

Research Article



Development and Evaluation of Cinnarizine Loaded Nanosponges: Pharmacodynamic and Pharmacokinetic Study on Wistar Rats

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ABSTRACT

The purpose of this study research was to improve bioavailability of Cinnarizine, a poorly water-soluble drug. The objective of this study is to formulate and evaluate Cinnarizine loaded nanosponges to increase the bioavailability of cinnarizine. Cinnarizine loaded nanosponges were prepared by quasi emulsion solvent diffusion method. The drug loaded nanosponge was characterized by FTIR, DSC, PXRD and SEM. Cinnarizine was loaded by using Eudragit RS 100, cold polyvinyl alcohol, dichloromethane, and distilled water. The prepared nanosponges were formulated and evaluated for powder properties, Pharmacodynamic and pharmacokinetic studies. Cinnarizine loaded nanosponges were white in color and odorless. FTIR study of formulation has shown that there were no interactions between drug and other excipients. X-ray diffraction (XRD) study of formulation confirmed amorphous nature of cinnarizine. For optimized batch (F5), percent yield (60.89±0.18%), entrapment efficiency (70.85±0.41%), particle size (492.33nm), Polydispersity index (0.08103±0.04), zeta potential (5.67mV), drug content (82.88 ±0.88%). Stability study of nanosponge was carried out as per ICH guidelines. The results of in vivo studies showed 1.4 times increases in bioavailability of Cinnarizine by nanosponges as compared to pure Cinnarizine powder. It was possible to prepare nanosponges of Cinnarizine by using quasi emulsion solvent diffusion method to obtain the best possible formulation.

Keywords: Eudragit RS 100, polyvinyl alcohol, Cinnarizine, nanosponges, Quasi emulsion solvent diffusion.

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INTRODUCTION

Low aqueous solubility of drug has always presented a major obstacle towards the development of drug delivery systems which often compromises patient compliance. Oral route is thought to be common and easy for drug administration. On oral administration of drug in its dosage form, it is expected to dissolve and release the drug into the gastrointestinal fluid before the absorption. Poor solubility may limit the dissolution of drug in gastrointestinal tract resulting to low bioavailability that can pharmacologically affect the therapeutic efficacy of drug.¹ The drugs belonging to BCS class II particularly fall in this category and have been extensively researched molecular optimization and development of novel efficacious dosage forms. Cinnarizine, (E)-1-(diphenylmethyl)-4-(3-phenylprop-2-enyl) piperazine, having molecular formula C₂₆H₂₈N₂ and molecular weight 368.51 g/mol, is white or almost white powder. It is originally obtained from wood-reed roots (*Cinna*). It is a weak base with poor aqueous solubility.² Nanosponges are porous polymeric delivery systems that are small

spherical particles with large porous surface. A sponge that has nano size holes, especially one used for bio-mimetic detoxification. The sponge acts as a three-dimensional network or scaffold, which consist of the backbone known as long-length polyester.³ Oral drug delivery has long been a problem for medical researchers how to get them to the right place in the body and how to control the release of the drug to prevent overdose. The development of new and complex molecules called nanosponges has the potential to solve these problems. Nanosponge is a novel and emerging technology which play a vital role in targeting drug delivery in a controlled manner. A wide variety of drugs can be loaded into nanosponge for oral drug delivery. These particles are capable of carrying both lipophilic and hydrophilic substances and of improving the solubility of poor water soluble molecule like cinnarizine. Nanosponges are small size and porous nature they can bind poorly soluble drugs within their matrix and improve their bioavailability. Cinnarizine is more commonly prescribed for nausea and vomiting due to motion sickness. Cinnarizine used in chemotherapy, vertigo or Meniere's disease. It is given in lower dose 25 mg & 75 mg higher doses of Cinnarizine. It can be taken by adults and by children over 5 years of age. Cinnarizine is used to treat problems affecting the inner ear and balance such as dizziness and sickness. It is also helpful in preventing travel sickness. There is a need to minimize the dose, avoid repetition of dosing frequency and minimize the side effects. Thus, there is a need to increase the efficacy of Cinnarizine. Formulating vesicular carriers like



nanosponges because nanosponges are more stable than other vesicular carrier system.⁴

In this study attempt has been carried out to improve the bioavailability of Cinnarizine (CN) by formulating it in the form of nanosponges; otherwise, its use would be limited due to poor aqueous solubility.

MATERIALS AND METHODS

Materials

Cinnarizine (CN) was provided as gift sample from Wallace Pharmaceuticals (Goa, India). Eudragit RS 100 was obtained from Analab fine chemicals (Mumbai, India), dichloromethane, polyvinyl alcohol, hydrochloric acid was supplied by Loba Chemicals (Mumbai, India). Gum arabic and hydrated aluminium silicate (Kaolin) was kindly supplied by Merck chemicals.

Formulation and optimization

Formulation of cinnarizine loaded nanosponge

The inner phase was prepared by using Eudragit RS 100 and dichloromethane (extra pure); Eudragit RS 100 (150 mg) was completely dissolved in dichloromethane (10 ml) with continuous stirring. The outer phase was prepared by using cold polyvinyl alcohol and distilled water. Using

magnetic stirrer, (cold) PVA (40 mg) was dissolved in distilled water. Cinnarizine (25mg) was added in inner phase, using ultra probe sonicator for 5-6 cycles (Time for one cycle=20sec). After adding inner phase into outer phase, a mixture formed was placed up to 3 hrs on magnetic stirrer, without heating. Magnetic stirring speed was kept 2000 rpm. Beaker containing formulation was kept in water bath, at 60°C then final formulation was obtained.^{5, 6, 7}

Design of experiment

Optimization of 3² factorial design of experiment was followed with 3 levels and 2 factors and runs 9 batches using the Design Expert software (version 8) was generated for the experimental design of Cinnarizine loaded nanosponges. According to 3² full factorial designs, possible combinations are mentioned in Table 1. The amount of nanosponges forming polymer, Eudragit RS 100 (X₁) and cold PVA (X₂), were independent variables and entrapment efficiency, particle size, percent yield, drug release, zeta potential were dependent variables. Two independent factors, the concentrations of Eudragit RS 100 and (cold) PVA were chosen at low, medium and high levels.

Table 1: Optimization by 3² factorial Batches

Sr. No	Ingredients	F1	F2	F3	F4	F5	F6	F7	F8	F9
1	CIN (mg)	25	25	25	25	25	25	25	25	25
2	Eudragit RS 100(mg)	100	100	100	150	150	150	200	200	200
3	Cold PVA(m)	10	10	10	40	40	40	75	75	75
4	DCM (mL)	10	10	10	10	10	10	10	10	10
5	Distilled water (ml)	10	10	10	10	10	10	10	10	10

Preformulation characterization

UV spectrum analysis of CN

The solution of Cinnarizine was prepared in 0.1 N HCl (pH 1.2). The sample was scanned between 200-400 nm to obtain the maximum wavelength of absorption and UV spectrum by using blank 0.1 N HCl of pH 1.2.

Standard plot of Cinnarizine in 0.1 N HCl

Accurately weighed 10 mg of Cinnarizine was added to 100mL volumetric flask. Volume was made up to 100 mL with stock solution. From this, 0.2mL was withdrawn and added into 10mL volumetric flask and volume was adjusted up to mark with 0.1 N HCl pH 1.2 (2µg/mL). Like this, from the stock solution 0.2, 0.4, 0.6, 0.8, 1.0, 1.2, 1.4 mL solution was withdrawn and added into volumetric flask of capacity 10mL and finally diluted up to 10mL with 0.1 N HCl (pH 1.2) to get the solutions with concentration of 2-14µg/mL respectively. The absorbance was measured by UV-visible spectrophotometer for each solution at 253 nm.^{8, 9, 10}

Drug polymer compatibility study

Three individual infrared spectra viz. Cinnarizine, Eudragit RS 100 and optimized formulation (F5) were recorded by Jasco M410 FTIR spectrophotometer. After baseline correction, the spectra of cinnarizine with potassium bromide, Eudragit RS 100 with potassium bromide and triturated nanosponges with potassium bromide were recorded. All samples were scanned from 4000 to 400 cm⁻¹ and finally the drug-polymer compatibility was confirmed.¹¹

Differential Scanning Calorimetry (DSC)

Cinnarizine loaded nanosponges (CLN) of the optimized batch was analyzed for thermal analysis by using differential scanning calorimetry. DSC measurement was performed on a differential scanning calorimetry equipped with an intra-cooler (DSC Mettler STAR SW 9.20, Switzerland). The analysis was carried out on a sample sealed in standard aluminum pans. An empty aluminum pan used as reference. Inert atmosphere was maintained by purging nitrogen gas at a flow rate of 25 ml /min at a scanning rate of 10°C per min from 50 to 300°C.¹²



Powder X-ray diffraction studies (PXRD)

X-Ray diffraction (XRD) measurement of pure cinnarizine was performed by using Philips (PW 3710) Expertpro MPD Diffractometer (PAN analytical Inc, Germany) with resolution of 0.001\AA . The pure drug sample of 10 mg was sprinkled on vacuum grease applied glass slide to make a layer having a thickness of 0.5 mm and slick the sample to slide before measurement. The samples were radiated using a Copper target tube. Scanning angles ranged from 5° to 60° of 2θ . The current used was 40 mA and voltage 40kV.¹³

Scanning electron microscopy (SEM)

The detailed surface characteristics of the optimized batch of Cinnarizine particles were observed by using a JEOL Scanning electron Microscope (Model: JSM 6360 A, Japan). The samples were attached to the specimen holder to aluminum stub by using a double coated adhesive tape and gold coated (20 nm thickness) under vacuum using sputter coater (Model : IB -2 , Hitachi , Tokyo , Japan) During gold coating process the samples were exposed to vacuum for 5-10 min at 40 mA and investigate a accelerating voltage of 15 kV and 10kV was applied and the image was photographed by Asia Pentax Camera.¹⁴

Formulation study**Determination of percentage yield**

The total percentage yield of nanosponges was determined by using formula percent yield = (practical yield of nanosponges/Theoretical yield of nanosponges) x 100. The percent yield of nanosponges can be determined by calculating initial weight of nanosponges and practical yield of nanosponges.¹⁵

Determination of entrapment efficiency

Nanosponge suspension was centrifuged then forms two parts i.e. supernatant and residue forms. Supernatant solution was selected and residue was separated by centrifugation at 2700 rpm for 30 min. The supernatant and residue was diluted by 0.1 N HCl. The concentration of cinnarizine in supernatant was determined spectrophotometrically (Jasco603) at 253nm. The entrapment efficiency was calculated through the formula % E.E = Entrapped drug/ (Entrapped drug + Drug in supernatant) x 100.

Determination of drug content

Drug content was analyzed by taking 10mg of sample and dissolved in 100 ml of 0.1N hydrochloric acid. Each of solution was further diluted in 0.1N hydrochloric acid. Solution was filtered to remove insoluble matter. Absorbance was measured by UV spectrophotometer at 253nm^{16,17}.

Particle size analysis

Particle size of prepared nanosponges was measured by particle size analyzer (NANOPOX, Symantec, Germany). Each sample was placed in transparent polystyrene

cuvette (path length =1cm) and placed in thermostatic sample chamber mean globule size and the span of resulting emulsion were determined by photon cross correlation spectroscopy (NANOPOX, Sympatec, Germany). Sample temperature was set at 25°C and runs of 60s were performed. Detection was carried out a scattering angle of 90° . From the resulting correlation curves second order analysis was performed to calculate the mean globule size. The globule size distribution was expressed in term of span^{18,19}.

Polydispersity index

Polydispersity index is measure of the heterogeneity of sizes of molecules or particles in a sample. A collection of objects was called uniform if the objects have the same size, shape, or mass. The data obtained from the particle size analysis using NANOPOX instrument can be used to derive the polydispersity index of respective formulation by using following formula $PDI = \Delta d/d_{avg}$ ¹⁹.

Zeta potential

The zeta of batches was measured by a Zeta sizer 2000 (Malvern Instrument UK). A sample was extemporaneously diluted in Milli-Q (Millipore Corp., USA) water ($1\mu/10$ ml) and injected in the apparatus. The measurements were carried out in the fully automatic mode. Each sample was analyzed twice²⁰.

In vitro diffusion study

Drug release from nanosponges was studied using a dialysis method. Dialysis bags were soaked before use in distilled water at room temperature for 12 hrs, followed by rinsing thoroughly in distilled water. In vitro release of cinnarizine from nanosponges was carried in dialysis sac (12,000 MW cut off; Sigma- Aldrich) with 150 ml of 0.1 N hydrochloric acid at 37 °C. Two end of the dialysis sac were tightly bound with thread. The sac was hung inside a conical flask with the help of glass rod so that the portion of dialysis sac with the formulation dipped into 0.1N hydrochloric acid. The beaker was kept on a magnetic stirrer (Matrex) and stirring was maintained at 100 rpm at 37 °C with a thermostatic control. Samples were collected at 5, 10, 15, 30, 45 min and then 1 hr, 2 hr,3 hr,4 hr and assayed spectrophotometrically for drug content at 253 nm and sample volume of 0.1 N hydrochloric acid maintained at the same temperature. The equal volume of fresh release medium was replaced at the same time intervals. The dissolution data analyzed for calculating the amount of drug released^{21,22}.

In vivo pharmacodynamics study

The protocol for the animal study in prescribed proforma was submitted to the Institutional Animal Ethics Committee (IAEC) of Modern College of Pharmacy, Nigdi, Pune. The protocol of animal study was approved by IAEC of Modern College of Pharmacy, Nigdi, Pune with approval no.MCP/IAEC/21/2016. The study was carried out on male albino wistar rats of weights 160-180g. The wistar rats were kept on fasting for 24 hrs prior to dosing. The study



of pharmacodynamics of CLN was carried out by using rat emesis model.

Rat emesis model

Male albino Wistar rats each weighing between 160 and 180 g are usually used in this study. In these animals which lack emetic reflex, pica (measured as kaolin intake) involves similar mechanisms as vomiting in humans and can be used to test the efficacy of anti-emetic drugs. It has been shown that pica is associated with 5-HT release from the enterochromaffin cells, increased c-fos labelling in the area postrema and the nucleus tractus-solitarius, and delay in gastric emptying as with emesis in humans. In this experimental model, an oral dose (40mg/kg) of copper sulfate was used for measuring pica in rats. Test sample (CLN suspension) is administered by oral route 10 min prior to emetic stimuli and pica was observed for a period of 120 hrs continuously²².

Preparation of Kaolin pellet

Gum Arabic (acacia) and hydrated aluminium silicate (kaolin) were mixed together (1:100 on a w/w basis) with distilled water to form a thick paste. Pellet of the resulting kaolin mixture were shaped to resemble the dimensions of the rats normal laboratory diet. The pellets were dried completely at room temperature²³.

In vivo pharmacokinetic study

Study design using Wistar albino rats

The protocol for the animal study was approved by Institutional Animal Ethics Committee (IAEC) of Modern college of pharmacy, Nigdi, Pune as mentioned earlier. The study was carried out on male albino Wistar rats of weights 160-180g. The Wistar rats were kept on fasting for 24 hrs prior to dosing. The rats were divided into two groups each containing six animals. Group A received Cinnarizine suspension in distilled water and Group B received nanosponges in distilled water. Two test groups were injected single dose of Cinnarizine equivalent 25 mg /kg given by oral route with the help of oral syringe. Blood samples were collected from the retro orbital plexus vein of each rat at time intervals of 1, 2, 3, 4 then 8, 12, 24, 36 (hrs) continuously. Blood samples were collected into heparinized tubes and RBCs were allowed to settle by centrifugation at 3000 rpm for 30 min. The supernatant plasma was collected in tube and analyzed by UV-visible spectrophotometer. The pharmacokinetic parameters for Cinnarizine following oral administration were determined from the plasma concentration- time data^{22, 23}.

Preparation of stock solution

Stock solution of Cinnarizine was freshly prepared in Acetonitrile to give a final concentration of 100µg/ml. Working solutions of Cinnarizine were prepared in Acetonitrile by appropriate dilution to give appropriate concentration of 2,4,6,8,10,12µg/mL²⁴.

Extraction of rat plasma

The blood samples of rat were obtained from its retro orbital plexus vein and collected in sodium EDTA tubes. These samples were centrifuged at 4500 rpm for 30 min. The plasma was separated by decanting it in other sodium EDTA tubes.

Preparation of standard curve in rat plasma

A standard curve was prepared by spiking 1 ml plasma sample with 1 ml of the working solution prepared above 2,4,6,8,10,12 µg/mL separately to produce calibration curve points equivalent to 1,2, 3,4,5,6 µg/mL respectively. In each mixture, Acetonitrile added and vortexed for 2 min then centrifuged at 3000 rpm for next 15 min. The organic layer transferred to another tube and evaporated to dryness. The residue was reconstituted in 1mL Acetonitrile and 20µl aliquot obtained.

Extraction of sample

Blood samples were collected at specific time interval into the tube containing disodium EDTA and centrifuged at 4500 for 10 min. A 0.5ml of the resultant plasma sample was mixed with 1.0 mL of Acetonitrile added and vortexed for 2 min and then centrifuged at 3000rpm for next 3min. The organic layer transferred to another tube and evaporated to dryness. The residue reconstituted in 1 ml of acetonitrile and 20µl aliquot was put into covette and at maximum wavelength of Cinnarizine observed the absorbance. The obtained results were used to determine for C_{max} , T_{max} , and Area Under curve ($AUC_{0-\infty}$) measured by using linear and logarithmic trapezoid method^{25, 26}.

Stability study of nanosponges

The stability study of Cinnarizine loaded nanosponges was carried out as per ICH guidelines. Samples maintained in a stability chamber under accelerated condition ($45^{\circ}C \pm 2^{\circ}C$, $75^{\circ}C \pm 5\% RH$) with humidity and temperature control, were taken at 0, 1, 2 and 3 month. Formulations were exposed to different thermal condition $45^{\circ}C \pm 2^{\circ}C$. CLN was stored in air tight glass container. Relative humidity for a period of a month and drug behavior of formulation was tested by measuring the entrapment efficiency, particle size and zeta potential of nanosponges. Samples were centrifuged for 2000 rpm for 30 min and measured the percent amount of drug entrapped in the nanosponges for periodic interval of one month. Total amount of the drug present in nanosponges was analyzed spectrophotometrically^{21, 27}.

RESULTS

Preformulation study

UV spectrum analysis of Cinnarizine

Calibration curve for Cinnarizine in 0.1 N HCl was shown in Fig.no.1 ($R^2=0.999$ and $y=0.067x+0.0173$)



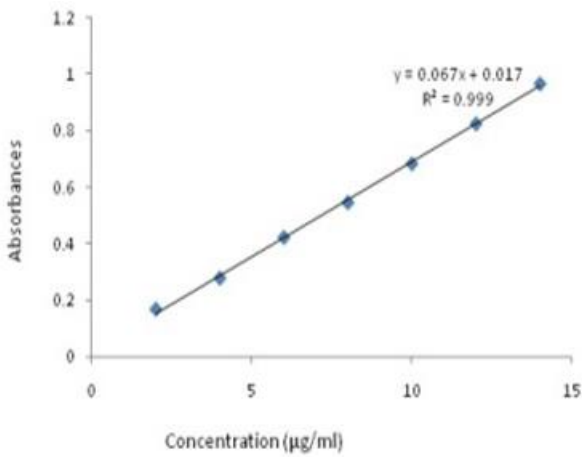


Figure 1: Calibration curve for Cinnarizine in 0.1 N HCl

Drug polymer compatibility study

FTIR spectrum showing chemical compatibility between Cinnarizine and Eudragit RS 100 is shown in Fig.no.2.

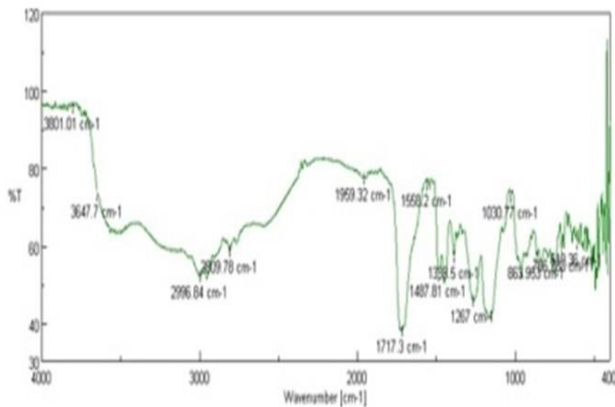


Figure 2: FTIR spectrum showing chemical compatibility between Cinnarizine and Eudragit RS 100

Differential scanning calorimetry

The DSC peak of cinnarizine was at 121°C which found to be slightly differing with standard i.e., within 118°C to 122°C shown in fig no.3 and DSC graph of Eudragit RS 100 and Optimized batch shown in fig.no.4 and fig. no 5.

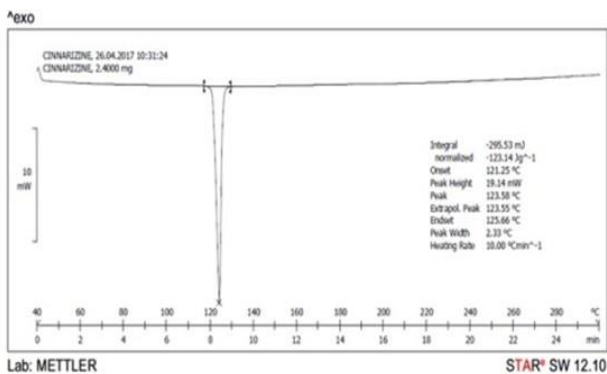


Figure 3: DSC peak of cinnarizine

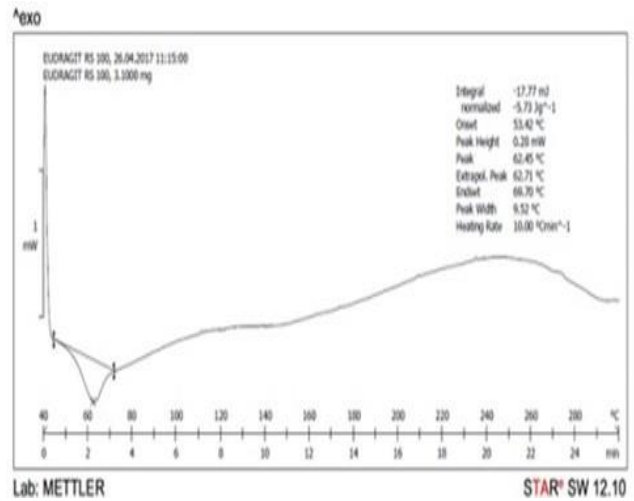


Figure 4: DSC graph of Eudragit RS 100

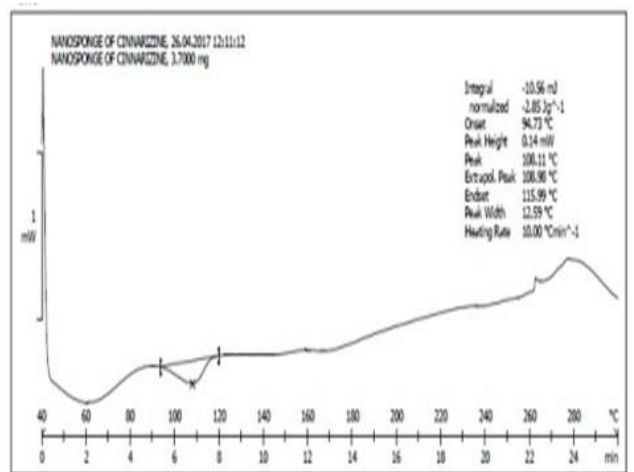


Figure 5: DSC graph of Optimized batch

Powder X-ray diffraction studies (PXRD)

The X-ray diffraction pattern of cinnarizine and Eudragit RS 100 suggests that it was amorphous powder shown in fig. no. 6 and fig. no.7.and Optimized batch (F5) PXRD shown in fig. no.8

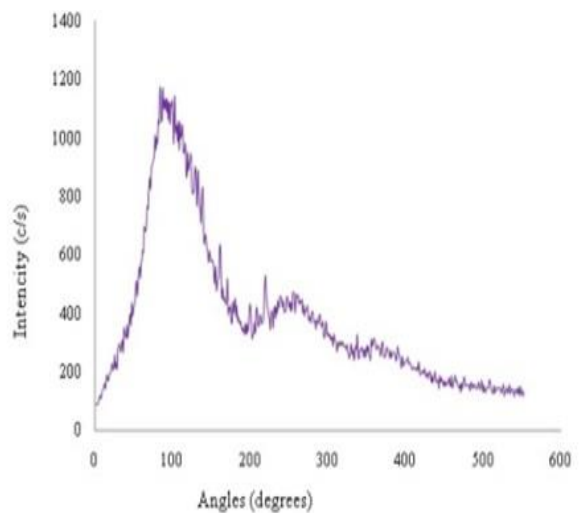


Figure 6: X-ray diffraction pattern of cinnarizine

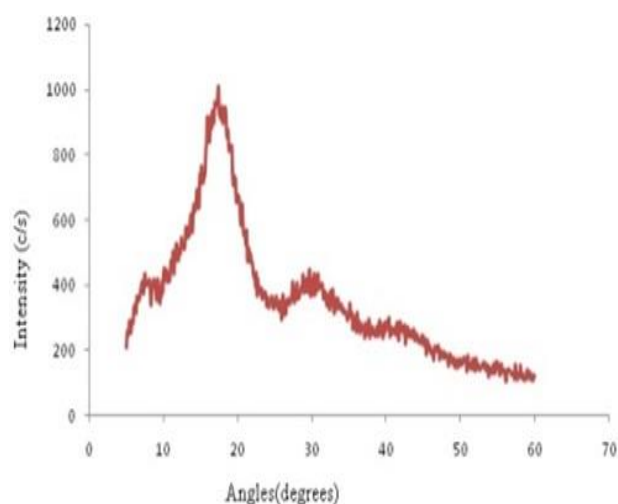


Figure 7: X-ray diffraction pattern of Eudragit RS 100

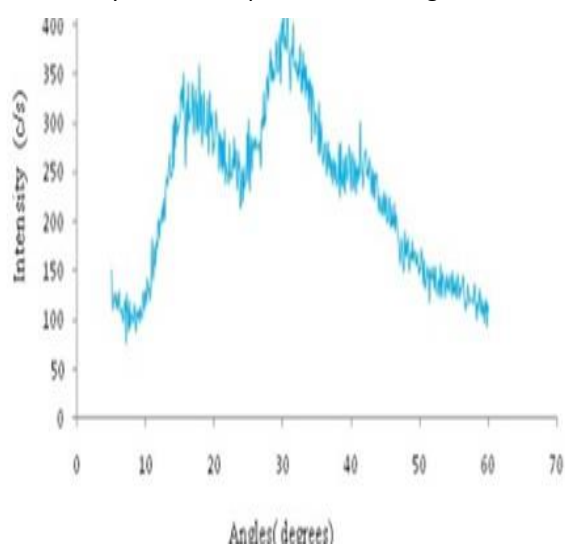


Figure 8: X-ray diffraction pattern of Optimized Batch

Scanning electron microscopy (SEM)

Scanning electron microscopy of cinnarizine, Eudragit RS 100 and Optimized batch (F5) were performed for study of surface morphology and topography shown in fig.no.9,10,11 respectively. CLN was found to be that discrete, spherical in shape.

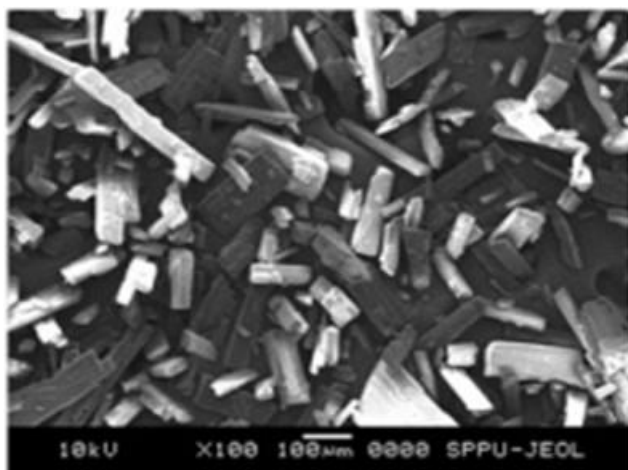


Figure 9: Scanning electron microscopy of cinnarizine

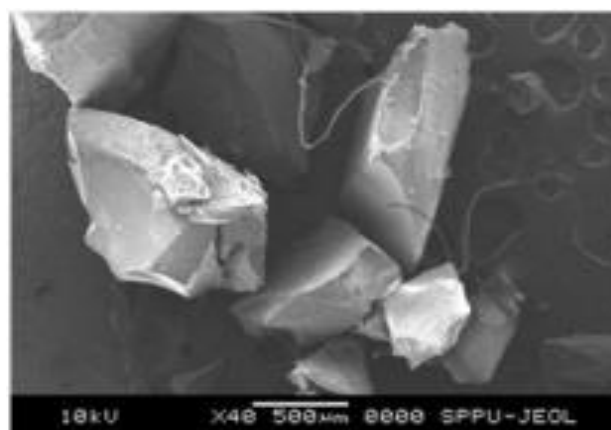


Figure 10: Scanning electron microscopy of Eudragit RS 100

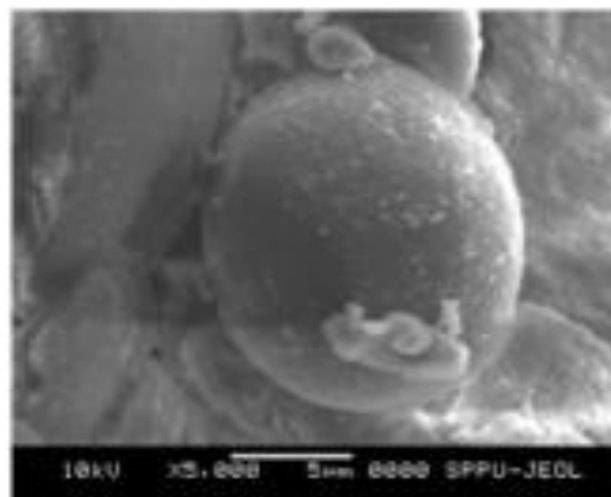


Figure 11: Scanning electron microscopy of Optimized batch (F5)

Formulation study

Determination of percent yield

Percent yield of nanosponges is shown in table no.2. The percent yield of nanosponges was in range of 50 to 72 %. Optimized batch (F5) percent yield was found to be $60.89 \pm 0.18\%$.

Determination of entrapment efficiency

The entrapment efficiency of cinnarizine loaded nanosponge formulation is shown in table no.2. The entrapment efficiency of nanosponges was in range of 50 to 72 %. Optimized batch (F5) entrapment efficiency was found to be $60.89 \pm 0.18\%$.

Determination of drug content

Drug content of F1 to F9 batches were found to be in range 50 to 82 %. The drug content of optimized batch (F5) was found to be $82.88 \pm 0.88\%$. Drug content of F1 to F9 batches shown in table no.2

Particle size analysis

The particle size of F1 to F9 batches were found to be 100nm to 500nm shown in table no.2

Table 2: Percent yield of nanosponges

Batches	Yield (%)	E.E. (%)	Particle size (nm)	PDI	Zeta potential (mV)	Drug content (%)
F1	50.56±0.11	78.69±0.24	465.75	0.2775±0.01	39.15	78.48±2.48
F2	42.56±0.28	78.54±0.22	279.12	0.2413±0.02	3.15	70.45±1.47
F3	50.75±0.32	74.32±0.25	506.32	0.2709±0.08	10.07	50.78±0.99
F4	55.89±0.14	69.97±0.37	240.94	0.2436±0.01	16.65	75.12±0.23
F5	60.89±0.18	70.85±0.41	492.33	0.08103±0.04	5.67	82.88±0.88
F6	65.11±0.16	80.88±0.39	382.73	0.2886±0.02	8.83	60.58±2.78
F7	72.11±0.17	72.11±0.22	111.08	0.0971±0.06	8.83	62.54±1.03
F8	68.00±0.16	78.81±0.14	329.46	0.3544±0.01	1.51	74.2±2.38
F9	55.12±0.17	82.36±0.34	381.80	0.2462±0.05	1.51	64.02±1.45

Polydispersity index of nanosponges

Polydispersity index of all batches was found to be less than one shown in table no.2

Zeta potential

Zeta potential of F1 to F9 in the range of 1.51 to 39.15mV. The zeta potential value of optimized batch was found to be +5.67.

In vitro diffusion study

The release of drug from nanosponge using medium 0.1N hydrochloric acid (1.2 pH). The drug release of optimized batch F5 was found to be 97.65±2.15%. (Table no.3)

Table 3: Drug release of optimized batch F5

Time (min)	0	5	10	15	30	45	60	120	180	240
F1	0	12.18±0.55	38.69±0.36	49.52±1.23	52.15±1.42	58.56±0.56	62.02±0.22	65.59±0.69	75.5±1.17	76.86±1.24
F2	0	16.06±0.41	23.79±0.36	43.26±0.98	46.89±0.77	47.78±0.78	54.18±0.40	57.59±0.53	58.23±1.24	60.88±1.56
F3	0	14.33±0.32	29.82±0.25	35.87±0.78	39.45±1.38	43.57±1.01	48.15±0.54	52.98±0.37	56.29±1.14	58.63±1.06
F4	0	8.1±0.74	14.92±0.59	43.45±0.69	57.01±1.21	57.15±0.64	58.97±0.29	62.19±0.80	64.78±	66.54±
F5	0	20.28±0.85	35.48±0.98	45.99±1.67	56.77±1.56	65.45±0.78	70.98±0.28	83.09±0.76	88.12±1.56	97.65±2.15
F6	0	16.35±0.63	34.25±0.29	48.63±1.46	53.69±1.42	64.52±0.64	72.98±0.30	75.98±0.63	78.94±1.20	80.45±1.56
F7	0	19.34±0.26	28.92±0.71	35.62±0.87	40.65±0.97	51.89±0.46	60.63±0.41	68.34±0.53	70.05±1.34	75.25±1.34
F8	0	17.56±0.62	30.87±0.12	40.98±0.85	56.8±0.86	60.08±0.82	64.05±0.35	67.08±0.61	75.25±1.06	78.98±1.57
F9	0	18.59±0.57	35.48±0.23	42.31±1.24	58.26±1.02	62.02±0.65	68.23±0.27	70.12±0.58	75.63±1.07	81.21±1.26

In vivo pharmacokinetic study**Table 4:** Concentration of CIS in rat plasma (µg/ml/hr)

Sr. No.	Time(hrs)	Concentration of Cinnarizine	
		Cinnarizine suspension in distilled water	Cinnarizine nanosponges in distilled water
1	1	0.9594	1.1351
2	2	3.7837	5.1621
3	3	4.9324	6.4864
4	4	5.4864	7.3243
5	8	4.5810	6.987
6	12	3.2432	4.7972
7	24	1.5945	1.6789
8	36	0.1216	0.098

The result of pharmacokinetic parameters of cinnarizine suspension and cinnarizine nanosponge suspension has shown in table no.4, 5 and fig. No 12 and fig. no 13.

Table 5: Pharmacokinetic parameters

Sr. No.	Pharmacokinetic parameters	Cinnarizine suspension in distilled water	Cinnarizine nanosponges in distilled water
1.	C _{max} (µg/ml)	5	7
2.	T _{max} (h)	4	7
3.	AUC(µg/ml/h)	336.32	396.41
4.	T _{1/2} (h)	2	3.5



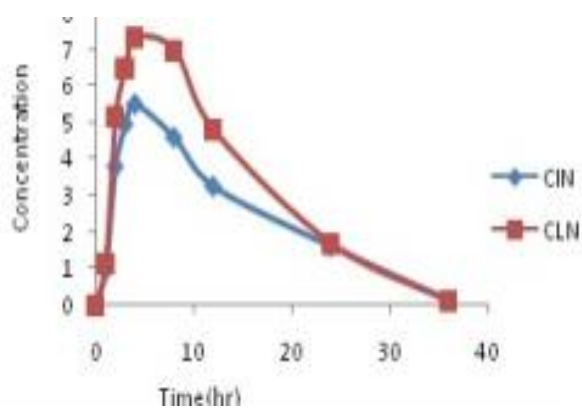


Figure 12: Pharmacokinetic parameters of cinnarizine suspension

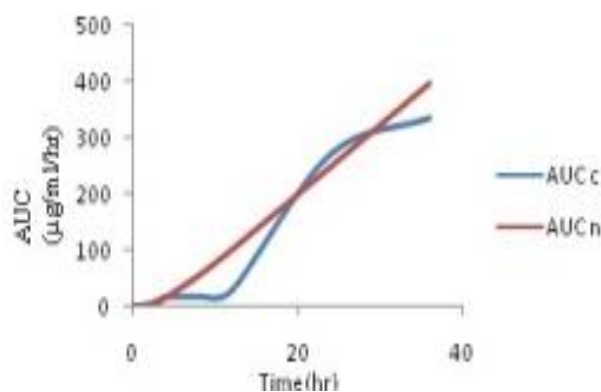


Figure 13: Pharmacokinetic parameters of cinnarizine nanosponge suspension

In Vivo pharmacodynamic study

After the five days observed that no vomiting occurred to albino wistar rat.

Stability study of CLN

Stability study optimized batch (F5) shown in table no.4

Parameters	Batch F5			
	Initial	After one month	After two months	After three months
Appearance	White color nanosponges	White color nanosponges	White color nanosponges	White color nanosponges
E.E. (%)	70.85±0.41	70.71±2.1	70.59±1.8	70.7±1.75
Drug content	82.88±0.88	80.17±1.2	77.88±1.05	75.46±2.01
Particle size(nm)	492.33	500-550	500-550	500-550
Zeta potential(mV)	5.67	6.70	6.87	6.98

DISCUSSION

Preformulation study

U.V spectrum analysis of Cinnarizine

From calibration curve it can concluded that it follows Beer's Lambert's law and thus gives linear equation of coefficient of correlation equal to 0.999.

Drug -polymer compatibility

Chemical compatibility between Cinnarizine and Eudragit RS 100 is shown in fig.no.3. Physical mixture shown two prominent peaks of piperazine and alkene group shown there is no incompatibility between the ingredients.

Differential scanning calorimetry

Cinnarizine has shown a prominent peak at 123°C due to slight change in concentration.

Powder X-ray diffraction study

The X-ray diffraction pattern of cinnarizine suggests that it is an amorphous powder. FTIR spectra of cinnarizine have given the idea about amorphous nature of cinnarizine due to 3066 cm⁻¹ stretch. PXRD study pattern of Eudragit RS 100 also showed amorphous nature.

Scanning Electron Microscopy

It was observed by SEM analysis that nanosponges were uniform spherical in shape.

Formulation study

Determination of percentage yield

It was noted that at higher stirring rate the production yield decreased and also polymer adhered to magnetic bead due to turbulence created within the external phase and hence production yield decreased.

Determination of entrapment efficiency

The E.E. was affected by internal phase and external phase ratio. The E.E. changed when drug and polymer ratio change. The highest loading efficiency was found for the F9 formulation where a greater amount of drug was encapsulated .Percentage entrapment depends on internal phase and external phase volume .It suggests that change in phase volume changed the entrapment efficiency.

Determination of drug content

The effect of drug: polymer ratio on the physical characteristics of the formulated nanosponges was examined for various drugs: polymer ratio of nanosponge

at stirring speed of 2000 rpm for 3hrs. It was observed that as drug: polymer ratio increases, the particle size decreased. This is probably due to the fact that at higher relative drug content, the amount of polymer available per nanosponge to encapsulate the drug becomes less, thus reducing the thickness of the polymer wall and hence smaller nanosponges.

Particle size analysis

Particle size of CLN was found to be below 500nm due to nanosize the surface area was significantly increase and it greatly increases rate of solubilization. An added benefit was that time taken by nanosponge system to traverse the small and large intestine was significantly increased thus maximizing the amount of drug that was absorbed. The author Dr. Prathima Srinivas et al studied were found to be volume of internal phase increased particle size in the polymers did not follow any particular pattern due to decreased viscosity of internal phase. Preparation of cinnarizine loaded nanosponges used 10 ml of dichloromethane therefore decreases viscosity of internal phase and formed appropriately sized nanosponges.

Polydispersity index of nanosponge

Polydispersity index of all batches was found to be less than 0.7. Thus, the particle size distribution sample was found to mid-range polydispers.

Zeta potential of nanosponge

The zeta potential value of optimized batch (F5) was +5.67 mV. The absolute value of zeta potential was lower than those values reported in the literature. This might be attributed to sorbitan monooleate, a nonionic surfactant which decreases the electrostatic repulsion between the particles and sterically stabilizes the nanosponge by forming a coat around their surface. The positive charge of cinnarizine loaded nanosponges may be attributed from cationic poly (meth) acrylates, viz. Eudragit RS 100, having quaternary ammonium group.

In vitro diffusion study

According to in vitro drug release study maximum time required to release drug (Cl) was found to be 4hrs.

In Vivo pharmacokinetic study

Cinnarizine suspension in distilled water was found to be C_{max} 5 μ g/ml in time "i.e."; T_{max} 4 of hrs suggesting the rapidly absorbed from gastrointestinal tract, with an absorption window in upper gastrointestinal tract. Whereas the CLN in which C_{max} of about 7 μ g/ml in time i.e., T_{max} 7 of hrs shown that delayed onset of action. This may indicate that plain cinnarizine was dominantly absorbed from stomach. Poor water solubility of cinnarizine its absorption resulting in lesser C_{max} but in case of cinnarizine nanosponges suspension it may due to its absorption from gastrointestinal tract and nanosponges approach improved solubility of cinnarizine resulting into better absorption i.e. C_{max} of additionally the increases in AUC shows the increased exposure of the drug to the body after

encapsulating pure drug in the nanosponges. Thus it is consider that there may be changes to increases bioavailability of the drug after using nanosponges approach.

In vivo pharmacodynamic study

Pharmacodynamics study of cinnarizine loaded nanosponges with the help of rat emesis model explain in experimental work. Test samples are administered by oral route 10min prior to emetic stimuli and pica is observed for a period of 120 hrs. After five days observation found that no vomiting occurred to albino wistar rat. It shows that cinnarizine loaded nanosponges shows the antiemetic activities.

Stability study of CLN

The effect of the accelerated testing was carried out for the formulation under storage i.e. F5 (optimized batch) for 3 months. There was no significance difference found between initial drug entrapment, drug content, particle size and zeta potential for the formulation after storage for 3 months.

CONCLUSION

It was possible to prepare nanosponges of cinnarizine by using quasi emulsion solvent diffusion method to obtain best possible formulation. According to in vitro study, maximum drug release (97.65 \pm 2.15%) was found in optimized batch (F5). In vivo pharmacokinetic study showed that C_{max} of cinnarizine loaded nanosponge is 1.4 times more than cinnarizine suspension. T_{max} of cinnarizine loaded nanosponge is 1.7 times more than cinnarizine suspension. AUC of cinnarizine loaded nanosponge is 1.1 times more than cinnarizine suspension. Cinnarizine loaded nanosponge increases efficacy of cinnarizine. According to in vivo pharmacodynamic study Cinnarizine loaded nanosponge show antiemetic activity.

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