Formulation And Evaluation Of Nisoldipine Antihypertensive Drug with Solubility Enhancement

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ABSTRACT

A nanoemulsion is a liquid dispersion comprising two immiscible liquid phases, such as an oil phase and a water phase, that is thermodynamically or kinetically stable . The technological approach for hydrophilic medium polar drugs is less effective, thus the use of a Poly δ-decalactonepolymer presents a potential strategy to improve this limitation.The optimized formulation obtained from formulation variables was then subjected to optimization with process variable. On varying stirring speed and time in respect to particle size was decreased. The optimized formulations have a particle size between 583-615 nm; PDI of0.657±1.8, 0.552±1.05, and 0.734±1.51were selected for loading of the drug for final formulations.The particle size and shape of nanoemulsions were not changed after drug encapsulation. the values of NNE1, NNE2, NNE3, and NNE5 formulation were found to be 6.3±0.04, 7.4±0.08, 6.7±0.06, and 7.0±0.09 units only. In all cases, pH showed the smallest changes. The pH value of the optimized nanoemulsion formulation NNE3 was found to be 6.6±0.06. demonstrating its suitability for oral administration. Drug entrapment efficiencies of different formulations i.e. NNE1, NNE2, NNE3, NNE4, and NNE5 were found to be 71.33±1.62%, 82.4±0.24%, 99.95±1.35%, 90.12±0.34%, and 79.03that showedto affect the encapsulation of drug. Stability studies were carried out at 4^0C and 25^0C.

Keywords: Nisoldipine, Solubility Enhancement, Bioavailability Enhancement, Tween-80

INTRODUCTION

NANOEMULSION

A nanoemulsion is a liquid dispersion comprising two immiscible liquid phases, such as an oil phase and a water phase, The Kelvin effect is responsible for Ostwald ripening, which is caused by a difference in Laplace pressure, which causes small oil droplets to increase oil solubility more than bigger droplets. The fundamental determinant of Ostwald ripening is oil solubility in the continuous phase. [1]

TYPES OF NANOEMULSIONS

Three types of nanoemulsion can be formed:

a. A nanoemulsion of oil in water in which the oil is disseminated in a continuous aqueous phase.

b. A water-in-oil nanoemulsion in which water droplets are scattered throughout the continuous oil phase

c. Bi-continuous nanoemulsions, in which oil and water microdomains are interleaved throughout the system.

A proper combination of surfactants and/or co-surfactants stabilizes the interface in all three forms of nanoemulsions**).**[2]

ADVANTAGES OF NANOEMULSION.

- Nanoemulsions are thermodynamically stable systems, allowing them to self-emulsify and have features that are independent of the procedure used.
- Drugs that are lipophilic and hydrophilic can be carried in the same Nanoemulsion.
- Using Nanoemulsion as a delivery mechanism can improve a drug's efficacy while also lowering the overall dose and reducing side effects [3]

DISADVANTAGES OF NANOEMULSION.

- Use of a large amount of surfactant and co-surfactant necessary for stabilizing the nanodroplet.
- Limited solubility capacity for high melting substances.

METHOD OF PREPARATION OF NANOEMULSION

Nanoemulsions have a very narrow particle size range, and they're best made with high-pressure equipment. Other technologies, such as ultrasonication and in-situ emulsification, can also be used to make nanoemulsions.

HIGH ENERGY METHODS:

- High-pressure homogenization
- Micro fluidization
- Ultrasonication

Low Energy Methods:[4]

- **Phase inversion emulsification method**
- **Transitional phase inversion (TPI)**
  - Phase inversion temperature (PIT)
  - Phase inversion composition (PIC)
- **Catastrophic phase inversion (CPI)**
  - Emulsion inversion point (EIP)
- **The self-nanoemulsification method**

High-pressure homogenization

The preparation of nanoemulsion requires high-pressure homogenization. This technique makes use of a high-pressure homogenizer/piston homogenizer to produce nanoemulsion of extremely low particle size (up to 1nm)

Micro fluidization

Micro fluidization is a mixing process that employs the use of a micro fluidizer. The product is forced through the interaction chamber, which is made up of small channels called micro channels, using a high-pressure positive displacement pump (500 to 20000psi).**).**

Ultrasonication

When it comes to lowering droplet size, ultrasonic emulsification is extremely effective. The energy for ultrasonic emulsification comes from sonotrodes called sonicator probes. [5]

LOW ENERGY METHODS

Phase transition temperature (PIT).

Phase inversion composition (PIC)

Catastrophic phase inversion (CPI)

Emulsion inversion point (EIP)

Theself-nanoemulsifying drug delivery systems (SNEDDS)

Characterization of nanoemulsion

- Droplet size analysis
- Viscosity determination.
- Dilution test
- Drug content
- Polydispersity Index
- Dye test.
- Refractive index
- pH

- Zeta potential

In vitro Release Studies

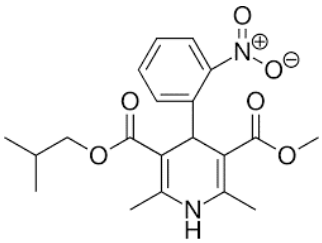
Application of nanoemulsions

- Topical Delivery:
- Parenteral nanoemulsions
- Oral drug delivery).
- Ocular and pulmonary drug delivery:).
- Intranasal drug delivery:
- Nanoemulsions in the commercial and clinical pipeline

DRUG PROFILE

DESCRIPTION OF THE DRUG:

Structure of Nisoldipine



Chemical Formula	$C_{20}H_{24}N_2O_6$
IUPAC NAME	3-O-methyl 5-O-(2-methylpropyl) 2,6-dimethyl-4-(2-nitrophenyl)-1,4-dihydropyridine-3,5-dicarboxylate
Synonyms	Nisoldipine, Nisoldipina, Nisoldipino, Nisoldipinum
Molecular Weight	388.4g/mol
Melting point	151-152(°C)
LogP	3.26
Solubility	5.77e-03g/L

Protein binding: 99%

Half-life:

The reported elimination half-life of nisoldipine is 7-12 hours.

Adverse effects:

- Dizziness.

- Swelling ankles/feet.
- Flushing (warmth, redness, or tingly feeling under your skin)

MATERIALS AND METHODS

Materials:

Materials
Nisoldipine
Monomer
Tween-80
Benzyl Alcohol
Benzoic Acid
Acetone
n-Octanol

EXPERIMENTAL WORK

Preformulation studies:

Preformulation study activities range from supporting the identification of new active agents to characterizing physical properties necessary for the design of dosage forms. Critical information provided during the preformulation can enhance the rapid and successful introduction of new therapeutic entities for humans.[6]

Hence, preformulation studies are essential to characterize drugs for the proper design of drug delivery systems. The objective of preformulation is the determination of those physical and chemical properties that will assist in developing a stable, safe and effective formulation with maximum bioavailability.

Physicochemical characterization of the drug:

Physical appearance:

The obtained drug sample of nisoldipine was a yellow powder. The drug substance color should be recorded by subjection description as well as by an objective means such as by comparison with standard color chips or spectro-photometric analysis if the compound's color intensity in solution is proportional to concentration.

The color of the nisoldipine was observed visually. Particle shape and crystalline characteristics were determined by microscopic evaluation using an optical microscope.

Capillary melting method: A melting point determination is a good indication of purity since the presence of a relatively small amount of impurities can be detected by lowering as well as widening in the melting point range. The melting point of the Nisoldipine was determined by a capillary method using digital melting point apparatus .[8-10]

Identification of drug:

Nisoldipine was identified by various techniques like ultraviolet spectroscopy, infrared spectroscopy, melting point determination, and solubility study.

Identification of Drug by Infrared Spectroscopy:

An FTIR spectrum of nisoldipine was obtained by using an FTIR spectrophotometer

Identification of Drug by Ultraviolet Spectroscopy:

- **λ_{max} Determination:**

For the determination of $\lambda_{max,}$ 1 ml of stock solution was scanned from 200-400 nm using a double beam UV spectrophotometer (Shimadzu UV-1700 series) and the λ_{max} was found to be 236 nm in ethanol. Mention in result and discussion section

- **Preparation of calibration curve in various solvents:**

Preparation of phosphate buffer, 6.8:

- **Potassium phosphate, monobasic, 0.2 M:**

27.22 g of potassium phosphate, monobasic was dissolved in distilled water and the volume was made up to 1000 ml.

- **Sodium hydroxide, 0.2 M:**

22.4 g of NaOH was dissolved in distilled water and the volume was made up to 1000 ml.

- **Preparation of calibration curve of nisoldipine at 6.8 pH phosphate buffer:**

UV spectrophotometric methods for the estimation of nisoldipine in pH 6.8 phosphate buffer were developed.

- **Preparation of stock solutions:**

Stock solutions containing 100 µg/ml were prepared. Accurately weighed quantities of 10 mg ofthe drug were transferred to a 100 mlcalibrated volumetric flask. The volume was made up to 100 ml with 6.8 pH (PBS). The resulting solution (1000 µg/ml) was further diluted ten times with the same media to get stock solutions (100 µg/ml).[10-12]

- **Preparation of standard solutions:**

Appropriate aliquots (0.2 to 1.0 ml) of the stock solutions of nisoldipine were transferred to 10 ml calibrated volumetric flasks and diluted up to the mark with respective media to obtain known final concentrations ranging from 2 to 10 µg/ml.

- **Determination of analytical wavelength:**

The spectrum scan of each standard solution was recorded using UV Visible spectrophotometer from 200 to 400 nm wavelength range against respective medium as blank The wavelengths with maximum absorbance (λmax) were selected as analytical wavelengths for respective media

- **Preparation of calibration curve:**

The calibration curve of Nisoldipine was prepared in ethanol by preparing 1 to 10µg/ml dilutions. The aliquots of 1, 2, 3, 4, 5, ….10 ml of stock solution (10µg/ml) were transferred quantitatively into a series of 10ml volumetric flask, and volume was made up to 10 ml to solve concentration ranging 1 to 10µg/ml. the absorbance of these solutions was determined at λ_{max} (236nm) against blank (distilled water).

Partition coefficient:

The partition coefficient of the drug was determined by allowing 10.0 mg of the drug to equilibrate in a mixture of n-octanol/water containing 10.0 ml of each by shaking on a vortex mechanical shaker for 24 hours and then storing it overnight at 37 ± 2^0C in a separating funnel. The two layers have separated the concentration of drug in the two layers was separated and the concentration of drug in the two layers was determined by UV spectrophotometer and the partition coefficient was determined using the formula as given below Table

$$P_{o/w} = \frac{\text{Concentration of drug in n-octanol}}{\text{The concentration of drugs in water}}$$

Solubility Study:

` The solubility study of the drug was performed in different solvents (e.g. acetone, methanol, ethanol, chloroform, distilled water). A known quantity of the drug was transferred in a series of volumetric flasks in different solvents. An excess amount of drug was added to different solvents till the solution became saturated and this volumetric flask was shaken by a mechanical shaker for 24 hours under constant vibration at constant temperature (25ºC). The prepared saturated solution was filtered, diluted, and then analyzed by UV spectrophotometer double beam Shimadzu-1700 Japan). There are three determinations were carried out before each sample to calculate the solubility of Nisoldipine in different solvents [12-14]

Fourier Transmittance Infra-red (FTIR):

` To check the integrity of the drug in the formulation FTIR spectra of formulations along with the pure drug and other excipients were obtained and compared using PerkinElmer FTIR spectrophotometer In the present study potassium bromide (KBr) pellet method was employed. The samples were thoroughly blended with dry powdered KBr crystals. The mixture was compressed to form a disc. The disc was placed in a spectrophotometer and the spectrum was recorded.

5X-ray Diffraction Technique:

X-ray diffraction analysis for pure Nisoldipine was performed by using a powder X-ray diffractometer with the copper anode (54 dot A) at 60 mA current and voltage of 60 kV. All samples were analyzed in scanning range at 20 angles of 5-50°, with a step size of

0.0017° using a scintillation counter detector. The change in characteristic peak of XRD, if any, was analyzed using previously published data

Differential Scanning Calorimetry (DSC):

Differential scanning calorimeter or DSC is a thermoanalytical technique in which differences in the amount of heat required to increase the temperature of a sample and reference are measured as a function of temperature. Both the sample and reference were mentioned at nearly sample temperature throughout the experiment. [15]

Drug Polymer interaction studies:

The identity and purity of excipients samples were determined by scanning the sample of excipients on an FTIR spectrophotometer.

The FTIR spectra showed the characteristic peaks which are identical with ranges of functional groups of the excipients respectively, spectra of a mixture of Nisoldipine, poly δ-Decalactone, and Tween 80.[16]

PREPARATION OF NANOEMULSION:

A nanoprecipitation method was used to prepare nanoemulsion using PDL polymer as oil and tween80 as surfactant. Drug-loaded oil-in-water nanoemulsions were prepared by dissolving drug (5 mg) and polymer (PDL25 mg) in solvent (acetone 1.5 ml). This organic mixturewas then added dropwise to the Milli Q water (3.5 ml), surfactant (Tween80, 1.5 ml) with stirring (1000 rpm). The solution was then stirred for at least 3 hrs.Room temperature (open vial) to ensure the complete removal of organic solvent. The nanoemulsion was finally filtered through a membrane syringe filter (pore size, 0.45μm) and use for further characterization.

Formulation parameters of Nanoemulsion:

The optimization studies were accomplished on the NNE formulation the parameters used for the preparation of this optimized formulation are shown in the following table6.10.After the optimization of all parameters final three formulations i.e. NNE1 1:1, NNE3 1:1.1, and NNE5 1:1.5 were prepared using different ratios of surfactant and the amount of PDL constant.[17]

EVALUATION OF PREPARED NANOEMULSION:[15-17]

The prepared Nanoemulsion of different formulations were evaluated for various physicochemical parameters such as:

- Particle size, Polydispersityindex. (PDI).
- Surface charge measurements. (ZP).
- Fourier transforms infrared spectroscopy. (FTIR)
- TEM
- Drug entrapment/Drug content

- In vitro drug release study
- Kinetics
- Stability studies

Dropletsize Analysis

Photon correlation spectroscopy was used to estimate the droplet size of the nanoemulsion. In a volumetric flask with 50 mL water, the formulation (0.1 mL) was spread and gently mixed by inverting the flask. At 25°C, at a 90° or 180° angle, measure with a Zetasizer and a light scattering monitor .

Zeta potential

Zeta potential (ZP. C-potential) is an important macroscopic measure of tremendous practical and scientific interest in the field of nanoparticles. In classical interpretation. The ZP is associated with the electrostatic potential that develops at an ill-defined boundary between an NP and surrounding fluid. A surface charge, formed when the solidsurface is exposed to an aqueous solution or another liquid, is compensated by counterions present in the solution. These oppositely charged layers form the so-called "electric doublelayer" (EDL).

pH measurement

A pH meter is employed for the determination of pH of Various formulations prepared.

Particle morphology (TEM)

The transmission electron microscope was used as visualizing aid for particle morphology. The sample (10µL)was placed on the grids and allowed to stand at room temperature for 90 sec. excess fluid was removed by touching the edge with the filter paper, All samples were examined under a transmission electron microscope (Philips Morgagni 268, Holland) at an acceleration voltage of 100 kV, and photomicrographs were taken at suitable magnification.

Drug entrapment:

A weighed amount of nisoldipine-loaded nanoemulsion was dispersed in methanol, centrifuged (Superspin R 1(Z.50) at 10,000 rpm for half an hour the supernatant withdrawn, suitably diluted in distilled water, and were subjected to UV spectroscopy for taking absorbance of the sample against blank distilled water at 236 nm wavelength.

With the help of absorbance, the concentration in the supernatant was determined by plotting the absorbance value against the concentration in the standard curve. The percentage of drug entrapment was calculated by the following equation:

$$\% \text{ Drug entrapment} = \frac{\text{Calculated drug content } 100}{\text{Theoretical drug content}}$$

RESULT

PREFORMULATION STUDIES:

Preformulation studies on the drug were carried out to confirm its identity and purity and also to confirm that there are no significant barriers to the development of the proposed formulation of the drug with the enlisted polymer and excipients. The studies besides confirming the identity and purity of the compound showed no significant interaction of the drug with the polymer used.

Physiochemical characterization of the drug:

Organoleptic property:

The physical appearance of the drug sample under investigation was found to be yellow powder and odorless which was similar as reported in the literature.

Organoleptic Property of Drug.

S.No.	CHARACTERISTIC	OBSERVATION
1.	Appearance	Crystalline
2.	Color	Yellow or almost Yellow
3.	Odor	Odorless

Identification of drug:

Melting point:

The melting point of the drug was determined by the capillary method and found to be 157 as the reported values (150-158 degrees). The description of the melting point is shown in the preformulation study.

Table-6.2: Melting point of Nisoldipine.

Reported	Observed
150-158	157 ±2.1

n=3, all values are expressed as mean±SD

Identification of Drug by Infrared Spectroscopy:

FTIR spectrum of the drug was obtained by potassium bromide method KBr using The principal peaks of the drug were identified and matched with the standard FTIR of the drug confirming the identity and purity of the drug.

Standard curve of Nisoldipine at 6.8 pH (PBS).

S. No.	Concentration(µg/ml)	Absorbance
1.	2.5	0.119±0.0-02

2.	5	0.226±0.001
3.	10	0.433±0.003
5.	15	0.612±0.006
6.	20	0.805±0.008
	25	0.992±0.003
7.	30	1.172±0.005

n=3, all values are expressed as mean±SD

Solubility studies:

Solubility studies of the drug were performed the drug was found to be soluble in ethanol, methanol, and acetone, slightly soluble in chloroform and distilled water are practically insoluble.

Table- Solubility of Nisoldipine in different solvents at room temperature

S.No.	Solvent	Solubility
1.	Acetone	Soluble
2.	Methanol	Soluble
3.	Ethanol	Soluble
4.	Chloroform	Slightly soluble
5.	Distilled water	Practically insoluble

Drug Solubility Determination in Various Components:

Table- Solubility of Nisoldipine in various components.

S.No.	Components	Function	Concentration (µg/ml)
1.	Poly δ-decalactone	Oil	100.13
2	Tween 80	Surfactant	43.21
3.	Tween 20	Surfactant	23.01
4.	Span 80	Surfactant	20.88
5.	Span 20	Surfactant	34.16

Evaluation of PDL Polymer:

Viscosity determination of PDL Polymer:

The shear rate vs shear stress curve suggested that the PDL polymer is a Newtonian fluid, and the infinite rate viscosity was found to be 62.20 Pa.s.

Formulation of the nanoemulsion:

The optimized formulation of nisoldipine nanoemulsion was prepared using polymer as oil and Tween 80 as surfactant by using Nanoprecipitation method.

OPTIMIZATION AND FORMULATION OF NANOEMULSION:

Optimization of formulation variables

On increasing the surfactant ratio, stirring speed and time the particle size of nanoemulsion gets reduce and also decrease in polydispersity index of nanoemulsion is seen consequently the zeta potential of nanoemulsion gets reduce in a similar way on increasing the ratio of Tween 80 enhancement in entrapment efficiency of nanoemulsion is seen. The best result was found to be in ratio 1.1.3 formulation NNE3 shown in table

EVALUATION OF FORMULATION:

FTIR study of prepared nanoemulsion (placebo and drug loaded nanoemulsion)

FTIR analysis helps us to analyze the presence of functional groups and chemical bonds thus suggesting chemical composition of prepared formulation. On comparing FTIR scans of the nisoldipine loaded nanoemulsion (NNE3) with the placebo nanoemulsion, it was observed there were no significant differences. Similar observation was made between nisoldipine and nisoldipine loaded nanoemulsion suggesting that the nisoldipine molecules were uniformly encapsulated in the prepared nanoemulsion

Table Optimization of formulation variables at different ratio.

S.NO	Polymer: ratio	Speed (rpm)	Time (hrs)	Particle size
NNE1	1:1	900	2:30	583.2±1.13
NNE2	1:1.2	950	2:45	573.8±1.55
NNE3	1:1.3	1000	3	506.1±1.01
NNE4	1:1.4	1050	3:15	605.3±2.03
NNE5	1:1.5	1100	3:30	615.23±1.21

n= 3, all values are expressed as mean

Table-pH of Formulation:

S.No.	Formulation	pH
1.	NNE1	6.3±0.04
2.	NNE2	7.4±0.08
3.	NNE3	6.6±0.06
4.	NNE4	6.7±0.01

5.	NNE5	7.0±0.09

n= 3, all values are expressed as mean±SD

Drug Entrapment/ Drug content:

The % encapsulation efficiency of nisoldipine in prepared inclusion complexes was found to be 71.33±1.62%, 79.4±0.24%, 99.95±1.35%, 90.12±0.34%, and 77.26±1.32%. It was found that for the NNE nisoldipine was loaded in the highest amount in NNE3 as much as 99.95% (w/w). This observation according to the entrapment efficiency % observed for nanoemulsion clearly demonstrates statistical difference among these comparisons. This observation undoubtedly suggest that PDL is playing a crucial role in enhancing aqueous solubility of drugs.

Table 6.19: %Drug entrapment

Formulation	% Drug entrapment
NNE1	71.33±1.62%
NNE2	79.4±0.24%
NNE3	99.95±1.35%
NNE4	90.12±0.34%
NNE 5	77.26±1.32%

n= 3, all values are expressed as mean±SD

In-vitro drug release study:

The release study was carried out as per the procedure described in materials and methods section…

The drug release was slow controlled and dependent upon the drug. Among the five formulations, NNE3 showed the maximum drug release value during the 12Hrs release study.

Table 6.20: In-vitro drug release study

Time (Hrs.)	Cumulative % Drug Release				
	NNE 1	NNE 2	NNE3	NNE4	NNE5
0	0±00	0±00	0±00	0±00	0±00
0.5	10.22±0.65	13.24±0.52	18.26±1.34	0±0.41	6.02±0.41
1	19.87±0.73	21.89±0.53	26.9±0.12	9.74±1.50	13.33±1.50
2	33.65±0.49	35.67±0.30	40.69±0.40	15.57±1.25	27.73±1.25

CONCLUSION

The 1, 4, dihydropyridine, nisoldipine, is a poorly water-soluble drug with low bioavailability and a short half-life of 7-12 hrs. Thus, nisoldipine has to be administered 2 or 3 times per day. Absorption is rapid and this, coupled with the short elimination half-life, can result in significant fluctuations in plasma drug concentrations. Nanoemulsions were prepared by Nanoprecipitation Method. Factors that may affect the formulation were optimized. Formulation variables such as polymer and surfactants ratio, stirring time, and stirring speed were optimized and formulation with optimum size, PDI with good morphology was selected.

Future prospects of the current study could be extended to the evaluation of the drug-loaded PDL nanoemulsion using in vivo studies like Pharmacokinetic studies including Cmax, Tmax, AUC, AUMC, MRT, and pharmacodynamics studies like regulation of increased blood pressure by nisoldipine loaded nanoemulsion could be performed. The data obtained from the above material studies could be well utilized for preclinical studies & possible large-scale commercial product development.

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