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Formulation and Evaluation of Nanoparticles of an Antianginal Drug for Transdermal Delivery

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Abstract: Solid Lipid Nanoparticles of Nifedipine were prepared by using Solvent emulsification evaporation method by using Compritol 888 ATO as Lipid, Span 60 and Tween 80 as Surfactants. The optimization was carried out using design expert software. The Central composite design (CCD) was selected to study the influence of critical formulation parameters. The prepared nanoparticles were evaluated for particle size, poly dispersity index, zeta potential, entrapment efficiency, drug content and In-vitro drug release studies using statistical methods. The particle size and entrapment efficiency of the nanoparticles was found to increase with increase in Compritol concentration and decrease in particle size with increasing tween 80. The optimized concentration for independent variables Compritol 888 ATO (X_1) and Span 60/Tween 80 (X_2) was found to be 2gm and 2gm respectively, with a desirability of 0.842 by Numerical optimization method. This was found to be in close agreement with the experimental response of formulation F1. It was concluded that the solid lipid nanoparticles of Nifedipine can be successfully formulated by solvent emulsification evaporation method.

Key words: Nanoparticles; Nifedipine; Compritol 888 ATO; Span 60; Tween 80; Optimization etc...

INTRODUCTION

Nanotechnology plays a significant role in advanced medicine/drug formulations, target specific delivery and controlled drug release. This can be consequently led to the improvement and development of convenient administration of drug, lower toxicity, fewer side effects, improved bio-distribution and extended drug life cycle. (1)

Transdermal route is developing as a greatly accepted route of drug administration due to its ability to apply the drug to the site of action without disrupting the skin membrane. The transdermal drug delivery system helps in the drug absorption via the skin which provides many advantages over conventional administration routes such as oral or intravenous administration for systemic and local drug delivery. In addition, it minimizes drug loss from first pass effect of the liver therefore delivering therapeutic drugs at a controlled rate. (2)

Stratum corneum acts as a natural barrier for drug delivery. This limits the transport of drugs. Nanocarriers are able to alter the stratum corneum as a function of shape, size, surface charges, and hydrophilicity lipophilicity balance, during drug delivery across the skin barrier. These nanocarriers shows a great potential for transdermal delivery. The nanocarriers can be classified into polymer based and lipid-based delivery carriers. The lipid based nanocarriers shows a great property in terms of safety, physical stability, biocompatibility, efficacy scale-up, ease of preparation, better entrapment of lipid soluble drugs, prolonged drug release of formulation. (2)

Nifedipine is a peripheral arterial vasodilator widely used to treat high blood pressure, prevent the chest pain caused by angina pectoris and also used to treat Raynaud's phenomenon and chilblains. (3) (4)

MATERIALS ANS METHODS

Materials

Nifedipine was a gift sample from Micro labs pvt.Ltd Hosur , India. Compritol 888ATO was a kind gift samples of lipid from Gattefosse India Pvt. Ltd Mumbai, India.

Methods

Pre-formulation studies:

Pre-formulation studies are the first step in the development of dosage forms of a drug substance. These studies are an essential

component of drug development process and it gives the physical, chemical and mechanical properties of a suitable excipients and drug which are used in the preparation of formulation.

Nifedipine is a photosensitive drug so precautions were taken to protect the drug from exposure to light.

Identification of pure drug:

Identification of Nifedipine was carried out by FT-IR Spectrophotometry.

Procedure:

Weighed amount of drug (Nifedipine) was mixed with IR grade KBr (1:10) and compressed under 10-ton pressure in a hydraulic press to form a transparent pellet. The pellet was scanned by IR spectrophotometer over a frequency of 4000 cm^{-1} to 400cm^{-1} range.

Melting point determination:

Melting point of Nifedipine was determined by Open Capillary Method. Fine powder of Nifedipine was filled in glass capillary tube one end of which was previously sealed. The capillary tube was inserted into the melting point apparatus which is containing the liquid paraffin and heated. At which temperature drug started to melt was observed by using the thermometer which was already immersed into the liquid paraffin in the apparatus. (5)

Preparation of calibration curve of Nifedipine

Stock-1 solution: 25mg of Nifedipine was weighed accurately and transferred in 25ml volumetric flask and dissolved in methanol and the volume was made up to the mark with methanol (1000µg/ml).

Stock-2 solution: 1ml from stock-1 solution was pipetted out and diluted to 10ml with phosphate buffer pH 7.4 containing 1% SLS and 10% methanol (100µg/ml). From this solution 0.2, 0.4, 0.6, 0.8, 1, 1.2 and 1.4ml was pipetted out and diluted to 10 ml using pH 7.4 phosphate buffer containing 1% SLS and 10% methanol to get aliquots of 2, 4, 6, 8, 10, 12 and 14µg/ml respectively. The absorbance was measured at 237nm using UV- Visible Spectrophotometer. (5) The obtained data was plotted by taking concentration on X-axis and absorbance on Y-axis. The concentration and absorbance are given in table no 3 and fig no 2.

Formulation of Solid Lipid Nanoparticles:

Solid lipid nanoparticles (SLN) are colloidal dispersions, which are made up of solid biodegradable lipids. Solid lipid nanoparticles (SLNs) are alternative carriers to colloidal systems, which helps for controlled and targeted drug delivery. Techniques such as High shear homogenization, Ultra-sonication and High- s p e e d homogenization, Cold homogenization, hot homogenization, Micro emulsion-based methods, Solvent emulsification evaporation technique, Solvent emulsification diffusion method, Solvent injection method, Supercritical fluid-based methods are used to prepare the Solid Lipid Nanoparticles.

The Solvent emulsification evaporation method was used to prepare Nifedipine loaded solid lipid nanoparticles. The method is as follows:

- The lipid phase containing Compritol ATO 888 and Span 60 was melted to 80℃ above its melting point (73℃ and 53℃ respectively).
- The Nifedipine was dissolved in acetone and added to the melted lipid phase.
- The aqueous phase was prepared by dissolving the Tween 80 in distilled water and heating up to the same temperature of the lipid phase.
- The lipid phase was poured into the aqueous phase and stirred for 6 hours under magnetic stirrer.
- Nanoparticles thus produced were evaluated for different parameters such as particle size, zeta potential, polydispersity index, entrapment efficiency, drug content and drug release. (6)

Table 1: Composition of Nifedipine solid lipid nanoparticles:

Sl. NO.	FORMULATION CODE	AMOUNT OF DRUG (MG)	COMPRITOL 888 ATO (GM)	SPAN 60 (GM)	TWEEN 80 (GM)	DISTILLED WATER (ML)
1	F1	250	2	2	4	45
2	F2	250	2	3	3	45
3	F3	250	2	4	2	45
4	F4	250	2.5	2	4	45
5	F5	250	2.5	3	3	45
6	F6	250	2.5	4	2	45
7	F7	250	3	2	4	45
8	F8	250	3	3	3	45
9	F9	250	3	4	2	45

Design of experiment

The Design of Expert (DOE) software version 12 was used for statistical study. Optimization by DOE was done to find out the values of controllable independent variables, that gives the most desired value of dependent variables. The most popular response surface method (RSM) designs are the Central Composite Design (CCD) also known as Box-Wilson design. Central Composite designs are based on 2-level factorial designs (2^n) augmented with axial or star points (2^n) and a center point to fit quadratic models. Central Composite Design was constructed where the amounts of two excipients COMPRITOL 888 ATO and the ratio of two surfactants SPAN 60 and TWEEN 80 were selected as the independent variables. The levels of the two factors were selected on the basis of preliminary trials carried out before implementing the experimental design and their levels (low and high considered as -1 and +1 respectively) for compritol 4% and 6% and the ratio of two surfactants tween 80 and span 60 1:2 and 2:1 ratio was considered. Here combination of span 60 and tween 80 were used, total amount of surfactant was 6 gm. The amount of span 60 used was 2, 3 and 4 gm and tween 80 was 4, 3 and 2gm respectively.

Face centered cube design results when the same positive and negative distance is taken from the center in a CCD. A center point was replicated to provide excellent prediction capability near the center of the factor space and to estimate experimental error, so there will be 3 levels for each factor where quadratic effect can be measured. Total 9 runs were obtained by Central Composite Design. (7)

Table 2: Factors:

Factor	Name	Units	Low	Medium	High
A	COMPRITOL 888ATO	GM	2	2.5	3
B	Span 60/TWEEN 80	GM	2	3	4

Evaluation of nanoparticles:

Drug content

0.5 ml formulation solution was dissolved in methanol and the solution was diluted with pH 7.4 Phosphate buffer to get the concentration in the standard graph range and the drug content was estimated by UV- Spectrophotometry at 237nm.

Particle size and Poly dispersity index

The Particle size of nanoparticles plays a very important role in drug permeation through the skin. Polydispersity index describes the particle size distribution of nanoparticles. It helps to know the average uniformity of particles size of the sample. High poly dispersity index value describes the high degree of non-uniformity of a distribution of the particles in the nanoparticle formulation.

By using the dynamic light scattering (DLS) technology, the average particle size and the poly dispersity index were evaluated in Horiba Scientific (Nano-partica) SZ-100 at an angle of 90˚ at 25℃. Before the measurement, 1ml of the sample diluted to 10ml with double distilled water and sonicated for 15 minutes and vortexed, kept in polystyrene cuvettes for the evaluation of the particle size and poly dispersity index(n=3). (7)

Zeta-Potential

Zeta potential (ZP) is the electric charge on the particle surface. It helps to predict the storage stability of colloidal dispersion. At higher zeta potential less particle aggregation occurs due to electrical repulsions. Zeta potential was measured using Horiba Scientific (Nano-partica) SZ-100. 1ml prepared SLN sample was diluted into 10ml with Double Distilled water and sonicated for 15 minutes. Then the sample was transferred into the cuvette which contains electrodes and kept inside the instrument and zeta potential of sample measured (n=3).

Entrapment efficiency

The Entrapment efficiency of prepared sample was determined by measuring the concentration of the drug in the dispersion medium. The free drug Nifedipine was determined by adding 0.2ml of Nifedipine loaded nanoparticles which is added with 2ml acetone transferred to centrifuge tubes and then this dispersion was centrifuged at 6000 rpm for 30min. The supernatant was diluted with buffer and absorbance measured spectrophotometrically at 237nm (n=3).

EE% = $(Wtotal - Wfree)/Wtotal \times 100$

In-vitro Drug release of Nifedipine from SLNs

In vitro release of drug from Nifedipine nanoparticles formulation was studied using dialysis bag method in phosphate buffer pH 7.4 containing 1% SLS and 10% methanol. The dialysis bag procured from HIMEDIA having molecular weight cut off between 12000 to 14000 Da and pore size is 2.4nm. 4cm of the dialysis bag were soaked in distilled water for 24 hrs. before the experiment. One end of the bag was tied with the help of the thread and Nifedipine nanoparticles sample (equivalent to 30mg of drug) was added into the dialysis bag from another end and tied to the burette stand with help of the thread. The dialysis bag containing nanoparticle sample placed in 100ml of dissolution medium (glass rod was used to immerse the dialysis bag and to avoid the floating of the bag) which was continuously stirred at 100 rpm at37℃ using magnetic stirrer. Aliquots were withdrawn at predetermined time intervals and the same volume of fresh dissolution medium was added to maintain a sink condition. The samples withdrawn were analyzed spectrophotometrically at 237nm (n=3). (8) (9)

Numerical Optimization

The goal of optimization is to find a good set of conditions that will meet all the goals. Numerical optimization will search the design space, using the models created during analysis to find factor settings that meet defined goals. Numerical Optimization will optimize any combination of one or more goals. The goals may apply to either factors or responses. The possible goals are: maximize, minimize, target, within range, none (for responses only) and set to an exact value (factors only). A minimum and a maximum level must be provided for each parameter included in the optimization.

RESULTS & DISCUSSION

Pre-formulation studies:

Identification of pure drug:

The IR spectrum of pure drug was found to be similar to the standard spectrum of Nifedipine. The spectrum of Nifedipine shows the functional groups as per the reference peaks shown in fig 1.

Melting point determination:

The melting point of Nifedipine was found to be 173℃, which complied with IP standards thus indicating purity of obtained drug sample.

Figure 1: FTIR spectra of Nifedipine

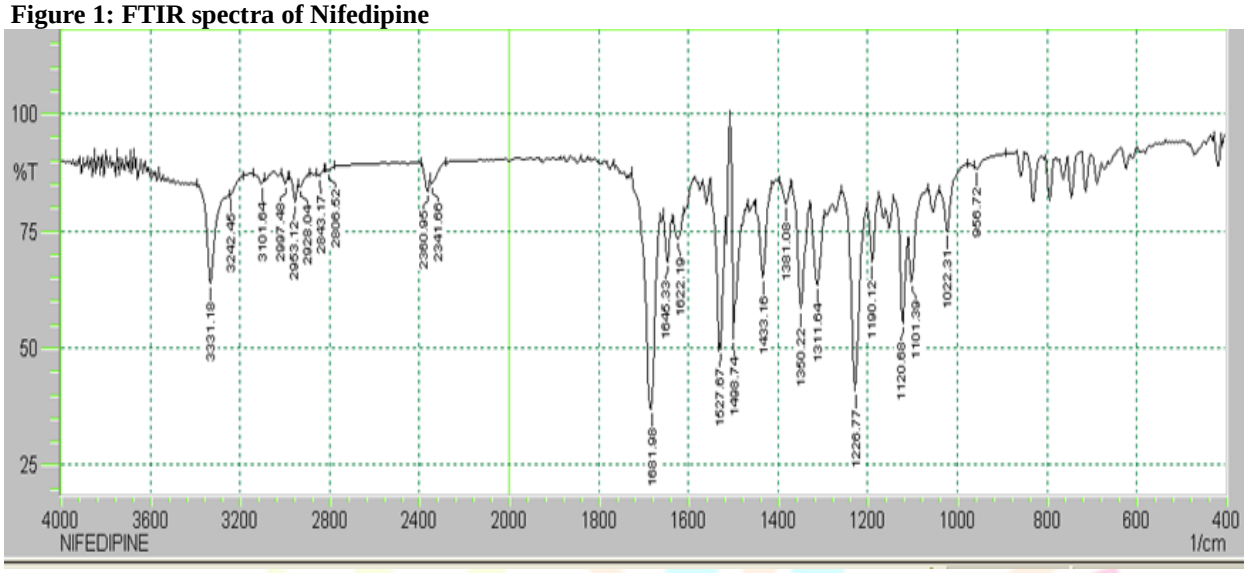
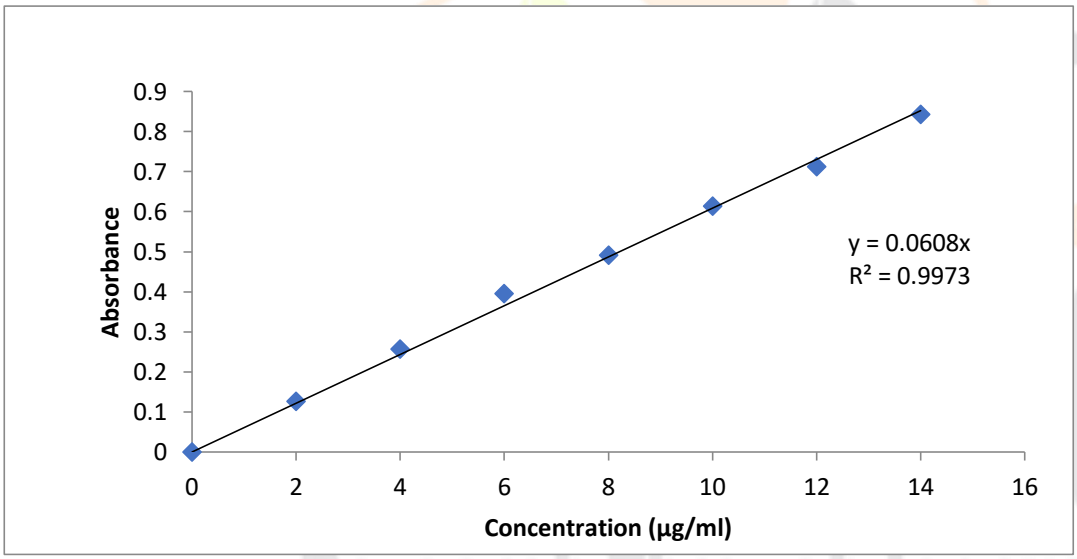


Figure 2: Calibration curve for Nifedipine:



Formulation of Solid Lipid Nanoparticles:

Nifedipine loaded solid lipid nanoparticles were successfully produced by solvent emulsification evaporation method. Different blends of solid lipid and ratio of surfactants were optimized in order to obtain uniform nanosized particles.

Table 3: Evaluated parameters for the nanoparticles of Nifedipine

Formulation code	Particle Size (nm)	Poly dispersity index (PDI)	zeta potential (mV)	Entrapment efficiency (%)	Drug content (%)	Drug release at 8h(%)
F1	101.7±0.23	1.29±0.25	-18.6±0.59	84.4±0.64	98.12±0.65	84.53±0.95
F2	123.4±0.63	0.22±0.37	-17.6±0.58	72.6±0.34	93.43±0.98	80.13±0.68
F3	135±0.52	0.19±0.36	-16.7±0.28	65.2±0.64	91±0.68	78.87±0.61
F4	146.1±0.35	0.54±0.48	-22.3±0.71	88.3±0.32	97.4±30.94	76.89±0.86
F5	156.5±0.16	0.15±0.59	-28.1±0.67	80.8±0.92	88.62±0.36	73.83±0.79
F6	169.4±0.19	0.30±0.27	-24.7±0.95	76.8±0.94	83.81±0.58	71.08±0.59
F7	207.2±0.64	0.43±0.59.	-29.9±0.61	95.1±0.62	87.13±0.39	69.28±0.81
F8	224.2±0.36	0.42±0.76.	-35.5±0.38	89.6±0.73	85.44±0.64	62.96±0.79
F9	250±0.95	0.46±0.94	-38.5±0.83	90.9±0.54	80±0.87	65.81±0.69

Evaluation of nanoparticles by DOE

Particle size

The particle size of obtained nanoparticles was ranged from 101.7 to 250nm.

The ANOVA (Analysis of Variance) for 2FI model was used to analyze the particle size of the prepared nanoparticles. The p-value and R^2 value was found to be 0.0004 and 0.9676 respectively, showing the model is significant.

DOE Results for particle size:

The **Model F-value** of 49.81 implies the model is significant. There is only a 0.04% chance that an F-value this large could occur due to noise.

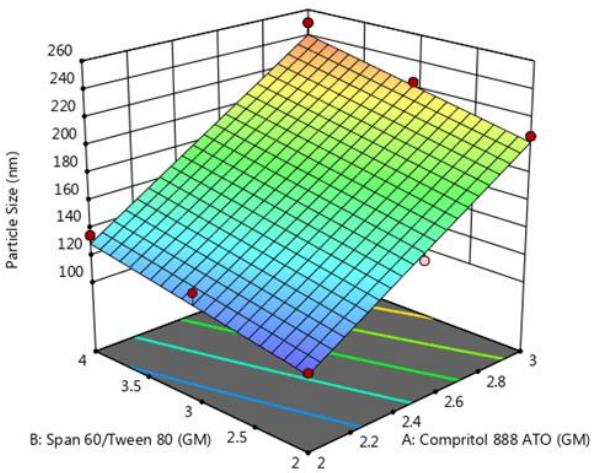
P-values less than 0.0500 indicate model terms are significant. In this case A, B are significant model terms.

Table 4: Fit Statistics of Particle size

Std. Dev.	11.24	**R^2**	**0.9676**
Mean	168.17	**Adjusted R^2**	0.9482
C.V.%	6.68	**Predicted R^2**	0.8763
		Adeq Precision	18.7158

The **predicted R^2** of 0.8763 is in reasonable agreement with the **Adjusted R^2** of 0.9482; i.e, the difference is less than 0.2.

Figure 3: 3-D graph of Particle Size



Equation in Terms of Coded Factors

Particle Size = +168.17+53.55×A+16.57×B+2.38×AB

Zeta Potential

The Zeta potential of obtained nanoparticles was ranged from -16.7Mv to -38.5Mv.

The ANOVA (Analysis of Variance) for 2 FI model was used to analyze the Zeta potential of the prepared nanoparticles. The p-value and R^2 value was found to be 0.0005 and 0.9636 respectively, showing the model is significant.

DOE Results

The **Model F-value** of 44.08 implies the model is significant. There is only a 0.05% chance that an F-value this large could occur due to noise.

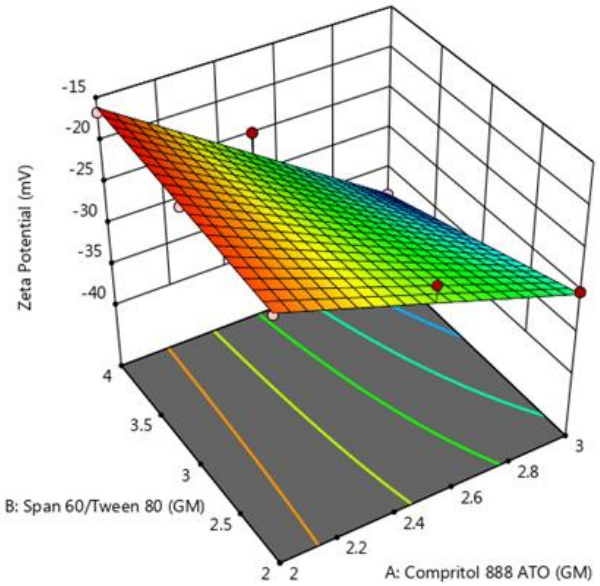
P-values less than 0.0500 indicate model terms are significant. In this case A, AB are significant model terms.

Table 5: Fit Statistics of Zeta potential

Std. Dev.	1.90	**R^2**	**0.9636**
Mean	-25.77	**Adjusted R^2**	0.9417
C.V. %	7.35	**Predicted R^2**	0.9302
		Adeq Precision	17.6118

The Predicted R^2 of 0.9302 is in reasonable agreement with the **Adjusted R^2** of 0.9417; i.e., the difference is less than 0.2**Adeq Precision** measures the signal to noise ratio. A ratio greater than 4 is desirable. The ratio of 17.61 indicates an adequate signal. This model can be used to navigate the design space.

Figure 4: 3-D graph for the Zeta potential



Equation in Terms of Coded Factors

Zeta potential = -25.77+8.50×A+1.52×B-2.63×AB

Entrapment efficiency

The percentage of incorporated drug in lipid matrix (Entrapment Efficiency) was evaluated. The results showed that the entrapment efficiency of Nifedipine loaded solid lipid nanoparticles ranged from 65.2 to 95.1%.

DOE Results

The **Model F-value** of 5.82 implies the model is significant. There is only a 3.35% chance that an F-value this large could occur due to noise.

P-values less than 0.0500 indicate model terms are significant.

Table 6: Fit Statistics of Entrapment Efficiency

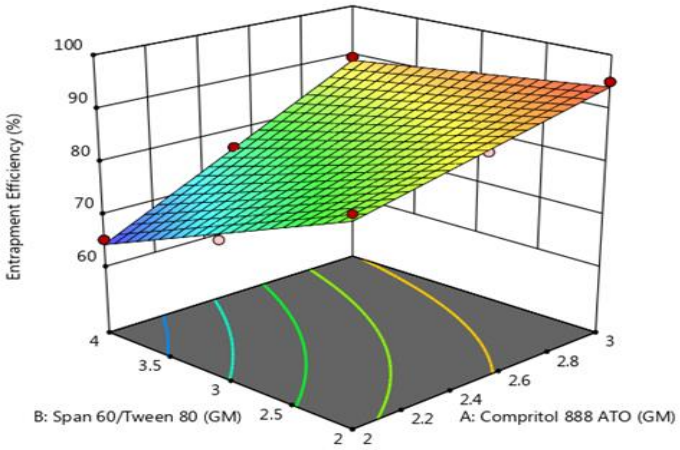
Std. Dev.	1.33	**R^2**	**0.9881**
Mean	82.63	**Adjusted R^2**	0.9810
C.V. %	1.62	**Predicted R^2**	0.9368
		Adeq Precision	33.5586

The **Predicted R^2** of 0.9368 is in reasonable agreement with the

Adjusted R^2 of 0.9810; i.e., the difference is less than 0.2.

Adeq Precision measures the signal to noise ratio. A ratio greater than 4 is desirable. The ratio of 33.559 indicates an adequate signal. This model can be used to navigate the design space.

Figure 5: 3-D graph of Entrapment Efficiency



Equation in Terms of Coded Factors

Entrapment Efficiency = +82.63+8.90×A–6.03×B+ 3.42×AB

In-vitro Drug release from SLNs

The In vitro drug release of obtained nanoparticles was ranged from 62.96% to 84.53%.

The ANOVA (Analysis of Variance) for 2 FI model was used to analyse the In vitro drug release of the prepared nanoparticles. The p-value and R^2 value was found to be 0.0007 and 0.9589 respectively, showing the model is significant.

DOE Results

The **Model F-value** of 38.90 implies the model is significant. There is only a 0.07% chance that an F-value this large could occur due to noise.

P-values less than 0.0500 indicate model terms are significant. In this case A, B are significant model terms.

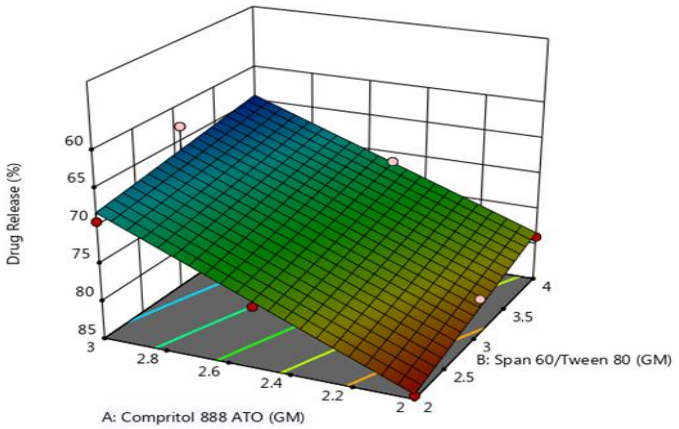
Table 7: Fit Statistics of Drug release

Std. Dev.	1.81	**R^2**	0.9589
Mean	73.71	**Adjusted R^2**	0.9343
C.V. %	2.46	**Predicted R^2**	0.8217
		Adeq Precision	16.6718

The **Predicted R^2** of 0.8217 is in reasonable agreement with the **Adjusted R^2** of 0.9343; i.e., the difference is less than 0.2.

Adeq Precision measures the signal to noise ratio. A ratio greater than 4 is desirable. The ratio of 16.672 indicates an adequate signal. This model can be used to navigate the design space.

Figure 6: 3-D Surface of Drug release



Final Equation in Terms of Coded Factors

Drug Release = +73.71-7.58×A – 2.49×B + 0.5475×AB

DISCUSSION

Pre-formulation studies

Identification of pure drug:

Identification of Nifedipine was carried out by using Fourier Transform Infra-Red spectroscopy. It was observed that all the characteristic peaks of Nifedipine were shown as of the standard peaks, so it was concluded that the drug is pure. (10)

Melting point

The melting point of the Nifedipine was determined by capillary method. The melting point was found to be 173℃ thus indicating the purity of the drug. The melting point of the Nifedipine is 172-174 0C. (3) as per literature.

Calibration curve of Nifedipine

Selection of suitable buffer for Nifedipine: Nifedipine is a poorly water-soluble drug. Freely soluble in acetone and methanol, so methanol is used as the cosolvent in the preparation of buffer to increase solubility of Nifedipine and to maintain sink conditions further in in-vitro drug release studies.

Preparation of Nifedipine calibration curve:

The calibration curve was linear between 2-14 µg/ml concentration range. It was constructed in pH 7.4 phosphate buffer containing the 1% SLS and 10% methanol, by plotting mean absorbance vs concentration at 237nm (n=3). The R^2 and slope was found to be 0.9999 and 0.0608 respectively.

Formulation of Solid Lipid Nanoparticles:

The solvent emulsification evaporation method was used to produce a Nanoparticles of Nifedipine and all the formulations were prepared using 2, 2.5 and 3 gm of compritol 888 ATO, 1:1, 1:2 and 2:1 ratio of surfactants (Span 60 and Tween 80) and 250 mg of Nifedipine respectively. Evaluation of Nanoparticles:

Drug content

The drug content of the formulation F1 to F9 was found to be 98.12%, 93.43%, 91%, 97.43%, 88.62%, 83.81%, 87.13%, 85.44% and 80% respectively.

Particle size and Polydispersity index

The mean particle size of colloidal carriers is important characteristic of SLNs. Average particle size and Polydispersity index of the nanoparticles are obtained. The results showed that all the SLNs are in the nano-size range and had low poly dispersity, which indicate relative narrow size distribution. Sizes ranged from 101.7 nm to 250 nm and the polydispersity index ranged from 0.15 to 1.29 respectively.

The ANOVA (Analysis of Variance) for 2FI model was used to analyze the particle size of the prepared nanoparticles. The p-value and R^2 value was found to be <0.0001 and 0.9665 respectively, showing the model is significant.

Final Equation in Terms of Coded Factors

Particle Size = +168.17+53.55×A+16.57×B

Were,

A= Compritol 888 ATO, B= Span 60/Tween 80

According to the coded factor equation of particle size, the Compritol have a positive effect on particle size. As the concentration of compritol increases particle size also increases and vice-versa. Whereas the amount Span 60 shows a positive effect on particle size, as

the concentration of Span 60 increases the size of the particles also increases which indirectly shows that tween has negative effect on the particle size i.e., as the concentration of Tween 80 increases the size of the particles decreases.

A possible explanation for the large particle size of Compritol based SLNs may be due to its high melting point (65–77 ^{0}C) and the long hydrocarbon chain of behenic acid (C22) which represents 85% of the acids esterifying glycerol in Compritol. High melting point results in an increase in the viscosity and leads to an inefficient homogenization which causes an inefficient reduction of the particle sizes. Moreover, the long hydrocarbon chains (HC) of behenic acid (C22) in Compritol resulted in bulkier molecules that are less susceptible to packaging into small size particulates. (11)
The type and concentration of surfactant are helpful in preventing aggregation of the droplets, thus maintaining a low polydispersity index. The addition of co surfactant helps to increase the efficacy of the surfactant. The inclusion of a co-surfactant may also reduce the amount of the surfactant needed, thus avoiding the potential toxicity issue due to the larger amount of the individual surfactant needed for preparing the formulation. Combination of surfactant and co surfactant beneficial in altering the physicochemical properties of the nanoparticles. (12)

The hydrophilic surfactant (Tween 80) shows a negative impact and hydrophobic surfactant (Span 60) shows a positive impact on particle size.

Zeta potential

The fit statistics of Zeta potential of obtained nanoparticles was ranged from -16.7Mv to -38.5Mv.

The ANOVA (Analysis of Variance) for 2FI model was used to analyze the Zeta potential of the prepared nanoparticles. The p-value and R^2 value was found to be 0.0005 and 0.9636 respectively, showing the model is significant.

Final Equation in Terms of Coded Factors

Zeta potential = -25.77+8.50×A-1.52×B-2.63×AB

According to coded factor equation of Zeta potential, Compritol shows the positive effect on zeta potential. As the concentration of compritol increases the zeta potential also increases. Span 60 shows the negative effect on zeta potential which indicates that concentration of Tween 80 has positive effect on zeta potential.

The fit statistics of zeta potential of Nifedipine SLNs is presented in table 5. The results showed a relatively good stability and dispersion quality.

According to the DLVO theory, a system can be regarded as stable if the electrostatic repulsion dominates the attractive van der Waals forces. The particles have to overcome an energy barrier of electrostatic repulsion to approach closely and form agglomerates. If their velocity or kinetic energy is high enough, they will collide. Higher temperatures as well as light increase the kinetic energy of a system, in combination with a reduced zeta potential this leads to SLN aggregation. (13)

A zeta potential value in the range of -30 mV to +30 mV is generally considered as the standard value in which particles shows the sufficient repulsive force to attain better physical colloidal stability. (14)

Entrapment efficiency (EE %)

The results showed that the entrapment efficiency of Nifedipine loaded solid lipid nanoparticles ranged from 65.2 to 95.1%. ANOVA for the 2 FI model of entrapment efficiency showed the p-value of <0.0001 and R^2 value of 0.9881 respectively showing the model is significant

Final Equation in Terms of Coded Factors:

Entrapment Efficiency = +82.63+8.90×A–6.03×B+ 3.42×AB

According to coded factor equation, the Compritol shows positive effect on the entrapment efficiency. As the concentration of compritol increases the entrapment efficiency also increases. The Span 60 showed the negative effect, by increasing the concentration of Span 60 the decrease in the entrapment efficiency observed. This indirectly showed the positive effect of Tween 80 on the entrapment efficiency, by increasing the concentration of Tween 80 the increase in the entrapment efficiency observed.

The results also showed that increasing the lipid concentration leads to a gradual increase in the entrapment efficiency. The higher amount of the compritol characterized to the high hydrophobicity due to the long chain fatty acids attached to the triglycerides, resulting in increased accommodation of drugs. A possible explanation for these observations is that the increase in lipid content can afford more space to encapsulate more drug, thus reducing drug partition in the outer phase which directly influence the lower entrapment efficiency. (15)

***In-vitro* Drug release from SLNs**

In vitro drug release of Nifedipine SLNs was performed in Phosphate buffer (pH 7.4) containing the 1% SLS and 10% Methanol using dialysis bag technique. The % drug release of Nifedipine from the prepared nanoparticles.
ANOVA for the 2 FI model of In-vitro drug release showed the p-value of 0.0007 and R^2 value of 0.9589 respectively, showing the model is significant.

Equation in Terms of Coded Factors:

Drug Release = +73.71-7.58×A – 2.49×B + 0.5475×AB

According to the coded factor equation, the lipid (Compritol 888 ATO) showing a negative effect on % drug release. As the concentration of lipid increases, the % drug release decreases and vice-versa. The Span 60 showing the negative effect on the drug release, as the concentration of the Span 60 increases the drug release decreases which indicate that Tween 80 has the positive effect on the % drug release which means as the concentration of the Tween 80 increases the % drug release also increases.
Increase in the concentration of lipid results in decrease in the drug release. In this, the drug core is surrounded by a lipid layer. At higher lipid concentration, drug will be coated by multiple layers of the lipid. Due to the increased diffusional distance and hindering effects by the surrounding solid lipid layers, the drug shows the slow drug release. (16)

The HLB value of Tween 80 is 15, which has a strong tendency to form oil-in-water emulsion and thus Tween 80 may migrate at oil/water interface along with the drug, thereby increasing the drug concentration at the surface of the nanoparticles. The amount of surfactant plays an important role, because it can avoid the coalescence of the oil globules. The surfactant molecules tend to align themselves at the droplet surface lowering the free energy at the interface between two phases and resisting coalescence of the droplets. Smaller nanodroplets have large surface area and thus helps in faster drug release. (12)

When the more amount of span 60 and less amount of tween 80 is used (in formulation F3, F6 & F9 4gm of span 60 and 2 gm of tween 80 was used) difference in drug release was more visible.

Numerical optimization

Numerical optimization technique was carried out using Design expert software version 12.0.9.0 to obtain an optimal concentration of process variables to minimize particle size, to maximize the entrapment efficiency and to enhance the % in- *vitro* drug release which serves as a basis for selection of the optimized best formulation.

The optimized formulation suggested the composition, Compritol 888 ATO (X_1) and

Span 60/ Tween 80 (X_2) were found to be 2gm and 2gm respectively, with a desirability of 0.842.

With these concentrations of process variables, suggested responses were 100.397nm for particle size, -18.375 for zeta potential, 83.19% for entrapment efficiency, 84.32% for drug release, respectively, the composition of the recommended formulation was found to be in close agreement with the experimental response of formulation F1. Thus, F1 can be chosen as an optimum formulation for further studies.

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