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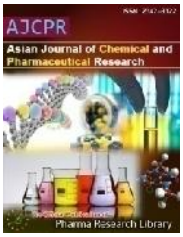

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Formulation and Evaluation of Nefidipine Nano Suspension

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A B S T R A C T

Polymeric nanoparticles (PNPs) are defined as particulate dispersions or solid particles with size in the range of 10-1000nm. Nanoparticles can offer significant advantages over the conventional drug delivery in terms of high stability, high specificity, high drug carrying capacity, ability for controlled release, possibility to use in different route of administration. Nano particles are prepared easily with the normally available ingredients. In presently study on nano particles was prepared by using solvent evaporation method with poly vinyl alcohol as a stabilizer. The drug release studies were performed and the dissolution data.

Keywords: Nanosuspension, Nifedipine, Poly Vinyl Alcohol

A R T I C L E I N F O

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1. Introduction

More than 40% of the new chemical entities being generated through drug discovery programmes are poorly water-soluble or lipophilic compounds. Formulating a poorly water-soluble drug has always been a challenging problem conformed by the pharmaceutical scientist. The usage of nano-sized particles can be implemented to all drug compounds belonging to biopharmaceutical classification system[1] (BCS) classes II and IV to increase the solubility and hence partition into gastrointestinal barrier. Nanotechnology can be used to solve the problems associated with these conventional approaches for solubility and bioavailability enhancement. Nanosuspension technology can also be used for drugs which are insoluble in both water and organic solvents, hydrophobic drugs such as Famitidine, Atorvastatin, Revaprazan, Acelofenac are formulated as nanosuspension. There are number of

formulation approaches that can be used to solve the problem associated with the low solubility and low bioavailability of class II drugs. Some of the approaches to increase solubility include micronization, solubilisation using co solvents, use of permeation enhancers, surfactant dispersions, salt formation, and precipitation techniques.

Most of these techniques for solubility enhancement have advantages as well as some limitations and hence have limited utility in solubility enhancement. Other techniques used for solubility enhancement like microspheres, emulsions, nanotechnology, micro emulsion, liposome's, supercritical processing, solid dispersion and inclusion complex using cyclo detrains show reasonable success but they lack in universal applicability to all drugs, which are not soluble in both aqueous and organic media. Oral route has been the commonly adopted and most convenient route for the drug delivery.

Oral drug delivery system has received more attention in the pharmaceutical field, because of its more flexibility in designing the dosage form than other drug delivery systems. In recent years novel drug delivery system like nanosuspension draws a considerable attention in the search for adverse drug reactions and improved patient compliance. As a nanosuspension it should posses optimum drug content and high entrapment efficiency.

Drug content in the amount of drug which is available for release in the drug delivery system. The amount of drug entrapped in the polymeric carrier is termed as entrapment efficiency. Entrapment efficiency affects the release of the drug from the delivery system. Various factors like type of polymer used, drug polymer ratio, Sonication time, agitation speed, surfactant concentration, solubility of drug and polymer may also affect the drug release and entrapment efficiency.

Definition of Nanosuspension

Nanosuspensions are colloidal dispersions of nano sized drug particles stabilized by surfactant. They can also be defined as biphasic system consisting of pure drug particles dispersed in an aqueous vehicle in which the diameter of the suspended particle is less than 1 µm in size. Nano is a Greek word which means 'dwarf[3]'. Nano means it is the factor of 10^{-9} or one billionth. some comparisons of nano scale are given below

0.1 nm = Diameter of one Hydrogen atom.
2.5 nm = Width of a DNA molecule
1 µm = 1000 nm.
1 nm = 10^{-9}m = 10^{-7} cm = 10^{-6} mm.
Micron = 10^{-6}m = 10^{-4}cm = 10^{-}3mm.

Need of Nanosuspension

- Poor bioavailability[4].
- Lack of dose-response proportionality.
- Use of harsh excipients i.e. excessive use of co-solvents and other excipients.
- Use of extreme basic or acidic conditions to enhance solubilization.
- Use of poorly water soluble as well as poorly organic soluble drugs.

Advantages

- Enhance the solubility and bioavailability of drugs. Suitable for hydrophilic drugs[5].
- Rapid dissolution and the tissue targeting can be achieved by IV route of administration.
- Higher drug loading can be achieved.
- Dose reduction is possible.
- Enhance the physical and chemical stability of drugs.
- Provides a passive drug targeting.
- Can be applied for poorly water soluble drugs.
- Can be given by any route.
- Reduced tissue irritation in case of subcutaneous.

Disadvantages

- Physical stability, sedimentation & compaction can cause problems.
- It is bulky sufficient care must be taken during handling & transport.
- Improper dose.
- Uniform & accurate dose cannot be achieved system[6].

Applications of Nanosuspensions

Oral[2]:

Oral drug delivery is the most widely preferred route of administration of drugs. But, some drugs possess the problem of limited bioavailability due to poor solubility and absorption which ultimately reduces its efficacy. In such cases, Nanosuspension can solve the problem as it helps in improving the dissolution rate and absorption due to increased surface area and enhanced adhesiveness. Nanosuspension can lead to increased mucoadhesion which can increase gastrointestinal transit time and lead to increased bioavailability. The enhancement in oral bioavailability can be attributed to increased surface area, saturation solubility and the adhesiveness of the drug Nanosuspension. Taste masking of particulate system is also easily possible.

Parenteral

Nanosuspensions can be used to transform poorly soluble non-injectable drugs into a formulation suitable for intravenous administration. Although the production of Nanosuspension for parenteral use is critical, current injectable formulations. The methods used for preparation of Nanosuspension are now precisely controlled, and are able to produce uniform particles with better control over maximum particle size. Various research reports are available which emphasize the applicability of Nanosuspensions for parenteral administration.

Ocular delivery[24]

Nanosuspension can prove to be a boon for drugs that exhibit poor solubility in lachrymal fluids. Nanosuspensions represent an ideal approach for ocular delivery of hydrophobic drugs due to their inherent ability to improve saturation solubility of drugs. Kassem et al., have developed Nanosuspension delivery system for certain glucocorticoid drugs.

Pulmonary

Nanosuspensions can be advantageous for delivering drugs that exhibit poor solubility in pulmonary secretion.

Currently available approaches for pulmonary delivery such as aerosols or dry powder inhalers possess certain disadvantages such as limited diffusion at required site, less residence time etc, which can be overcome by Nanosuspensions. Fluticasone and budesonide have been successfully formulated as Nanosuspension for pulmonary Delivery.

Dermal: The nanocrystalline form possesses increased saturation solubility resulting in enhanced diffusion of the drug into the skin. Nanocrystals also exhibit various properties such as increased penetration into a membrane, enhanced permeation and bioadhesiveness which could be very useful for dermal application.

Targeting[25]

The uptake of drug nanoparticles depends on their particle size. By changing the surface properties of the nanoparticles, their in vivo behavior can be altered and can be used as targeted delivery system. The phagocytotic uptake of nanocrystals can be avoided by preparing stealth nanocrystals or by preparing smart crystals i.e. drug particles below particle size of 100nm, which can be used as a targeted drug delivery system. Due to method simplicity, development of nanosuspension is a commercially viable option for targeted delivery. Thus, Tween 80 coated nanocrystals can be used for brain targeting. Atovaquone nanocrystals coated with Tween80 were used to treat toxoplasmosis; the parasites could be efficiently eradicated in brain.

Mucoadhesion of the Nanoparticles

Nanoparticles orally administered in the form of a suspension diffuse into the liquid media and rapidly encounter the mucosal surface. The particles are immobilized at the intestinal surface by an adhesion mechanism referred to as "bioadhesion." From this moment on, the concentrated suspension acts as a reservoir of particles and an adsorption process takes place very rapidly. The direct contact of the particles with the intestinal cells through a bioadhesive phase is the first step before particle absorption[26].

Future Prospects

- Nanosuspension technology is a unique and novel approach to overcome drug problems such as poor bioavailability that are related with the delivery of hydrophobic drugs, including those that are poorly soluble in aqueous as well as organic media[31].
- Production methods like media milling and high-pressure homogenization have been successfully employed for large scale production of Nanosuspensions.
- Nanosuspension technology can be combined with dosage forms: tablets, traditional capsules, pellets, and can be used for parenteral products.
- To take advantage of Nanosuspension drug delivery, simple formation technologies and variety applications, Nanosuspensions will continue to be of interest as oral formulations and non-oral administration develop in the future[32].
- In consideration to data available Nanosuspensions can be considered as renaissance in formulation technologies for coming years.

Drug Profile

Nifedipine[10]

Nifedipine is a calcium channel blocker

Chemical name: dimethyl 2,6-dimethyl-4-(2-nitrophenyl)-1,4-dihydropyridine-3,5-dicarboxylate.

Structural formula:

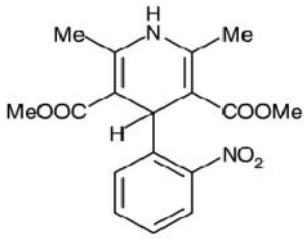

Figure 1: Dimethyl 2,6-dimethyl-4-(2-nitrophenyl)-1,4-dihydropyridine-3,5-dicarboxylate

Molecular Formula: $C_{17}H_{18}N_2O_6$, **Molecular Weight:** 346.3, **CAS Registry Number:** 21829-25-4

Description: Nifedipine is a yellow, crystalline powder which is practically insoluble in water and sparingly soluble in absolute ethanol. It is sensitive to light.

Mechanism of action:

Nifedipine decreases arterial smooth muscle contractility and subsequent vasoconstriction by inhibiting the influx of calcium ions through L-type calcium channels. Calcium ions entering the cell through these channels bind to calmodulin. Calcium-bound calmodulin then binds to and activates myosin light chain kinase (MLCK). Activated MLCK catalyzes the phosphorylation of the regulatory light chain subunit of myosin, a key step in muscle contraction. Signal amplification is achieved by calcium-induced calcium release from the sarcoplasmic reticulum through ryanodine receptors. Inhibition of the initial influx of calcium inhibits the contractile processes of smooth muscle cells, causing dilation of the coronary and systemic arteries, increased oxygen delivery to the myocardial tissue, decreased total peripheral resistance, decreased systemic blood pressure, and decreased afterload. The vasodilatory effects of nifedipine result in an overall decrease in blood pressure.

Indication:

For the management of vasospastic angina, chronic stable angina, hypertension, and Raynaud's phenomenon. May be used as a first line agent for left ventricular hypertrophy and isolated systolic hypertension (long-acting agents).

Pharmacokinetics

Absorption : Rapidly and fully absorbed following oral administration

Protein binding : 92-98%

Metabolism : Hepatic metabolism via cytochrome P450 system. Predominantly metabolized by CYP3A4, but also by CYP1A2 and CYP2A6 isozymes.

Route of elimination:

Nifedipine is extensively metabolized to highly water-soluble, inactive metabolites accounting for 60 to 80% of the dose excreted in the urine. The remainder is excreted in the feces in metabolized form, most likely as a result of biliary excretion.

Half life: 2 hours

Side effects

- Headache, dizziness
- Drowsiness, tired feeling
- nausea, diarrhea mild constipation or stomach pain
- sleep problems (insomnia)
- mild rash or itching
- joint pain, leg cramps
- warmth, redness, or tingly feeling under your skin
- Urinating more than usual.

Contraindications

- Known hypersensitivity to Nifedipine or related dihydropyridine calcium channel blockers or to any of the excipients.
- Pregnancy and lactation.
- Cardiogenic shock.
- Kock pouch (ileostomy after proctocolectomy).
- Concomitant administration with rifampicin.
- Within the first eight days of an acute episode of myocardial infarction

Dosage: 10 mg/day

Uses: Nifedipine belongs to a class of medications known as calcium channel blockers. It works by relaxing blood vessels so blood can flow more easily. Used for the long-term treatment of hypertension (high blood pressure) and angina pectoris. In hypertension, recent clinical guidelines generally favour diuretics and ACE inhibitors.

Polymer Profile (Polyvinyl Alcohol)

Nonproprietary Names:

PhEur: Poly (vinylis acetas) USP: Polyvinyl alcohol

Synonyms: Airvol; Alcotex; Elvanol; Gelvatol; Gohsenol; Lemol; Mowiol; Polyvinol; PVA; vinyl alcohol polymer.

Chemical Name and CAS Registry Number

Ethanol, homopolymer [9002-89-5]

Empirical Formula and Molecular Weight

$(C_2H_4O)_n$ 20 000–200 000, Polyvinyl alcohol is a water-soluble synthetic polymer represented by the formula $(C_2H_4O)_n$. The value of n for commercially available materials lies between 500 and 5000, equivalent to a molecular weight range of approximately 20 000–200 000.

Structural Formula

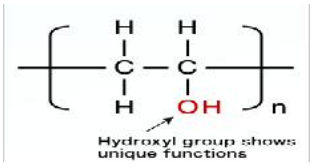

Figure 2

Description: Polyvinyl alcohol occurs as an odorless, white to cream-colored granular powder.

Melting point: 228^{0}C for fully hydrolyzed grades; 180–190^{0}C for partially hydrolyzed grades.

Refractive index: n_D^{25} = 1.49–1.53

Solubility: Soluble in water, slightly soluble in ethanol (95%). insoluble in organic solvents. Dissolution requires dispersion (wetting) of the solid in water at room temperature followed by heating the mixture to about 90^{0}C for approximately 5 minutes. Mixing should be continued while the heated solution is cooled to room temperature.

Specific gravity: 1.19–1.31 for solid at 25^{0}C; 1.02 for 10% w/v aqueous solution at 25^{0}C.

Specific heat: 1.67 J/g (0.4 cal/g)

Functional Category: Coating agent, lubricant, stabilizing agent, viscosity-increasing agent.

Applications in Pharmaceutical Formulation or Technology: Polyvinyl alcohol is used primarily in topical pharmaceutical and ophthalmic formulations .It is used as a stabilizing agent for emulsions (0.25–3.0% w/v). Polyvinyl alcohol is also used as a viscosity-increasing agent for viscous formulations such as ophthalmic products. It is used in artificial tears and contact lens solutions for lubrication purposes, in sustained-release formulations for oral administration, and in transdermal patches. Polyvinyl alcohol may be made into microspheres when mixed with a glutaraldehyde solution.

Stability and Storage Conditions:

Polyvinyl alcohol is stable when stored in a tightly sealed container in a cool, dry place. Aqueous solutions are stable in corrosion-resistant sealed containers. Preservatives may be added to the solution if extended storage is required. Polyvinyl alcohol undergoes slow degradation at 100^{0}C and rapid degradation at 200^{0}C; it is stable on exposure to light.

Incompatibilities:

Polyvinyl alcohol undergoes reactions typical of a compound with secondary hydroxy groups, such as esterification. It decomposes in strong acids, and softens or dissolves in weak acids and alkalis. It is incompatible at high concentration with inorganic salts, especially sulfates and phosphates; precipitation of polyvinyl alcohol 5% w/v can be caused by phosphates. Gelling of polyvinyl alcohol solution may occur if borax is present.

Safety: Polyvinyl alcohol is generally considered a nontoxic material. It is nonirritant to the skin and eyes at concentrations up to 10%; concentrations up to 7% are used in cosmetics. Studies in rats have shown that polyvinyl alcohol 5% w/v aqueous solution injected subcutaneously can cause anemia and infiltrate various organs and tissues.

LD50 (mouse, oral): 14.7 g/kg

LD50 (rat, oral) : >20 g/kg

Handling Precautions:

Observe normal precautions appropriate to the circumstances and quantity of material handled. Eye protection and gloves are recommended. Polyvinyl alcohol dust may be an irritant on inhalation. Handle in a well-ventilated environment.

Regulatory Status: Included in the FDA Inactive Ingredients Guide (ophthalmic preparations and oral tablets). Included in nonparenteral medicines licensed in the UK. Included in the Canadian List of Acceptable Non-medicinal Ingredients.

Dichloromethane[12]

Synonyms: Freon30; Nevolin; Driverit; Freon30; Metaclen

Molecular Formula: CH_2Cl_2

Molecular Weight: 84.93

Description: A colorless liquid with a sweet, penetrating, ether-like odor. Noncombustible by if exposed to high

temperatures may emit toxic chloride fumes. Vapors are narcotic in high concentrations. Used as a solvent and paint remover.

Melting point: -97 °C, **Boiling point:** 39.8-40 °C mm Hg (lit.), **Density: 1.325** g/mL at25 °C (lit.),
Vapor density: 2.9(vs air),
Vapor pressure: 24.45 psig (55°C),
Refractive index: n^{20}_{D} 1.424(lit.),
Water Solubility: 20 g/L (20 ºC)
Storage temperature: Store at Room temperature.
Hazards: Anesthetic effects, nausea and drunkenness. Skin and eyes irritation.
Usage: Suitable for HPLC, spectrophotometry, environmental testing

2. Materials and Methods

All the chemicals used were of analytical grade. The drug Nifedipine was procured as a gift sample from Matrix laboratories. Other ingredients procured were of analytical grade, Poly vinyl alcohol 10% (w/v) (PVA) mol. wt. 125000.

- Ethanol
- Dichloro methane
- Distilled water

Calibration Curve of Nefidipine[13]

The standard curve of Nifedipine was prepared by using 6.8pH phosphate buffer.

Spcectrophotometric method for the estimation of Nifedipine

The standard curve of Nifedipine was prepared in phosphate buffer of ph 6.8 at 238nm.

Standard solution

Stock solution of 100µg|ml was prepared by dissolving accurately weighed quantity of 10mg Nifedipine in 10ml of ethanol.

Working solution

From the stock solution aliquots of 0.5, 1, 1.5, 2and 2.5ml of stock solution were pipetted out into 10ml volumetric flask. The volume was make up to the mark with of phosphate buffer ph 6.8.These dilutions give 5,10,15,20 and 25 5µg|ml concentration of Nifedipine respectively. The absorbance of prepared solutions of Nifedipine in phosphate buffer ph 6.8 was measured at 238nm in UV-spectrophotometry against an appropriate blank. The standard calibration curve yields a straight line, which shows that drug follows Beer's law in the concentration range of 5 to 25 µg|ml. A stander graph was plotted by keeping the known concentration on X-axis and obtained absorbance on Y-axis.

Formulation of Nanosuspension

Formulation

Nanosuspension formulation requires basically stabilizer or surfactant, proper solvent system and others ingredients for its preparation.

Stabilizer[14]

Stabilizer plays an important role in the formulation of nanosuspension. In the absence of an appropriate stabilizer, the high surface energy of nano-sized particles can induce agglomeration or aggregation of the drug crystals. Stabilizer is used to wet the surface of solute or drug particle and retard the Ostwald ripening and agglomeration in order to provide high physical stability which further reflects to its performance. Commonly used stabilizers are Polysorbate (Tween/Span series), povidone, cellulosic, Poloxamer and lecithin. The type and amount of stabilizers has a pronounced effect on the physical stability and in-vivo behaviour of nanosuspension. In some cases a mixture of stabilizers is required to obtain a stable nanosuspension.

Organic solvents[15]

Organic solvents may be required in the formulation of nanosuspension if they are to be prepared using an emulsion or micro emulsion as a template. As these techniques are still in their infancy, elaborate information on formulation considerations is not available. These solvents are very hazardous in physical and environment. The pharmaceutically acceptable and less hazardous water miscible solvents, such as ethanol and isopropanol, and partially water-miscible solvents, such as ethyl acetate, ethyl format, butyl lactate, tri acetin, propylene carbonate and benzyl alcohol.

Other additives

Formulation considerations Nanosuspension may contain additives such as buffers, salts, polyols, osmogent and cryoprotectant[27], depending on either the route of administration or the properties of the drug moiety.

Preparation Methods[16]

Technically preparations of nanosuspensions are simpler alternative than liposome's and other conventional colloidal drug carriers but reported to be more cost effective. it is particularly for poorly soluble drugs and to yield a physically more stable product. Mainly there are two methods for preparation of nanosuspension. They are;

- Top-down process technology,
- Bottom-up process technology.

In bottom-up technology the drug is dissolved in a solvent, which is then added to non-solvent to precipitate the crystals. This technique is that during the precipitation procedure the growing of the drug crystals needs to be controlled by addition of surfactant to avoid formation of micro particles.

Examples include;

- Solvent-anti solvent method,
- Super critical fluid process,
- Emulsification solvent evaporation technique,
- Lipid emulsion/micro-emulsion template.

In top-down process technology follows disintegration approach from large particles, micro particles to nanosized particles.

- Examples include;
- Pressure homogenization.
- Media milling {nano crystals}.
- Nano edge.
- Nano pure.

Bottom up Technology: The term "Bottom up technology" means that one starts from the molecular level, and goes via molecular association to the formulation of a solid particle. That means that we are discussing classical precipitation techniques by reducing the solvent quality, for example, by pouring the solvent into a non solvent or changing the

temperature or a combination of both. Precipitation is a classical technique in pharmaceutical chemistry and technology.

Advantages: use of simple and low cost equipment. Higher saturation solubility is the advantage for precipitation compare to other methods of Nanosuspension preparation.

Disadvantages

- The drug needs to be soluble in at least one solvent (Thus excluding all new drugs that are simultaneously poorly soluble in aqueous ad in orgaic media.
- The solvent needs to be missible to with atleast one nonsolvent.
- A solvent residue needs to be removed, thus increase production costs.

Top down Technology:

The top down technologies include

- Media milling
- High pressure homogenization

Media Milling

Nano suspensions are produced by using high shear media mills or peral mills. The mill consists of a milling chamber, milling shaft and a recirculation chamber. An aqeous suspension of the drug is then fed into the mil containing small grinding balls/perals. As these balls rotate at a very high shear rate under control temperature, they fly through the grinding jar interior and impact against to the sample on the oppisite grinding jar wall. The milling media or balls are made of ceramic sintered aluminium oxide or zirconium oxide or highly cross linked polystyrene resin with high abrasion resistance .planetary ball mills[30] (PM100 and PM200;Retsch Gmbh and co., KG, Han ,Germany) is one example of an equipment that can be used to achieve a grind size below 0.1µm.The major drawbacks of this technology include the erosion of balls/pearls that can leave residues as contaminants in the final product , degradation of the thermolabile drugs due to heat generated during the process and presence of relativey high proportions of particles ≥5µm.

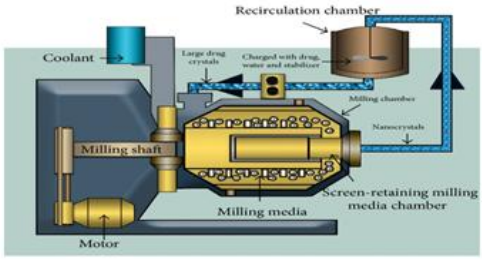

Figure 3: Schematic representation of the media milling process

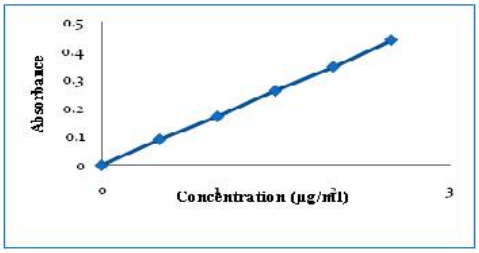

Figure 4: calibration plot of Nifedipine

Advantages

- Simple technology.
- Low cost process regarding the millig it self.
- Large scale production possible to some extent (batch process).

Disadvantages

- Potential erosion from the milling material leading to product contamination.
- Duration of the process not being very production friendly.
- Potential growth of germs in water phase when milling for a long time.
- Time and cost associated with the separation milling material from the drug nanoparticle suspension, especially when producing parenteral sterile products.

Table 1: calibration of Nifedipine

S.No	concentration	Absorbence
1	0	0
2	0.5	0.09
3	1	0.17
4	1.5	0.26
5	2	0.343
6	2.5	0.430

Table 2: Optical characteristics of Nifedipine

S.No	Parameters	values
1	Absorption maxima	238
2	Beer's law range	0.5-2.5µg/ml
3	Regression equation	y=0.173x+0.333
4	Correlation coefficient	0.999

High Pressure Homogenization

It is most widely used method for preparing nanosuspensions of many poorly aqueous soluble drugs. It involves three steps. First drug powders are dispersed in stabilizer solution to form presuspension, and then the presuspension[29] is homogenized in high pressure homogenizer at a low pressure for premilling, and finally homogenized at high pressure for 10 to 25 cycles until the nanosuspensions of desired size are formed. Different methods are developed based on this principle for preparations of nanosuspensions are Disso cubes, Nanopure, Nanoedge and Nanojet.

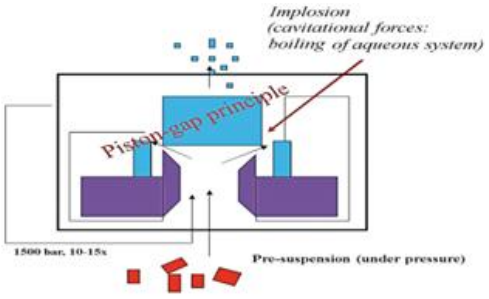

Figure 5: Schematic representation of the high-pressure homogenization process.

Homogenization in Aqueous Media (DISSO CUBES)

This technology was developed by R.H. Muller using a piston-gap type high pressure homogenizer in 1999. In this method, the suspension containing a drug and surfactant is forced under pressure through a Nano sized aperture valve of a high pressure homogenizer.

Principle

This method is based on cavitation principle. The dispersion present in 3cm diameter cylinder is suddenly passed through a very narrow gap of 25µm.According to Bernoulli's law the flow volume of liquid in a closed system per cross section is constant. It leads to increase in dynamic pressure and decrease of static pressure below the boiling point of water at room temperature due to reduction in diameter from 3cm to 25µm.Then water starts boiling at room temperature and forms gas bubbles, which implode when the suspension leaves the gap (called cavitation) and normal air pressure is reached. The particles cavitation forces are sufficiently high to convert the drug micro particles into nanoparticles.

Advantages

- It does not cause the erosion of processed materials.
- It is applicable to the drugs that are poorly soluble in both aqueous and organic media.

Disadvantages

- Pre-processing like micronization of drug is required.
- High cost instruments are required that increases the cost of dosage form.

Homogenization in Nonaqueous Media (Nanopure)

Nanopure is suspensions homogenized in water-free media or water mixtures like PEG 400, PEG 1000 etc. The homogenization can be done at room temperature, 00C and below freezing point (-200C), hence it is known as "deep freeze" homogenization.

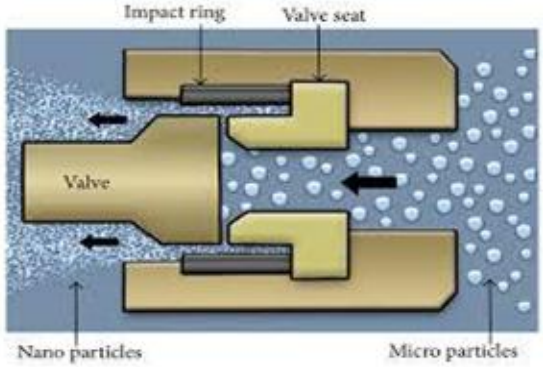

Figure 6: Schematic Cartoon of the High-Pressure Homogenization Process

Nano Edge

Nano edge technology is the combination of both precipitation and homogenization. The basic principle is same as that of precipitation and homogenization. The major disadvantage of precipitation technique such as crystal growth and long term stability can be overcome by using the Nan edge technology[28]. Particles of smaller size and better stability in short time can be achieved.

Solvent Evaporation Method[17]

In the solvent evaporation method, the solutions of polymer are prepared in volatile solvents and emulsions. The emulsion is converted into a nanoparticles suspension on evaporation of the solvent for the polymer, which is allowed to diffuse through the continuous phase of the emulsion. The particle size was influenced by the concentration of polymer, stabilizer and the speed of homogenizer. In the conventional methods, two main strategies are being used for the formation of emulsions, the preparation of single-emulsions, e.g., oil-in-water (o/w) or double-emulsions, e.g., (water-in-oil)-in-water, (w/o)/w. These methods require high-speed homogenization or ultrasonication, followed by evaporation of the solvent, either by continuous magnetic stirring at room temperature or under reduced pressure. By ultracentrifugation the solidified nanoparticles are collected which was washed with distilled water to remove the additives like surfactants, and then it was lyophilized. Single-emulsions, e.g., oil-in-water (o/w) or double-emulsions, e.g., (water-in-oil)-in-water, (w/o)/w.

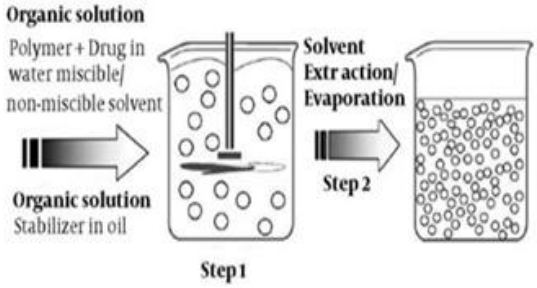

Figure 7: Process of Solvent evaporation method

Nano Edge

Nano edge technology is the combination of both precipitation and homogenization. The basic principle is same as that of precipitation and homogenization. The major disadvantage of precipitation technique such as crystal growth and long term stability can be overcome by using the Nan edge technology. Particles of smaller size and better stability in short time can be achieved.

Table 3: Formula for preparation of Nano suspension

S.no	Ingredients	Quantity
1.	Nifedipine	10mg
2.	PVA	100mg
3.	DCM	5ml
4.	ethanol	5ml

Evaluation methods

In-vitro evaluation[18]

In vitro release studies are carried out by using dialysis tubes with an artificial membrane. The prepared nanoparticles and 10ml of phosphate buffer is added to the dialysis tube and subjected to dialysis by immersing the dialysis tube to the receptor compartment containing 250 ml of phosphate buffer. The medium in the receptor is agitated continuously using a magnetic stirrer a temperature is

maintained at 37±5^{0}c. 5ml of sample of receptor of compartment is taken at various intervals of time over a period of 24h and each time fresh buffer is replaced. The amount of drug released is determined by using spectrophotometrically.

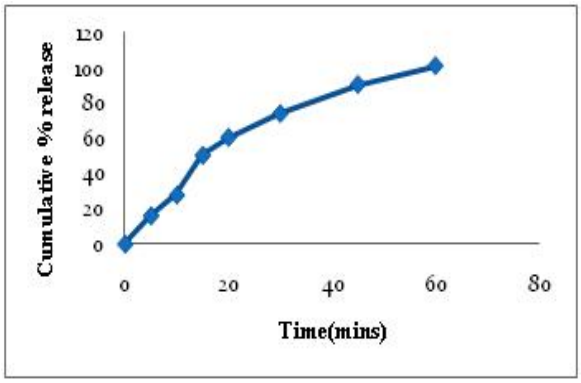

Figure 8: Cumulative % drug release

Particle charge (zeta potential)[19]

The determination of the zeta potential of a nanosuspension is essential as it gives an idea about the physical stability of the nanosuspension. The zeta potential of a nanosuspension is governed by both the stabilizer and the drug itself. In order to obtain a nanosuspension exhibiting good stability, for an electro statically stabilized nanosuspension a minimum zeta potential of -30mV is required where as in the case of a combined electrostatic and steric stabilization, a minimum zeta potential of -20mV is desirable.

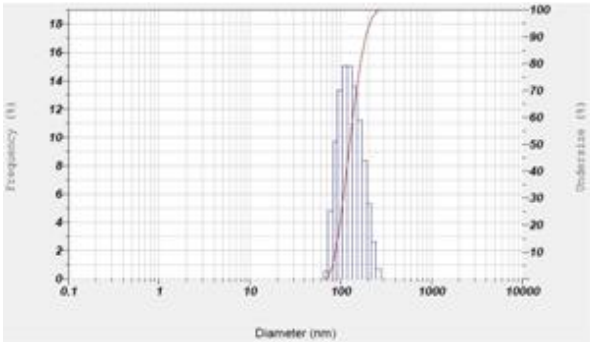

Figure 9: particle size determination

Z-Average: 1780.8 nm
PI : 1.278

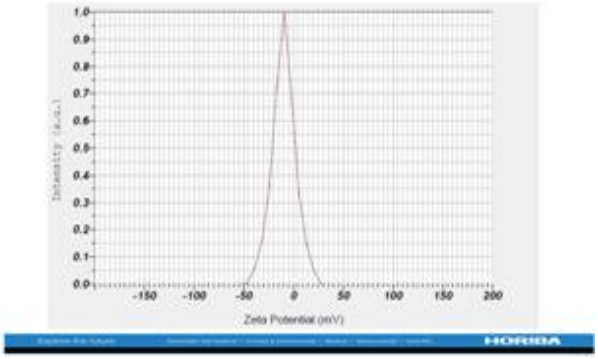

Figure 10: Particle size analysis by using zeta potential

Zeta Potential (Mean): -9.5 mV
Electrophoretic Mobility Mean: -0.000074 cm2/Vs

6.3.2 Scanning Electron microscopy[20]

Scanning electron microscopy is giving morphological examination with direct visualization. The techniques based on electron microscopy offer several advantages in morphological and sizing analysis; however, they provide limited information about the size distribution and true population average. For SEM characterization, nanoparticles solution should be first converted into a dry powder, which is then mounted on a sample holder followed by coating with a conductive metal, such as gold, using a sputter coater. The sample is then scanned with a focused fine beam of electrons. The surface characteristics of the sample are obtained from the secondary electrons emitted from the sample surface. The nanoparticles must be able to withstand vacuum, with results obtained by dynamic light scattering.

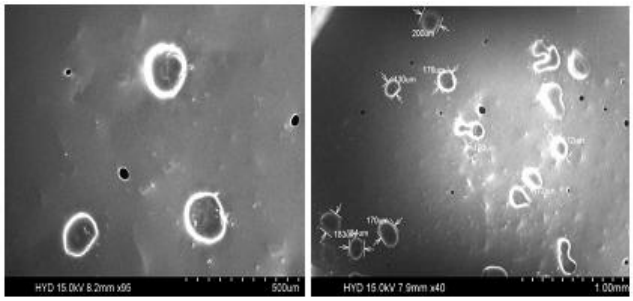

Figure 10: SEM Analysis of Nifedipine nano suspension

3. Results and Discussion

Nifedipine belongs to a class of medications known as calcium channel blockers majorly used in hypertension. . 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 present investigation is solvent evaporation method and poly vinyl alcohol as stabilizing agent[21]. The Nano suspensions are novel promising target and controlled released dosage form which is gaining importance because of ease of manufacturing and diversified applications. The present trend of pharmaceutical research lies in the usage of biodegradable polymer because of its availability and low toxicity.In the present study we have selected Nifedipine as a drug which can be easily soluble in ethanol which is stabilized by polyvinyl alcohol. After maintained room temperature subsequently stirred on homogenizer (stirrer) to allow the volatile solvent to evaporate. Organic solvents were left to evaporate off under a slow stirring of the nanosuspension at room temperature for 1 hour. Drug release from the nano suspension was studied by using 8 station dissolution rate test apparatus (LAB INDIA DS8000) employing a paddle stirrer at 50rpm and at 37±1ºc .pH 6.8 phosphate buffer as dissolution medium samples of 5 ml each were withdrawn at different time intervals over a period of 60 min and it is replaced with equal amount of fresh dissolution medium.

Samples were diluted and analyzed at 224 nm for n using Systronics UV Visible double beam Spectrophotometer 2202. The results of the dissolution studies showed that 16mg of Nifedipine was released.

Table 4: Drug release studies of Nifedipine

Time (min)	Cumulative % drug release studies					
	NF1	**NF2**	**NF3**	**NF4**	**NF5**	**NF6**
0	0	0	0	0	0	0
5	14.682	15.423	16.123	14.230	13.562	17.429
10	30.827	32.462	27.826	29.280	31.938	33.268
15	46.425	48.420	50.421	49.236	44.259	42.990
20	56.626	59.728	60.620	66.420	68.423	69.421
30	70.926	71.120	74.128	72.289	77.236	79.235
45	88.486	88.281	90.682	86.420	84.426	91.884
60	95.240	96.896	101.283	92.328	94.289	101.28

4. Conclusion

Nanosuspension solved poor bioavailability problem of hydrophobic drugs and drugs which are poorly soluble in aqueous and organic solutions. Productions techniques such as solvent evaporation method, media milling and high pressure homogenizer are used for large scale production of Nanosuspensions. Nanosuspensions can be administered through oral, parenteral, pulmonary, ocular and topical routes. Since nanotechnique is simple, fewer requirements of excipients increased dissolution velocity and saturation solubility many poor bioavailability drugs are formulated in nanosuspension form. The advances in production methodologies using emulsions or micro emulsions as templates have provided still simpler approaches for production but with limitations. Further investigation in this regard is still essential. Attractive features, such as increased dissolution velocity, increased saturation solubility, improved bioadhesivity, versatility in surface modification and ease of post-production processing, have widened the applications of nanosuspensions for various routes.

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