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RESEARCH ARTICLE

PREPARATION AND EVALUATION OF NANOSUSPENSION OF NIFEDIPINE

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ABSTRACT

Processing a poorly soluble drug is a challenging task during formulation development. Nano suspension is one of the techniques that can improve the dissolution of poorly soluble drug. Nanosuspension is the fine dispersion of uniform-sized solid particles in an aqueous vehicle. The present work was aimed at the formulation and evaluation of nanosuspension of Nifedipine, a poorly water soluble anti-hypertensive drug. The nanosuspensions were prepared by nanoprecipitation alone and in combination with ultrasonication method using acetone as solvent and water as antisolvent. The prepared nanosuspensions were characterized for particle size, zeta potential, polydispersity index, Scanning electron microscopy (SEM), drug entrapment efficiency and release behaviour. The effect of variable concentration of drug, stabilizer, extent of ultrasonication, and solvent to antisolvent ratio on the physical, morphological and dissolution properties of Nifedipine were studied. The average particle size of Nifedipine nanoparticles was found to be in the range of 13–230 nm. It was further confirmed by SEM photograph. The particle size varies with increase in concentration of drug and stabilizer. The preparations showed negative zeta potential and polydispersity index in the range of 0.3-0.9. The dissolution of prepared Nifedipine nanoparticles markedly increased as compared to the pure drug. The dissolution profiles of nanosuspension formulation showed up to 74.4 % release in 4 h. It may be concluded that the nanoprecipitation with ultrasonication have potential to formulate homogenous nanosuspensions with uniform-sized stable nanoparticles of Nifedipine. The prepared nanosuspension showed enhanced dissolution which may lead to enhanced oral bioavailability of Nifedipine.

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INTRODUCTION

The formulation of poorly water-soluble drugs has always been a challenging problem faced by pharmaceutical scientists and it is expected to increase because approximately 40% or more of the new chemical entities being generated through drug discovery programmers are poorly water-soluble (Vishvajit et al., 2010; Jain et al., 2010; Mukherjee et al., 2009 and Sahu, 2013). Several techniques have been developed concerning the optimization of the dissolution rate of these drugs. Such methods include particle size reduction, solubilization, salt formation and preparation of solid dispersion systems. Nevertheless, there are several disadvantages and limitations in the use of these techniques. Specifically, the particle size reduction technique is practically limited regarding the minimum size that could be achieved and producing very fine powders deteriorates their flow properties and wettability, while it advances the development of electrostatic forces, leading to problematic formulations.

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The solubilization techniques correspond to liquid preparations usually do not assure the patient's acceptability and normally appear to have poor stability. Salt formation is a complicated process, while it is not feasible for neutral compounds. In addition, the dissolution enhancement is not always predictable due to the high correlation of the salt's solubility with the pH value which appears to be a highly variable in the gastrointestinal tract. In fact, for biopharmaceutic class II drugs, the bio-absorption process is rate-limited by dissolution in gastrointestinal fluids. According to the Noyes–Whitney equation, the dissolution rate of poorly water-soluble drugs could be increased by reducing the particle size to the micro or nano-scale thus increasing the interfacial surface area. Nanosuspensions is the colloidal dispersions of nano-sized drug particles that are produced by a suitable method and stabilized by a right stabilizer. They can also be defined as the biphasic system consisting of pure drug particle dispersed in an aqueous vehicle in which the diameter of the suspended particle is less than 1um in size. Techniques of drug nanosuspensions preparation can be categorized into two principle classes; top-down and bottom-up technologies. The "top-down" technologies are the mechanical communication

processes of larger drug particles, as in milling (jet mills and pear-ball mills) and homogenization (high-pressure homogenizers). The "bottom-up" technologies begin with the molecules which are dissolved and then precipitated through on-solvent addition as in supercritical fluid (SCF) technology, spray-freezing into liquid process, evaporative precipitation into aqueous solution (EPAS)and liquid solvent change process. Although "top-down" approaches are widely employed, the drawbacks associated with mechanical attritions processes, such as time consumption, intensive-energy use, introduction of impurities, inadequate control of particles size and electrostatic effects, promote greater interest toward "bottom-up" creation of nanoparticles (Vemula, 2010).

Characterization of nanosuspension of nifedipine

Fourier Transform – Infrared (FT-IR) Spectroscopic Analysis

Drug excipients interactions were studied by FTIR spectroscopy. The spectra were recorded for Nifedipine, HPMC, PVA individually and physical mixtures (drug & PVA; drug & HPMC). Samples were prepared in KBr pellets using 2 mg drug in 8 mg KBr with a hydrostatic press at a force of 8 t cm^{-2} for 2 min. The scanning range was 450–4,000 cm^{1} and resolution was 2 cm^{1}.

Table 1. Formulae of various nano suspensions

Ingredients	FF1	FF2	FF3	FF4	FF5	FF6	FF7	FF8	FF9
Drug	60	60	60	60	20	40	80	40	60
PVA	0.15%	0.25%	0.5%	1%	0.5%	0.5%	0.5%	-	-
HPMC	-	-	-	-	-	-	-	0.5%	0.5%
Acetone	1ml	1ml	1ml	1ml	1ml	1ml	1ml	1ml	1ml
Water	20ml	20ml	20ml	20ml	20ml	20ml	20ml	20ml	20ml

In the last decade, bottom-up techniques that rely on dissolving the drug in a solvent and precipitating it by the addition of a non-solvent, like supercritical fluid (SCF) technique and liquid precipitation, have been widely investigated to obtain ultrafine drug particles, such as cephradine, ce Amsa furoxime axetil and danazol (Kalpesh, 2011; Aswathy, 2012; Ekambaram, 2011 and Tripathi, 2003). Nifedipine, a highly potent calcium-channel blocker belonging to the group of 1,4-dihydropyridines widely used in the treatment of vascular diseases such as hypertension and angina pectoris (http://www.healthstats.com/en/hypertension-statistics).
Nifedipine has low aqueous solubility (~20 µg/ml) and could be classified as a BCS class II drug (Ekambaram, 2012). For poorly soluble drugs, the dissolution in the gastrointestinal fluid media is the rate limiting step for the absorption of the drugs. So, nanonisation has been employed for treating Nifedipine. In this work the production of nanosuspensions was intended for oral use of Nifedipine using Precipitation – Ultrasonication technique.

MATERIALS AND MATERIALS

Nifedipine, Polyvinyl alcohol (PVA) and Hydroxy Propyl Methyl Cellulose (HPMC) were obtained as gift samples from Dr. Reddy's Laboratories, Hyderabad. Acetone used was of analytical grade from SD Fine Chemicals Pvt. Ltd, Mumbai, India. All the other reagents and chemicals used were of analytical reagents obtained from local source.

METHODS

Preparation of Nifedipine nano particles: Nifedipine nanoparticles were produced by precipitation–ultrasonnication technique. The required amount of drug was completely dissolved in water-miscible solvent acetone. Different concentrations of drug in solvent (20, 40, 60, 80 mg/ml) were used. The obtained drug solution was then injected into the water containing the stabilizer such as HPMC and PVA under stirring at 1,000 rpm. Precipitation of solid drug particles occurred immediately upon mixing. The suspension was then ultrasonicated for 15 min under cold condition (Ekambaram, 2012).

Size Measurement and Zeta Potential Analysis

The particle size and the polydispersity Index (PI) of the drug particles was measured immediately after precipitation by dynamic laser light scattering (Zetasizer, Malvern Instruments, Malvern). The measurement was done in triplicate and size Z-Average (d.nm) and PI was reported. The zeta potential of the preparations was also measured using (Zetasizer, Malvern Instruments, Malvern) (Amsa, 2014).

Scanning Electron Microscopy (SEM)

Particle morphology was observed using scanning electron microscopy (SEM) JSM-6360 (JEOL Inc., Japan). A small drop of the suspension was air dried followed by oven drying and was fixed on an SEM stub using double-sided adhesive tape and coated with Au at 20 mA for 6 min through a sputter-coater (Ion sputter JFC 1100). A scanning electron microscope with a secondary electron detector was used to obtain digital images of the samples at an accelerating voltage of 15 Kv (Amsa, 2014).

Drug Entrapment Efficiency (DEE)

The freshly prepared nanosuspension was centrifuged at 20,000 rpm for 20 min at 5 °C temperature using cooling ultracentrifuge. The amount of unincorporated drug was measured by taking the absorbance of the appropriately diluted 25 ml of supernatant solution at 350 nm using UV spectrophotometer against blank/control nanosuspension. DEE was calculated by subtracting the amount of free drug in the supernatant from the initial amount of drug taken. The experiment was performed in triplicate for each batch and the average was calculated. The entrapment efficiency (EE %) could be achieved by the following equation (Smitha, 2012).

Entrapment efficiency (%)=$W_{initialdrug}$ $W_{freedrug}/W_{initialdrug}\times100$.

X-ray Diffraction studies (XRD)

X-ray diffraction analysis was employed to detect the effect on crystallinity of precipitated nifedipine nanopartilces which was conducted using a XRD-6000 diffractometer (Shimadzu,

Japan) [14]. The powder was placed in a glass sample holder. CuK radiation was generated at 30 mA and 40 kV. Samples were scanned from 10° to 90° with a step size of 0.02°

Invitro drug release studies

The dialysis membrane diffusion technique was used. One millilitre of the nanosuspension was placed in the dialysis membrane (Mw cutoff 12,000–14,000 Hi-media), fixed in a Franz diffusion cell with the receptor volume of 20 ml. The entire system was kept at 37 °C with continuous magnetic stirring. Sample of 1 ml was withdrawn from the receptor compartment at predetermined time intervals and replaced by fresh medium. The amount of drug dissolved was determined using UV spectrophotometer at 350nm (Ding, 2011).

Physical stability study

Three temperature conditions were applied in the stability study of the nanosuspensions,4 °C (refrigerator), room temperature and 40 °C (Stability Chamber). Physical stability of the nanosuspension was evaluated after 3 months of storage. The particle size was and settling behaviour was analyzed by visual examination (Jawahar, 2009).

RESULTS AND DISCUSSION

Preparation of Nanoparticles

Nifedipine nanoparticles were produced by precipitation–ultrasonnication technique. The aqueous phase containing a suitable stabilizer has been used as the antisolvent and the use of water miscible solvent (acetone) having good solubility of Nifedipine has been explored as solvent. The effect of various variables like diffusing drug concentration, solvent:antisolvent ratio (S:NS), type of stabilizer, concentration of stabilizer, stirring time and ultrasonication has been observed. The results have been summarized in Table 2. Acetone was tried as solvent for the preparations. Acetone as solvent produced very uniform-sized nanoparticles on precipitation (Gavade, Hany, 2009). Nanosuspensions were prepared by using different solvent:antisolvent ratios (1:10, 1:15, 1:20). Preparations with solvent:antisolvent ratio of 1:20 were a bit more homogenous, hence were taken for the preparation as suitable solvent:antisolvent ratio. The effect of stirring time (5, 10, 30 and 60 min) on the particle size was studied [18]. No sign of aggregation due to stirring was observed, and the particle size was not dependent on stirring time. HPMC or PVA at various concentrations such as 0.1, 0.25, 0.5, 1.0 % was used as stabilizer for the preparations. From the preliminary studies, 0.5 % concentration of stabilizer was found to be optimum. The PVA-based formulations at concentration 0.5 % produced smaller and more uniform nanoparticles as observed by particle size analysis whereas preparations based on 0.5 % HPMC as stabilizer were a bit bigger and cuboidal in size. The preparations have been made with different diffusing drug concentration (20, 40, 60 and 80mg/ml). Preparations with 60 mg/ml diffusing drug concentration were found to be optimum for PVA-based formulations whereas in case of HPMC it was found at 40 mg/ml.

Characterization of Nanosuspension

Drug Entrapment Efficiency (DEE)

The formulations showed drug entrapment in the range of 86–98 %. Formulation (F3) containing drug concentration 60 mg/ml stabilised with PVA showed highest entrapment up to [19] 98 % w/v. Formulations containing PVA showed better entrapment in comparison to HPMC. The results have been shown in Table 2.

Table 2. Effect of drug concentration and surfactant concentration on polydispersity index, drug entrapement and drug release

Formulation code	Diffusing drug concentration (mg/ml)	PVA in %	HPMC in %	Polydispersity index (PI)	Drug entrapment efficiency (%)	Drug release in 4 h (%)
F1	60	0.15		0.776	85.4	58.5
F2	60	0.25		0.756	92.6	65.4
F3	60	0.5		0.975	97.5	74.4
F4	60	1		0.584	88.4	59.2
F5	20	0.5		0.371	94.9	55.3
F6	40	0.5		0.487	95.3	58.5
F7	80	0.5		0.748	86.7	61.1
F8	40		0.5	0.624	94.3	63.6
F9	60		0.5	0.893	91.2	57.8

Size Measurement and Zeta Potential Analysis

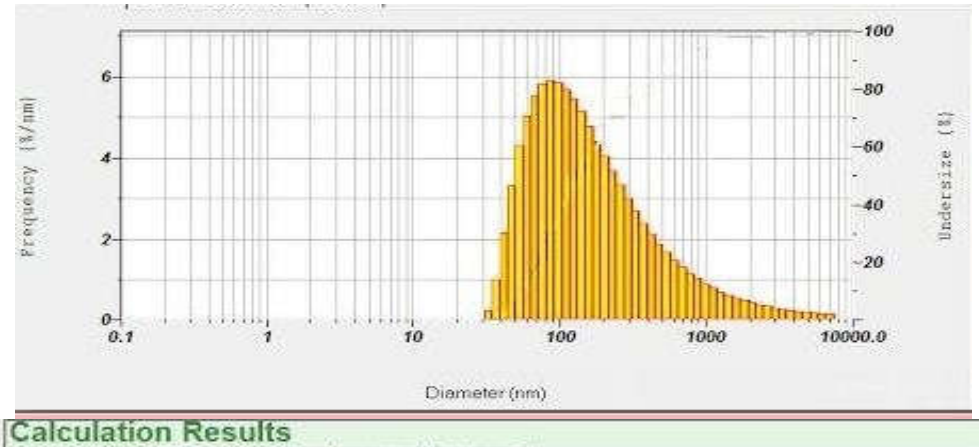
The particle size and PI of the formed drug particles was measured immediately after precipitation by dynamic laser light scattering (Zetasizer Ver. 6.11Malvern). The average particle size of Nifedipine nanoparticles was found to be in the range of 13–230 nm. The formulations containing 0.5 % PVA as stabilizer showed particle size in the range of 322.3 nm at various drug concentrations. The zeta potential of the nanoparticles was found to be -15, negative which may be due to the presence of terminal carboxylic groups. High potential values should be achieved to ensure a high-energy barrier and favour a good stability (Ofokansi, 2010).

Scanning Electron Microscopy (SEM)

Morphology of precipitated drug particles in the suspension after air drying followed by oven-drying. The drug particles precipitated with the PVA as stabilizer are spherical in shape and the size ranges from 100 to 500 nm. The particle size of PVA based based nanosuspension was found to be 0.5µm. The particles are discrete and uniform in size and there is no sign of agglomerations (Hecq, 2006).

Fourier Transforms Infrared Spectroscopy

Infra red spectra of Nifedipine and physical mixture of Nifedipine and HPMC, Nifedipine and PVA were comparable and the peaks of Nifedipine in the physical mixture are of lower intensity than pure drug.The results have been shown below. FTIR of drug-polymers interaction studies are shown in Fig 4-8 and the datas are reported in Table 3.



Calculation Results

Peak No.	S.P.Area Ratio	Mean	S.D.	Mode
1	1.00	322.3 nm	44.3 nm	37.6 nm
2	---	--- nm	--- nm	--- nm
3	---	--- nm	--- nm	--- nm
Total	1.00	322.3 nm	44.3 nm	37.6 nm

Histogram Operations

% Cumulative (1)	: 10.0 (%) - 55.7 (nm)
% Cumulative (2)	: 50.0 (%) - 134.4 (nm)
% Cumulative (3)	: 90.0 (%) - 647.6 (nm)
Mean	: 322.3 nm

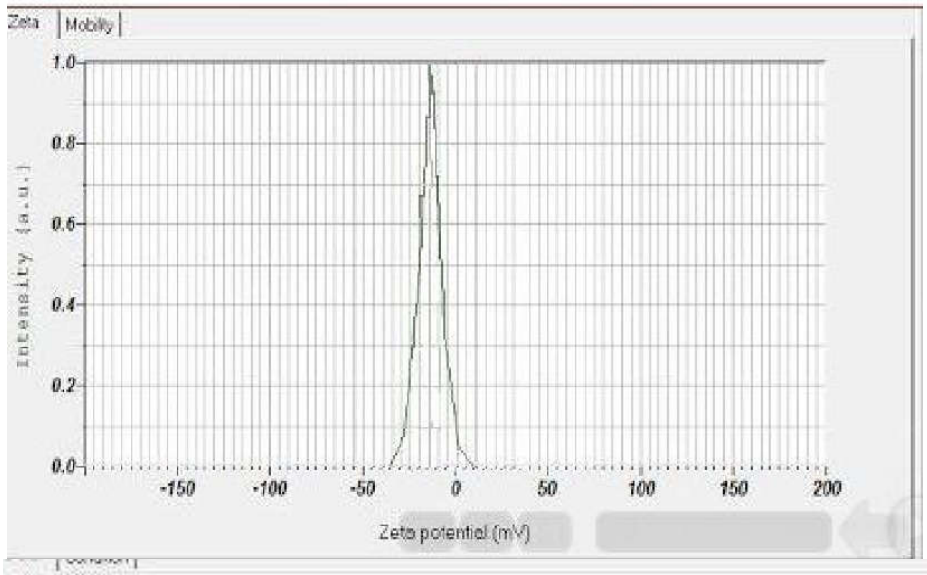
Cumulant Operations

Z-Average	: 13.1 nm
PI	: 0.975

Molecular weight measurement

Molecular weight	: ---
Mark-Houwink-Sakurada parameters	: ---

Figure 1. Particle analyzer results showing particle size and polydispersity index



Measurement Results

Date	: Tuesday, May 06, 2014 3:07:59 PM
Measurement Type	: Zeta Potential
Sample Name	: sample-1-zeta
Temperature of the holder	: 25.1 °C
Viscosity of the dispersion medium	: 0.893 mPa·s
Conductivity	: 0.196 mS/cm
Electrode Voltage	: 3.3 V

Calculation Results

Peak No.	Zeta Potential	Electrophoretic Mobility
1	-15 mV	0.000002 cm2/Vs
2	--- mV	--- cm2/Vs
3	--- mV	--- cm2/Vs

Zeta Potential (Mean)	: -15 mV
Electrophoretic Mobility mean	: 0.000002 cm^2/Vs

Figure 2. Particle analyzer results showing zeta potential

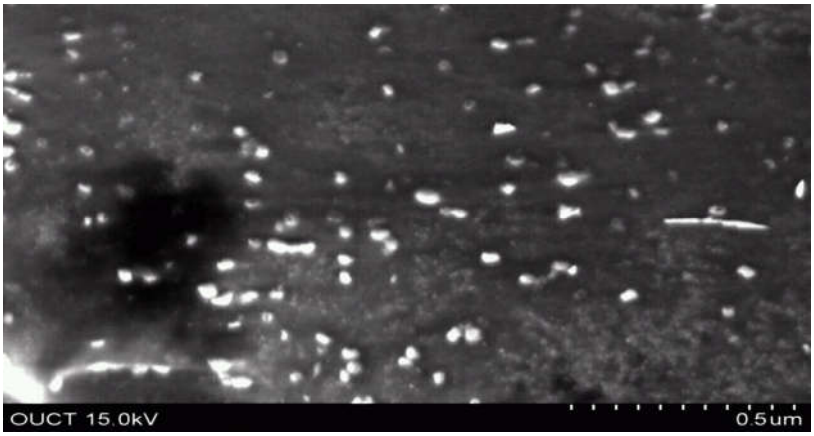


Figure 3. SEM photomicrograph of PVA based Nifedipine Nanoparticles

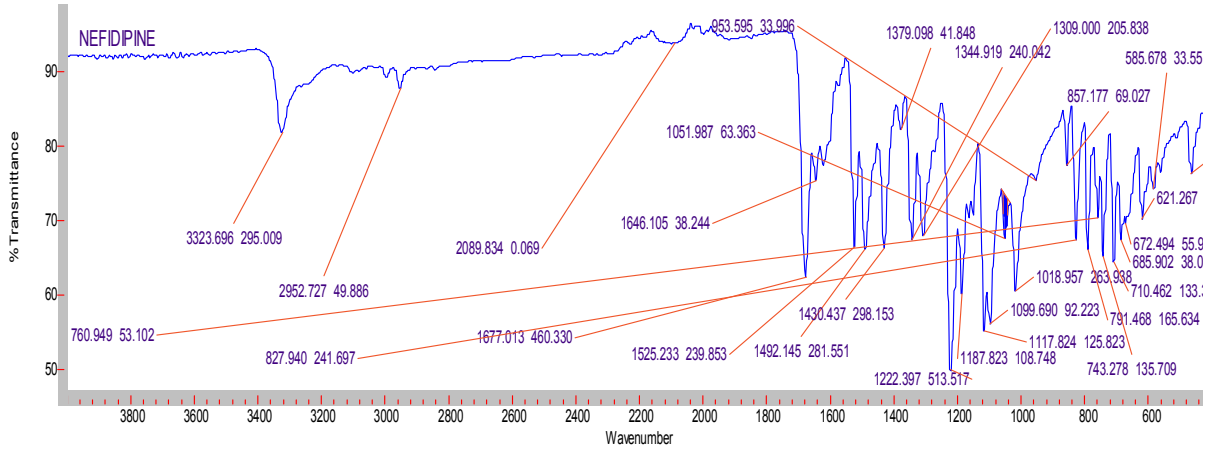


Figure 4. FTIR spectra of Nifedipine

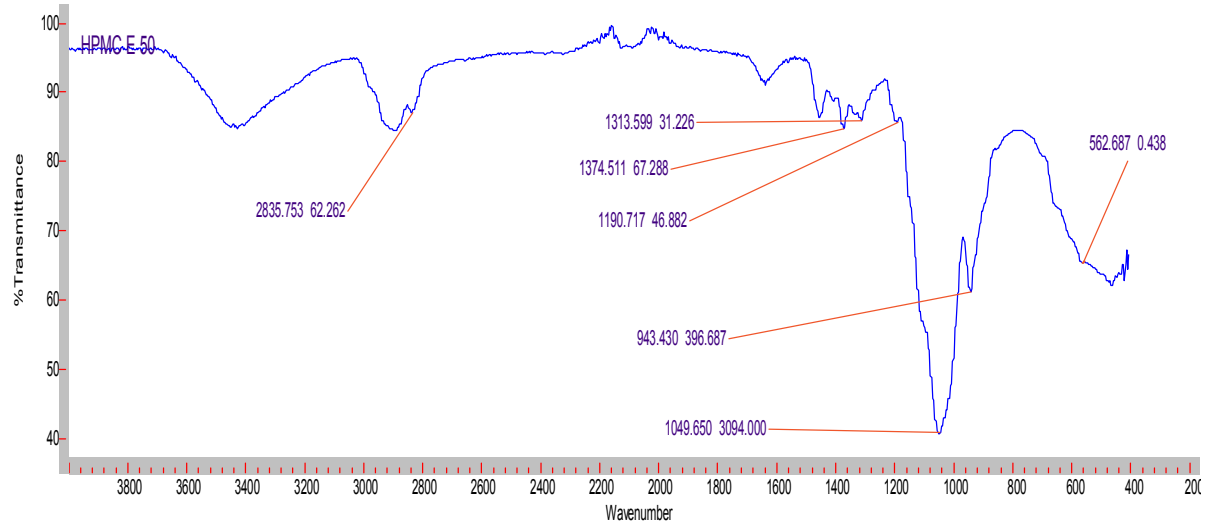


Figure 5. FTIR spectra of HPMC

It was found that Nifedine was compatible with each other and with polymers used in the formulation and there were no extra peaks observed.

In vitro release

It needs to be verified *in vitro* that nanoparticles were able to release incorporated drugs to achieve a biological effect. Membrane diffusion techniques are the most widely used experimental methods for the study of the *in vitro* release profiles of drugs incorporated in nanoparticles.

The release profile of Nifedipine nanosuspensions shows up to 74.4% (F3) release in 4 h. The dissolution of prepared Nifedipine nanoparticles markedly increased as compared to the original drug. The comparative release results of selected formulations have been shown. Formulations based on PVA showed better release than that of HPMC as stabilizer (Sudhir Verma, 2011).

Physical stability study

The Particle size of the formulations after 3 months was observed.

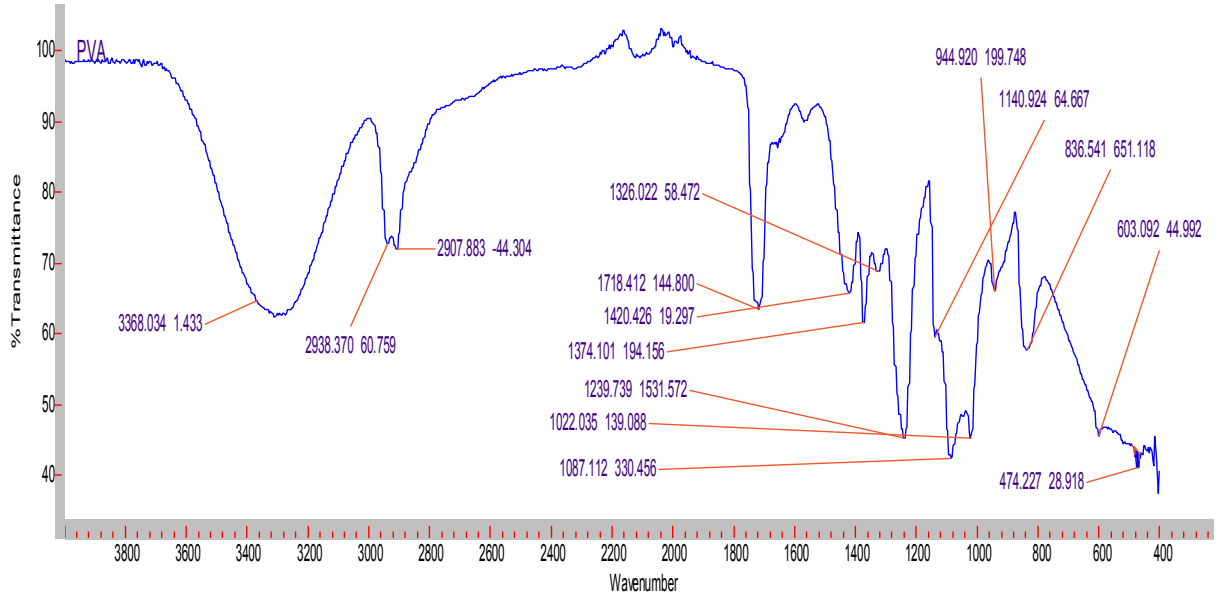


Figure 6. FTIR spectra of PVA

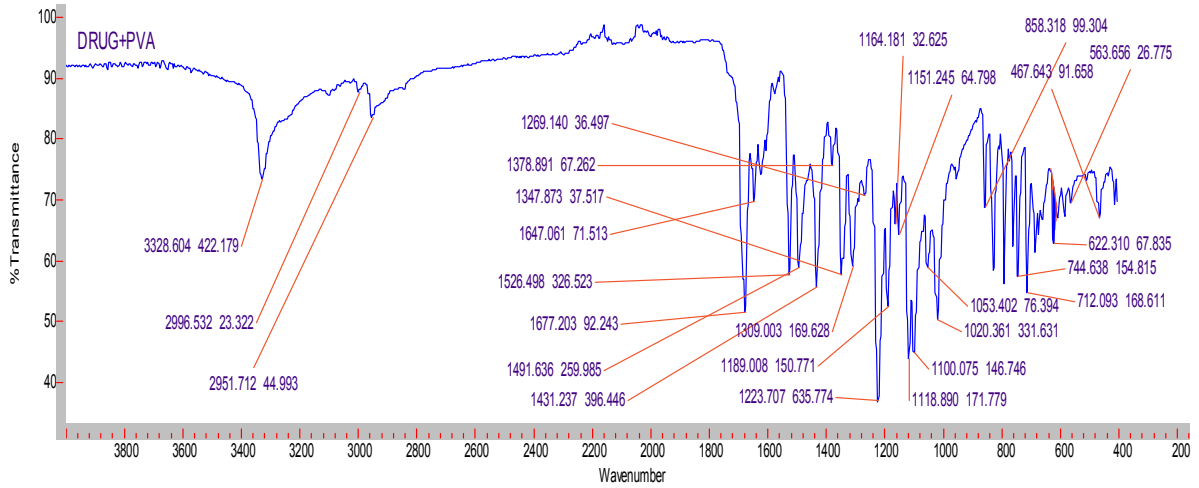


Figure 7. FTIR spectra of Drug+PVA

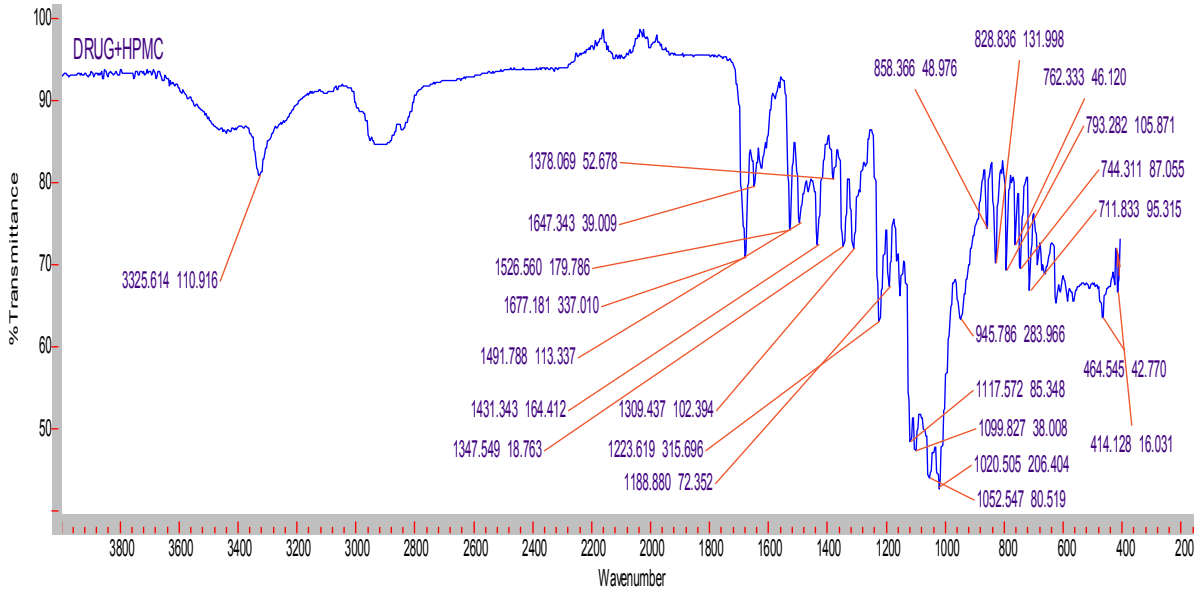


Figure 8. FTIR spectra of Drug+HPMC

Table 3. List of wavenumbers of individual drug,poymers and physical mixture

S.No	Wavenumbers(cm^{-1}) in physical mixture	Wavenumbers in individual(cm^{-1})			Wavenumbers (cm^{-1}) range
		Nifedipine	HPMC	PVA	
1	3328.604	3323			3500-3100
2	1526.498	1525			1550
3	1309.003	1309			1320-1210
4	2915.712			2938	3000-2850
5	1151.245			1140	1300-1000
6	3325.694	3323			3500-3100
7	1526.360	1525			1550
8	1188		1190		1300-1000
9	945		945		1000-650

Table 4. Invitro drug release profile of Nifedipine of F1-F5

S.No	Time (mins)	Cummulative drug release for formulations F1-F5(%)				
		F1	F2	F3	F4	F5
1	0	0	0	0	0	0
2	10	12.5	19.7	12.5	18.3	11.3
3	20	19.6	21.5	17.3	23.4	23
4	30	22.4	28.7	33.2	29.3	31.4
5	40	28.6	32.4	42.6	33	34.5
6	60	31.8	49.2	53.9	36.8	38.3
7	120	36.7	52.8	58.4	42.5	41.6
8	180	47.5	60.2	63.8	48.4	48.2
9	240	58.5	65.4	74.4	59.2	55.3

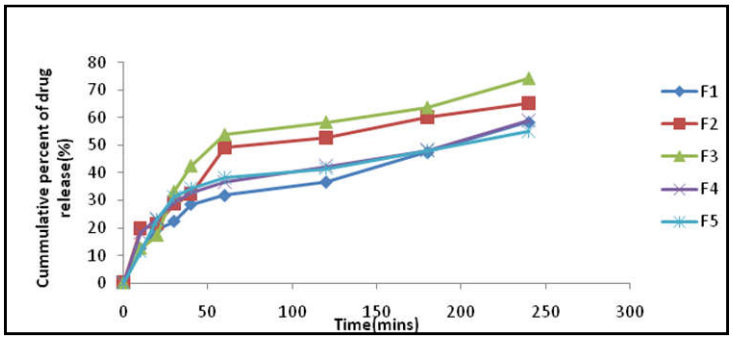


Figure 9. Drug realease pattern from formulations F1-F5

Table 5. Invitro drug release profile of Nifedipine of F6-F9

S.No	Time (mins)	Cummulative drug release for formulations F6-F9(%)			
		F6	F7	F8	F9
1	0	0	0	0	0
2	10	12.9	23.7	15.4	11.5
3	20	25.7	26.4	21.9	18.5
4	30	29.2	30.7	27.2	27.9
5	40	30.2	32.6	35.8	32.6
6	60	41.7	39.5	42.3	37.9
7	120	45.8	41.7	48.4	48.9
8	180	53.2	55.8	53.5	51.1
9	240	58.5	61.1	63.6	57.8

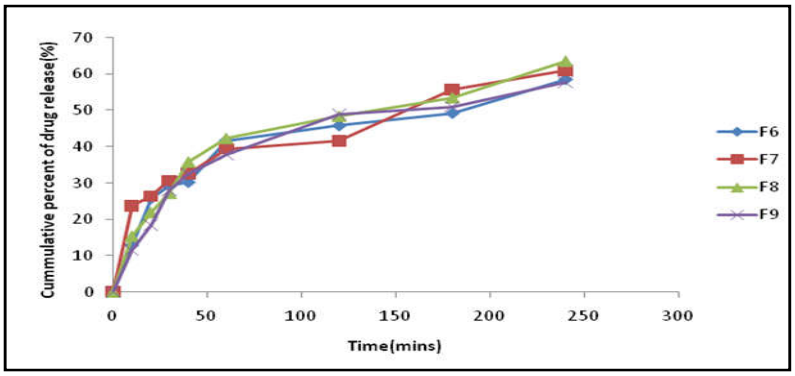


Figure 10. Drug realease pattern from formulations F6-F9

Table 6. Physical Stability evaluation of the nanosuspensions

Formulations	Storage temperature conditions	Initial particle size(nm)	Particle size after 3 months (nm)
F3	4 ºC Room temperature 40 ºC	322.3	322 325.6 338.5

The formulations at 4 °C remained stable, whereas that at room temperature showed slight increase in particle size, stored at 40 ºC showed increase in particle size. The results of physical stability study was shown below (Vishvajit, 2010).

Conclusion

It may be concluded that the nanoprecipitation with ultrasonication have potential to formulate homogenous nanosuspensions with uniform-sized stable nanoparticles of Nifedipine. The reduced particle size of the precipitated particles may enhance the solubility of Nifedipine. The study also showed that the nanosuspension showed enhanced dissolution of Nifedipine. Since the limited oral bioavailability of Nifedipine is due to its poor dissolution hence the increase solubility, and thereby the dissolution of Nifedipine in the form of nanosuspension may enhance the oral bioavailability of Nifedipine. The preparations were found to physically stable with PVA and HPMC as stabilizer.

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