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Formulation and Evaluation of Solid Lipid Nanoparticles of Felodipine

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ABSTRACT

The purpose of this study was to develop and characterize Felodipine solid lipid nanoparticles to improve dissolution rate, and hence bioavailability. Felodipine is a BCS class II drug and has a limited bioavailability of 15–20% due to extensive first-pass metabolism. The solubility of the drug was carried out in various lipids. the maximum solubility of the felodipine was seen in stearic acid and hence was chosen as a lipid for the formulation of SLNs. Tween 80 and polyvinyl alcohol (PVA) were the surfactants employed. The felodipine solid lipid nanoparticles were prepared by the emulsion solvent diffusion and melt sonication methods. The SLN prepared by using the melt sonication method was not stable and precipitated. Hence, the emulsion solvent diffusion method was employed. The F4 formulation consisting of PVA, tween 80 (1%), and drug: fat (1:3) was selected as the best formulation as these nanoparticles exhibited high entrapment efficiency, good drug loading, and high drug release (92.6±1.62% for 24 hours). The average particle size was found to be 444.5 nm with a narrow PDI of 0.357, and zeta potential of -14.4 mV.

INTRODUCTION:

Lipid-based drug delivery (LBDD) has attained more importance in recent years as it improves the solubility and bioavailability of drugs belonging to BCS class II and class IV. In general, lipid-based drug delivery systems are used as carriers because of high stability, high carrier capacity, feasibility of incorporating both lipophilic and hydrophilic drugs, and feasibility of various routes of administration including oral, topical, parenteral, and pulmonary routes. The lipid-based formulations increase the drug solubilization for water-insoluble drugs through various mechanisms such as affecting the intestinal environment, stimulating the lymphatic transport of active ingredients, and interacting with enterocyte-based transport. The lipid-based drug delivery systems were broadly classified as emulsion-based systems, vesicular systems, solid lipid particulate dosage forms, and solid lipid tablets. The modification of these types includes solid lipid nanoparticles, lipospheres, nanostructured lipid carriers, Pickering emulsions, self-emulsifying formulations, etc [1].

Solid lipid nanoparticles (SLNs) were introduced in 1991 and are defined as a colloidal carrier system that comprises a high melting point lipid as a core that is surrounded by an aqueous surfactant. These are the same as nanoemulsions but the only difference is liquid lipid is replaced by solid lipid. The solid lipid nanoparticles act as an alternative to traditional colloidal carrier systems such as liposomes, emulsions, and polymeric microparticles. They are biodegradable, biocompatible, and used for controlled drug delivery and specific targeting. SLN consists of a lipid matrix which should be solid at room temperature and have a particle size from 50 nm to 1000 nm. The lipids used in SLN are triglycerides, hard fats, partial glycerides, fatty acids, and waxes. Solid lipids are very beneficial as they offer high stability of incorporated therapeutic agents and facilitate the controlled release of drugs. Along with the solid lipid, another major excipient used in the SLN preparation is surfactant which is of aqueous type and serves as an emulsifier to form o/w type emulsion and stabilizer for dispersion containing solid lipid nanoparticles. Solid lipid nanoparticles can be prepared by various methods such as high-pressure homogenization, ultrasonication, double emulsion method, solvent evaporation, solvent injection method, and emulsion solvent diffusion method [2]. The method of preparation of SLNs is very important apart from the ingredients used in preparation as it shows the impact on particle size, drug loading, and drug stability. The cold homogenization method can be used to prepare SLNs of water-soluble and thermolabile drugs [3]. The homogenization method and ultrasonication methods are the most

widely used methods for the preparation of SLNs but these methods have drawbacks like high-energy input, time consumption, temperature sensitivity, etc. To avoid these drawbacks, the emulsion solvent diffusion method can be employed for the preparation of solid lipid nanoparticles and it has many advantages easy to implement, reduced tendency of agglomeration, no involvement of the heating process, and is cost-effective.

MATERIALS AND METHODS:

Materials:

Felodipine was a kind gift sample from Aizant Drug Research Solutions Pvt. Ltd. Stearic acid, and polyvinyl alcohol was procured from SD Fine Chem Limited, Mumbai. Tween 80 was purchased from the Sisco Research Laboratories Pvt. Ltd, Maharashtra, and dichloromethane was procured from Finar Limited, Ahmedabad. Dialysis membrane was purchased from HiMedia Laboratories Pvt. Ltd, Maharashtra.

Methodology:

Analytical method development for felodipine:

Determination of λmax of Felodipine:

10µg/ml solution of felodipine was prepared in methanol and pH 6.8 phosphate buffer and scanned using a UV VIS Spectrophotometer in the range of 200-400nm to determine the λmax of Felodipine in methanol and pH 6.8 phosphate buffer.

The blank solution i.e., pH 6.8 phosphate buffer was also scanned for absorbance using a UV double beam spectrometer (T60 UV VIS Spectrophotometer) and the peak obtained for blank and peak obtained for 10µg/ml (0.01mg/ml) solution of drug prepared with the pH 6.8 phosphate buffer was compared to check for interference in analytical study.

Construction of calibration curve of Felodipine:

Dilutions of felodipine were prepared from 100µg/ml felodipine solution in methanol and in pH 6.8 phosphate buffer. The absorbance of these solutions was measured at 238nm with the aid of a UV-VIS spectrophotometer. The calibration curve was plotted between concentration on the X-axis and absorbance on the Y-axis [4].

PREFORMULATION STUDIES:

Determination of the melting point of felodipine:

The melting point of felodipine was determined by the capillary tube method [5] and the obtained melting point of the drug was compared to the reported value mentioned in the literature.

Lipid screening:

Lipids are the main ingredient in the formulation of solid lipid nanoparticles. Before the formulation, the drug solubility in the lipid was screened. Drugs with good lipid solubility can be encapsulated more effectively in SLNs in large quantities and offer good stability.

The solubility of felodipine was studied in glyceryl monostearate, stearic acid, and oleic acid. A small quantity of lipids was weighed, melted, and transferred into glass vials. A known quantity of the drug was added to it and kept on a Rota shaker and the saturation solubility of the drug in lipids was assessed visually [6].

Fourier transform infrared spectroscopy (FTIR):

The FTIR analysis was carried out for the drug (Felodipine) and the Felodipine SLN dispersion using Aligent Cary 630 FTIR. The sample was placed onto the ATR crystal and pressed down using a swivel press for optimal contact between the sample and the crystal. In contrast, the SLN formulation was analyzed by placing a small drop of SLN dispersion onto the ATR crystal, and the measurement was taken. After completion of the measurement, the crystal is wiped clean by using a suitable solvent. The spectrum was recorded in the frequency range 4000-650 cm^{-1} and the key peaks were analyzed [7].

APPROACHES OF PREPARATION OF SOLID LIPID NANOPARTICLES:

Melt sonication method:

The lipid was heated up to 85^0C and the drug was dissolved in it (lipid phase). Similarly, the surfactant was dissolved in water (40ml) and heated to the same temperature (aqueous phase). The aqueous phase was added to the lipid phase to get the primary emulsion under magnetic stirring for 15 minutes. The primary emulsion obtained was sonicated using a bath sonicator

for 20 minutes. The nanoemulsion formed was cooled and diluted with the distilled water and the dispersion was passed through a 0.45µm membrane filter.

Table 1: Formulation table of felodipine solid lipid nanoparticles by melt sonication method

Name of the Component	F1	F2	F3	F4	F5	F6
API (mg)	10	10	10	10	10	10
Stearic acid (mg)	100	150	200	100	150	200
Poloxamer 188 (% w/v)	1	1	1	-	-	-
Tween 80 (g)	-	-	-	0.4	0.4	0.4
Dichloromethane (ml)	2.5	2.5	2.5	2.5	2.5	2.5
Distilled water (ml)	q. s	q. s	q. s	q. s	q. s	q. s

Emulsion solvent diffusion method:

Lipid was dissolved in 2.5ml of dichloromethane as the internal oil phase and the drug was dissolved in it. This solution was added into 25ml of an aqueous solution of PVA (polyvinyl alcohol) dropwise and homogenized at 3000rpm for 30 min to form a primary o/w emulsion. The formed emulsion was poured into 75ml of ice-cold water (2-3ºc) containing tween 80 to extract organic solvent into continuous phase for proper solidification of SLNs and stirring was continued for 2.5hrs at 3000rpm. The final emulsion was sonicated using a probe sonicator for 20 min to get uniform particle size.

Table 2: Formulation table of felodipine solid lipid nanoparticles by melt sonication method

Name of the Component	F1	F2	F3	F4	F5	F6	F7	F8	F9
API (mg)	10	10	10	10	10	10	10	10	10
Stearic acid (mg)	20	20	20	30	30	30	40	40	40
PVA (% w/v)	1	1.5	2	1	1.5	2	1	1.5	2
Tween 80 (% w/v)	1	1.5	2	1	1.5	2	1	1.5	2
Dichloromethane (ml)	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5
Distilled water (ml)	q. s	q. s	q. s	q. s	q. s	q. s	q. s	q. s	q. s

EVALUATION OF SOLID LIPID NANOPARTICLES:

Entrapment efficiency and drug loading:

The entrapment efficiency of solid lipid nanoparticles was determined by measuring the free drug present in the supernatant obtained by centrifugation. 10ml of SLN dispersion was taken and centrifuged at 6000 rpm for 45 minutes. The supernatant obtained was checked for free drug using a UV spectrometer at 238nm.

The entrapment efficiency can be calculated by using the following formula:

Entrapment efficiency (EE%) = $(W_T - W_S / W_T) \times 100$

Drug loading can be calculated by using the following formula:

Drug loading (DL%) = $(W_T - W_S / W_T - W_S + W_L) \times 100$

Where W_T is the total amount of drug added to the formulation, W_S is the amount of drug in the supernatant after centrifugation and W_L is the weight of lipid used in the formulation [8].

In-vitro release studies:

The *in-vitro* drug release studies were carried out using the dialysis bag method. The dialysis membrane with a molecular weight cut off between 12000 to 14000 Da. was used. The dialysis membrane was soaked in double distilled water overnight before the drug release study. About 5ml volume of SLN formulation was placed in the dialysis bag and placed in a beaker containing 100ml of pH 6.8 phosphate buffer. An aliquot of 5ml samples was withdrawn from the receptor compartment at the regular time intervals for 24 hours and the medium was replaced with freshly prepared buffer simultaneously to maintain sink conditions. The collected samples were analyzed by UV spectrophotometer at 239nm after calibration with the respective blank [9].

CHARACTERISATION OF FELODIPINE SOLID LIPID NANOPARTICLES:

Particle size and Zeta potential:

The particle size of the F4 formulation was determined by Dynamic Light Scattering (DLS) at 25^0C under an angle of 90^0. The zeta potential of SLN was determined by using Zetasizer (HORIBA SZ-100). The surface charge of the particles can be known by measuring the zeta

potential. The sample was diluted and placed in the disposable cell and the mobility of particles was determined. The stability of the SLN dispersion depends on zeta potential [10].

Morphology of SLN:

The shape and surface morphology of the SLN formulation was examined by Scanning Electron Microscopy (SEM) (Hitachi S-3700N). The three-dimensional images of the nanoparticles were observed by SEM. One drop of SLN dispersion was mounted on a stub covered with clear glass and air-dried. The gold coating was applied using sodium aurothiomalate to examine under SEM 10,000 magnifications.

RESULTS AND DISCUSSION:

Analytical method development for felodipine:

The determination of λmax for a drug is an important analytical tool that supplies information about drug identity, purity, and concentration of the drug.

Determination of λmax in methanol:

Felodipine solution of concentration 10µg/ml was prepared with methanol and scanned using a UV-visible spectrometer in the range of 200-400nm. Felodipine showed λmax at 238nm in methanol.

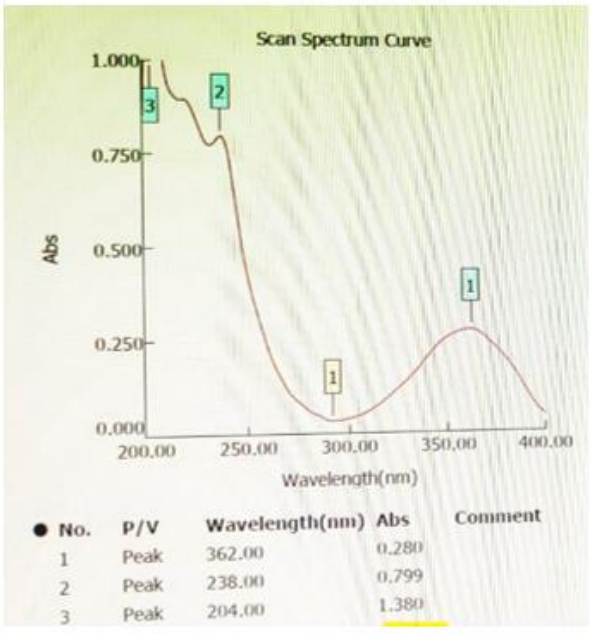

Figure 1: λmax of felodipine in methanol

Determination of λ max pH 6.8 phosphate buffer:

Felodipine solution (10µg/ml) was prepared with pH 6.8 phosphate buffer and was scanned using a UV-visible spectrometer in the range of 200-400nm. Buffer solution without the drug was also scanned for comparison. Felodipine showed λmax at 239nm in pH 6.8 phosphate buffer.

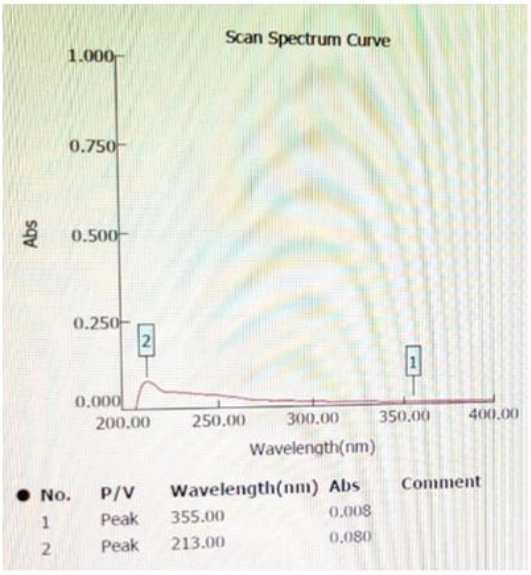

Figure 2(a)

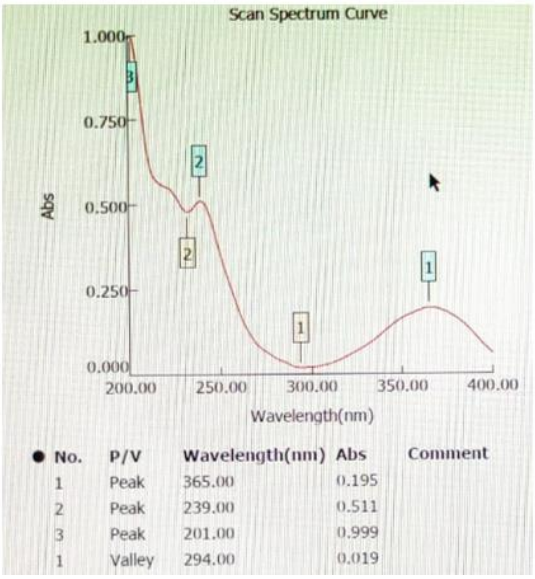

Figure 2(b)

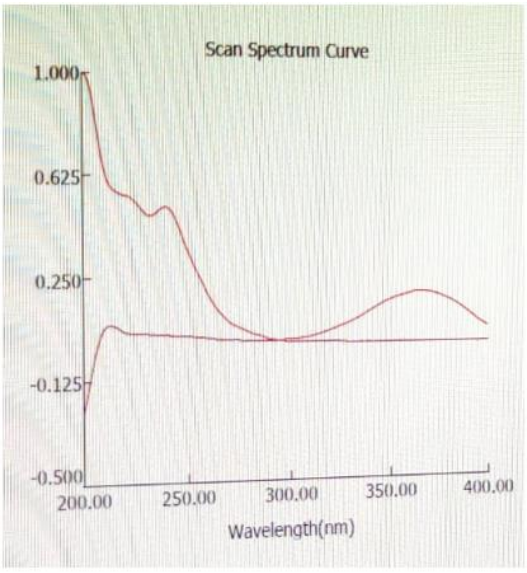

Figure 2(c)

As seen in Fig.2(c) there was no interference with the solvent in the analysis of the drug.

Construction of calibration curve of felodipine:

The standard stock solutions of felodipine in the range of 2 to 12 µg/ml and 3 to 15 µg/ml were prepared by using methanol and pH 6.8 phosphate buffer respectively. The absorbance was measured at 238nm and 239nm by using a UV spectrometer. The calibration curve of felodipine in methanol and pH 6.8 phosphate buffer are displayed in Figures 3 and 4. As seen in Figures 3 and 4, the equation for the standard graph of felodipine in methanol was found to be y= 0.0626x + 0.0603 with an R^2 value of 0.9978 and the equation for the standard graph of felodipine in pH 6.8 phosphate buffer is y= 0.0459x + 0.0712 with an R^2 value of 0.9969.

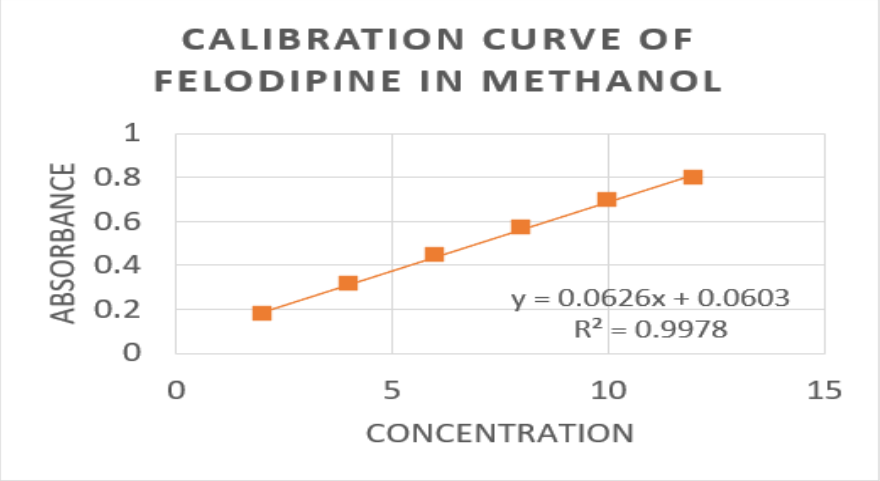

Figure 3: Calibration curve of Felodipine in methanol.

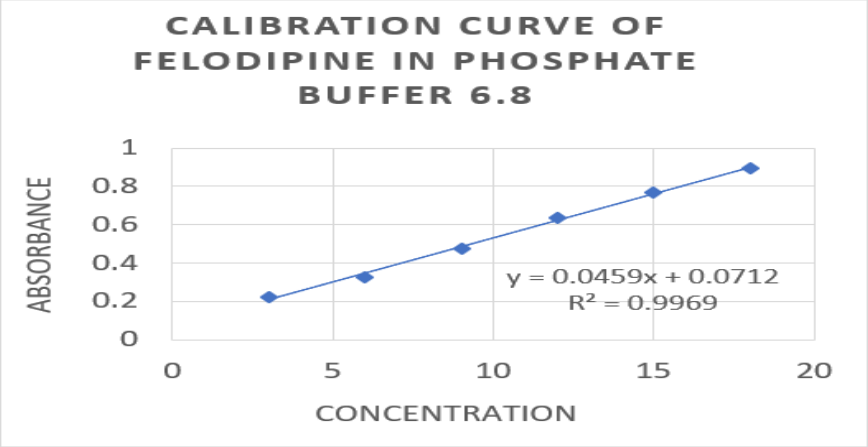

Figure 4: calibration curve of felodipine in pH 6.8 phosphate buffer

PREFORMULATION STUDIES:

Determination of the melting point of felodipine:

The physical property of a drug like melting point determination provides useful information about sample identification or sample purity.

The melting point of the Felodipine obtained is mentioned in Table 3. The observed value was found close to the reported value which indicated that the drug was pure.

Table 3: Melting point of felodipine.

MELTING POINT	
Reported value	**Observed value**
142-145^{0}C	141^{0}C

Lipid screening:

The solubility of felodipine was studied in Glyceryl monostearate (GMS), Stearic acid, and Oleic acid. Among these lipids, the Felodipine solution was clear in stearic acid. Hence stearic acid was selected as a lipid to formulate solid lipid nanoparticles.

Fourier transform infrared spectroscopy (FTIR):

The FTIR analysis was carried out to determine the trace of any intermolecular interactions between the drug and excipients. The FTIR spectra of the felodipine and the Felodipine SLN dispersion are displayed in Fig 4 (a), (b). The wave numbers of the absorption bands shown by Felodipine and the Felodipine SLN dispersion are mentioned in Table 4.

Table 4: Wave numbers of peaks obtained for drug and SLN dispersion

Functional groups	Wave number (cm $^{-1}$) of peaks obtained for drug	Wave number (cm $^{-1}$) of peaks obtained for SLN dispersion
N-H (amine)	3369	3391
C-H (alkane)	2981	2966
C-H (aromatic)	2948	2929
CH_3COOH	1688	1617
$COOC_2H_5$	1640	1617
C=C	1490,1416	1498, 1405
C-Cl (chlorine)	767.8	779
=C-H (alkene)	670.9	663

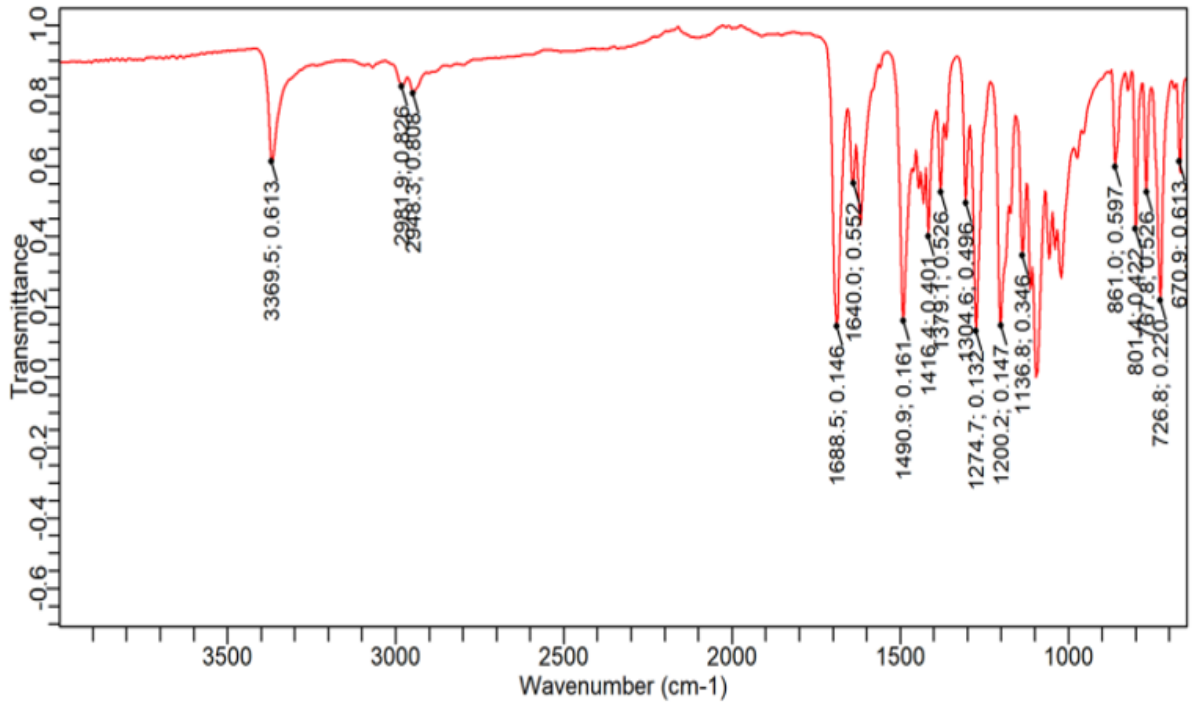

Figure 4:(a) FTIR spectrum of Felodipine

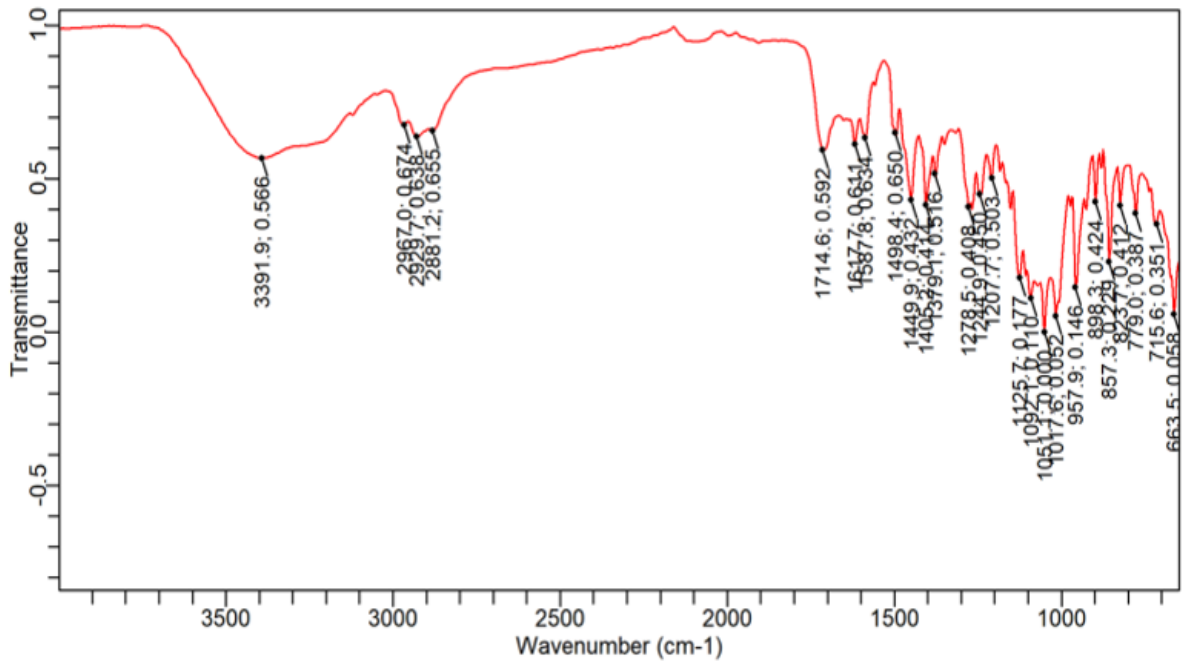

Figure 4(b) FTIR spectrum of Felodipine SLN dispersion

As seen in Figure 4 (a) and (b) i.e., in the FTIR spectra of Felodipine and Felodipine SLN dispersion, showed no new peaks. The absence of new peaks reveals that there was no intermolecular interaction but, a slight decrease in the intensity of the peaks in the spectrum of Felodipine SLN dispersion was observed compared to the peaks obtained for the drug.

PREPARATION OF SOLID LIPID NANOPARTICLES:

The Felodipine Solid Lipid Nanoparticles were formulated by using the Melt sonication method and the Emulsion solvent diffusion method. The Felodipine SLN Formulation prepared by the melt sonication method was not stable and precipitated after the preparation. The melt sonication method also has disadvantages like less encapsulation efficiency, susceptibility to thermal degradation, and oxidative degradation. Hence, the Emulsion solvent diffusion method was employed to overcome these disadvantages.

The emulsion solvent diffusion method has many advantages like ease of preparation and prevention of drug exposure to high temperature and physical stress. It enables the entrapment of both lipophilic and hydrophilic drugs.

EVALUATION OF SOLID LIPID NANOPARTICLES:

Entrapment efficiency and drug loading:

The entrapment efficiency and drug loading are defined as the amount of drug incorporated within the solid lipid nanoparticles compared to the total amount of drug used in the formulation. The entrapment efficiency and drug loading are crucial parameters that are associated with the bioavailability, drug delivery, dose loading, and stability of the solid lipid nanoparticles. The high entrapment efficiency of SLNs results in a controlled release pattern and reduces the side effects of the drug.

The entrapment efficiency values of formulations F1 to F9 were obtained in the range of 87.2% to 92.9% and are mentioned in Table 5. The entrapment efficiency value was low for the F9 formulation i.e., 87.2±0.14%, and the high entrapment efficiency was obtained for the F4 formulation i.e., 92.9±0.63%.

The drug loading values were obtained in the range of 17.8% to 31.5% (Table 5). The drug loading was found highest for F1 formulation i.e., 31.5±0.14%, and the lowest value was found for F9 i.e., 17.8±0.35%.

Table 5: Percentage of Entrapment efficiency and Drug loading of F1 to F9.

Formulation	Entrapment efficiency (%) ± S. D	Drug loading (%) ± S. D
F1	92±0.35	31.5±0.14
F2	87.9±0.21	30.5±0.21
F3	87.9±0.35	30.5±0.14
F4	92.9±0.63	23.6±0.07
F5	87.8±0.21	22.6±0.141
F6	92.7±0.42	23.5±0.070
F7	92.5±0.14	18.7±0.42
F8	90.6±0.42	18.4±0.21
F9	87.2±0.14	17.8±0.35

In-vitro drug release studies:

The drug release studies were performed for formulations F1, F3, F4, F6, and F8 as the entrapment efficiency and drug loading percentage of these formulations were high.

The drug release studies were carried out by using the dialysis bag method. The drug release from the formulation was observed for 24 hours in pH 6.8 phosphate buffer and the cumulative percentage of drug release was calculated. The cumulative amount of drug release was higher for the F4 formulation i.e., 92.6±1.62%. About 50-60% of the drug was released in 8 hours and the drug release was about 90% after 24 hours. The drug release pattern exhibited by the formulations is displayed in Fig.5.

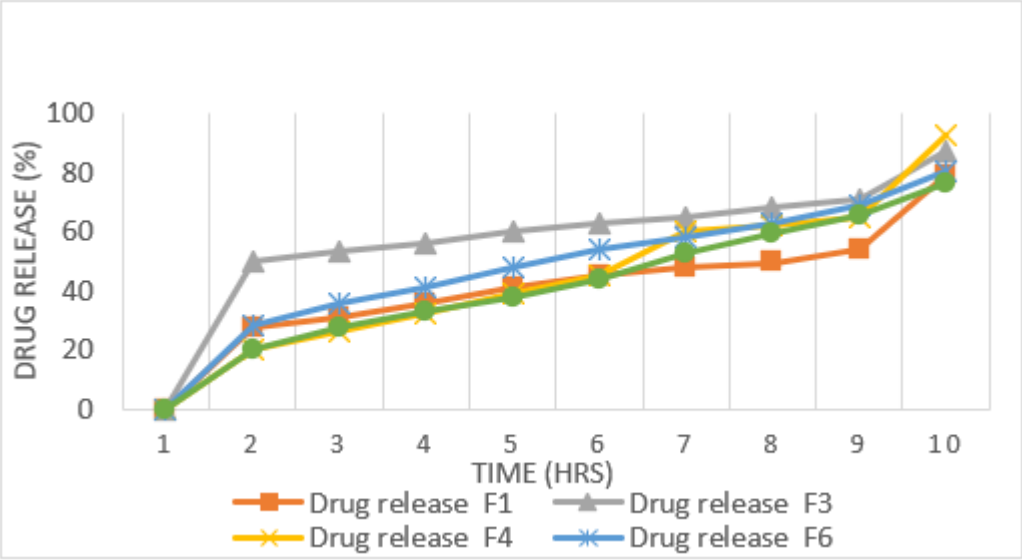

Figure 5: Drug release profile of formulations F1, F3, F4, F6, and F8

CHARACTERISATION OF FELODIPINE SOLID LIPID NANOPARTICLES:

The particle size, polydispersity index (PDI), and zeta potential were determined for F4 formulation as the cumulative percentage of drug release was obtained high for it.

Particle size and Polydispersity index (PDI):

The average particle size of F4 formulation was found to be 444.5 nm and the polydispersity index was found to be 0.357. The polydispersity index is an important parameter that determines the sample particle size distribution. The PDI measures the degree of heterogeneity in particle size in a given sample. The polydispersity index value was obtained in a moderate range i.e., 0.357 depicting that there was a slight variation in particle size distribution. The particle size distribution of the F4 formulation is neither monodisperse nor highly polydisperse. Hence it is confirmed that the formulation F4 contains particles of both small and large sizes moderately.

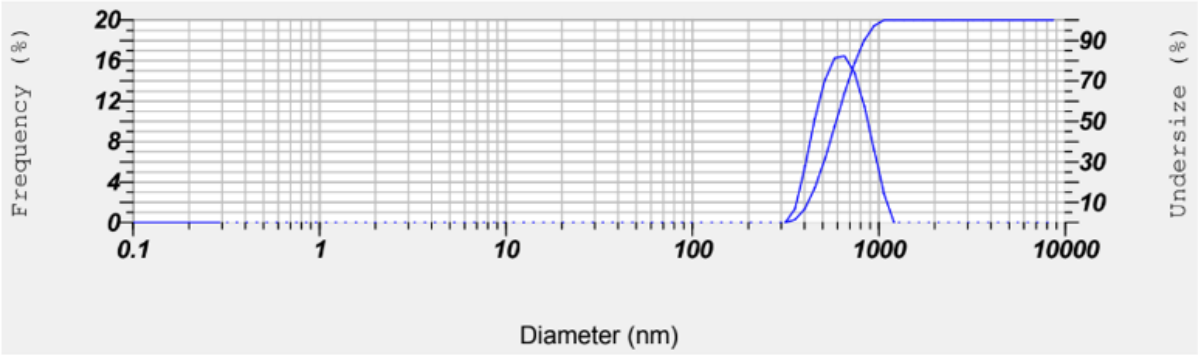

Figure 6: Average size distribution of formulation F4.

Zeta potential:

The zeta potential of SLN formulation F4 was determined by using Zetasizer (HORIBA SZ-100). The F4 formulation exhibited a zeta potential value of -14.4mV which was slightly negative due to the presence of a carboxylic acid group of stearic acid. The negative zeta potential value indicates that the particle surface was negatively charged causing repulsion between the particles and thus preventing the aggregation of particles and thus maintaining the stability of the formulation.

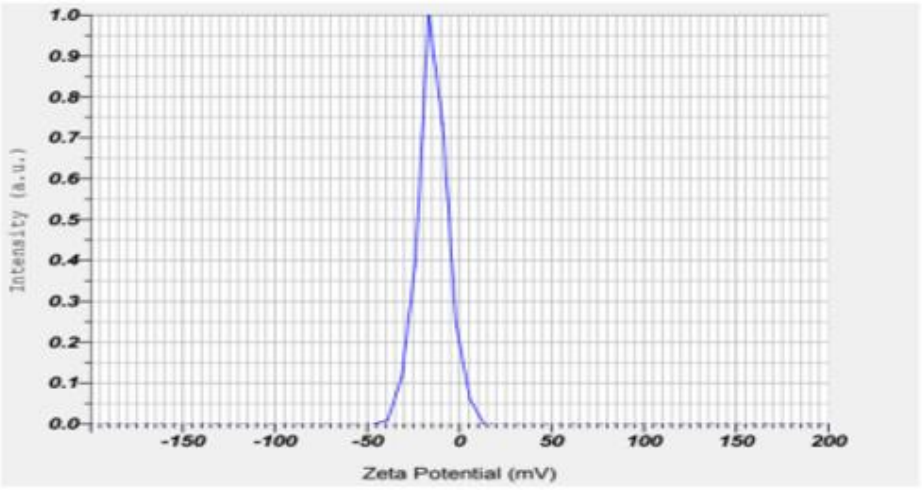

Figure 7: Graph showing the zeta potential of F4 formulation.

Morphology of SLN:

The surface morphology of prepared SLNs was examined by Scanning Electron Microscopy. It was observed that the nanoparticles were spherical with smooth surfaces (displayed in Fig.8).

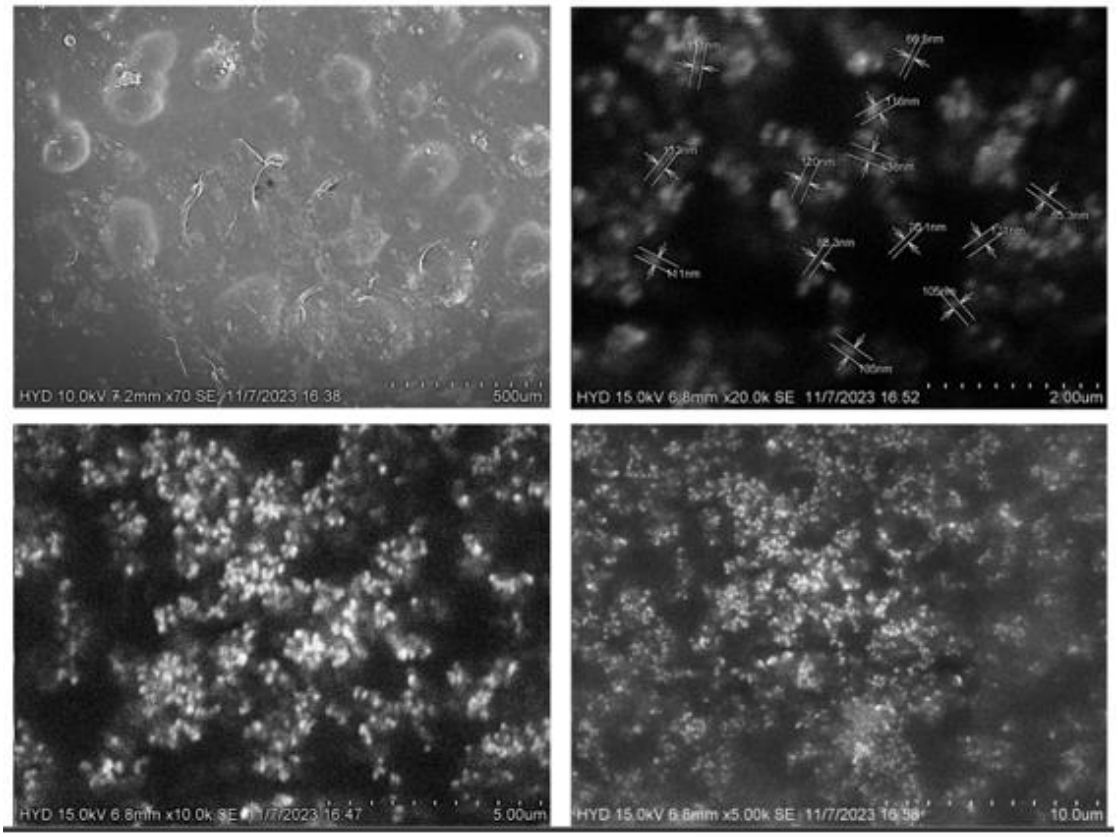

Figure 8: SEM images of Felodipine SLN

CONCLUSION:

The felodipine solid lipid nanoparticles were prepared to improve its dissolution profile and hence bioavailability. The solid lipid nanoparticles were prepared by the emulsion solvent diffusion method. Based on the results obtained for entrapment efficiency, drug loading, and *in-vitro* drug release F4 was selected as the best formulation and it was characterized for particle size, PDI, zeta potential, and SEM. The F4 formulation represented the particle size (444.5±1.58 nm), PDI (0.357), zeta potential (-14.4 mV), entrapment efficiency of 92.9±0.63%, and drug release of 92.6% for 24 hours. From the results, it was concluded that there was an increase in the dissolution profile of felodipine solid lipid nanoparticles.

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