).

No	Independent Variables		Dependent Variables			
	A: lipid/lecithin	B: Drug (mg)	Size (nm)	PDI	Zeta (mV)	%EE
F ₁	1	30	352 ± 32	352 ± 32	-22.70 ± 2.52	88.20%
F ₂	0.2	30	253 ± 27	253 ± 27	-5.40 ± 3.37	85.40%
F ₃	0.6	5.86	1075 ± 19.8	1075 ± 19.8	-16.40 ± 1.48	84.70%
F ₄	0.6	20	257 ± 15.9	257 ± 15.9	-14.60 ± 5.21	90.30%
F ₅	0.6	20	235 ± 26.5	235 ± 26.5	-13.80 ± 2.21	99.10%
F ₆	0.6	20	241 ± 20.5	241 ± 20.5	-14.10 ± 4.20	85.70%
F ₇	0.03	20	143 ± 26.7	143 ± 26.7	-3.70 ± 3.61	91.40%
F ₈	0.6	20	252 ± 24.7	252 ± 24.7	-14.70 ± 3.21	98.60%
F ₉	0.2	10	371 ± 24.6	371 ± 24.6	-10.70 ± 3.62	87.50%
F ₁₀	1	10	843 ± 25.9	843 ± 25.9	-34.25 ± 4.33	88.10%
F ₁₁	1.17	20	677 ± 14.61	677 ± 14.61	-31.10 ± 2.50	93.40%
F ₁₂	0.6	34.14	344 ± 12.4	344 ± 12.4	-12.10 ± 4.42	95.90%
F ₁₃	0.6	20	237 ± 24.6	237 ± 24.6	-13.90 ± 2.21	89.90%

was determined on the basis of comparison of several statistical parameters including coefficient of variation (CV), R-Squared (R²), adjusted R-Squared (adjusted R²) and adequate precision provided by design-expert® software. The level of significance was considered at p<0.05. Co-efficient of each significant effect was further used to develop a reduced equation by step-wise multiple regression analysis. Some of the interactions between independent variables were visually explained by using 3-D response surface plots.

Model validation: In order to validate the experimental model and evaluation of prediction errors, the optimized formulation suggested by the software was prepared experimentally in five times and characterized in terms of particle size, polydispersity index (PDI), zeta potential (mV), entrapment efficiency (EE%) and drug loading (DL%) using Malvern Zetasizer - Nanosizer and HPLC as previously discussed.

Freeze drying of nanoparticles: Freeze drying of SLNs was performed on experimentally prepared optimized formulation of nanoparticles. Before freeze-drying process, the settled down optimized nanoparticles were reconstituted by sucrose 5% (w/v) as the cryo-protectant. Then, the re-dispersed nanoparticles were freeze-dried using Operon™ freeze dryer (FDB 5503, Korea). After freeze drying, the lyophilized SLNs were reconstituted in mili-Q water for re-evaluation of parameters including particle size, PDI and zeta potential. The significance of the effects of the lyophilization process on physico-chemical characteristics of nanoparticles was also studied by comparing the characteristics of nanoparticles before and after lyophilization using two sample independent t-test statistical analyses performed by SPSS® software (V16.0.0).

Determination of the morphology of the particles: Lyophilized nanoparticles were examined by transmission electron microscopy (TEM) performed by a Zeiss EM10C 100kV, Germany. Samples for TEM were diluted in deionized water and the sonicated with Misonix S3000, USA for 5 minutes. Then the samples got coated with Holey carbon coated grid Cu Mesh 300 and were placed under TEM and were examined.

In vitro release studies: The optimized and experimentally developed formulation of SLN was subjected to *in vitro* release

studies by using a dialysis bag under sink condition at 37 ± 2°C in phosphate buffer saline (PBS, pH=5.6) (the acidic pH of dermis). Proper volume of Tramadol-SLNs colloidal suspension equivalent to 2.5 mg of Tramadol was placed in dialysis bag (MWCO 12 KDa). The dialysis bag was subsequently immersed in 200 ml of the pre heated PBS and incubated at 37 ± 2°C while stirring at 100 rpm using Heidolph™ shaker incubator (Heidolph™ Unimax 1010 / Incubator 1000, Germany). At predetermined time intervals, 1ml of release medium was withdrawn and was replaced by the equal volume of freshly prepared pre-heated buffer. Drug concentration in the samples was determined using HPLC (Shimadzu, LC-20AD) at 270 nm. All experiments were carried out in triplicates.

In order to evaluate the release kinetic of nanoparticles, release data were fitted to various release models such as zero order, first order, Higuchi, Korsmeyer-Peppas and Hixson Crowell and their appropriate correlation coefficients were investigated using Sigma-plot® software (version 10.0.0.54).

STATISTICAL ANALYSIS

In this study all experiments were performed in triplicate except otherwise stated which were carried out experimentally in five times. Comparison of two groups of data performed using two sample independent t-test by SPSS® software (V.16.0). Fractional factorial design and model fitting were accomplished using design expert® software (V.7.0.0). The significance level was set as 0.05.

RESULTS AND DISCUSSION

Preparation and characterization of solid lipid nanoparticles

Selection of a suitable technique for preparation of SLNs depends on the physicochemical characteristics of the drug to be encapsulated. While numerous methods have been successfully developed for the incorporation of lipid-soluble compounds into SLNs, producing formulations consisting of lipid-insoluble drugs is more problematic. Drugs with poor lipid solubility are substantially expelled from the hydrophobic matrix into the dispersing aqueous

phase during SLN preparation. The DESE method in which the inner aqueous phase serves as a reservoir is typically employed to encapsulate water-soluble drugs that are not soluble in organic phase, whereas the oil phase plays the role of a diffusion barrier, preventing drug leakage from internal to external aqueous phase. The DESE method was employed for loading of tramadol hydrochloride into SLNs.

In this study, Central-composite design was employed for preparation of solid lipid nanoparticles to evaluate all main effective and possible binary interactions to determine which independent variables and interactions have significant influence on the defined responses.

In this case, 13 formulations of Tramadol-loaded SLNs were fabricated by Double emulsification - Solvent evaporation method. The values of independent variables and the related experimental data in suggested formulations (i.e., F1-F13) are summarized in Table 2.

In this study, analysis of responses performed using design-expert software showed that dependent variables including particle size (Y_1), pDI (Y_2) and zeta potential (Y_3) were fitted to quadratic models with the model p-value of 0.0002 and <0.0001, respectively. The values of R^2 , adjusted R^2 , Adeq precision, SD and CV% are summarized in Table 3.

Size of nanoparticles

The particle size with mean diameter ranging, were between 235 ± 26.5 (Run 5) and 1075 ± 19.8 nm (Run 3) as shown in Table 2. Statistical analysis performed by Design-Expert® based on central-composite design was applied to establish the best significant fitted model for prediction of size of particles. The characteristics of fitted model are summarized in Table 3. Analysis of variance for data revealed that the linear coefficients of all independent factors except for the interaction coefficients of A.B were significant ($p < 0.05$). The coefficients of significant variables on particle size (Y_1) have shown in Eq. 3 as follows:

$$Y_1 = + 283.74 + (165.77 \times A) - (205.35 \times B) - (93.25 \times A \times B) + (198.92 \times B^2) \quad \text{(Equation 3)}$$

where:

Y_1 : Particle size

A: Coefficient of GMS/ Lecithin concentration ratio

B: The amount of drug

B^2 : Second power square of drug

AB: Interaction of Coefficient of GMS/Lecithin concentration ratio and the amount of the drug

As shown in the equation, the coefficient of GMS/Lecithin and the amount of drug and its second power square and also the interaction of both of the factors (AB), showed a positive effect on size of

nanoparticles (Y_1), but it is undeniable that the coefficient of GMS/ Lecithin interaction effect is the highest. It means that particle size would expect to be increased with increasing concentration ratio of GMS/Lecithin (A). The effect of Glyceryl Monostearate (GMS) itself on the size is undeniable and by increasing the GMS/Lecithin ratio up to 5% to 10%, the particle size rise in a large amount (even up to micro particles). GMS as the central core, forms the lipid matrix of the SLNs. Therefore, it is sensible that an increased particle size can be observed with higher concentration ratios of GMS/lecithin [10]. This phenomenon was in well accordance with previous studies which showed the dependency of the size of SLN on the concentration of GMS as the lipid matrix and it was previously reported that increased amount of GMS caused an increase in particle size which can be explained in terms of tendency of the lipid to coalesce at high concentration [11] (Figure 1). Based on Stoke's law, this behavior can be defined by the difference in density between internal and external phase. Moreover, the study of Mehnert and Mader demonstrated that particle size of SLN would be increased by increasing the lipid content of the nanoparticles [12]. This can be due to a decrease in homogenization efficiency by increasing the viscosity of inner phase followed by increasing the lipid concentration [13]. Moreover, at lower amounts of lipids, the ability of surfactant to stabilize particles could be enhanced [14]. In order to study the interaction patterns between variables, 3D response surface curves were plotted by model prediction of the particle size (Y_1) at different levels of effective two variables while keeping the other variables at their center levels (Figure 2) as it is seen in the curve, by increasing the amount of drug no matter what is the ratio of the GMS/Lecithin is, at first the particle size decreases enormously until an equilibrium point. After that a slight increase is seen in the particle size. It is also shown that the least particle size is when the amount of drug is in the average area (i.e., 20.0 mg) when we use the least constant amount of drug (i.e., 5.86 mg), by increasing the GMS/Lecithin ratio the particle size increases slightly, but there isn't any meaningful changes in the particle size when the ratio is increasing at the higher amount of drug. The largest particle size is seen when we use the highest GMS/Lecithin ratio (i.e., 1.0) and the lowest amount of the drug.

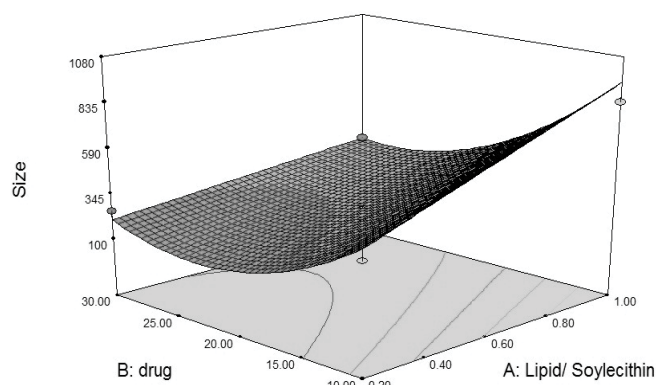


Figure 1: 3D plots of effective binary interactions on particle size.

Table 3: Characteristics of best fitted mathematical model.

Dependent variable (responses)	Best fitted model	Lack of fit	Adeq percision	Pred R-squared	Adj R-squared	R-squared
Size (Y_1)	Quadratic	Significant	15.741	0.7024	0.8864	0.9243
PdI (Y_2)	Quadratic	Insignificant ($p > 0.1$)	22.288	0.8516	0.9499	0.9666

Table 4: Optimized independent variables and predicted responses.

Desirability	Predicted dependent Variables (Responses)			Optimized independent variables		No
	Y_3 =Zeta	Y_2 =Pdl	Y_1 =Size (nm)	Drug (B)	Lipid/lecithin (A)	
0.744	-10	0.361	351.91	12.62	0.27	1

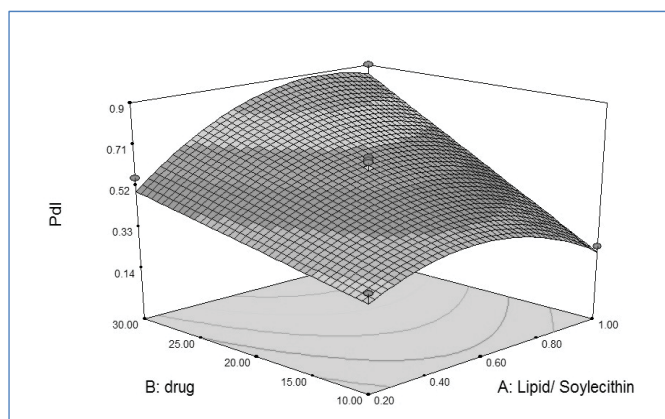


Figure 2: 3D plots of effective binary interactions on pdI.

It is notable that the amount of surfactant (Tween 80) was constant (i.e., 1%) in all of the sample runs.

Polydispersity index (PdI) of nanoparticles

As shown in Table 2, the experimentally observed PdI is ranged from 0.145 ± 0.002 to 0.899 ± 0.001 . Homogeneity of nano-suspension becomes higher as the PdI approach to zero [15]. Statistical analysis performed by Design-Expert® based on Central composite design was applied to establish the best significant fitted model for prediction of PdI. The characteristics of the best fitted model are summarized in Table 3. Analysis of variance for data revealed the linear coefficients of all independent factors were significant ($P < 0.05$). The coefficients of significant variables on pdI (Y_2) have shown in Eq. 4 as follows:

$$Y_2 = +0.62 + (0.072 \times A) + (0.21 \times B) + (0.11 \times A \times B) - (0.16 \times A^2) \quad (\text{Equation 4})$$

Where:

Y_2 : pdI of particles

A: Coefficient of GMS/ Lecithin concentration ratio

B: The amount of drug

A^2 : Second power square of GMS/Lecithin ratio

As it is shown in the equation the coefficient of GMS/Lecithin concentration and its second power square and also the amount of the drug has a positive effect on the particles pdI. In order to study the interaction patterns between variables, 3D response surface curves were plotted by model prediction of the pdI (Y_2) at different levels of effective two variables while keeping the other variables at their center levels (Figure 2) in the areas where the GMS/Lecithin ratio is low, there is a slight increase in particle pdI when the drug is getting higher. And at the highest drug amount (i.e., 30.0 mg) the slope is also getting high. And despite the drug amount, increasing in the GMS/Lecithin ratio leads to a greater pdI at the beginning and its decrease in the end. The second power square of the lipid/SL ratio (A^2) had a significant effect on the PdI which explains the importance of the lipid amount on the heterogeneity of particles.

Table 5: The observed responses for predicted optimized formulations (n=3).

Dependent Variable	Predicted Response	Experimental Response	Predicted Error
size	220.67	131	12.01%
PDI	0.23	0.21	10.48%
Zeta	-15.9	-19.8	19.70%
EE	91.30%	89.40%	2.12%

Also when the greatest drug (i.e., 30 mg) is used and the GMS/Lecithin ratio is the highest (i.e., 1.0) the particle pdI is also at its greatest amount.

Optimization and model validation

The optimization of the physicochemical characteristics of SLN was carried out according to statistical analysis of experimentally obtained data using the Central composite design. The optimized and predicted conditions for preparation of SLN are shown in Table 4. To determine the model validation and calculation of the appropriate prediction error, the suggested optimized formulation were prepared and characterized experimentally ($n=6$). The observed responses and value of predicted errors are indicated in Table 5. As shown in the table, the calculated prediction errors were well below 10% for all items demonstrating the proper predictability, efficiency and adequacy of the fitted models.

Entrapment efficiency (EE%), drug loading (DL%) and zeta potential are considered as the most important physicochemical characteristics of submicron systems. Accordingly, EE% of optimized SLNs formulation was calculated and determined as high as $89.4 \pm 2.38\%$ which demonstrates that Tramadol HCl can be successfully loaded into the nanostructures. Drug loading (DL%) is also an effective parameter in determining the suitability of a drug carrier system which is ordinarily defined in percent related to the lipid phase that was determined to be 9.74 ± 1.62 (Table 5). Zeta potential of the particles is considered as the well indicator for prediction of the stability of the colloidal dispersion [15]. Accordingly, particle aggregation is less likely to occur in high zeta potentials (either positive or negative) due to high electrostatic repulsion force between particles [16]. Therefore, nanoparticles with zeta potential values greater than +20 mV or less than -20 mV exhibit high degrees of stability [17]. As shown in Table 5, zeta potential of the optimized SLNs was determined as -19.8 ± 1.04 mV which can ensure proper stability for the Tramadol HCl loaded SLN. According to the previous studies, the negative charge of zeta potential is related to the lipids that incorporate into SLN structure [18,19]. In this study, surface accumulation of GMS and soy lecithin as the main lipids in structures of SLN lead to high negative zeta potential of particles (Table 6).

Lyophilization of nanoparticles

The effect of freeze drying process in the presence of the lyoprotectant

(i.e. sucrose 5% w/v) on physico-chemical characteristics including particle size, PDI and zeta potential of nanoparticles was investigated and the appropriate results are illustrated in Table 3. Previous studies revealed that di-saccharides such as sucrose are more efficient cryo-protectant compared to mono-saccharides such as mannitol, sorbitol and terhalose and consequently exhibit higher efficiency in conserving the physico-chemical features of nanoparticles during lyophilization [20].

As shown in Table 7, although the size of nanoparticles was slightly increased from $(131 \pm 61.31 \text{ nm})$ to $(197 \pm 57.25 \text{ nm})$ during lyophilization, but statistical analysis of data using two independent sample t-test revealed no significant difference in size of nanoparticles before and after freeze-drying ($p \text{ value} > 0.05$). Table 7 revealed that the PDI of nanoparticles was significantly increased from (0.21 ± 0.013) to (0.22 ± 0.018) during the lyophilization ($p \text{ value} < 0.05$). Determination of zeta potential is an effective method to consider the eventual interactions between the cryo-protectant molecules and the nanoparticles surface [21,22]. It was showed that the zeta potential of the particles were significantly decreased from $(-19.8 \pm$

Table 6: Parameters of drug release kinetics.

Model	Equation	R ² Value Total Release (%)	Adjusted R ² Value (%)
Zero order	$Q=Kt$	0.873	0.733
First order	$Q_0 - Q=Kt$	0.891	0.736
Hixon-Crowell	${}^3\sqrt{Q_0} - {}^3\sqrt{Q}=K_{HC}t$	0	0
Higuchi	$Q=K_H t^{1/2}$	0.848	0.694
Korsmeyer-peppas	$\text{Log}Q=\text{Log}K + n\text{Log}t$	0.891	0.768

Table 7: Influence of lyophilization on SLNs characteristics, a) particle size (nm) b) pDI c) Zeta potential (mV).

SLNs Characteristics	Before Lyophilization	After Lyophilization
Particle size	$131 \pm 61.31 \text{ nm}$	$191 \pm 57.25 \text{ nm}$
PDI	0.21 ± 0.017	0.22 ± 0.018
Zeta potential	$-19.8 \pm 1.04 \text{ mV}$	$-37.4 \pm 1.98 \text{ mV}$

Data represent Mean \pm SD, n=3. *Results are significantly different, $p < 0.05$.

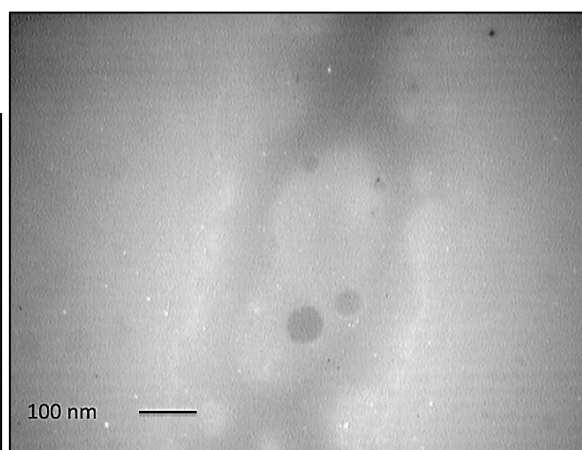
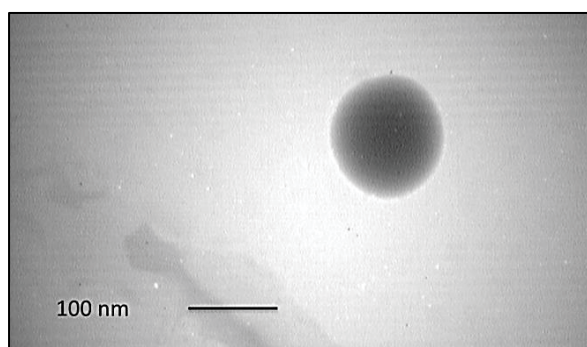


Figure 4: TEM images of nanoparticles.

1.04 mV to $-37.4 \pm 1.98 \text{ mV}$) ($p \text{ value} < 0.05$) due to accumulation of sucrose as lyoprotectant to the surface of the nanoparticles due to establishment of hydrogen bonds and masking the negative charges of lipids (Figure 3) [23,24].

In vitro release study

The *in vitro* release of Tramadol HCl from optimized nanoparticle formulation was evaluated in phosphate buffer saline (PBS) adjusted to a pH value of 5.6 (equal to the skin's pH). The results are illustrated in Figure 3. As shown in the figure, $30.01 \pm 2.48\%$ and $41.34 \pm 5.76\%$ of entrapped Tramadol HCl was released in 2 h and 24 hr, respectively from optimized SLN formulation indicating a slow and sustained release behavior. Similarly, various studies reported slow and sustained release behavior of drugs encapsulated into SLN preparations [25-28]. Therefore, SLNs are suggested as the suitable carriers for prolonged and sustained drug release and this can be achieved when drug is homogenously dispersed into the lipid matrix and can only be released through diffusion [29]. Moreover, in the study performed by Kushwaha et al. it was suggested that the slow release of drug from solid lipid nanoparticles may be due to increased diffusional distance and hindrance effects of lipid shells which does not allow surrounding aqueous medium to penetrate inside the particles and release the encapsulated through dissolution mechanism [28].

The drug release data were fitted to various mathematical kinetic models including zero order, first order, Higuchi, Hixon-Crowell and Korsmeyer-Pepas using Sigma-plot® software (version 10.0.0.54). As shown in Table 7, release kinetic of both optimized SLN formulation and DPI preparation were best fitted to the first

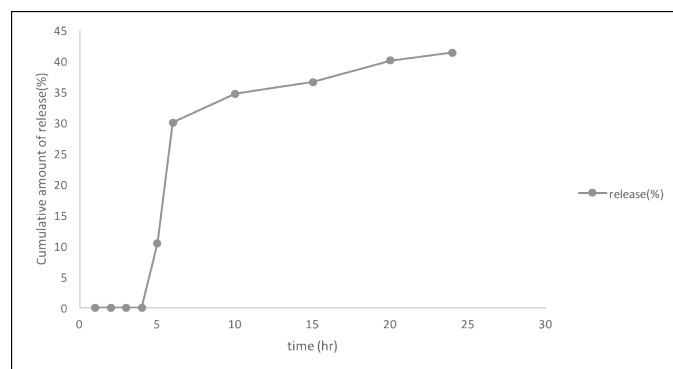


Figure 3: Cumulative Tramadol HCl release profile from solid lipid nanoparticles formulation (pH=5.6) (n=3).

order kinetic model. In this study, the sustained release of Tramadol HCl from the optimized SLNs describes the diffusion of drug from homogenous matrix system which is in well accordance with Fickian diffusion mechanism explained by the first order release kinetic model. Studies performed by Priyanka et al. revealed first order release kinetic of all montelukast-loaded SLNs formulations. The study of Kakkar et al. showed first order release kinetic of curcumin from SLNs [29,30].

Morphology of particles

Transmission electron micrographs of SLNs formulations are illustrated in Figure 4. As shown in Figure 4, TEM images revealed a spherical shape and smooth surface particle with diameters in accordance with data obtained by photon correlation spectroscopy (PCS).

CONCLUSION

This study focuses on the preparation and *in vitro* characterization of SLN containing Tramadol HCl optimized by Central composite design. The effects of formulation variables including concentration ratio of GMS/lecithin, concentration of tween 80 along with emulsification time and cooling time on physicochemical properties of nanoparticles were also studied. Optimized nanoparticles were characterized as smallest in size and lowest in PDI. Morphological study of nanoparticles revealed formation of non-aggregated, uniformly sized and spherical shape particles with smooth surfaces. Lyophilization technique was employed successfully to stabilize nanoparticles for the preparation of topical anesthetic gels. *In vitro* release studies were performed on nano-suspensions preparation containing solid lipid nanoparticles encapsulating Tramadol HCl and the results have showed sustained release profile of Tramadol HCl from nanoparticles up to 48 hours and the kinetic of release was best fitted to of first order kinetic model.

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DECLARATION OF INTEREST

The authors report no declaration of interest.

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