Advances in Bioresearch
Adv. Biores., Vol 14 (1) January 2023: 225-238
©2023 Society of Education, India
Print ISSN 0976-4585; Online ISSN 2277-1573
Journal's URL:http://www.soeagra.com/abr.html
CODEN: ABRDC3
DOI: 10.15515/abr.0976-4585.14.1.225238

Advances in Bioresearch

ORIGINAL ARTICLE

Formulation, Evaluation and Stability Studies of Nicardipine Hydrochloride Microspheres

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ABSTRACT
Nicardipine Hydrochloride is having vasodilation action. It is also a potent calcium channel blocker. It acts by interfering with the release of calcium from the sarcoplasmic reticulum, by inhibiting ion-control gating mechanisms. The Nicardipine Hydrochloride Microspheres were subjected to physicochemical studies, in-vitro drug release, and stability studies. In-vitro release studies show that 91% of the drug was released from all the formulations were within 12 h. DSC and FTIR studies showed there was no interaction between drugs and polymers. The formulation showed optimum hardness & sustained drug release within 12 h. The formulations were subjected to stability studies and shown there were no significant changes in drug content, physicochemical parameters, and release patterns. Developed Nicardipine Hydrochloride Microspheres shows effective drug release over a prolonged time frame that led to greater therapeutic efficacy.
Keywords: Nicardipine HCL, Microsphere, Formulation, Evaluation, Stability Studies.

Received 19.10.2022 Revised 05.11.2022 Accepted 22.11.2022

How to cite this article:
Fayyaz A, Aejaz A, Quazi M, Pravin G, Ansari Y A, Kabir S, Mohd.Razi A, Pathan M. Formulation, Evaluation and Stability Studies of Nicardipine Hydrochloride Microspheres. Adv. Biores. Vol 14 [1] January 2023.225-238

INTRODUCTION

The most favored dosage forms include tablets, capsules, and solutions because of their ease of manufacture and administration. Failure to systemically deliver select compounds through the oral route led to research on alternate routes of drug delivery. Lack of adequate absorption through the gastrointestinal tract was the single most predominant reason for such efforts. Researchers resorted to the parenteral route as an easy solution to the problem but have major disadvantages such as patient compliance, health hazards, higher cost of therapy due to the use of highly qualified health care workers, and expensive equipment/ tools. The use of the nasal route for the administration of drugs has engaged the attention of mankind since ancient times. Psychotropic drugs & hallucinogens have been used as snuffs by the natives in South America for centuries (Ranade and Hollinger, 2010). Nasal therapy has been a recognized form of treatment in the Ayurvedic systems of Indian medicine, it is called "NASAYA KARMA". The use of intranasal (IN) administration to target therapeutics to the central nervous system (CNS) has many benefits in the treatment of neurologic disorders. (1-3)

Nicardipine Hydrochloride

Chemical name of Nicardipine is 3-{2-[benzyl (methyl) amino] ethyl} 5-methyl 2,6-dimethyl-4-(3-nitrophenyl)-1,4-dihydropyridine-3,5-dicarboxylate. Nicardipine Hydrochloride is a potent calcium channel blocker with marked vasodilation action. It acts by inhibiting ion-control gating mechanisms, and/or interfering with the release of calcium from the sarcoplasmic reticulum. It inhibits the influx of extracellular calcium across the myocardial and vascular smooth muscle cells, causing dilation of coronary and systemic arteries. (4-6)

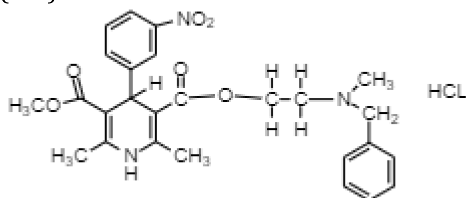

Figure 1: Structure of Nicardipine HCL

MATERIAL AND METHODS

Materials

Nicardipine Hydrochloride was obtained as a gift sample from Kwality Pharmaceutical Pvt. Ltd, Amritsar. Soya oil and n-octanol were obtained from Research-Lab Fine Chem Industries, Mumbai. Dialysis membrane (cut off MW 1200) was purchased from Hi-media lab, Mumbai. Chitosan, Span 80, and Potassium hydroxide were of Analytical Research (AR) Grade obtained from Loba Chemie Pvt. Ltd, Mumbai.

EQUIPMENTS

Table 1: Equipments and instruments used in present research work

Sr.No.	Equipment/ Instrument	Model	Maker
1	UV Visible double beam spectrophotometer	V-630	Jasco, Japan
2	Infrared Spectrophotometer	FTIR-4100	Jasco, Japan.
3	Differential Scanning Calorimetry (DSC)	DSC6220, ETSTA R6000 Series	Shimadzu, Kyoto, Japan
4	Optical Microscope	OPTIK KG-5	Micron Instrument Industries, Ambala
5	Vacuum Pump	VE155	VALVE Pumps, India.
6	Digital Optical Microscope	DMB-1	Motic China Group Co.
7	Electronic Weighing Balance	D455003609	Schimadzu Corporation, Japan
8	High precision balance	PGB 200	Wensar, India
9	Magnetic Stirrer	1MLH an 2 MLH	Remi motors, India.
10	pH meter	CL180	Chemiline, India
11	Ultra sonicator	D-Compact	Labhosp, India
12	Hot air oven	L187-D	Labin, India

Preformulation study

Preformulation studies are performed to ensure the development of a stable as well as the effective and safe dosage form. It is a stage of development during which the pharmacist characterizes the Physico-chemical properties of the drug substances and their interaction with various formulation components with the following objective:

i. To determine the necessary physicochemical parameter of a new drug substance.
ii. To establish its compatibility with the excipients of the formulation.

Identification and characterization of drug

Organoleptic characterization of drug

Nicardipine Hydrochloride was observed for organoleptic characteristics viz., appearance, color and odor.

Melting point determination

The melting point of Nicardipine Hydrochloride was determined by the melting point apparatus using the capillary method.

UV spectroscopy

Nicardipine Hydrochloride solution (100 µg/mL) in methanol was prepared. This solution was subjected to scanning over the wavelength range of 200-400 nm using a double beam UV spectrophotometer against Methanol as a blank. The plot of absorbance versus wavelength was recorded. (7-9)

Solubility

To 5ml solvent Excess of the drug was added and kept for shaking for few minutes and observed.

Preparation of acetate buffer pH 5.5 (USP, 2008)

A. Acetic acid, 2N:

Acetic acid (116 ml) was added to water and diluted with water to 1000 mL.

B. Acetate buffer pH 5.5:

Sodium acetate (5.98 gm) in a 1000 ml volumetric flask, added 2N acetic acid solution (3.00 ml) then add water to volume and mix. The solubility of the drug was checked by visual observations in different solvents like acetate buffer pH 5.5, dichloromethane, chloroform, alcohol.

2.3.1.5 Infrared spectroscopy (IR)

Nicardipine Hydrochloride was examined by IR absorption spectral analysis using the KBr disk method (1 mg drug in 100 mg KBr). The spectrum was recorded by scanning in the wavelength region of 4000-400 cm^{-1} using an IR spectrophotometer.

Differential scanning calorimetry (DSC)

The DSC thermogram of simvastatin was recorded using a Differential scanning calorimeter (DSC6220, EXSTAR 6000 SERIES, Shimadzu, and Kyoto, Japan). Approximately 5 mg of sample was heated in a closed pierced aluminum pan from 30 °C to 300 °C at a heating rate of 10 °C/min under a stream of nitrogen at a flow rate of 40ml/min.

Preparation of standard calibration curve of Nicardipine Hydrochloride in acetate buffer pH 5.5 with 0.3% sodium lauryl sulfate (SLS)

An accurately weighed quantity of Nicardipine Hydrochloride (10mg) was placed in a 100 mL volumetric flask and dissolved in acetate buffer pH 5.5 with 0.3% SLS. Then it was diluted with acetate buffer pH 5.5 to give stock solution (100 (µg/mL). The aliquots (0.5-3 mL) of stock solution were further diluted to 10 mL to produce a standard solution having concentrations in the range 5-30 (µg/mL the absorbance of the solutions was measured at 237.8 nm using a double beam UV visible spectrophotometer against acetate buffer pH 5.5 as a blank. The plot of concentration (µg/ml) versus absorbance was plotted and data were subjected to linear regression analysis in Microsoft Excel.

Drug-excipient compatibility study

A drug and physical mixture of drug substances with chitosan (1:1) was placed in amber-colored glass vials, capped with rubber closures, and sealed with aluminum caps. These samples were kept for two weeks at 55˚C and 75% RH. Samples were observed physically for caking, liquefaction, discoloration, and odor or gas formation.

FTIR spectroscopy

IR absorption spectral analysis of drug and drug with chitosan (1:1) was carried out using the KBr disk method (1 mg drug in 100 mg KBr) and the spectrum was recorded by scanning in the wavelength region of 4000-400 cm^{-1} using an IR spectrophotometer. The spectrum of the drug was compared with that of the physical mixture of drug and excipients to check for any possible drug excipient interaction.

Formulation and development

General method of preparation of chitosan microspheres

Chitosan microspheres were prepared by a simple w/o emulsification-cross linking process using soya oil as an external phase. Briefly, an accurately weighed quantity of chitosan was dissolved in 0.5% aqueous acetic acid solution by continuously stirring until a homogeneous solution was obtained. The drug was added in chitosan solution and the dispersion was added slowly through a syringe to soya oil containing 5% v/v of span 80 as a stabilizer under high speed stirring for 1 hr using a Remi (1 MLH) high-speed magnetic stirrer. To this W/O emulsion, appropriate quantities of potassium hydroxide (1% solution in n-octanol, as cross-linking agent) 0.75 mL/15 mint were added for 1.5 hr. The hardened microspheres were separated by vacuum filtration and washed several times with hexane to remove the oil. Finally, microspheres were washed with distilled water to remove unreacted potassium hydroxide. The microspheres were air-dried for 24 h and then stored in a vacuum desiccator until further use.

Preliminary trials for preparation placebo chitosan microspheres and selection of processing variables for preparation of microspheres:

The preliminary studies were carried out by preparing various batches of microspheres with different process parameters to optimize the formulations for obtaining microspheres with proper physical characteristics and of particle size ranging from 10-50 µm which is ideal for nasal delivery. The following

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are the process variables that were studied to standardize the method for preparation of the microspheres:

1. Emulsifier concentration (Span 80)
2. Chitosan concentration
3. Aqueous: oil phase ratio

Emulsifier concentration (Span 80)

Five different formulations namely A_1, A_2, A_3, A_4 and A_5were prepared by varying the emulsifier concentration from 3%, 4%, 5%, 6%, and 7% v/v respectively, by keeping other process variables like the volume of cross-linking agent (4.5 ml) and cross-linking time (1.5h) as shown in Table 2.

Chitosan concentration

Five different formulations namely A_6, A_7, A_8, A_9, and A_{10} were prepared by varying the concentration of chitosan i.e. 1.5%, 2%, 2.5%, 3%, and 3.5% w/v respectively while keeping all other process variables like the volume of cross-linking agent (4.5 ml) and cross-linking time (1.5h) as shown in Table 2.

Aqueous: oil phase ratio

Three batches of microspheres were prepared namely A_{11}, A_{12}, and A_{13} by varying the different ratio of aqueous: oil phase i.e. 1:5, 1:10, and 1:15 respectively, while keeping all other process variables like the volume of cross-linking agent (4.5 ml) and cross-linking time (1.5h) as shown in Table 2

Table 2: Formulation batches of placebo microspheres

Sr. No.	Formulation batches and process variables	Constant parameters
1	Emulsifier concentration (Span 80) A1 (3%) A2 (4%)A3 (5%)A4 (6%) A2 (4%)A3 (5%)A4 (6%) A4 (6%) A3 (5%)A4 (6%) A4 (6%) A4 (6%) A4 (6%) A4 (6%)	Cross-linking time (1.5 h) The volume of cross-linking agent (4.5 ml)
2	Chitosan concentration A6 (1.5%)A7 (2%)A8 (2.5%)A9 (3%) A7 (2%)A8 (2.5%)A9 (3%) A8 (2.5%)A9 (3%) A9 (3%) A10 (3.5%)	Cross-linking time (1.5 h) The volume of cross-linking agent (4.5 ml)
3	Aqueous: oil phase ratio A11 (1:5)A12 (1:10) A12 (1:10) A13 (1:15)	Cross-linking time (1.5 h) The volume of cross-linking agent (4.5 ml)

Evaluation of placebo chitosan microspheres:

Morphological characterization

All batches of microspheres were preliminarily checked for shape and size by optical microscopy at 10X magnification. The samples were prepared by suspending a small number of microspheres in paraffin oil.

Particle size determination:

A microscopic image analysis technique for the determination of particle size was applied. The morphology and particle sizes were determined in a Motic DMW2-223 digital microscope equipped with a 1/399 CCD camera imaging accessory and computer-controlled image analysis software (Motic images plus, 2.0 version). The microspheres were dispersed on a microscope slide. A microscopical field was scanned by a video camera. The images of the scanned field are analyzed by the software. In all measurements at least 100 particles were examined for their mean particle diameter (m). The average particle size was determined by using Edmundson's equation.

$$\text{Dmean} = \frac{\sum nd}{\sum n} \quad \text{......... (7.1)}$$

Where, n: Number of microspheres observed, d: mean size range

Formulation of drug-loaded chitosan microspheres

Drug: polymer ratio

Based on the results of preliminary investigation, the different process parameters like emulsifier concentration, chitosan concentration, and aqueous: oil phase ratio were optimized, and drug-loaded three formulations D_1, D_2, and D_3 were designed by varying drug: polymer ratio i.e. 1:1, 1:2 and 1:3 and formulation procedure were followed as described in section 7.4.1 as shown in Table 3.

Volume of cross-linking agent

Three different batches namely D_4, D_5, and D_6 were formulated with varying the amount of cross-linking agent (1% KOH) i.e. 3 ml, 4.5 ml, and 6 ml respectively, and the formulation procedure was followed as described in section 7.4.1 while other conditions such as drug: polymer ratio (1:2), chitosan concentration, concentration of emulsifier and aqueous: oil phase ratio was constant as shown in Table 3.

Cross-linking time

Three different batches namely D_7, D_8, and D_9 were formulated by varying cross-linking time i.e. 1h, 1.5h, and 3h respectively, and the formulation procedure was followed as described in section 7.4.1. while other conditions such as drug: polymer ratio (1:2), volume of cross-linking agent (4.5 ml), chitosan concentration, concentration of emulsifier, and aqueous: oil phase ratio were constant as shown in Table 3.

Table 3: Formulation of drug-loaded microspheres

Sr. No.	Formulation batches and process variables	Constant parameters
1.	**Drug: polymer ratio** D1 (1:1)D2 (1:2) D2 (1:2) D3 (1:3)	Emulsifier concentration (5% v/v) Chitosan concentration (2.5% w/v) Aqueous: oil phase ratio (1:10)
2.	**The volume of cross-linking agent** D4 (0.4 mL/2h)D5 (0.2 mL/2h) D5 (0.2 mL/2h)D6 (0.1 mL/2h) D6 (0.1 mL/2h) D7 (0.1 mL/1h)	Emulsifier concentration (5% v/v) Chitosan concentration (2.5% w/v) Aqueous: oil phase ratio (1:10) Drug: polymer ratio (1:2)
3.	**Cross-linking time**	Emulsifier concentration (5% v/v) Chitosan concentration (2.5% w/v) Aqueous: oil phase ratio (1:10) Drug: polymer ratio (1:2) Volume of cross-linking agent (4.5 ml)

Evaluation of drug-loaded chitosan microspheres

Percentage yield

The practical percentage yield was calculated from the weight of dried microspheres recovered from each batch about the sum of the initial weight of starting materials. The percentage yield was calculated using the following formula:

$$\% \text{ yield} = \frac{\text{Practical yield}}{\text{Theoretical yield}} \times 100 \quad \text{.......(7.2)}$$

Morphological characterization

The same procedure was used as described in section 7.4.3.1.

Particle size determination

The same procedure was used as described in section 7.4.3.2.

Drug loading and incorporation efficiency

The Nicardipine hydrochloride-loaded microspheres (10 mg) were crushed in a glass mortar and pestle and the powdered microspheres were suspended in 10 mL glacial acetic acid and methanol mixture (1:4) under stirring. The solution was filtered and analyzed for the drug content using a UV visible spectrophotometer at 235 nm. The drug loading and % incorporation efficiency was calculated using the following equations

$$\text{Drug Loading} = \frac{\text{M actual}}{\text{Wt. of microspheres}} \times 100$$

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$$\% \text{ incorporation efficiency} = \frac{M \text{ actual}}{M \text{ theoritical}} \times 100$$

Where, M actual is the actual drug content in the weighed quantity of powder of microspheres and Mtheoretical is the theoretical amount of drug in microspheres calculated from the quantity added in the cross emulsification process.

***In vitro* drug release study**

In vitro release of drugs from the microspheres was carried out in a modified Franz diffusion cell with a dialysis membrane (cut-off 12,000-14,000 kDa). For the preparation of the artificial membrane, the dialysis membrane was soaked in acetate buffer pH 5.5 for 24h before mounting on the diffusion cell. After a preincubation time of 20 min., accurately weighed of drug-loaded microspheres equivalent to 10 mg Nicardipine hydrochloride was placed in the donor compartment with 2 ml and 20 ml acetate buffer pH 5.5 was placed in donor and receptor compartment respectively. The temperature of the receptor compartment was maintained at 37±1˚C during the experiment. At set time interval, 1 ml sample was withdrawn from the receptor compartment; replacing the sampled volume with acetate buffer pH 5.5 after each sampling for a period of 12h. Sample withdrawn were suitably diluted and analyzed using a UV visible spectrophotometer at 237.8 nm. (11-12)

Stability study

The purpose of stability testing is to provide evidence on how the quality of a drug substance or drug product varies with time under the influence of a variety of environmental factors such as temperature, humidity, and light, and to establish a re-test period for the drug substance or shelf life for the drug product and recommended storage conditions (ICH guideline Q1A (R2)).

Stability studies were carried out on microspheres according to ICH (International Conference on Harmonization) guidelines. A sufficient quantity of microspheres in amber-colored sealed vials was stored in a desiccator containing a saturated solution of sodium chloride, which gave a relative humidity of 75±5%. The desiccator was placed in a hot air oven maintained at 40±2^{0}C, and samples were withdrawn at 0, 1st, 2^{nd,} and 3rd months. The optimized formulation was evaluated at a predetermined time interval for particle size, drug content, and in vitro drug release. (13-14)

RESULTS AND DISCUSSION

Preformulation study

Identification and characterization of drug

Organoleptic characterization of drug

The organoleptic characteristics of drug-like appearance, color, and odor were studied and shown in Table 4.

Table 4: Organoleptic characterization of drug

Sr.No	Test	Observation
1	Appearance	Crystalline
2	Color	Greenish-Yellow
3	Odor	Odorless

Melting point determination

The melting point of Nicardipine hydrochloride was determined by the melting point apparatus using the capillary method and DSC. The melting point of the drug was found to be in the range of 180-184˚C, which matches with the reported value i.e.182-186 ˚C.

Solubility

Nicardipine hydrochloride was freely soluble in Chloroform, Methanol, Glacial acetic acid, and acetate buffer pH 5.5, sparingly soluble in ethanol and slightly soluble in water.

UV Spectroscopy

UV spectrum of Nicardipine hydrochloride solution (100 µg/mL) in methanol was scanned and wavelength (λmax) was found to be at 237 and 258 nm.

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Infrared spectroscopy

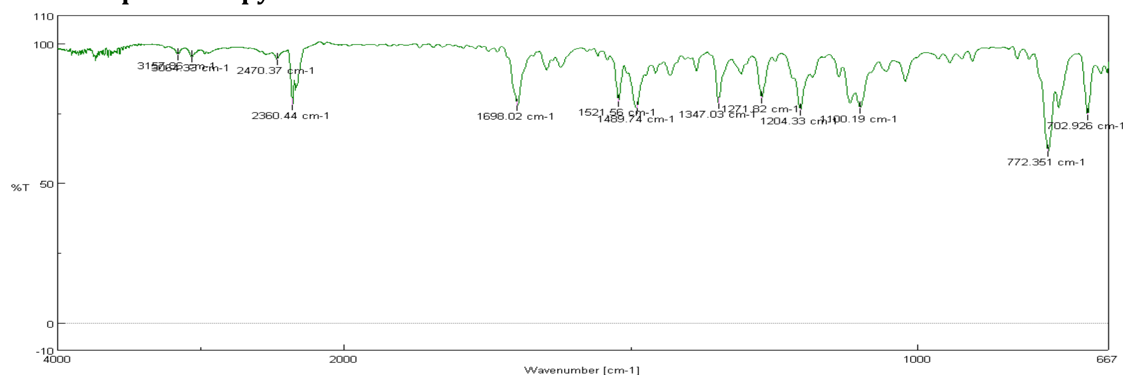

Figure 2: IR spectrum for Nicardipine hydrochloride

The IR spectrum of the Nicardipine hydrochloride was taken by using the KBr disk method (1 mg drug in 100 mg KBr), the drug was scanned over the wavelength region of 4000-400 cm^{-1} and the functional groups were interpreted as per the spectra and were found to match the structure of the drug. The IR spectrum of the Nicardipine hydrochloride is shown in figure 2.

Principal peaks obtained in the IR spectrum along with their corresponding functional groups are given in Figure 2.

Table 5: The functional groups along with their wavenumbers of the simvastatin

Wave number (cm^{-1})	Functional groups
3548.38	Stretching (Alcohol H bonded)
2951.52	Stretching Alkanes (C-H)
1696.09	Ester group
1389.46	-C-O- of ester
868.774	Aromatic ring

Differential scanning calorimetry (DSC)

DSC thermogram showed a sharp endothermic peak at 186.4ºC which confirms a melting point similar to the reported. DSC thermogram of simvastatin is shown in Figure 3.

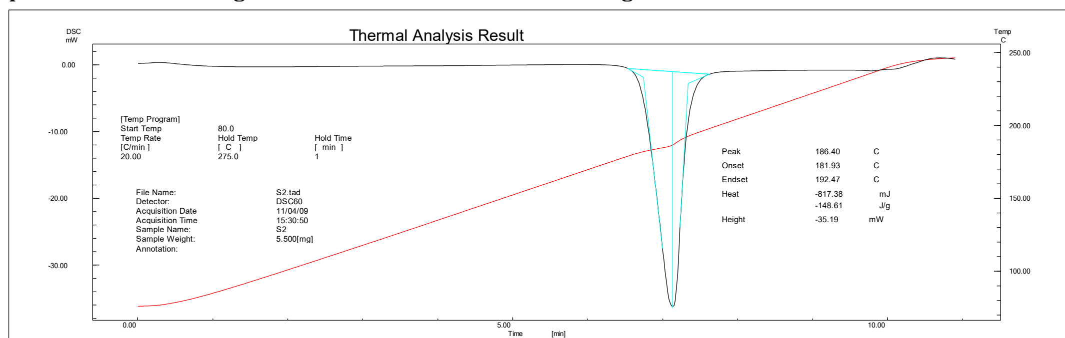

Figure 3: DSC thermogram of Nicardipine hydrochloride

Preparation of standard calibration curve of Nicardipine hydrochloride in acetate buffer pH 5.5 with 0.3% sodium lauryl sulfate (SLS) at $\lambda_{max.}$ 237.8 nm

The standard calibration curve data and a standard calibration curve of Nicardipine hydrochloride in acetate buffer pH 5.5 are shown in Table 6 and Figure 4 respectively. It was found that the Nicardipine hydrochloride shows absorbance in the UV range of 200 to 400 nm. The absorbance of serial dilution of Simvastatin in acetate buffer pH 5.5 was taken and the concentration (g/ml) versus absorbance was plotted. It was found to be linear over the range of 5-30 g/ml indicating its compliance with Beer's Lambert's law.

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Table 6: Standard calibration curve of Nicardipine hydrochloride in acetate buffer containing pH 5.5 at $\lambda_{max.}$ 238 nm

Concentration (g/ml)	Absorbance
5	0.233±0.008
10	0.445±0.004
15	0.673±0.006
20	0.892±0.007
25	1.117±0.019
30	1.364±0.020

Mean ± S.D. (n=3)

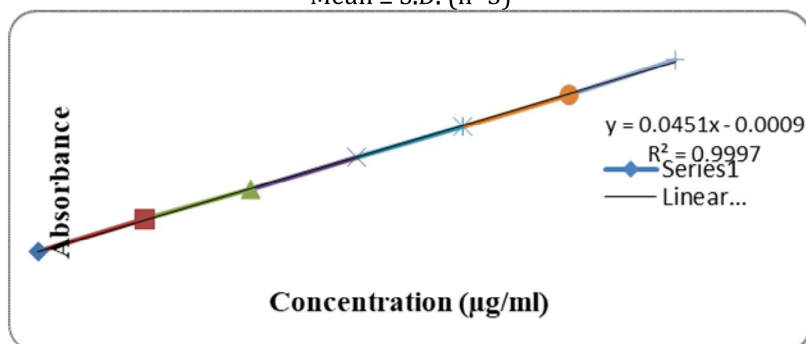

Figure 4: Standard calibration curve of Nicardipine hydrochloride in acetate buffer pH 5.5

Drug excipient compatibility study

All the physical mixtures of drugs and excipients (1:1) were placed in amber-colored glass vials, capped with rubber closures, and sealed with aluminum caps. These samples were kept for two weeks at 55°C. Samples were observed physically for caking, liquefaction, discoloration, and odor or gas formation and were found to be without any physical change (Table 7). Therefore it is confirmed that all the active and inactive excipients ingredients which were kept under compatibility study are compatible with each other. Hence all these ingredients were selected and used in the present work.

Table 7: Drug excipient compatibility study

Sample	**Period**	**Caking**	**Liquification**	**Discoloration**	**Odor and Gas**
Drug	Initial	Free-flowing	Solid	White	Odorless
	14 Days	No change	No change	No change	No change
Chitosan (1:1)	Initial	Free-flowing	Solid	Creamy White	Odorless
	14 Days	No change	No change	No change	No change
Chitosan	Initial	Free-flowing	Solid	Creamy White	Odorless
	14 Days	No change	No change	No change	No change

Fourier transform infrared spectroscopy

An IR spectrum for Nicardipine hydrochloride, chitosan, and physical mixture of Nicardipine hydrochloride and chitosan is shown in Figure 2, Figure 5, and Figure 6 respectively. From IR spectra it can be seen that the principal peaks of Nicardipine hydrochloride, chitosan, and physical mixture of Nicardipine hydrochloride: chitosan (1:1) as shown in Table 10, are present in both, the spectra of pure drug and physical mixture with chitosan. FTIR studies suggest the absence of any significant interaction between the polymers and the drug in the formulation.

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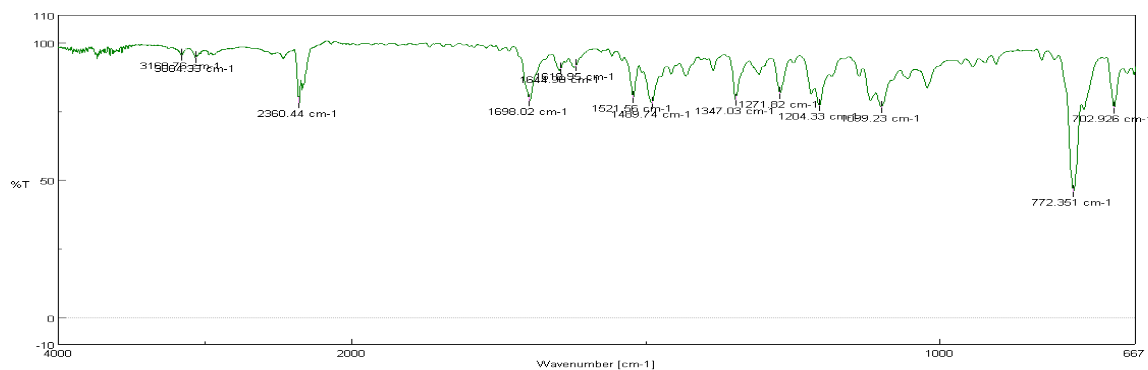

Figure 5: IR spectra for chitosan

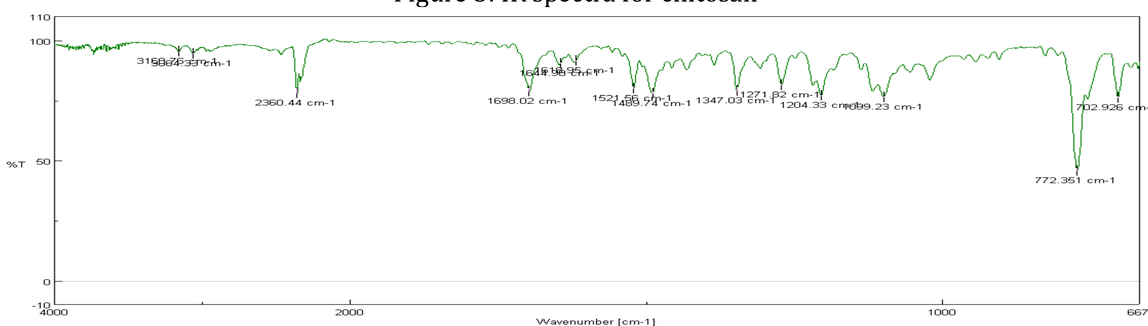

Figure 6: IR spectra for the physical mixture of Nicardipine hydrochloride: chitosan (1:1)

Table 8: Principle peaks of drug / drug: polymer

Drug/Drug: Polymer	Principle peaks of IR (cm^{-1})
Nicardipine HCL	702, 772, 1204, 1551, 1688, 2360, 2470
Chitosan	772, 1022, 1219, 2360, 3732
Nicardipine HCL: Chitosan (1:1)	702, 772, 1204, 1233, 1521, 1698, 2360

Formulation and development

Preliminary trials for preparation placebo chitosan microspheres and selection of processing variables for preparation of microspheres:

The different preliminary trials were taken as mentioned in Table 3 for the selection of different processing variables for the preparation of microspheres and subjecting the formulations to particle size analysis and physical appearance. The formulations were studied for the effect of emulsifier concentration, chitosan concentration, and Aqueous: oil phase ratio. Based on the results of these preliminary studies (Table 9), the design of the final formulation was made by varying the drug: polymer ratio, the volume of cross-linking agent (Potassium hydroxide), and cross-linking time. While keeping the other process variables constant.

Emulsifier concentration (Span 80)

As the concentration of surfactant increased from 3 to 7% v/v for batches A1 to A5 the morphology of particle changes from spherical to the irregular shape. Complete emulsification was not achieved with emulsifier 3% v/v. Microspheres fabricated with 4% v/v emulsifier showed the lowest particle size but also aggregates formed while those prepared with 5% v/v showed particle size in the range i.e. 16.8-41.1 µm and spherical. An increase in surfactant concentration led to the formation of microparticles irregular in shape. Microspheres fabricated with 5% v/v showed good results and it showed particle size in an acceptable range.

Chitosan concentration

Preliminary trials viz. A6, A7, A8, A9, and A10 were carried out at different chitosan concentrations i.e. 1.5%, 2%, 2.5%, 3%, and 3.5% w/v respectively and batches fabricated with concentration 1.5 and 2% w/v of chitosan showed particle size within the range but irregular in shape. The batch fabricated with 2.5% w/v chitosan showed particle size within the range and spherical. As the concentration of chitosan increased from 2.5% w/v the particle size increased. Batches containing 3% and 3.5% w/v concentration of chitosan had poor syringe ability, due to the high viscosity of chitosan solution and it causes difficulty to passes through the syringe and produced large droplets.

Aqueous: oil phase ratio

The three batches of microspheres were prepared namely A11, A12, and A13 by varying the different ratios of aqueous: oil phase i.e. 1:5, 1:10, and 1:15 respectively. Batch with 1:10 showed good sphericity, uniform in size, and free-flowing where batches fabricated as 1:5 and 1:15 showed irregular particle size so that measurement was difficult, and batch having 1:10 ratio selected for further study.

Table 9: Optimized process parameters by placebo microspheres trails

Parameters	Optimized Values
Emulsifier concentration (Span 80)	5% v/v
Chitosan concentration	2.5%
Aqueous: oil phase ratio	1:10

Evaluation of placebo chitosan microspheres

The placebo microspheres were evaluated for morphological characterization and particle size determination.

Morphological characterization

All batches of microspheres were preliminarily checked for shape and size by optical microscopy at 10X magnification. Some batches of microspheres obtained were quite spherical, some are irregular in shape. The results of all batches from A11 to A13 were discussed in the above different selected variables. The photographs of the placebo chitosan microspheres taken by a digital optical microscope are shown in figure 7.

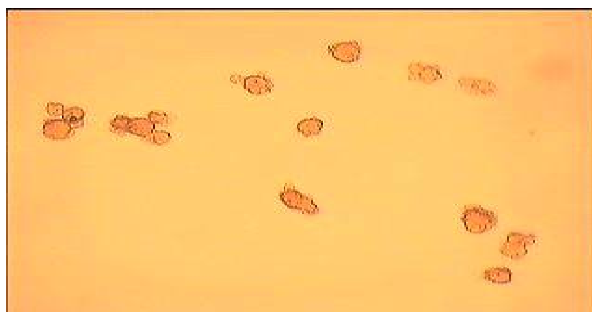

Figure 7: Photomicrograph of placebo microspheres

Particle size determination

A microscopic image analysis technique for the determination of particle size was applied. The morphology and particle sizes were determined in a motic DMB1 digital microscope equipped with a 1/399 CCD camera imaging accessory and computer-controlled image analysis software (Motic images plus, 2.0 version). The result of all batches from A1 to A13 was discussed in the above different selected variables. The particle size of batch A1, A2, A3, A4, and A5 were found to be 20.94 ± 5.1, 17.42 ± 4.0, 27.91 ± 6.4, 29.66 ± 3.97 and27.91 ± 2.88 µm respectively. Batch A2 having the lowest particle size but aggregates formed. The particle size of batch A6 and A7 were found to be 37.47 ± 6.61, and 32.88 ±7.8 µm respectively. The batch A8 having particle size 36.10 ± 9.39 µm and spherical, batch A9 particle size found to be 47.47 ± 6.61 µm, and batch A10 having particle size 49.84 ± 12.43 µm but irregular in shape. In batch A11, A12 and A13 particle size were found to be 35.18 ± 5.65, 27.47 ± 6.61, and 41.48 ± 4.32 µm respectively. Batch A12 showed good sphericity, uniform size, and free-flowing.

Formulation of drug-loaded chitosan microspheres

Selection of drug-polymer ratio

Based on the result of the preliminary investigation, the different process parameters like emulsifier concentration, chitosan concentration, and aqueous: oil phase ratio were optimized, and the drug-loaded three formulations were mentioned in Table 9. D1, D2, and D3 were designed by varying drug: polymer ratios i.e. 1:1, 1:2, and 1:3, and formulation procedure was used as described in section 2.4.1. Three sets of formulations were prepared while keeping other process variables as per preliminary trials such as emulsifier concentration (5% v/v) chitosan concentration (2.5% w/v) and aqueous: oil phase ratio (1:10). The obtained microspheres were evaluated for physical appearance, particle size, drug loading, and incorporation efficiency.

The % incorporation efficiency of 1:1, 1:2, and 1:3 batches were found to be 68.82%, 89.08%, and 71.28% respectively and drug loading was found to be 43.51%, 34.7%, and 22.32% respectively and particles were spherical in shape and particle size in the range as shown in Table 11.

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Drug: polymer ratio affects the drug content & % incorporation efficiency of microspheres. In the above study, there is a slight increase in the % incorporation efficiency with a decrease in drug to polymer ratio but the batch D2 (1:2) shows better drug content & % yield, so it was selected for further studies shown in Table 10.

Selection of the volume of cross-linking agent (1% KOH) and cross-linking time

Three different batches namely D4, D5, and D6 were formulated with varying the amount of cross-linking agent i.e. 3 ml, 4.5 ml, and 6 ml respectively, and other conditions such as emulsifier concentration (5% v/v), chitosan concentration (2.5% w/v), aqueous: oil phase ratio (1:10) and drug-polymer ratio (1:2) were kept constant as shown in Table 8.6. and obtained microspheres were evaluated for *in vitro* drug release study.

Batch D4 was not prepared due to an insufficient number of cross-linking agents. The number of cross-linking agents affects the release of drug from microspheres, as the cross-linking agent concentration increases, the drug release time extends. Batch D5 with cross-linking agent 4.5ml showed a good drug release pattern i.e. at 12 hr-85.14% so it was selected for further studies.

Selection of crosslinking time

Three different batches namely D7, D8, and D9 were formulated with varying the cross-linking time i.e. 1 hr, 1.5 hr, 2 hr respectively, and other conditions such as emulsifier concentration (5% v/v), chitosan concentration (2.5% w/v), aqueous: oil phase ratio (1:10), drug-polymer ratio (1:2) and volume of cross-linking agent (4.5 ml) were kept constant as shown in Table 10 and obtained microspheres were evaluated for *in vitro* drug release study.

Cross-linking time affects the release of drug from microspheres, as the cross-linking time increases, the drug release time extends and the D8 batch with 1.5 hr cross-linking showed a good drug release pattern i.e. at 12h- 85.14% drug release so it was selected for further studies shown in Table 12.

Table 10: Optimized process parameters by drug-loaded microspheres

Parameter	Optimized Values
Drug: Polymer ratio	1:2
Conc. of Chitosan Solution	2.5% w/v
Conc. Of emulsifier	5% v/v
Aqueous: oil phase ratio	1:10
Vol. of cross-linking agent	4.5 ml
Cross-linking time	1.5 hr

Evaluation of the drug-loaded microspheres

The drug-loaded microspheres were evaluated for percentage yield, morphological characterization, size and shape, drug loading, incorporation efficiency, and *in vitro* drug release study. Results are shown in Table 11 and Table 12.

Table 11: Results of evaluation of drug-loaded microspheres

Batch	Drug: polymer ratio	Percentage yield	Particle size* (µm)	Drug loading (%)	Incorporation efficiency (%)
D1	1:1	65.30%	20.79 ± 6.93	43.51	68.8
D2	1:2	67.17%	25.95 ± 6.82	34.76	89.08
D3	1:3	60.57%	27.32 ± 6.92	22.32	71.28

*Mean±S.D., n=3

Percentage yield

It was observed that as the polymer ratio in the formulation increases, the product yield also increases. The low percentage yield in some formulations may be due to microspheres lost during the washing process. A 100% yield could not be achieved principally due to the adhesion of microspheres to the stirring rod and filtration assembly. The percentage yield was found to be in the range of 60.57 to 67.17 % of drug-loaded microspheres were shown in Table 11.

Morphological characterization

All batches of microspheres were preliminarily checked for shape and size by optical microscopy at 10X magnification. The microspheres obtained were quite spherical, some are irregular in shape. Results were shown in Table 8.8 and the photographs of the drug-loaded microsphere taken by digital optical microscope are shown in figure 8.

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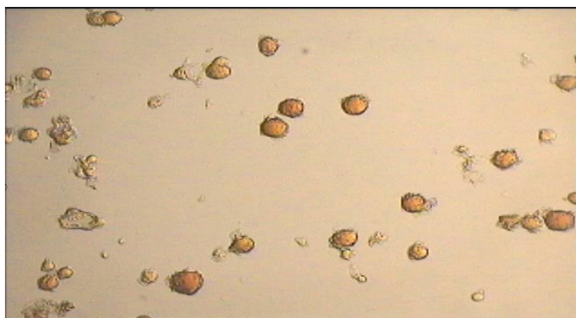

Figure 8: Photomicrograph of drug-loaded microspheres

Particle size

The prepared microspheres were in a size range suitable for nasal delivery. The mean size increased with increasing polymer concentration which is due to a significant increase in viscosity, thus leading to an increased emulsion droplet size and finally a higher microspheres size. Drug-loaded chitosan microspheres were found in the size range of 20 µm to 27 µm.

The particle size distribution of microspheres obtained depends on the size of the emulsion droplets that are determined by a balance between the dispersive and the surface tension forces. The former tends to disperse the emulsion and the latter causes coalescence. The interfacial tension between the polymeric droplets and the oil in the continuous phase is reduced in the presence of an emulsifier and microsphere size is decreased. Mean size decreased at higher stirring speeds, thus an optimal stirring speed was chosen to prepare microspheres with a narrow size distribution suitable for nasal drug delivery.

Drug loading and incorporation efficiency

Drug loading and incorporation efficiency of drug-loaded microspheres is shown in Table 8.8 The incorporation efficiency of D1, D2, and D3 batches were found to be 68.8%, 89.08%, and 71.28% respectively. The incorporation efficiency of D1 to D2 increases due to decreasing the drug: polymer ratio. The low percentage yield in D3 may be due to microspheres lost during the washing process. The % drug loading and incorporation efficiency ranged from 22.32% to 43.51% and 68.8% to 89.08% respectively for chitosan microspheres.

***In vitro* drug release study**

The drug release study of batches D5, D6, D7, D8, and D9 is shown in Table 8.9 and Figure 8.10. Formulation batch D4 was found to have a poor drug release of 42.81%. Each batch shows a different drug release. It may be due to a change in the volume of glutaraldehyde and the cross-linking time of each trial. In the D4 batch, drug release was 42.81% possible reason must be a high amount of crosslinking agent. The D6 batch, 0.1 ml glutaraldehyde with 2hr cross-linking shows good drug release of 89.17%. The D7 batch shows 96.29% drug release at 8h shows as crosslinking time decreased drug release was increased. The D6 batch shows a good drug release selected for the optimization study.

Table 12: Data of *in vitro* drug release study

Sr.No	Time (h)	% CDR				
		D5	D6	D7	D8	D9
1	1	11.34	9.42	9.95	10.57	13.06
2	2	22.45	14.45	20.84	23.50	19.20
3	3	31.56	21.67	32.97	34.72	26.09
4	4	39.95	30.76	44.09	41.45	29.86
5	5	52.47	38.76	54.71	54.43	31.95
6	6	60.65	45.89	66.26	61.93	36.57
7	7	65.57	52.56	68.09	63.42	43.86
8	8	72.39	58.07	71.02	74.66	52.35
9	9	79.37	62.81	74.97	78.93	55.51
10	10	81.94	65.34	77.02	81.23	59.29
11	11	83.48	67.42	80.62	85.25	63.46
12	12	83.67	67.60	82.42	85.31	68.17

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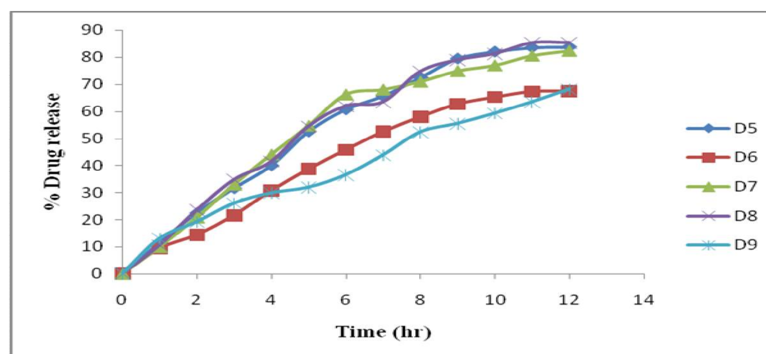

Figure 9: *In vitro* drug release study

CONCLUSION

The Nicardipine HCL Microspheres were subjected to physicochemical studies, *in-vitro* drug release, kinetic studies, and stability studies. DSC and FTIR studies showed there was no interaction between drugs and polymers. *In-vitro* release studies show that 91% of the drug was released from all the formulations were within 12 h. The optimization of the formulation was done based on the results obtained in the above formulation and the required drug release. The optimized batch (O) having the composition containing drug: polymer ratio (1:1), the volume of potassium hydroxide (4.5 ml), and cross-linking time (2h) showed good desired release patterns. The optimized formulation was prepared and subjected to evaluation. This formulation was then kept for stability studies at 40°C and 75% RH for three months. The composition of the optimized formulation is shown in Table 15. The optimized formulations were subjected to stability studies and shown there were no significant changes in drug content, physicochemical parameters, and release patterns. 32 full factorial design optimization techniques were successfully used in this research work. Developed Nicardipine Hydrochloride Microspheres shows effective drug release over a prolonged time frame that led to greater therapeutic efficacy.

CONFLICT OF INTEREST: Authors having no conflict of interest.

ACKNOWLEDGEMENT

Authors are thankful to Maulana Gulam Md Vastanvi President, J.I.I.U's Jamia College of Pharmacy, Akkalkuwa, and teaching and non-teaching staff of Jamia and Ali Allana College of Pharmacy, Akkalkuwa, Dist: Nandurbar, Swami Vivekanand Sanstha's IOP, Malegaon, Singhania University, Pacheri Bari for support and motivation during research work.

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