FORMULATION AND EVALUATION OF NIFIDIPINE BY NANOSUSPENSION

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ABSTRACT : To prepare and evaluate the suitable nanosuspensions of Nifedipine to increase its solubility and dissolution. The Nifedipine nanosuspensions were prepared by emulsification solvent evaporation technique by applying ultrasonic energy through Sonicator, where the organic phase of drug solution in methanol was emulsified in aqueous phase containing hydroxy propyl methyl cellulose as solubilizer and sodium lauryl sulphate as stabilizer. The prepared nanosuspensions were characterised for particle size, surface morphology by SEM, drug excipient compatibility by FTIR and in-vitro drug release studies. Results showed that the prepared nanosuspensions having Scanning electron microscopic pictures revealed that the obtained nanosuspension particles were spherical in shape with surface smoothness and in-vitro drug release studies notified that the prepared nanosuspensions showed increase in solubility and dissolution of Nifedipine when compared with the pure form and F2 formulation is obtained as optimized formulation. The nanosuspensions of Nifedipine could be successfully prepared and can be concluded that the nanosuspension formulation is a promising approach to increase the solubility and dissolution of drugs like Nifedipine.

Keywords: Nifedipine, Nanosuspensions, Emulsification and Solvent Evaporation, polymers,FTIR, invitro drug release.

I.INTRODUCTION

Pharmaceutical industries are always looking for new methods in order to obtain adequate oral bioavailability, as most of the biological properties exhibiting NCEs are poorly water-soluble.[1] The increasing frequency of poorly water-soluble NCEs exhibiting therapeutic activity is of major concern to the development of new formulations in the pharmaceutical industry, which leads to low turnout in the development of new molecular entities as drug formulations are poor solubility and poor permeability of the lead compounds[2]. Recently, the formulation of such drugs as nanoscale systems (which have a size below 1μm) has quickly grown as a new and novel drug delivery system. The major distinctive of these systems is the quick dissolution rate, which improves bioavailability after oral administration[3]. The present article aims to review the nano-suspensions as an emerging and promising tool for the formulation of poorly soluble drugs. A Pharmaceutical nanosuspension is described as "very finely colloid, biphasic, discrete solid drug particles in an aqueous vehicle, stabilized by way of surfactants, for either parenteral and pulmonary administration, oral and topical use or, with decreased particle size, leading to a better dissolution rate and therefore increased bioavailability"[4]. The diameter of the suspended particle is less than 1 μm in size (*i.e.* 0.1nm-1000 nm). The particle size distribution of the solid particles in nanosuspensions is usually less than one micron with an average particle size ranging between 200 and 600 nm.[5] An upturn in the dissolution rate of micronized particles (particle size < 10 μm) is related to an increase in the surface area and consequently the dissolution velocity. Nanosize particles can increase dissolution velocity and dissolution velocity because of the vapor pressure effect [6].More than 40% of drugs are poorly soluble in water, so they show difficulties in formulating them in conventional dosage forms. Also, for class II drugs that are poorly soluble in aqueous and organic media, the problem is more difficult [7]. Nanosuspensions can be used to improve the solubility of drugs that are poorly soluble in aqueous as well as lipid media. As a result, the rate of flooding of the active compound rises, and the maximum plasma level is reached faster (*e.g.*, oral or intravenous (IV) administration of the nanosuspension). This is one of the typical advantages that it has over other approaches for increasing solubility. It is useful for molecules with poor solubility, poor permeability or both, which poses an important challenge for the formulators. Major issues associated with poorly water-soluble compounds [8]. Development, Formulation and

Evaluation of Nifedipine by Nanosuspension. Nifedipine is in a group of drugs called calcium channel blockers. It works by relaxing the muscles of your heart and blood vessels. Nifedipine is used to treat hypertension (high blood pressure) and angina (chest pain). Nifedipine may also be used for purposes not listed in this medication guide.[9]

II. MATERIALS AND METHOD

2.1 MATERIALS

Nifedipine was collected as a gift sample from Hetero labs,Hyd, polymers and other excipients were purchased from AR Chemicals, Hyd.

2.2 METHODODOLOGY

Compatibility studies:

The drug-polymer compatibility was ascertained by subjecting the drug and homogenates of drug and polymer to Infrared spectrophotometric study.

Fourier Transform Infrared Spectroscopy (FTIR)[10,11]

Assessment of possible incompatibilities between an active drug substance and different excipients forms an important part of the preformulation stage during the development of a dosage form. The use of FTIR technique allows pointing out the implication of the different functional groups of drug and excipients by analysing the significant changes in the shape and position of the absorbance bands. In this method individual samples as well as the mixture of drug and excipients were ground mixed thoroughly with potassium bromide (1:100) for 3-5 mins in a mortar and compressed into disc by applying pressure of 5 tons for 5 mins in hydraulic press. The pellet was kept in the sample holder and scanned from 4000 to 400 cm-1 in FTIR spectrophotometer. Then the characteristics peaks were obtained of all sample as well as mixtures.

Method of preparation of Nifedipine loaded nanosuspension:

Nanosuspension formulations were prepared by emulsion solvent evaporation method. The various different amount of polymers was dissolved in solvent mixture of methanol (5 ml) and dichloromethane (10 ml) very slowly on a magnetic stirrer and Nifedipine (500mg) was added to it and the contents were allowed to stand at room temperature for 30 to 45 minutes with occasional vortexing to allow complete solubilisation of drug and polymer. This solution was poured into 5 ml of each different concentration of surfactant solution. subject the mixture to Sonicator for 15 minutes The resulting solution was homogenized by using high pressure homogenizer for 20 minutes to form emulsion. Then the contents were stirred for 1 hours at room temperature with a magnetic stirrer to evaporate organic volatile solvent, The suspension was filtered through membrane filter. The filtrate was centrifuged (1000 rpm for 10 minutes) and nanosuspension was collected[12]

Table-: 1 Composition of the Nanosuspension

Ingredients	Batch no			
	F1	F2	F3	F4
Nifedipine (mg)	100	100	100	100
HPMC K15 M (mg)	100	200	-	-
Eudragit RLPO	-	-	100	200
SLS(mg)	50	50	50	50
Methanol (ml)	5	5	5	5
Dichloromethane (ml)	10	10	10	10
Water	q.s	q.s	q.s	q.s

Evaluation of Nifedipine loaded nanosuspension:

Particlesize:

All the prepared batches of nanosuspension were viewed under microscope to study their size. Size of nanosuspension from each batch was measured at different location on slide by taking a small drop of nanoparticle dispersion on it and average size of nanosuspension were determined.[13]

SEM analysis

The morphology of Nanosuspension was studied by a scanning electron microscope. For this purpose, the sample was lyophilized and placed on aluminum stubs and the surface was coated with a layer of gold particles using a sputter coater. The shape of the Nanosuspension was determined by scanning electron microscopy (SEM) (XL30, Philips, the Netherlands) at 15 kV and 750 mA[14].

Drug encapsulation efficiency:

Nanosuspension 50mg were dissolved in 100ml of phosphate buffer and the drug amount was determined by UV analysis. The encapsulation efficiency was determined as the mass ratio of entrapped Nifedipine in nanosuspension to the theoretical amount of the drug used in the preparation. The entrapment of the Nifedipine nanosuspension was expressed as loading capacity[15].

$$\text{Entrapment Efficiency (\%)} = \frac{\text{Amount entrapped}}{\text{Total drug loaded}} \times 100$$

In-vitro drug release studies:

The release studies were carried out by franz diffusion cell. It containing 10 ml Phosphate buffer. Phosphate buffer pH 7.4 (100 ml) was placed in a 10 ml of beaker. The beaker was assembled on a magnetic stirrer and the medium was equilibrated at 37±5^{0}C. Dialysis membrane was taken and one end of the membrane was sealed. After separation of non-entrapped Nifedipine dispersion was filled in the dialysis membrane and other end was closed. The dialysis membrane containing the sample was suspended in the medium. 1ml of aliquots were withdrawn at specific intervals, filtered after withdrawal and the apparatus was immediately replenished with same quantity of fresh buffer medium[16].

Percentage of drug release was determined using the following formula.

$$\text{Perentage drug release} = \frac{Da}{Dt} \times 100$$

Where, Dt = Total amount of the drug in the patch

Da = The amount of drug released

Stability studies:

Selected Formulation was subjected to stability studies as per ICH guidelines.

Following conditions were used for Stability Testing.

1. 25^{0}C/60% RH analysed every month for period of three months.
2. 30^{0}C/75% RH analysed every month for period of three months.
3. 40^{0}C/75% RH analysed every month for period of three months[17].

III. RESULTS AND DISCUSSION

Drug - excipient compatibility studies (FT-IR):

The compatibility between the drug and the selected lipid and other excipients was evaluated using the FTIR peak matching method. There was no appearance or disappearance of peaks in the drug-lipid mixture, which confirmed the absence of any chemical interaction between the drug, lipid, and other chemicals.

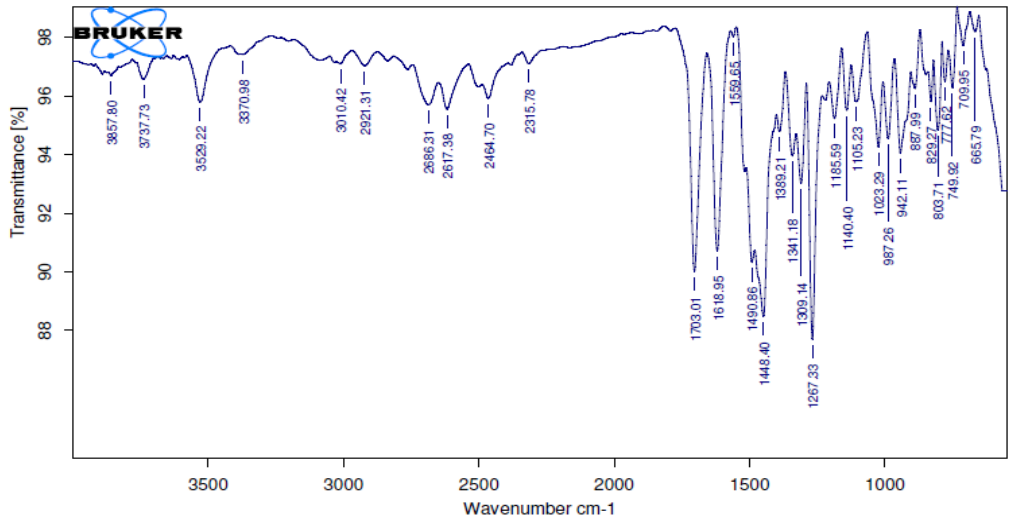

Fig.1. FT-IR Sample for Nifedipine

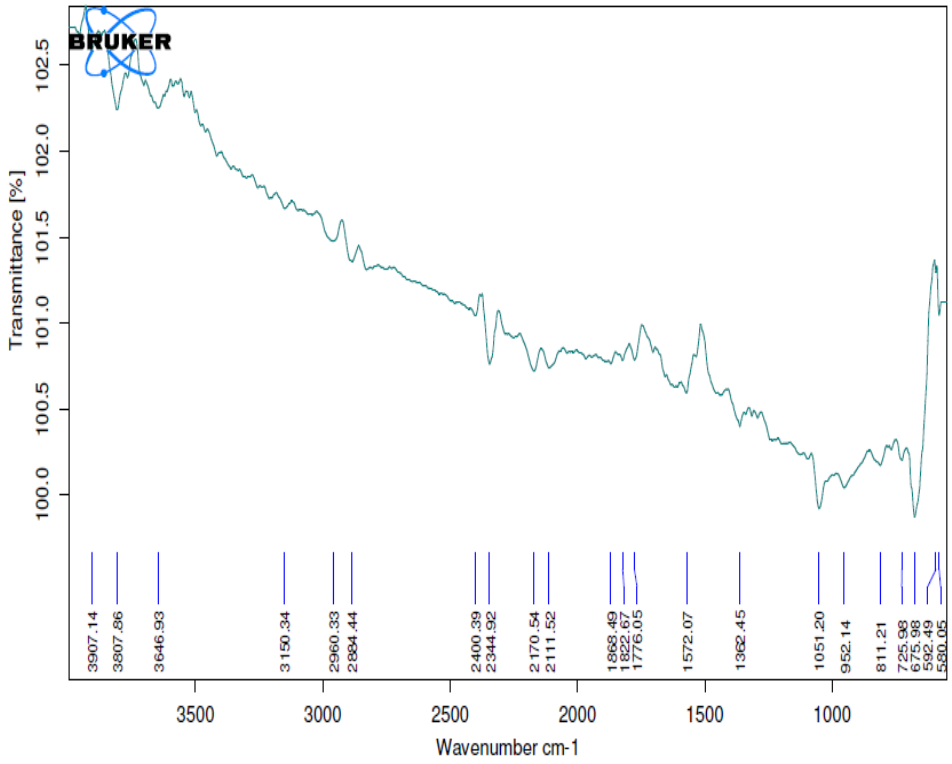

Fig.2. FT-IR Sample for nano suspension mixture

EVALUATION PARAMETERS:

Entrapment Efficiency:

Separation of the unentrapped drug from Nano suspension was done by exhaustive dialysis method. A measured quantity of Nano suspension was placed in a dialysis tube to which osmotic cellulose membrane was attached securely on one side and the dialysis tube was suspended in 100ml of phosphate buffer pH 7.4 which was stirred continuously using a magnetic stirrer. Through the osmotic cellulose membrane, the unentrapped drug was separated into the medium. For every one hour, the whole medium was replaced with the same quantity of fresh medium and continued for about 80 mins till the absorbance of the collected medium reaches a constant reading indicating complete separation of the unentrapped drug. The Nano suspension in the dialysis tube was further lysed with propane–1–ol and the entrapped drug was estimated with the help of a double beam UV spectrophotometer at 275 nm. The entrapment efficiency was measured in % with the help of the following equation,

$$\% \; Entrapment \; efficiency = \frac{A}{T}$$

Table-: 2 Drug entrapment efficiency of all formulation

F.no	Drug entrapment efficiency
F1	78.24
F2	96.32
F3	89.41
F4	94.45

Determination of Vesicle morphology and Size

The morphological characteristics of formulated Nano suspension were carried by using Scanning electron microscopy (SEM). A small drop of Nano suspension was placed between two rivets fixed on a gold plated copper sample holder. The whole system was slushed under a vacuum in liquid nitrogen. The sample was heated to -85^{0}C for 30 min to sublime the surface moisture. Finally, the sample was coated with gold and allowed the SEM to capture the images at a temperature of -120^{0}c and voltage of 5kV.

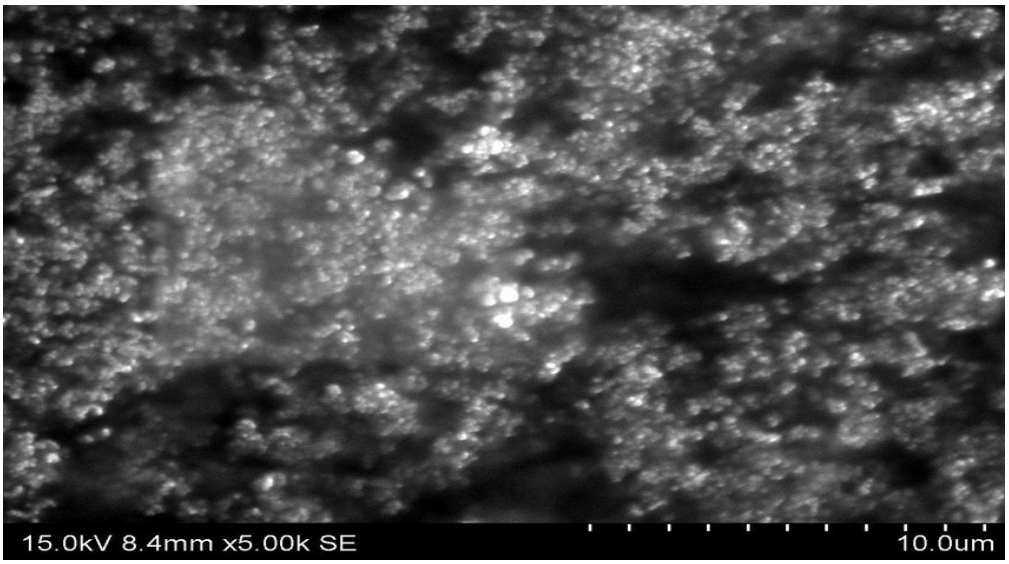

Fig.3. SEM analysis of Optimized Nano suspension

Table-:3 Evaluation Studies of Vesicle size Nano suspension

Batch No	Particle size (nm)
F1	255.86
F2	302.26
F3	299.36
F4	285.10

***In vitro* drug release studies:**

The release of the drug from Nano suspension was investigated using the dialysis tubing method. All the formulations were separately placed in a dialysis membrane of 5cm length with closed ends which were washed and soaked in phosphate buffer pH 7.4 for about 15min. The membrane was suspended in a beaker containing 500ml of phosphate buffer pH 7.4 as diffusion medium maintained at a temperature of 37 ± 0.5^0 C and stirred continuously using a magnetic stirrer at a constant speed. At a regular time interval of one hour, 5ml of diffusion medium was withdrawn periodically for about 80 mins and immediately replaced with the same amount of fresh diffusion medium to maintain sink condition. The collected samples were measured spectrophotometrically at 275 nm.

Table-:4 *In- vitro* drug release studies of (F1-F4) formulation

Time (mins)	F1	F2	F3	F4
0	0	0	0	0
10	12.18	21.63	20.12	17.33
20	23.42	30.25	29.68	27.85
30	34.67	42.16	39.15	39.90
40	41.98	49.80	44.85	45.82
50	55.18	59.86	55.18	56.89
60	70.46	79.54	75.12	77.35
70	76.81	88.96	86.15	83.66
80	88.69	96.84	93.86	94.55

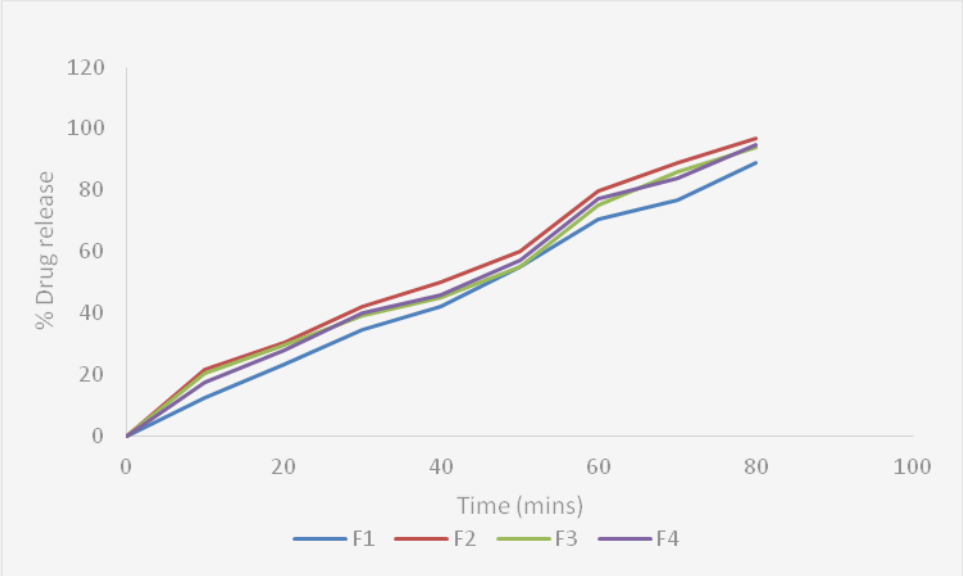

Fig.4. In vitro drug release studies of (F1-F4) formulation

Stability studies:

There was no significant change in physical and chemical properties of the Nano formulation F-2 after 3 months. Parameters quantified at various time intervals were shown.

Table-: 4 Results of stability studies of optimized formulation F-2

Formulation Code	Parameters	Initial	1st Month	2nd Month	3rd Month	Limits as per Specifications
F-2	**25^{0}C/60%RH % Release**	96.84	96.55	95.20	95.02	**Not less than 85 %**
F-2	**30^{0}C/75% RH % Release**	96.84	95.99	95.12	94.99	**Not less than 85 %**
F-2	**40^{0}C/75% RH % Release**	96.84	95.25	95.10	94.45	**Not less than 85 %**

IV.CONCLUSION

The present research proposed a novel formulation Nano suspension for controlled release. Investigation of the preparation, characterization and in-vitro release of the Nano suspension was carried out. The different formulations of with various ratios of drug-polymer and surfactant were evaluated and optimized. In this research, a drug encapsulation efficiency as high as 96.32% has been achieved. The method used for the formulation of Nifedipine containing HPMC K15 M and Eudragit RLPO. Nano suspension formulations showed good results in terms of the assayed drug content and encapsulation efficiency. This indicates that the method used for the formulation produced good yield and it was suitable and reproducible in nature. It was found that as the concentration of Eudragit RLPO, the % of encapsulation efficiency was also increased. Permeation studies with dialysis membrane were carried out as per the method reported. The formulations showed good drug release from the polymer, the *in vitro* drug release profiles of all the formulations showed an initial burst effect, and followed by a slow drug release. The burst release of drug is associated with those drug molecules dispersing close to the nanoparticle surface, which easily diffuse in the initial incubation time. The Nifedipine release was faster for those Nanosuspension with higher drug content. Formulation (F-2) showed the highest encapsulation efficiency.

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