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FORMULATION OF NANONASAL SPRAY OF ANTI-MIGRAINE DRUGS TRIPTANS BY ION GELATION METHOD

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ABSTRACT

Background: Triptans are effective medicines used to treat migraine and certain other headaches. This study has been done to prepare nanoparticles of triptans by ion gelation technique using different drug to polymer ratios and cross linking agent and then evaluated. The aim of this study was to prepare and evaluate intranasal spray of Chitosan loaded Triptan nanoparticles (TNP) having high entrapment efficiency (EE) and low particle size (PS) as a new specific nasal spray for anti-migraine activity to achieve faster and higher levels of drug absorption in the brain through olfactory route, bypass the blood- brain barrier and allow the direct transport of the drug from nose to the brain. **Methods:** We developed a specific Triptan nano nasal spray (TNNS) by ion gelation method for anti-migraine activity. Chitosan as polymer and sodium tripoly-phosphate (TPP) as a cross linking agent, were used at different levels to prepare Triptan nanoparticles with high entrapment efficiency and low particle size. Completion of the reaction was confirmed by Infrared spectroscopy (IR spectra). Nanoparticles were evaluated for shape, surface morphology, polydispersity index, zeta potential, particle size distribution and entrapment efficiency. After filling the nanoparticles of triptans in metered dose nasal spray containers along with suitable excipients, *in-vitro* analysis of the drug loaded chitosan nanoparticles from the metered dose spray device (MDS) was carried out for clarity test, pH, viscosity, assay of the drug, content uniformity, plume geometry and spray pattern analysis. **Results:** Among all the formulations, formulation of Almotriptan-loaded chitosan nanoparticles (A_{C5}) have small particle size of 75.3 ± 3.5 nm and high entrapment efficiency of 75.4 ± 0.4%. **Conclusions:** In this study, we have found that the chitosan nanoparticles in presence of 0.75 % TPP as a cross linking agent could be used as delivery vehicle of triptans.

KEYWORDS: Triptan, Nanoparticles, Chitosan, Ion-gelation Method.

BACKGROUND

Migraine headache is a neurological disorder often initiated by a trigger and characterized by a headache, which may be accompanied by a variety of multiple organ/system symptoms, such as nausea, allodynia, vomiting, and urinary frequency.

Triptans are serotonin 5-hydroxytryptamine (5-HT) receptor agonists that are generally effective, well tolerated, safe, and used to treat migraine and certain other headaches. They may be taken subcutaneously, orally as tablets, capsules, or quick-dissolving wafers, or intranasally as a spray.[1]

The use of polymeric nanoparticles (PNP) in drug delivery, generally increases the stability of the pharmaceutical agents and it can be easily and cheaply fabricated in large quantities by a multitude of methods.[2] Nanoparticles (NP_S) have been extensively investigated in biomedical and biotechnological areas and, especially, in drug delivery systems for drug targeting. The advantages of targeted drug delivery to the specific site of the body, paved the way for applying NPs to achieve this type of drug delivery. Much attention has been provided to non-parenteral routes like oral, pulmonary, nasal and opthalmic delivery of the drugs.[3]

The present work aims to advantages of targeted nano nasal spray of drugs such as Almotriptan, Naratriptan, Frovatriptan and Zolmitriptan, (second-generation triptans) by using ion gelation technique, using chitosan (biodegradable polymer) for anti-migraine activity. The nanoparticles so formed have high entrapment efficiency (EE) and low particle size (PS), filled in MDS device along with best excipients for the nasal drug delivery systems.[4]

METHODS

Instrumentation and materials: Almotriptan, Zolmitriptan, Naratriptan and Frovatriptan, were received as a gift samples from Apotex research private limited, Bangalore. Chitosan and Sodium Tripoly-

Phosphate (TPP) were procured from Sigma Aldrich private limited company (Pvt. Ltd). India. Phenyl ethyl alcohol was procured from Avra synthesis Pvt. Ltd. Pune, India. Polysorbate 80 (Tween 80), Sodium hydroxide, Menthol, Microcrystalline Cellulose (MCC), Glycerin, and Dextrose were purchased from S. D. Fine Chemicals Ltd. Mumbai, India.

Drug Excipient Compatibility Study

Drug-excipient interaction plays a vital role in the release of drug from the formulation. Fourier transform infrared spectroscopy (FTIR) has been used to study the physical and chemical interactions between the drug and the excipients used. Fourier transform infrared (FTIR) spectra of Chitosan and physical mixture of Triptans with Chitosan and Sodium tri-poly phosphate were recorded using Potassium bromide (KBr) pellet method on FTIR instrument.

Preparation of Triptan-loaded Chitosan Nanoparticles: Chitosan nanoparticles containing the drug Almotriptan (A) were prepared by the ionotropic gelation technique.[5,6] Accurately weighed chitosan was dissolved in 1% v/v acetic acid solution, to which Polysorbate 80 (Tween 80) used as surfactant and the drug were added.

Sodium tri-poly phosphate (TPP) was dissolved in distilled water. To the chitosan-drug solution, TPP solution was added dropwise through a No.4 syringe needle and continuously stirred using a mechanical stirrer (Remi Motors- RO-123, RPM 4000) at room temperature for 30 min, which led to the formation of nanoparticles. Subsequently, the pH was adjusted to 5.5 with the help of a required amount of 1 N sodium hydroxide solution (NaOH) and then centrifuged at 12000 Revolutions per minute (rpm) using a refrigerated Eppendorf Centrifuge 5430R (Table 1).

Table. 1: Experimental control factors and different levels of Polymer (Chitosan) and Cross linking agent (TPP), for Almotriptan loaded Chitosan nanoparticles (A_{C1}-A_{C9}) formulation.

S. No	Ingredients	A_{C1}	A_{C2}	A_{C3}	A_{C4}	A_{C5}	A_{C6}	A_{C7}	A_{C8}	A_{C9}
1	Drug: polymer concentration (mg/ml)	1:1	1:2	1:3	1:1	1:2	1:3	1:1	1:2	1:3
2	TPP (w/v)	0.5	0.5	0.5	0.75	0.75	0.75	0.1	0.1	0.1
3	Tween 80 (v/v)	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
4	Stirring speed (rpm)	1500	1500	1500	1500	1500	1500	1500	1500	1500

0.1N NaOH is added till the pH of the solution reaches 5.5.

The best formulation procedure has been repeated with the Zolmitriptan (Z), Naratriptan (N) and Frovatriptan (F).

After preparation of triptan loaded chitosan nanoparticles, the following excipients were added and filled in metered dose nasal spray (Fig. 1).

Table. 2: Excipients used in triptan loaded Chitosan nanoparticles nasal spray.

S. No	Ingredients	Function	Percentage
1	Drug	Active	5 mg
2	Glycerin	Humectant	0.05 %
3	MCC	Suspending agents	0.05 %
4	Phenyl ethyl alcohol	Preservative	0.01 %
5	Dextrose	Tonicity adjustment	0.05 %
6	Sodium hydroxide	pH adjustment	0.01 %
7	Menthol	Flavoring agent	0.05 %

Characterization of triptan loaded chitosan nanoparticles

Shape and Surface morphology

The shape and surface morphology of the Chitosan nanoparticles was visualized by scanning electron microscopy (SEM) LEO-430 Cambridge and U.K. The samples were prepared by lightly sprinkling nanoparticles on double-sided adhesive tape on an aluminum stub. The stubs were then coated with gold to a thickness of 200 to 500 Å under an argon atmosphere using a gold sputter module in a high vacuum evaporator.[7] The samples were then randomly scanned and photomicrographs were taken at different magnifications with SEM.

Particle Size and Zeta Potential Measurement

Particle size was measured with the help of HORIBA Scientific Nano Partica, nanoparticle analyzer SZ-100. For the determination of particle size, samples were prepared by tenfold dilution of 1 milliliter (ml) of the Nano particulate suspension with distilled water. The analysis was carried out in triplicate.[8]

A Zeta potential was used to measure the surface charge of nanoparticles. The average particle size, Polydispersity index (used to describe the degree of uniformity of a distribution) and Zeta potential were measured by HORIBA scientific nano Partica, nanoparticle analyzer SZ-100.

Drug Entrapment Efficiency

The entrapment efficiency of the formulation was determined upon the centrifugation of a fixed quantity of the aqueous nanoparticulate suspension (about 2 ml) at 12000 rpm for 30 minutes at 20°C (SIGMA 3-18K, Sartorious). The absorbance of the unencapsulated drug in the supernatant was evaluated using UV-VIS spectrophotometer (UV-1800 Pharma Spec, Shimadzu).

The drug entrapment by the nanoparticles was calculated using the following equation.[8]

$$\%\text{Drug entrapment efficiency} = \frac{\text{Initial amount of the drug added} - \text{Amount of drug in supernatant}}{\text{Initial amount of drug added}} \times 100$$

Characterization of triptan nano nasal spray

***In-vitro* Bioequivalence Testing**

For the assessment of *in-vitro* bioequivalence, the food and drug administration (FDA) guidance requires *in-vitro* testing to demonstrate comparable delivery characteristics of drug products.[9]

A description of the study design and results were given for the *in-vitro* tests such as emitted dose uniformity, droplet size distribution, spray pattern, plume geometry, and priming/re-priming and tail-off profile, of Triptan Nano Nasal Spray (TNNS).

Appearance, Color, and Clarity Test

The appearance of the contents of the container (i.e., formulation) and the container closure system (e.g., pump components, inside of the container) should conform to their respective descriptions as an indication of the drug product integrity. If any color is associated with the formulation (either present initially or from degradative processes occurring during shelf life) then, a quantitative test with appropriate acceptance criteria should be established for the drug product by the manufacturer.[10]

pH

The pH of the nasal formulation is very important mainly to avoid irritation of the nasal mucosa, to prevent the growth of pathogenic microorganisms, to sustain normal physiological ciliary movement. Lysozyme which is present in nasal secretion, that is responsible for destroying certain microorganisms at acidic pH.

Under alkaline pH, Lysozyme is deactivated and the nasal tissue is susceptible to microbial infection.[10]

It is therefore advisable for the pH of the formulation to be adjusted between 4.5 - 6.5.

The pH of the all prepared formulations was measured using digital pH meter.

Pump Delivery

A pump delivery test to assess pump-to-pump reproducibility in terms of drug product performance and to evaluate the delivery from the pump should be performed.[11]

For pump delivery analysis first the empty container having a single nozzle (0.2 millimeter diameter), Weighed then the formulation was filled into the container and reweighed. After 10 of times actuation, the weight of the weighing bottle was reweighed again and the difference was calculated.

Viscosity

For formulations containing an agent contributing to the viscosity, this parameter should be tested. The contact time between the drug and the nasal mucosa is increased by higher viscosity of the formulation thereby increasing the time for permeation.

Viscosity measurement of different polymeric dispersions (for triptan loaded nasal spray solution, in phosphate buffer, pH 5.5) was measured in order to find out the effect of viscosity of vehicles on drug release using Brookfield Viscometer DV-E.[12]

Drug Content (Assay)

The assay of drug substance in the entire container should be determined analytically with a stability indicating procedure. This test provides assurance of consistent manufacturing (e.g., formulation, filling, sealing). An assay procedure designed to find out any degradation of the drug substance, adherence of the drug substance to the container and closure components, and the potential effect of formulation evaporation and/or leakage.[9]

100 mg of Almotriptan was dissolved in pH 5.5 phosphate buffer in a 100 ml volumetric flask and made up to 100 ml with the same. Aliquots from this stock solution were taken and dilutions were done using phosphate buffer to obtain concentrations of 2, 4, 6, 8 and 10 µg/ml. Absorbances of these solutions were measured at 227 nm (λ_{max} of Almotriptan). The data is presented in Tables 8, 9, 10 and 11, were the average of triplicate readings. The linear regression analysis (LRA) was performed. LRA equation for calibration curve of Almotriptan was utilized for Almotriptan determination by the UV method.

Graphical representation is given in Figures 7.

The same procedure has been repeated with the, Zolmitriptan, Naratriptan and Frovatriptan at suitable λ_{max} (222, 223 and 244 nm respectively).

Content per spray (CPS)

The container of metered dose nasal spray contains 5 ml of Zolmitriptan-loaded Chitosan Nanoparticles (Z-CNP). Individually 250 mg doses of drug were present inside nasal spray pump with intermittent shaking for 5 seconds. After each actuation it delivers 0.1 ml of (Z-CNP) which delivers 5 mg of the drug.

1 puff of these solutions were introduced into 100 ml volumetric flasks and were diluted to 100 ml with phosphate buffer pH 5.5. Then 1 ml of the above solutions were transferred to 10 ml volumetric flasks to determine the amount of drug in each puff of metered dose nasal spray. Absorbances of these were measured at 222 λ_{max} of Zolmitriptan the absorbance was recorded at 222 nm using UV method.

The concentration of the drug present in the formulation was computed from the calibration curve.

The same procedure has been repeated with the Almotriptan, Naratriptan and Frovatriptan at suitable λ_{max} 227, 223 and 244 respectively.

Emitted dose uniformity, priming, priming/re-priming, and tail-off profile

The uniformity of content of active ingredient was determined using the method similar to that mentioned in the content per spray test for the drug loaded in metered dose nasal sprays. In order to check the extent of variation doses sprayed from the device were analyzed for the content of active ingredient in each spray.

Three individual lots of test products and reference products are evaluated. For each lot, ten samples were then tested for pump priming, unit spray content through life, and tail-off studies. Then, additional samples for each lot were evaluated for the prime hold study (re-prime study). For each sample unit, spray samples were collected for sprays 1-8 and analyzed in order to determine the minimum number of actuations required before the pump delivers the labeled dose of drug (sprays 1-8). To characterize emitted dose uniformity at the beginning of unit life, sprays 9-14 were wasted by the automatic actuation station. Sprays 15-17 are collected and analyzed to determine the middle of unit life. Sprays 18-20 are wasted. Sprays 21-23 are collected and analyzed to determine the tail off profile at the end of the unit life. Ten additional samples are drawn randomly from each lot of the drug product for the pump prime hold study. For each unit, the first 12 sprays (sprays 1-12) were wasted. Sprays 13 and 14 are collected as fully primed sprays. The unit is then stored undisturbed for 24 hours. Within each lot, five samples are placed in the upright position, while the other five samples are placed in a side position. After that, sprays 15-17 are collected. The unit is then stored undisturbed in its former position for another 24 hours. After that, the doses emitted by sprays 18-20 are collected. All spray samples are weighted in order to obtain re-priming characteristics.[13-15]

Plume geometry

To determine the characteristics of nasal spray, droplet size and size distribution, spray pattern and plume geometry were analyzed.[16]

Plume geometry describes the shape of the discharged sample parallel to the axis of plume after actuation of nasal pump device. Plume geometry was performed on the nasal spray plume that was allowed to develop into an unconstrained space that far exceeds the volume of nasal cavity. It represents a frozen moment in spray plume development that was viewed from two axes perpendicular to the axis of plume development. The samples should be actuated vertically. Prime the pump with 10 actuations until a steady fine mist was produced from the pump. A fast-speed video camera was placed in front of the sample bottle and it starts recording. The test was repeated by rotating the actuator 90 degree to the previous actuator placement so that two side views were at 90 degrees to each other (two perpendicular planes) and relative to the axis of the plume of the spray, were captured when actuated into space.

For plume geometry, the light-sheet dissects the plume along the Centre line, and is parallel to the flow of the device (Fig. 2a).

Laser light scattering from spray droplet was recorded using high-speed camera at 500 Hz.[17]

Spray pattern

A spray pattern produced by a nasal spray pump evaluates in part the integrity and the performance of the orifice and pump mechanism in delivering a dose to its intended site of deposition. Measurements can be made on the diameter of the horizontal intersection of the spray plume at different distances from the actuator tip. Spray patterns were measured at two distances (3 cm and 6 cm). As a result, a spray pattern was collected for each sample unit. For each spray pattern image, the diameters (the longest and shortest diameters) and the Ovality (which was defined by the ratio of the longest to the shortest diameters) were measured.

A high-speed camera, captures images of the Almotriptan loaded nanoparticles moving through the light-sheet. The captured images are processed by the Photron, Fastcam-SA5 to provide detailed data on the plume geometry, spray pattern at 3 cm and 6 cm from the device and event duration. (Fig. 2b).

Spray pattern is measured only for Almotriptan. Based on cone angle measurements, it will be similar for all the liquids.[15]

RESULTS AND DISCUSSION

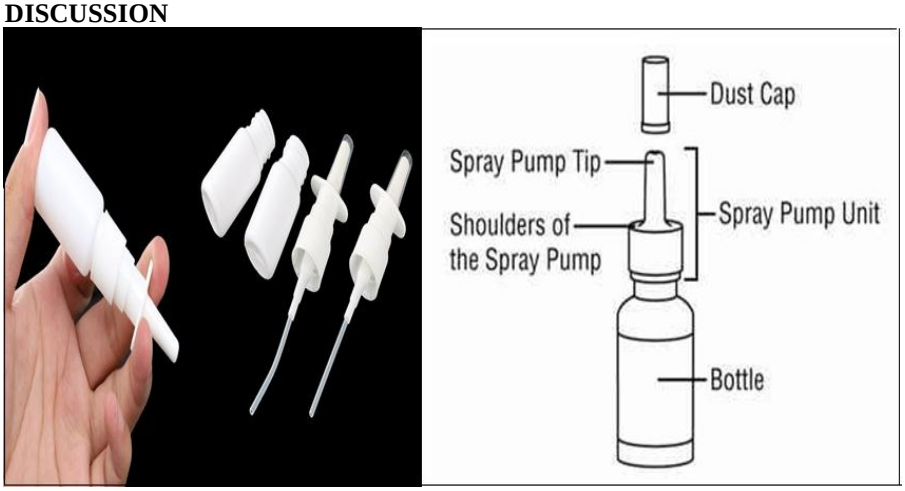

Fig. 1: Metered-Dose Pump Sprays.

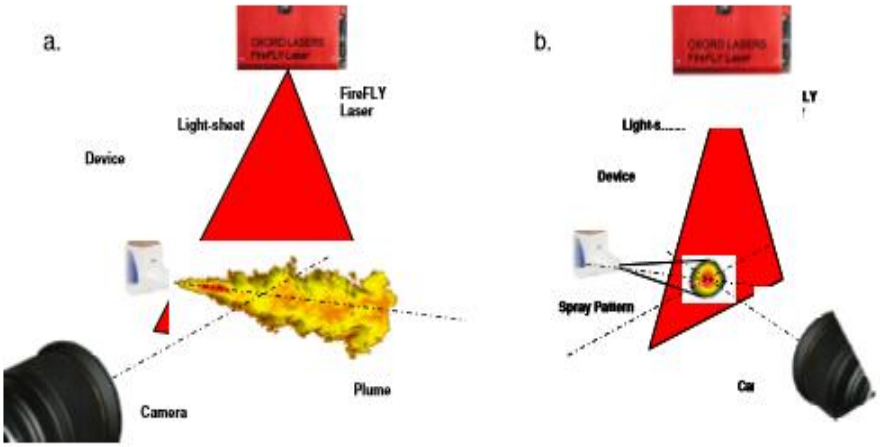

Fig. 2: Represents the plume geometry (a) and spray pattern (b) analysis from metered dose spray device (MDS).

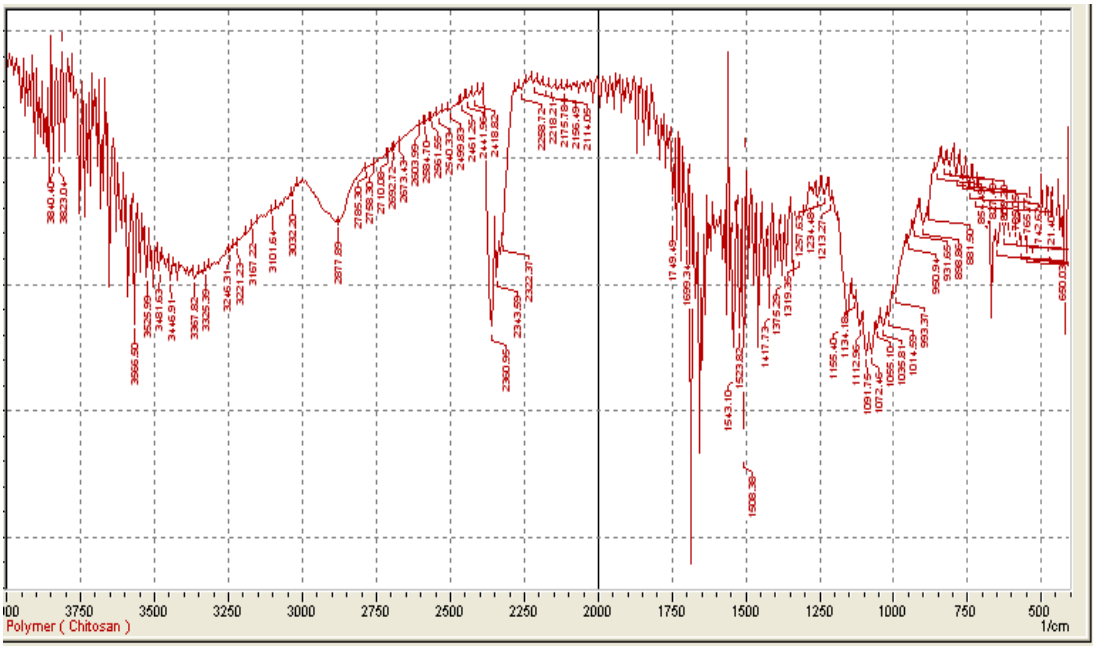

Fig. 3 IR spectra of Chitosan polymer.

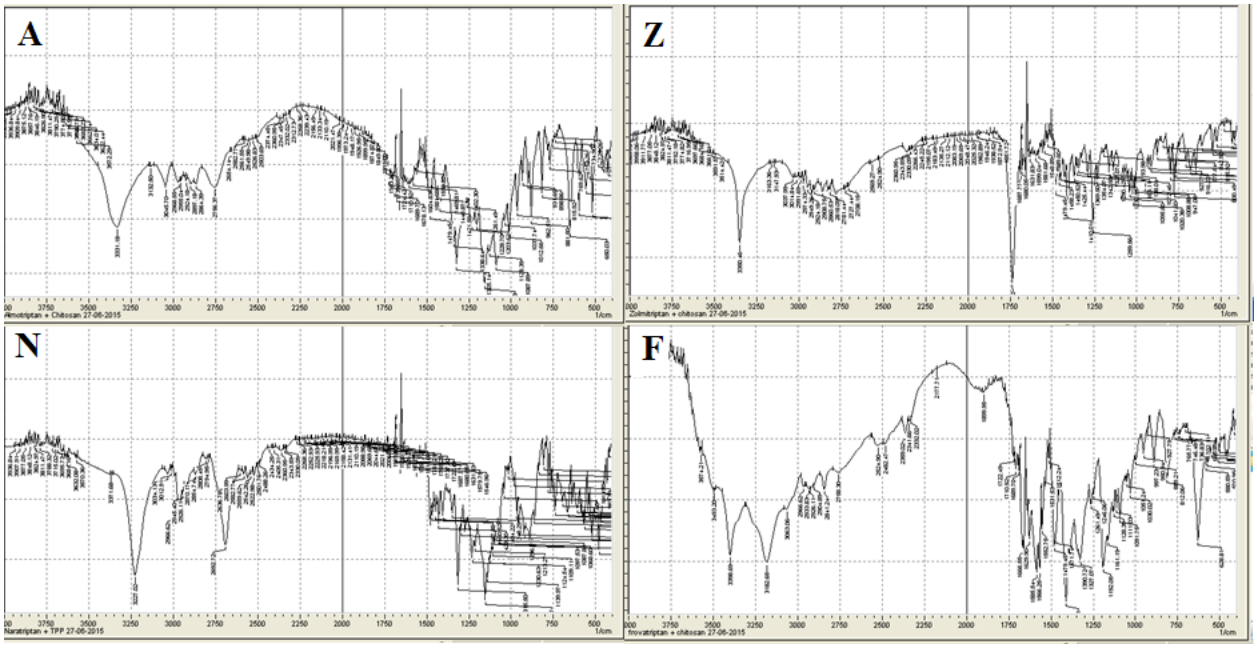

Fig. 4: IR spectra of Almotriptan (A), Zolmitriptan (Z), Naratriptan (N) and Frovatriptan with Chitosan polymer and TPP.

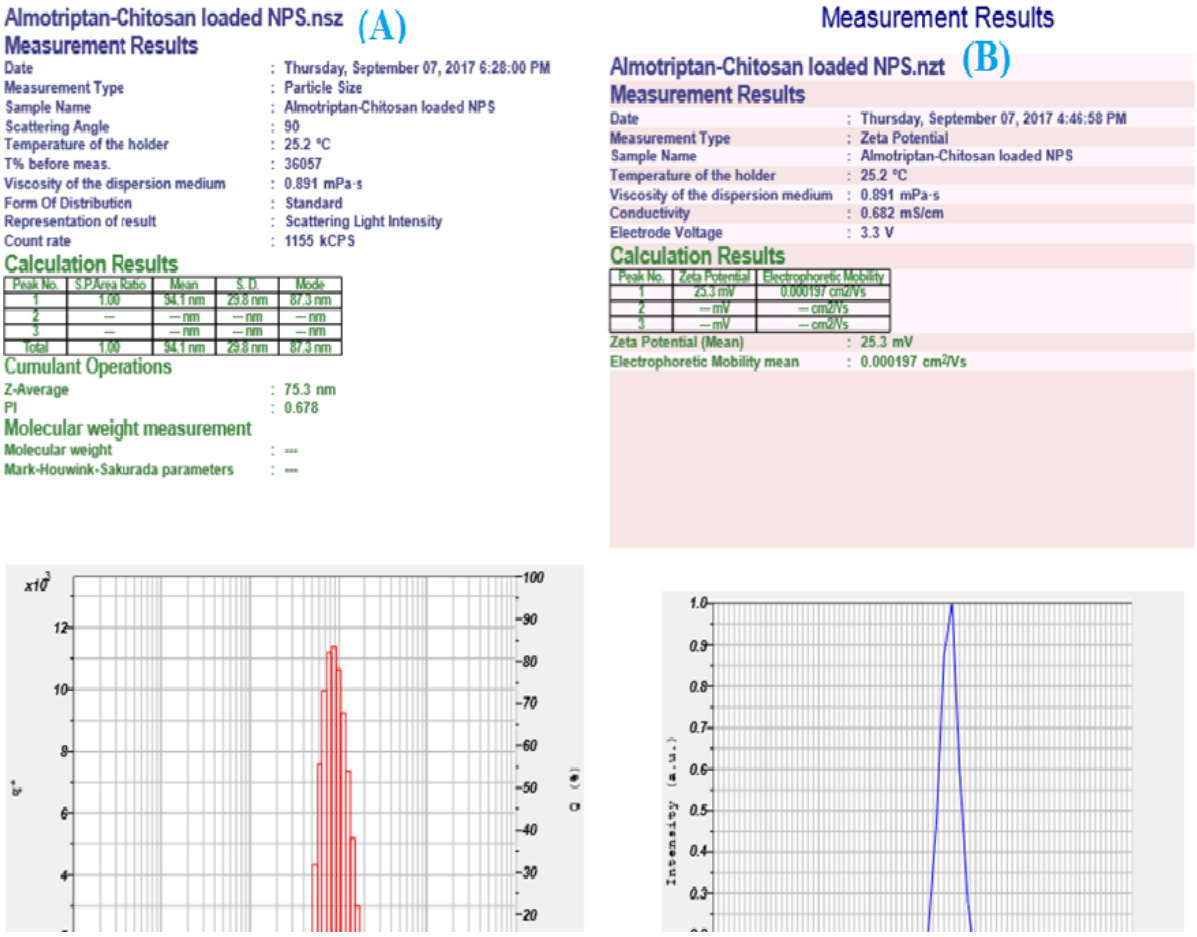

Fig. 5: Average particle size (A) and Zeta Potential (B) of Almotriptan loaded Chitosan Nanoparticles (A-CNP$_S$).

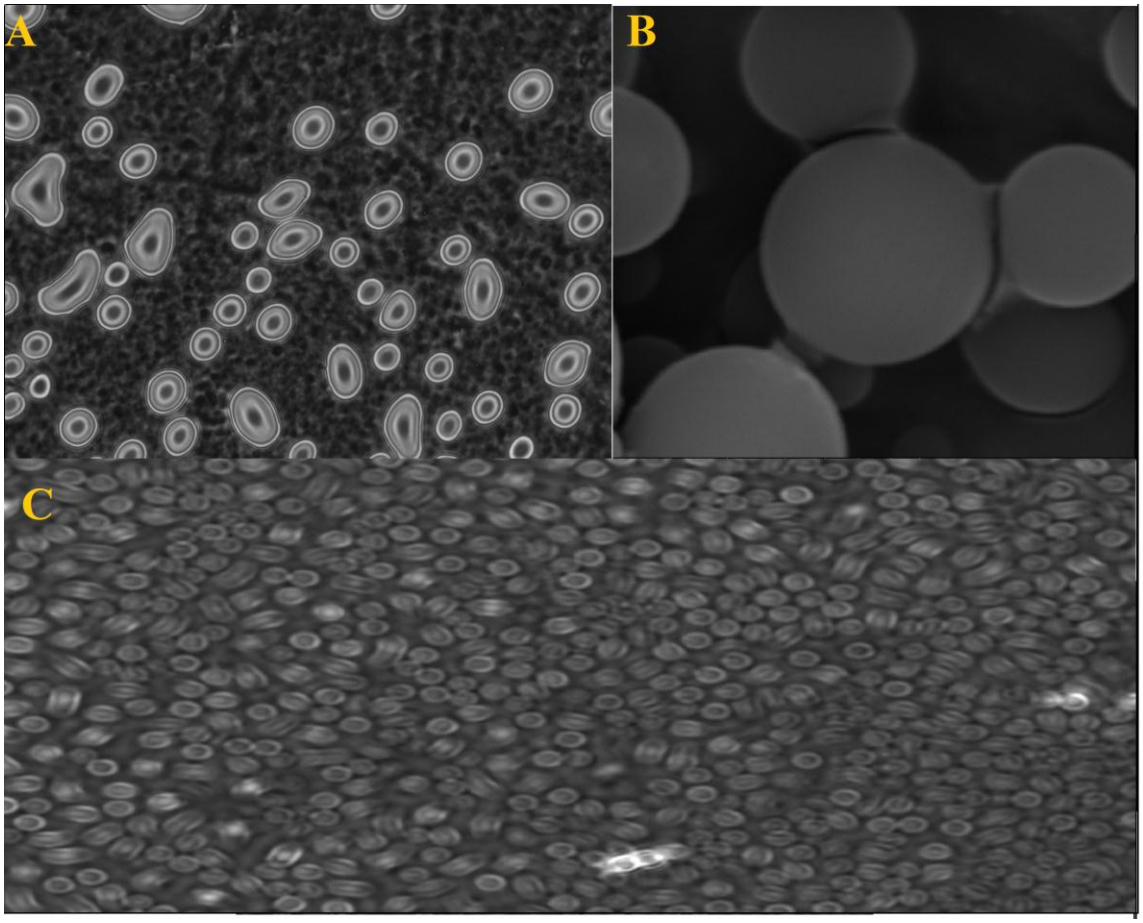

Fig. 6: SEM images of Chitosan (A), Almotriptan (B) and Almotriptan loaded Chitosan Nanoparticles (C).

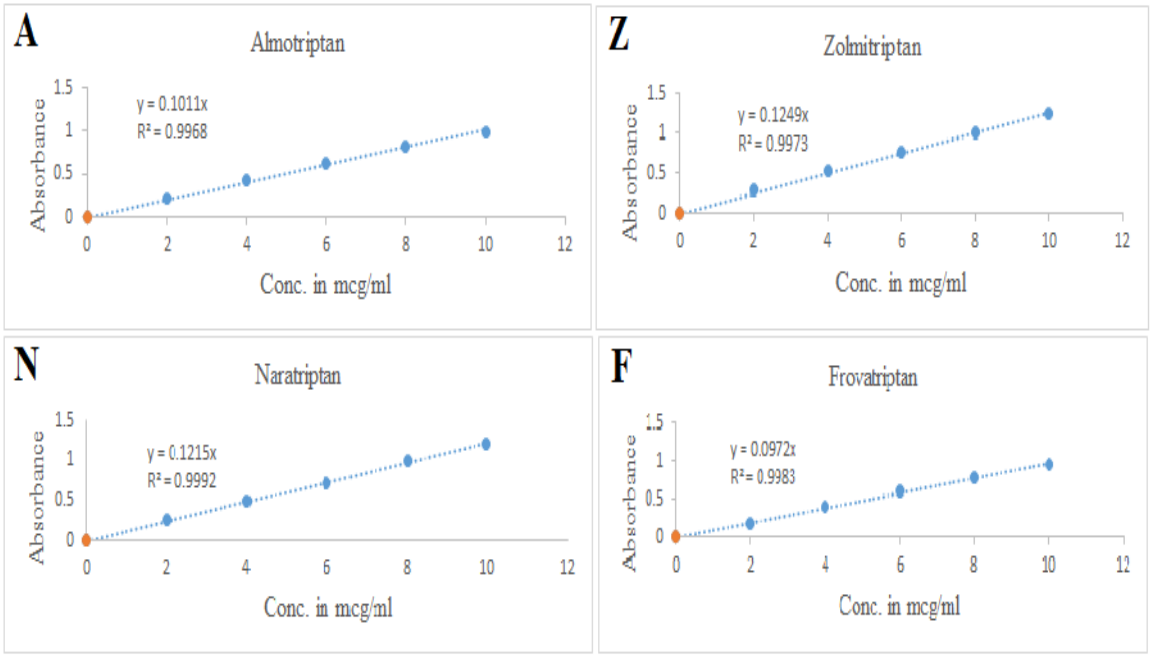

Fig. 7: Calibration curve of Almotriptan (A), Zolmitriptan (Z), Naratriptan (N) and Frovatriptan (F) *(Mean ± SD, n=3).

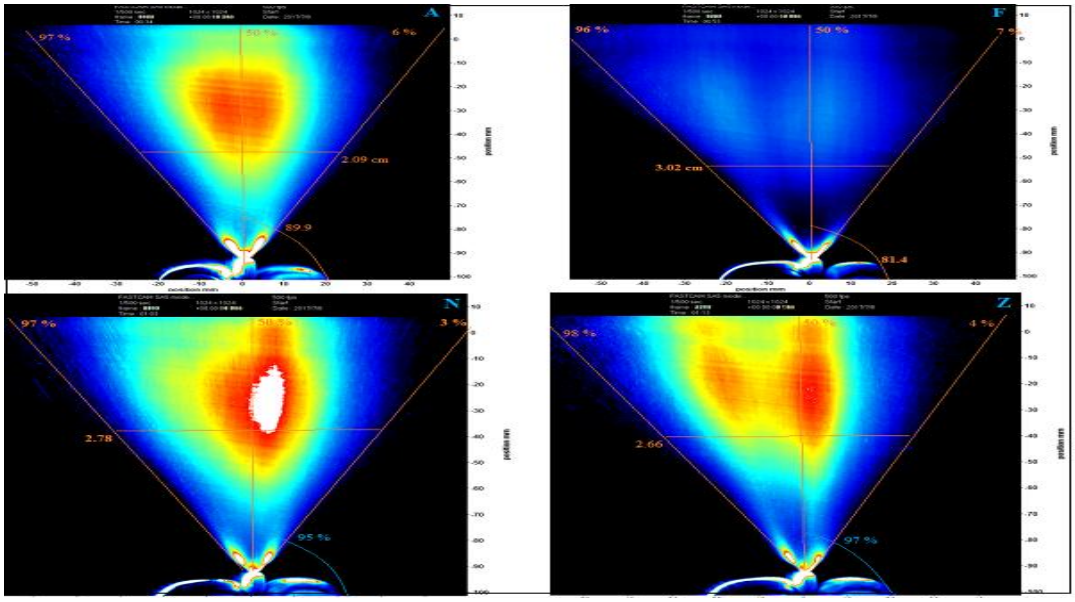

Fig. 8: Images of Plume Geometry of Almotriptan (A), Zolmitriptan (Z) and Frovatriptan (F) loaded chitosan nanoparticles from MDS device.

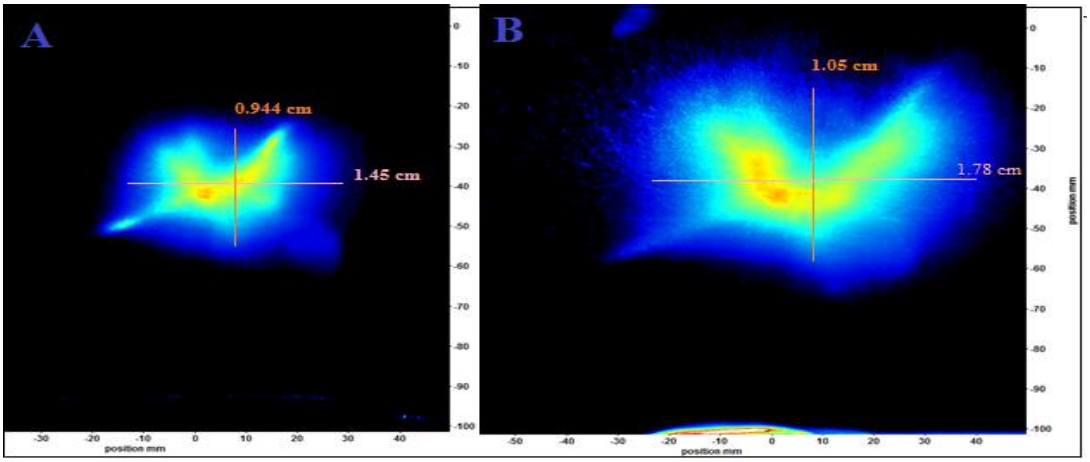

Fig. 9: Image of Spray Pattern of Almotriptan loaded chitosan nanoparticles from MDS device at 2 positions, (A) 3 cm, (B) 6 cm.

Drug-Excipients compatibility studies by FT-IR

FTIR techniques have been used here to study the physical and chemical interactions between drug and excipients used.

Fig. No 3 presented the IR spectrum of Chitosan polymer.

In the present study, it has been observed that there is no chemical interaction between Almotriptan, Zolmitriptan, Naratriptan, Frovatriptan and the excipients used, thus indicating compatibility of the drugs and excipients (Fig. 4).

Particle size and Zeta potential measurement of nanoparticles

The values for the average particle size, zeta potential, and polydispersity index are tabulated in Tables 3 and 5.

The drug and chitosan polymer in the ratio of 1:2 with 0.75% of Sodium tri-poly phosphate (TPP) as cross linker (A_{C5}) showed particle size of 75.3 nm, Polydispersity index of 0.67 and zeta potential of +25.3 medium voltage (mV).

Results were shown in Fig. 5.

From the above formulations the drug and polymer ratio of 1:2 and 0.75% of Sodium tri-poly phosphate (TPP) showed the best results for Chitosan nanoparticles formulations for all the drugs.

Therefore, we decided to repeat the best procedure of Chitosan nanoparticles (A_{C5}), for the formulation of Zolmitriptan, Naratriptan and Frovatriptan. The results of which are shown in table 5.

Table. 3 Data for Particle Size, Entrapment Efficiency, Zeta Potential and Polydispersity Index for the formulation of Almotriptan, at variable levels of Chitosan, and Cross linking agent (TTP).

Formulation	Average particle Size (nm) ± S.D	Zeta Potential (mV) ± S.D	Polydispersity Index (PdI) ± S.D	Entrapment Efficiency (%) ± S.D
1.Almotriptan (A_{C1})	136.9 ± 4.8	20.1 ± 1.0	0.58 ± 0.28	52 ± 0.4
2.Almotriptan (A_{C2})	151.1 ± 1.6	21.7 ± 0.6	0.58 ± 0.32	65 ± 1.7
3.Almotriptan (A_{C3})	142.3 ± 2.9	19.5 ± 0.9	0.64 ± 0.24	55 ± 1.1
4.Almotriptan (A_{C4})	103.7 ± 2.6	29.1 ± 1.4	0.39 ± 0.14	67 ± 1.7
5.Almotriptan (A_{C5})	75.3 ± 3.5	25.3 ± 2.3	0.67 ± 0.19	75 ± 0.4
6.Almotriptan (A_{C6})	106.6 ± 2.3	26.6 ± 1.4	0.56 ± 1.14	71 ± 1.4
7.Almotriptan (A_{C7})	111.6 ± 0.4	22.6 ± 1.4	0.62 ± 1.33	68 ± 1.4
8.Almotriptan (A_{C8})	124.6 ± 1.9	23.6 ± 1.4	0.59 ± 0.34	69 ± 1.4
9.Almotriptan (A_{C9})	171.3 ± 4.6	20.5 ± 0.2	0.54 ± 0.54	74 ± 1.5

n=3; Values are mean ± standard deviation

As formulation No. 5, (A_{C5}) gave best result for Almotriptan, similarly for the drugs Zolmitriptan, Naratriptan and Frovatriptan, Z_{C5}, N_{C5} and F_{C5} respectively were chosen as the best formulations (Table 3).

Table. 4 Data for formulation of Zolmitriptan (Z_C), Naratriptan (N_C) and Frovatriptan (F_C) loaded chitosan nanoparticles.

S.No	Ingredients	Z_C, N_C, F_C
1	Drug: Polymer (Chitosan) concentration (mg)	1:2
2	TPP (w/v)	0.75
3	Tween 80 (v/v)	0.2
4	Stirring speed (rpm)	1500

0.1N NaOH is added till the pH of the solution reaches 5.5.

Table. 5 Data for Particle Size, Entrapment Efficiency, Zeta Potential, Polydispersity Index, formulation of Zolmitriptan, Naratriptan, Frovatriptan based on best formulation.

Formulation	Average particle Size (nm) ± S.D	Zeta Potential (mV) ± S.D	Polydispersity Index (PdI) ± S.D	Entrapment Efficiency (%) ± S.D
1. Zolmitriptan (Z_{C5})	79.7 ± 4.5	25.4 ± 0.6	0.57± 0.23	68 ± 1.1
2. Naratriptan (N_{C5})	99.5 ± 2.4	22.2 ± 0.7	0.51 ± 0.14	65 ± 1.7
3. Frovatriptan (F_{C5})	85.5 ± 5.7	24.1 ± 0.1	0.64 ± 0.17	72 ± 0.4

n=3; Values are mean ± standard deviation

Entrapment Efficiency

Entrapment efficiency of the Triptan-loaded Chitosan nanoparticles was determined and the data were shown in Tables 3 and 5. A_{C5} showed an average drug entrapment efficiency of 75.4 ± 0.8%, which is highest amongst all the formulations.

Scanning Electron Microscopy (SEM)

The SEM analysis of prepared TNP was performed for morphological studies. The shape and surface morphology of the Almotriptan-loaded Chitosan nanoparticles (C-NP) were visualized by scanning electron microscopy (SEM). The SEM images of chitosan polymer, pure drug (Almotriptan) and Almotriptan-loaded chitosan nanoparticles are presented in Fig. 6/ A, B, C respectively.

It revealed that the nanoparticles were spherical in shape and have a smooth surface (Fig. 6C).

Appearance, Color, and Clarity test The appearance of the content of the container and closure system was analyzed for all the formulations. There was no change in color, size, shape, texture and clarity of the formulation. It is an indication of the drug product integrity in all batches and was presented in table 6.

Table. 6 Characterizations of the Triptan-Loaded Chitosan Nano Nasal Spray.

Parameters	Zolmitriptan	Almotriptan	Naratriptan	Frovatriptan
Clarity	T	T	T	T
pH	5.6 ± 0.04	5.5 ± 0.07	5.6 ± 0.1	5.3 ± 0.3
Viscosity (cps)	4.4 ± 0.1	4.4 ± 0.5	4.4 ± 0.1	4.3 ± 0.4
Drug content (%)	95 ± 0.2	96 ± 0.3	92 ± 0.2	97 ± 0.5

T=Transparent; *(Mean ± SD, n=3).

pH: To stabilize the Triptan loaded nasal spray delivery system, pH was preferably adjusted to a pH of 4.5 - 5.5, so as to increase the chemical stability of the active ingredient and to aid in the prevention of growth of microorganism (Table 6).

Pump delivery: The average pump delivery of formulation by using MDS device, after calculation was found to be 0.098 g per actuation. Individual spray delivery was within 15% of the target weight and their mean weight was within 10% of the target weight in 10 actuations. Total net content from 10 containers was found to be not less than 90% of the labeled amount. There was no loss of weight in the product stored in an upright, inverted and horizontal position (Table 7).

Table. 7: Average pump delivery weight for all formulation.

Parameters	Almotriptan	Zolmitriptan	Naratriptan	Frovatriptan
Weight of empty bottle (g)	27.09 ± 0.04	27.08 ± 0.01	27.09 ± 0.01	27.08 ± 0.03
Weight of bottle with formulation (g)	32.07 ± 0.21	32.09 ± 0.19	32.06 ± 0.16	32.07 ± 0.24
Weight of bottle after 10 actuation (F5)	31.29 ± 0.12	31.27 ± 0.11	31.23 ± 0.14	32.19 ± 0.12

* Each value represents an average of three determinations. (Mean ± SD, n=3).

Viscosity

The viscosity of the formulation is low hence the spray from container has good spread ability of the solution within the nose. All the formulations were lying within the acceptable limits (3-6 cps), which its present in table 6.

Drug Content (Assay) and Content per spray (CPS)

The calibration curve for Almotriptan, Zolmitriptan, Naratriptan and Frovatriptan in pH 5.5 buffer was linear in the concentration range 2–10 µg/ml at λ_{max} of 227, 222, 223 and 244 nm respectively.

The R^2 values for Almotriptan, Zolmitriptan, Naratriptan and Frovatriptan were found to be 0.9968, 0.9973, 0.9992 and 0.9983 respectively. The average slope of Almotriptan, Zolmitriptan, Naratriptan and Frovatriptan were found to be 0.1011, 0.1249, 0.1215 and 0.0972 respectively.

The data for Drug Content (Assay) and Content per spray (CPS) are presented in Tables 8, 9, 10 and 11.

Graphical representation of the calibration curve of Almotriptan (A), Zolmitriptan (Z), Naratriptan (N) and Frovatriptan (F) is given in Figure No. 7.

Table 8 Calibration curve data for Almotriptan in phosphate buffer pH 5.5 at λ_{max} of 227 nm, Vs. 1 Actuation (puff) of Almotriptan from Nano nasal spray (containing 0.1ml).

Concentration in µg/ml	Absorbance at λ_{max} 227 nm
0	0 ± 0.01
2	0.209 ± 0.01
4	0.430 ± 0.02
6	0.622 ± 0.01
8	0.823 ± 0.01
10	0.978 ± 0.01
Sample	0.665 ± 0.02

* Each value represents an average of three determinations. (Mean ± SD, n=3).

Table. 9: Calibration curve data for Zolmitriptan in phosphate buffer pH 5.5 at λ_{max} of 222 nm Vs. 1 puff of Zolmitriptan Nano nasal spray (containing 0.1ml).

Concentration in µg/ml	Absorbance at λ_{max} 222 nm
0	0 ± 0.01
2	0.280 ± 0.02
4	0.536 ± 0.01
6	0.759 ± 0.01
8	0.991 ± 0.02
10	1.230 ± 0.01
Sample	0.650 ± 0.02

* Each value represents an average of three determinations. (Mean ± SD, n=3).

Table. 10: Calibration curve data for Naratriptan in phosphate buffer pH 5.5 at λ_{max} of 223 nm Vs. 1 puff of Naratriptan Nano nasal spray (containing 0.1ml).

Concentration in µg/ml	Absorbance at λ_{max} 223 nm
0	0 ± 0.01
2	0.189 ± 0.02
4	0.407 ± 0.01
6	0.594 ± 0.03
8	0.790 ± 0.02
10	0.950 ± 0.01
Sample	0.670 ± 0.01

* Each value represents an average of three determinations. (Mean ± SD, n=3).

Table. 11: Calibration curve data for Frovatriptan in phosphate buffer pH 5.5 at λ_{max} of 244 nm Vs. 1 puff of Frovatriptan Nano nasal spray (containing 0.1ml).

Concentration in µg/ml	Absorbance at λ_{max} 244 nm
0	0 ± 0.01
2	0.255 ± 0.01
4	0.488 ± 0.02
6	0.719 ± 0.01
8	0.993 ± 0.02
10	1.202 ± 0.02
Sample	0.579 ± 0.01

*Each value represents an average of three determinations. (Mean ± SD, n=3).

Emitted dose uniformity, priming, priming/re-priming, and tail-off profile

Drug content was found to be uniform among all the different batches of triptan loaded chitosan nanoparticles formulations from MDS device. The drug content ranged from 92 ± 0.1% to 95.8 ± 0.2% for priming (sprays 1-8), 98 ± 0.1% to 99.9 ± 0.2% for re-priming (sprays 15-17) and 96 ± 0.1% to 98.5 ± 0.2% at the tail-off profile (sprays 21-23).

Table. 12: Emitted dose uniformity, priming, priming/re-priming, and tail-off profile for Almotriptan nasal spray using metered dose spray device (MDS).

Concentration in µg/ml	Absorbance at λ_{max} = 227 nm
1	0.141 ± 0.13
2	0.243 ± 0.16
3	0.502 ± 0.14
4	0.601 ± 0.03
5	0.604 ± 0.23
6	0.607 ± 0.12
7	0.605 ± 0.12
8	0.605 ± 0.03
9-14 wasted	-
15	0.608 ± 0.11
16	0.605 ± 0.11
17	0.606 ± 0.02
18-20 wasted	-
21	0.604 ± 0.13
22	0.602 ± 0.12
23	0.602 ± 0.03

* Each value represents an average of three determinations. (Mean ± SD, n=3).

Plume geometry

Results showed consistent data across the 3 replicates. For plume geometry, results in table 13 show, 8.9 % variation in the data for the plume angle, from 15.05 degrees to 17.52 degrees. At 6 cm, the mean width from the device was 2.15 cm. The mean plume length was 16.1 cm. Figure 8 shows plume geometry details from the Metered Dose Spray (MDS) device. An average spray cone angle of 46.5° can be taken for all the cases.

Table. 13 Plume Geometry data for 3 event from a single MDS device.

No.	Plume Angle	Plume Width at 6 cm	Length (cm)	Duration time (ms)
A	48.52 ± 0.21	2.09 ± 0.71	16.87 ± 0.31	97 ± 0.51
F	45.32 ± 0.41	3.02 ± 0.41	15.05 ± 0.21	96 ± 0.21
N	49 ± 0.15	2.78 ± 0.11	17. 52 ± 0.11	94 ± 0.61
Z	44 ± 0.13	2.66 ± 0.21	17.15 ± 0.61	97 ± 0.41

* Each value represents an average of three determinations. (Mean ± SD, n=3).

Spray pattern

The spray pattern results shown in Table 14 provide details on D_{min}, D_{max}, Area & Ovality ratio of Almotriptan nano nasal spray. Comparisons of the Ovality ratios show a 12% variation at 3 cm, whereas the variation is reduced to 6% at 6 cm. The area results show variation of 10.08% and 9.49% at 3 cm and 6 cm respectively. The data showed that the spray pattern is more consistent at 6 cm than at 3 cm (Fig. 9).

Table. 14: Spray Pattern results of Almotriptan loaded chitosan nanoparticles for 3 repetitions at 3 & 6 cm from the MDS device.

	Spray Pattern at 3 cm				Spray Pattern at 6 cm			
REP	**Area($cm^{2)}$**	**D_{max}(cm)**	**D_{mix}(cm)**	**Ovality Ratio**	**Area($cm^{2)}$**	**D_{max}(cm)**	**D_{mix}(cm)**	**Ovality Ratio**
1	0.95 ± 0.23	1.45 ± 0.31	0.94 ± 0.01	1.82 ± 0.31	1.34 ± 0.01	1.76 ± 0.21	1.09 ± 0.01	1.71 ± 0.02
2	0.89 ± 0.11	1.51 ± 0.61	0.82 ± 0.51	1.85 ± 0.21	1.24 ± 0.81	1.78 ± 0.11	1.05 ± 0.01	1.57 ± 0.31
3	0.98 ± 0.41	1.42 ± 0.21	0.97 ± 0.11	1.45 ± 0.11	1.15 ± 0.61	1.61 ± 0.41	0.97 ± 0.61	1.75 ± 0.51
MEAN	0.93 ± 0.21	1.46 ± 0.11	0.85 ± 0.01	1.63 ± 0.01	1.27 ± 0.11	1.68 ± 0.41	1.04 ± 0.31	1.54 ± 0.11
STDEV	0.09 ± 0.01	0.04 ± 0.11	0.10 ± 0.31	0.32 ± 0.01	0.14 ± 0.41	0.08 ± 0.61	0.08 ± 0.41	0.11 ± 0.01
%RSD	10.08 ± 0.11	2.47 ± 0.71	11.19 ± 0.21	12.7 ± 0.41	9.49 ± 0.31	4.81 ± 0.11	7.54 ± 0.51	6.27 ± 0.31

*Each value represents an average of three determinations. (Mean ± SD, n=3).

Draft guidance developed by the MDS Nasal devices for the characterization of spray pattern and plume geometry has been applied successfully to metered dose nasal spray containing triptan loaded chitosan nanoparticles device.

Using La-Vision, system with MDS test chamber, the system captured high quality image of the spray pattern and plume geometry.

DISCUSSION

Ion gelation technique is a method by which can be prepared stable nanoparticles were prepared with a Zeta potential of +29.1 mV which was confirmed through HORIBA Scientific Nano Partica, nanoparticle analyzer SZ-100 (Tables 3 and 5). Electrophoresis confirms the particles are positively charged in Chitosan nanoparticles.

Preformulation studies such as standard graph and compatibility studies between polymer and drug were determined.

Triptan-loaded Chitosan nanoparticles were successfully formulated by the ion gelation method and the effects of process parameters on NPs size, zeta potential and polydispersity were characterized using the HORIBA scientific nano Partica, nanoparticle analyzer SZ-100.

Results indicated that the triptan loaded Chitosan nanoparticles, A_{C5} formulation has entrapment efficiency of 75 ± 0.4%, particle size of 75.3 ± 3.5 nm, Polydispersity index of 0.67 and zeta potential of +25.3 mV, which may be attributed to the positive charges on the polymer matrices (Chitosan) and surfactant.

The effect of different drug to polymer ratios and the amount of cross linker, on average particle size, zeta potential, Polydispersity Index and entrapment efficiency of nanoparticles is shown in Tables 3, and 5. The particle size ranged from 75.3 ± 3.5 nm (Formulation A_{C5}) to 171.3 ± 4.6 nm (Formulation A_{C9}) which was prepared using the higher amount of polymer in the organic phase. The increase in amount of polymer in the organic phase resulted in significant differences in particle size.

The shape and surface morphology of both the formulations were visualized by scanning electron microscopy (SEM). The nanoparticles were spherical in shape and have a smooth surface (Fig. 6).

Nanoparticles of triptans (Chitosan nanoparticles prepared by using ion gelation method) were filled in metered dose nasal spray containers along with suitable excipients, and then *in-vitro* analysis of the drug loaded chitosan nanoparticles from the metered dose spray device (MDS) was carried out.

The tests carried out are clarity test, pH, viscosity, assay of the drug, content uniformity, plume geometry and spray pattern analysis.

The characterization of spray pattern and plume geometry has been applied successfully to a metered dose spray (MDS) device. Using La-Vision, with MDS test chamber, the system captured high quality image of the spray pattern and plume geometry (Fig. 8 and 9).

CONCLUSION

Triptan-loaded Chitosan nanoparticles was successfully formulated via the ion-gelation technique. Among all the formulations, formulation A_{C5} containing drug and Chitosan in the ratio of 1:2 and 0.75 % of TPP as a cross linking agent was selected as the optimum formulation. The optimum A_{C5} formulation had the particle size of 75.3 ± 3.5 nm, Polydispersity index of 0.67 and zeta potential of +25.3 mV, with the high entrapment efficiency 75 ± 0.4 % in nanoparticle formulation.

Abbreviations

TPP: Sodium tripoly-phosphate; IR: Infrared spectroscopy; MDS: Metered dose spray; 5-HT: 5-hydroxytryptamine; PNP: Polymeric nanoparticles; TNP: Triptan nanoparticles; NPS: Nanoparticles; EE: Entrapment efficiency; PS: Particle size; MCC: Microcrystalline Cellulose; Pvt-Ltd: privet limited company; FTIR: Fourier transform infrared spectroscopy; KBr: Potassium Bromide; NaOH: Sodium hydroxide; TNNS: Triptan Nano Nasal Spray; RPM: Revolutions per minute; N: Normality; VS: Versus; NM: Nanometer; ML: Milliliter; MV: Medium Voltage; LRA: Linear regression analysis; SEM: Scanning electron microscopy; UV: Ultraviolet; T: Transparent; FDA: Food and Drug Administration; CPS: Content per spray; A-CNP / A_C: Almotriptan loaded chitosan nanoparticles; Z-CNP / Z_C: Zolmitriptan-loaded Chitosan Nanoparticles; N-CNP / N_C: Naratriptan loaded chitosan nanoparticles; F-CNP / F_C: Frovatriptan loaded chitosan nanoparticles.

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Availability of data and materials

All data generated or analyzed during this study are included in this published article.

Authors' Contributions

FA participated in design of the study, carried out all experiments and drafted the manuscript. LCSR participated in design of the study, interpretation and analysis of data and major contributor in writing the manuscript. LVG Principal Nargund college of pharmacy, Bangalore, India, participated in management and immense support in accomplishing the studies. All authors read and approved the final manuscript.

Competing Interests

The authors declare that they have no competing interests.

Consent for Publication

Not applicable.

Ethics approval and consent to participate

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