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Formulation and *In-vitro* Evaluation of Nifedipine Nanosuspensions

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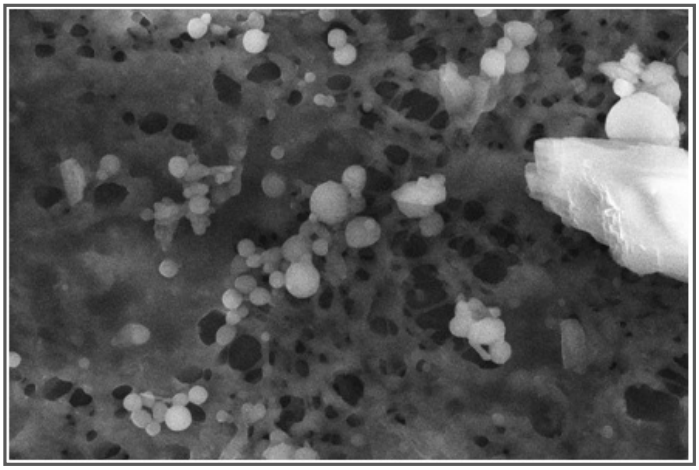
ABSTRACT

Nifedipine nanosuspensions were prepared by solvent evaporation method using various polymers such as SLS, Polaxomer, PVP-K30, urea and acetone. Optimized formulations of nanosuspension displayed zero order release kinetics and drug release. IR spectroscopic studies indicated that there are no drug-excipient interactions. NF8 is the best formulation which showed effective drug release of 99.75% within 30 min following zero order release kinetics. Thus nanosuspension can be a better alternative for the solubility and dissolution rate enhancement of nifedipine.

Graphical Abstract



SEM image of optimized nifedipine nanosuspension

Keywords: Nifedipine, nanosuspensions, SEM, dissolution studies.

INTRODUCTION

Drug delivery systems are becoming increasingly sophisticated as pharmaceutical scientists acquire a better understanding of the physicochemical and biological parameters pertinent to their performance. Despite tremendous advantages in drug delivery, the oral route remains the most preferred route for the administration of therapeutic agents because of the low cost of therapy and ease of administration leads to high-level of patient compliance. On the other hand, this high-throughput screening process has done little to address the issue of poor bioavailability of orally administered drug candidates.

Formulating a poorly water soluble drug has always been a challenging problem confronted by the pharmaceutical scientist. The formulation of nano-sized particles can be implemented to all drug compounds belonging to biopharmaceutical classification system (BCS) classes II and IV to increase their solubility and hence partition into gastrointestinal barrier [1]. Micronization is used for class II drugs of (BCS), i.e. drugs having a good permeability and poor solubility [2]. There are many conventional methods for increasing the solubility of poorly soluble drugs, which include micronization, solubilization using co-solvents, salt form, surfactant dispersions, precipitation technique, and oily solution [3]. Other techniques are like liposomes, emulsions, microemulsion, solid dispersion and inclusion complexation using cyclodextrins show sensible achiever, but they lack in universal applicability to all drugs [4]. These techniques are not applicable for those drugs which are not soluble in aqueous and organic solvents.

Nanotechnology opens up new vistas of research in the development of novel drug delivery systems [5]. “Nano” word comes from the Greek word ‘nanos” which means dwarf. Nano means it is the factor of 10^{-9} or one billionth. Nanosuspension is submicron colloidal dispersion of drug particles [6]. A pharmaceutical nanosuspension is defined as very finely colloid, biphasic, dispersed solid drug particles in an aqueous vehicle, size below 1 µm stabilized by surfactants and polymers prepared by suitable methods for drug delivery applications [7]. Nanosuspension has revealed their potential to solve the problem associated with the delivery of poorly water soluble and poorly water and lipid soluble drugs. It enhances the absorption and bioavailability and help to reduce the dose of conventional oral dosage forms [8].

Nifedipine is a selective hypertensive calcium channel protein inhibitor which targets L-type voltage-sensitive calcium channels [9]. Nifedipine is a vasodilator that is selective for inotropic over chronotropic cardiac effects [10]. It has also been shown to induce apoptosis in human glioblastoma cells and inhibit advanced glycation end product-induced mesangial cell damage *in vitro* [11]. Additionally, nifedipine has been suggested to work as an antioxidant and antiinflammatory agent against AGEs in tubular cells by suppressing RAGE expression via PPAR-γ activation [12].

For successful treatment of hypertension it is essential to maintain constant plasma drug concentration which can be achieved by giving the drug in target and controlled release dosage form which can improve the patient compliance. So, Nifedipine is a suitable candidate to design target and controlled release dosage form. In the present research work an attempt was made to improve the solubility and dissolution rate of Nifedipine.

MATERIALS AND METHODS

Nifedipine was obtained as a gift sample from Dr Reddy’s Laboratories, Hyderabad. Polaxomer, urea, PVP K30, acetone were purchased from Rankem, Mumbai. All other chemicals and solvents used in this study were of analytical grade.

Preparation of Nifedipine nanosuspension by solvent evaporation method: Nanosuspension was prepared by the solvent evaporation technique [13]. Nifedipine was dissolved in acetone at

room temperature (organic phase). This was poured into water containing different stabilizers of PVP K30, urea, polaxomer and SLS maintained at room temperature and subsequently stirred on magnetic stirrer which is stirred at rpm 800-1000 for 30 min allowing the volatile solvent to evaporate. Addition of organic solvents by means of a syringe positioned with the needle directly into stabilizer containing water. Organic solvents were left to evaporate off under a slow magnetic stirring of the nanosuspension at room temperature for 1 hour followed by sonication for 1 h. The composition of Nifedipine nanosuspensions were given in table 1.

Table 1. Composition of Nanosuspension of Nifedipine

Ingredients (mg tablet^{-1})	NF1	NF2	NF3	NF4	NF5	NF6	NF7	NF8	NF9	NF10	NF11	NF12
Nifedipine	10	10	10	10	10	10	10	10	10	10	10	10
SLS (mg)	5	--	--	10	--	--	15	--		7.5	--	7.5
PVP K-30	--	5	--	--	10	--	--	15	--	7.5	7.5	--
UREA	--	--	5	--	--	10	--	--	15	--	7.5	7.5
Polaxomer	10	10	10	10	10	10	10	10	10	10	10	10
Acetone (mL)	5	5	5	5	5	5	5	5	5	5	5	5
Water (mL)	40	40	40	40	40	40	40	40	40	40	40	40

Evaluation of nifedipine nanosuspensions

FT-IR spectroscopy: Fourier Transform Infrared (FT-IR) spectra of nifedipine and optimized formulation mixture were recorded in a FTIR spectrophotometer. Potassium bromide pellet method was employed and background spectrum was collected under identical conditions. Each spectrum was derived from 16 single average scans collected in the range of 400-4000 cm^{-1} at the spectral resolution of 2 cm^{-1}.

SEM: The external morphology of nanosuspension formulations was analyzed by a scanning electron microscope (Jeol, JSM-840 A, Japan). The sample was adhered to the brass specimen club using double sided platinum coated electrically conductive adhesive tape for 300 s at 15 Ma.

Drug content uniformity: 10 mL of each formulation was taken and dissolved in 10 mL isotonic solution and kept overnight. 10 mg (similar as in formulation) of drug was taken and dilution was made to 10 µg mL^{-1}. The dilutions were filtered and analyzed using UV for their content uniformity. The absorbance of the formulations were read using one cm cell in a UV-Vis spectrophotometer (T60 UV Spectrophotometer). The instrument was set at 238 nm. The drug content in each formulation was calculated based on the absorbance values of known standard solutions.

Entrapment efficacy: The freshly prepared nanosuspension was centrifuged at 20,000 rpm for 20 min at 5°C temperature using cool ultracentrifuge. The amount of unincorporated drug was measured by taking the absorbance of the appropriately diluted 25 mL of supernatant solution at 238 nm using UV spectrophotometer against blank/control nanosuspensions. DEE was calculated by subtracting the amount of free drug in the supernatant from the initial amount of drug taken. The entrapment efficiency (EE %) could be achieved by the following equation:

% Entrapment efficiency= Drug content *100/Drug added in each formulation

Particle size and shape: Average particle size and shape of the formulated nanosuspension was determined by using Malvern Zetasizer ZS using water as dispersions medium. The sample was scanned 100 times for determination of particle size.

in vitro drug release study.

In–vitro dissolution studies were performed in USP apparatus-II (LAB INDIA DS 8000), employing paddle stirrer at rotation speed of 50 rpm and 200 mL of pH 6.8 phosphate buffer as dissolution medium. Accurately weighed bulk drug and nanosuspensions were dispersed in dissolution medium. The release study is performed at 37 ± 0.5°C. Samples of 5 ml are withdrawn at predetermined time intervals and replaced with fresh medium to maintain sink condition. The samples were filtered through 0.22 µm membrane filter disc (Millipore Corporation) and analyzed for nifedipine after appropriate dilution by measuring the absorbance at 238 nm. The results of in vitro release profiles obtained for the NDDS formulations were fitted into different release kinetic models like 1. Cumulative percent drug released versus time (zero order kinetic model). 2. Log cumulative percent drug remaining versus time (first- order kinetic model).

RESULTS AND DISCUSSION

FTIR: Compatibility studies were performed using IR spectrophotometer. The IR spectrum of pure drug and physical mixture of drug and excipients were studied. The peaks of nifedipine at 3381 cm^{-1} indicates the presence of –CONH-, 2921 cm^{-1} indicates the presence of –R-COOH, 1564 cm^{-1} indicates C=N-. The nifedipine nanosuspension showed the peaks in the range representing the presence of original finger print regions. From the drug excipient compatibility studies it was observed that there are no interactions between the pure drug (Nifedipine) and excipients used in the formulation figure 1.

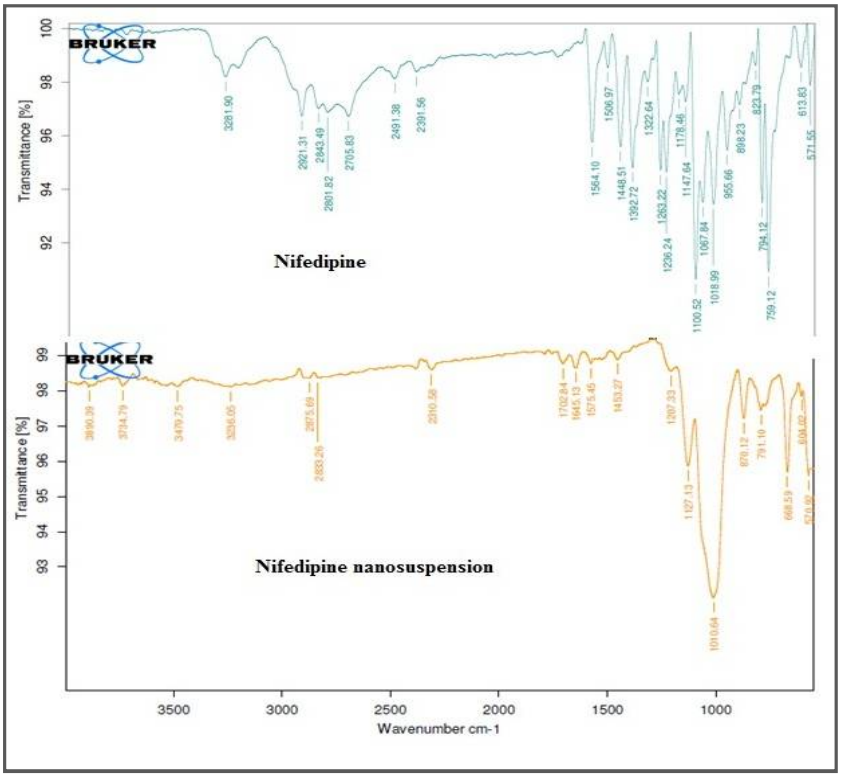


Figure 1. FTIR spectra of nifedipine and its nanosuspension.

Drug content and entrapment efficacy: The drug content of the formulated nanosuspension was found in the range of 78.23 to 98.47 %. The entrapment efficacy of the formulated nanosuspension was found to be in the range of 84.16% - 97.58%. The drug content and entrapment efficiency of nanosuspensions were given in the table 2.

Table 2. Drug content and entrapment efficiency of nanosuspensions

Formulation	Drug content	Entrapment efficiency
NF1	78.23±0.67	84.16±0.33
NF2	80.14±0.42	86.12±0.42
NF3	82.24±0.54	88.42±0.65
NF4	84.22±0.21	91.30±0.82
NF5	82.64±0.73	89.64±0.19
NF6	84.31±0.47	90.48±0.37
NF7	88.42±0.98	92.38±0.41
NF8	86.76±0.29	94.92±0.77
NF9	86.45±0.85	83.38±1.02
NF10	89.81±0.54	89.62±0.09
NF11	94.46±0.65	92.76±0.87
NF12	98.47±0.71	97.58±0.40

Values are expressed as mean ± S.D.

Dissolution studies: The *in-vitro* dissolution profiles of nifedipine and its suspension were depicted in figure 2. The % of drug release of NF1 was 98.22% at 60 min, NF2 was 96.32% at 55 min, NF3 was 96.21% at 60, NF4 was at 98.64% 45, NF5 was 97.20% at 45min, NF6 was 96.32% at 50min, NF7 was 98.26% at 35min, NF8 was 99.75% at 30min, NF9 was 97.49% at 40min, NF10 was 98.71% at 35min, NF11 was 99.74% at 50min, NF12 was 99.57% at 50. Based on this results NF8 formulation was chosen as the best formulation and evaluated for particle size determination and SEM analysis. *In-vitro* drug release data of all the nanosuspension formulations of nifedipine was subjected to goodness of fit test by linear regression analysis according to zero order, first order kinetics and according to equations of drug release. The drug release from the nanosuspension was explained by using mathematical model equations such as zero order, first order, and equation methods. Based on the regression values it was concluded that the optimized formulation NF8 follows Zero order kinetics.

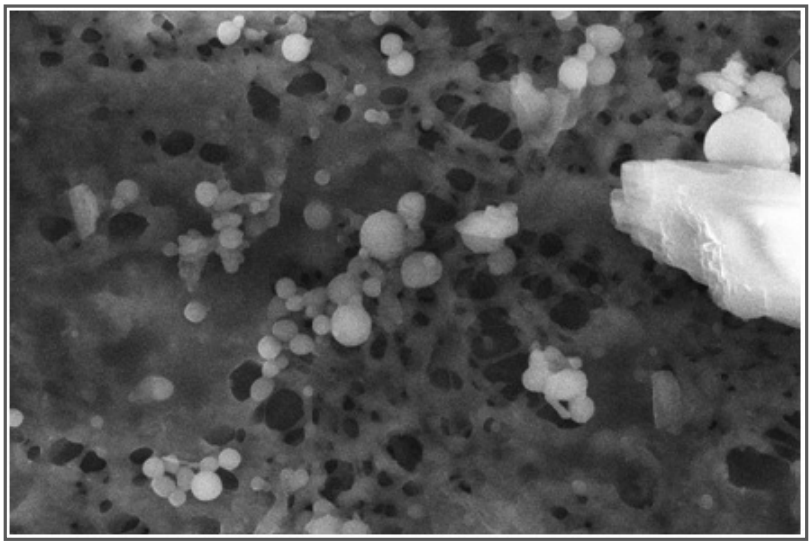


Figure 2. SEM image of optimized nifedipine nanosuspension

SEM: SEM images of nifedipine nanosuspension indicate well-separated particles with definite shape in nanosize range as represented in figure 3.

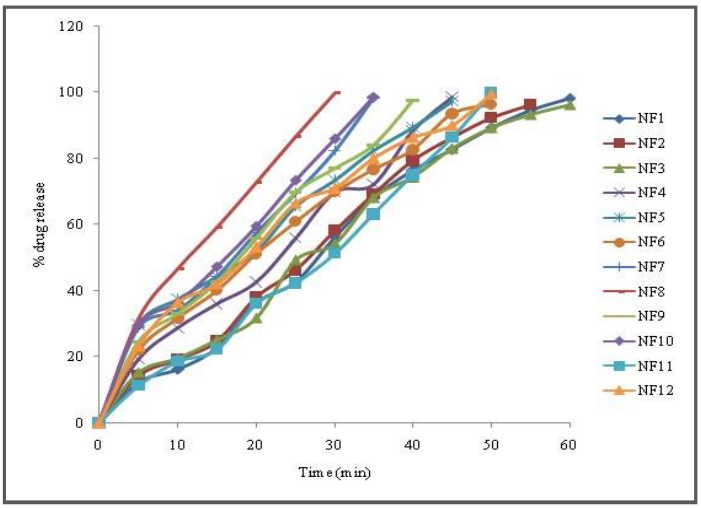


Figure 3. % Drug release of nifedipine and its nanosuspension.

Particle size: The particle size of the optimized formulation (NF8) depicted in figure 4 was found to be 371.8 ± 90.2 nm.

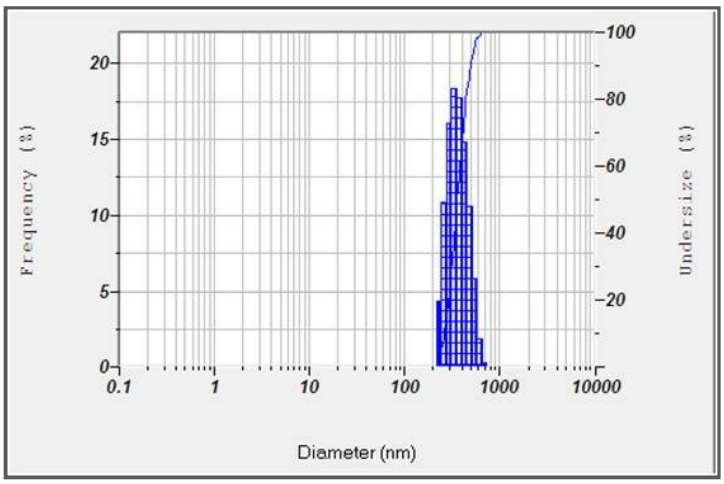


Figure 4. Particle size distribution of optimized nifedipine nanosuspension.

APPLICATION

Nifedipine, used to manage angina, high blood pressure, Reynaud's phenomenon and premature labour. It is one of the treatments of choice for Prinz metal angina. Used to treat severe high blood pressure in pregnancy. Its use in preterm labour may allow more time for steroids to improve the baby's lungs and time to transfer the mother to a well qualified medical facility before delivery. Nifedipine is taken by mouth and comes in fast and slow release formulations. Common side effects include light headedness, headache feeling tired, leg swelling, cough, and shortness of breath. Serious side effects may include low blood pressure and heart failure. It is a calcium channel blocker.

CONCLUSION

Nifedipine nanosuspensions were prepared by solvent evaporation method using various polymers such as SLS, Polaxomer, PVP-K30, urea and acetone. As the polymer is increases, the drug release rate decreases, whereas nanosuspension strength increases. Optimized formulations of

Nanosuspension displayed zero order release kinetics and drug release. IR spectroscopic studies indicated that there are no drug-excipients interactions. NF8 is the best formulation which showed effective drug release of 99.75% within 30 min following zero order release kinetics. Thus nanosuspension can be a better alternative for the solubility and dissolution rate enhancement of nifedipine.

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