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Formulation and Characterization of Nasal Spray of *Mesua ferrea* for Asthma Management

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ABSTRACT

This study focuses on the development and evaluation of a novel nasal spray formulation containing friedelin-loaded chitosan nanoparticles for the effective management of asthma. Mesua ferrea was extracted using ethanol, and phytochemical screening confirmed the presence of key bioactive compounds. Friedelin was isolated through column chromatography and characterized using spectroscopic techniques. Nanoparticles were prepared by ionotropic gelation and optimized based on particle size, zeta potential, entrapment efficiency, and drug-release behavior, exhibiting a biphasic release pattern suitable for sustained delivery. The optimized nasal spray formulation (MNS5) was developed by evaluating surfactant and cosurfactant concentrations and showed acceptable clarity, pH, viscosity, drug content, and diffusion properties. Stability, sterility, spray content uniformity, priming, and repriming studies further confirmed its robustness and suitability for nasal administration. Overall, the friedelin-loaded nanoparticle nasal spray shows significant promise as a patient-friendly, targeted, and efficient therapeutic approach for asthma management.

Keywords: Asthma, Nanoparticles, Mesua ferrea, nasal spray

INTRODUCTION

Asthma is a chronic inflammatory disorder of the airways, characterized by bronchoconstriction, airway hyperresponsiveness, and excessive mucus production, leading to recurrent episodes of wheezing, coughing, and shortness of breath. Despite the availability of conventional therapies, including inhaled corticoste[1] roids and bronchodilators, challenges such as systemic side effects, poor patient compliance, and suboptimal drug bioavailability persist. [2, 3] Consequently, there is growing interest in developing novel, targeted drug delivery systems that improve therapeutic efficacy while minimizing adverse effects. [4]

Natural products have long been explored for their therapeutic potential due to their bioactive compounds with anti-inflammatory, antioxidant, and immunomodulatory properties. [5] Mesua ferrea, a medicinal plant belonging to the family Calophyllaceae, is rich in triterpenoids, flavonoids, and other phytochemicals, with friedlin identified as a potent bioactive compound exhibiting anti-inflammatory and antioxidant activity. [6]

Nanoparticle-based drug delivery offers significant advantages, including enhanced solubility, stability, and targeted delivery to the site of action. Nasal delivery, in particular, provides a non-invasive route with rapid absorption and direct access to the systemic circulation and respiratory tract, making it an attractive option for asthma management. This study focuses on the formulation and characterization of friedlin-loaded nanoparticles in a nasal spray, aiming to develop a novel, effective, and patient-friendly therapy for asthma.

MATERIALS & METHODS

Materials: The materials used in this study included Mesua ferrea collected from the local region of Bareilly district. Various chemical reagents and solvents were procured from reputable manufacturers, including Molish's Reagent, Fehling Solution A and B, hydrochloric acid, and sulphuric acid from Nice Chemicals Private Limited, Chennai. Silica gel was obtained from Hi Pure Fine Chem Industries, Chennai, while ethanol and lead acetate were sourced from Sigma Aldrich Chemie, Germany. Sodium hydroxide used in the experiments was sourced from Ranken, India. All materials were of analytical grade and used as received without further purification.

Extraction and Preliminary Phytochemical Screening:: The powered plant materials of *Mesua ferrea* were separately extracted with 95% ethanol in Soxhlet extractor (hot continuous extraction). The extracts were evaporated under reduced pressure using a Rotary flash evaporator. The extracts thus obtained extract was stored separately in airtight containers and maintained at 4 °C for further use. [7] The extract of *Mesua ferrea* was subjected to a preliminary phytochemical screening to identify the active chemical constituents like alkaloids, glycosides, saponins, steroids, tannin and phenolic compound.

Isolation of active compound by column chromatography: Column chromatography is an adsorption-based separation technique that operates on the principle of differential affinity of compounds toward the stationary and mobile phases. The procedure begins with preparing the stationary phase, usually silica gel or alumina, properly wetted to ensure a uniform interface with the mobile phase. The sample mixture is then carefully loaded without disturbing this layer, and as the mobile phase flows through the column, components migrate according to their polarity: non-polar compounds elute first, followed by moderately polar, and finally the most polar compounds, which interact strongly with the adsorbent. [8] Fractions are collected sequentially, illustrating the effectiveness of column chromatography in isolating pure compounds for further experimental use. [9]

Preparation Of Isolated Compound (Friedelin) Loaded Chitosan Nanoparticles: The isolated compound, Friedelin, was loaded into chitosan nanoparticles using the ionotropic gelation method with sodium tripolyphosphate (STPP) anions at various drug-to-polymer ratios. Chitosan was dissolved in 1% v/v aqueous acetic acid, and the pH was adjusted to 4.8 using 10 N NaOH. Nanoparticles were formed by adding an aqueous STPP solution (1 mg/mL) to the chitosan solution (2 mg/mL) under magnetic stirring at 1000 rpm for 2 hours at room temperature. Friedelin was associated with chitosan by incubating for 20 seconds prior to STPP addition. [10] The resulting mixture was then filtered through a 0.45 µm membrane to remove residual particles. The nanoparticles were concentrated by ultracentrifugation at 12,000 rpm and 4 °C for 20 minutes using a Remi cooling centrifuge (C-24, Mumbai, India). The supernatant was collected, and the unbound Friedelin was quantified using a UV-Visible spectrophotometer (UV-1601, Shimadzu, Japan) at 270 nm.

Evaluation Of Nanoparticles: The particle size, size distribution, and polydispersity index (PDI) of the Friedelin-loaded chitosan nanoparticles were determined using a Zetasizer Nano ZS (Malvern Instruments Ltd, Malvern, UK). A constant sample volume of 1 mL was used for analysis. The nanoparticles exhibit Brownian motion, which scatters light intensity; this change in intensity is detected using suitable optics and a photomultiplier. Samples were appropriately diluted with filtered distilled water to prevent multi-scattering, and the PDI was assessed to evaluate the uniformity of particle size distribution. The zeta potential, indicating surface charge and stability, was measured using the same instrument. [11] Each sample was diluted fivefold with filtered distilled water and placed in a disposable zeta cell. Electrophoretic mobility was converted to zeta potential via the Helmholtz-Smoluchowski equation, and the average of three measurements was reported, with zeta potential limits ranging from -200 to +200 mV. The encapsulation efficiency (EE) and loading capacity (LC) of Friedelin in the nanoparticles were calculated using the formulas:

$$\%EE = [(A-B)]/A*100$$

$$\%LC = [(A-B)]/C*100$$

Where A is the total amount of Isolated compound (Friedelin), B is the free amount of Isolated compound (Friedelin) and C is the weight of nanoparticles.

In vitro drug release studies were conducted using the dialysis bag diffusion technique. Friedelin nanoparticles and drug solution, each containing 5 mg of Friedelin, were placed in a cellulose dialysis bag (MWCO 12,000 g/mol) with minimal dissolution medium, sealed at both ends, and immersed in methanolic phosphate-buffered saline (pH 6.4, 30% v/v methanol) under continuous stirring at 100 rpm at 37°C. Samples were withdrawn at predetermined intervals, replaced with fresh medium, and analyzed by UV spectrophotometry at 258 nm. [12, 13] Experiments were performed in triplicate, and average values were reported.

Formulation Development of Nasal Spray by Nanoparticles

Preparation of Nasal Spray Formulation: The 10 ml sterile nasal nanoparticles was packed into the sterile spray container closure system. The container closure system procured from Aptar Pharma, which was qualified with tip seal technology, with 360 degree possible applications. [13]

Preliminary study of Formulation Parameters of Nasal Solution: The preliminary formulation trials were designed for the selection of surfactant concentration, and co-surfactant amount. The preliminary parameters like clarity, pH, drug content, %diffusion at 10 minutes and viscosity were optimized by varying one parameter at a time, while keeping the others constant, so that the effect of varied parameter could be evaluated. Each batch was repeated thrice (n=3).

Table 1: Composition of preliminary trial for selection of surfactant concentration

Ingredient	Formulation code			
	MNS1	MNS2	MNS3	MNS4
Nanoparticles (ml)	10	10	10	10
Sodium cholate (gm)	0.05	0.10	0.15	0.20
Sodium CMC (gm)	0.01	0.01	0.01	0.01
PEG 400 (ml)	1.50	1.50	1.50	1.50
Glycerin (ml)	0.25	0.25	0.25	0.25
Methyl paraben (gm)	0.035	0.035	0.035	0.035
Water q.s (ml)	10 ml	10 ml	10 ml	10 ml

Table 2: Composition of preliminary trial for selection of cosurfactant concentration

Ingredient	Formulation code			
	MNS5	MNS6	MNS7	MNS8
Nanoparticles (ml)	10	10	10	10
Sodium cholate (gm)	0.10	0.10	0.10	0.10
Sodium CMC (gm)	0.01	0.01	0.01	0.01
PEG 400 (ml)	1.00	1.50	2.00	2.50
Glycerin (ml)	0.25	0.25	0.25	0.25
Methyl paraben (gm)	0.035	0.035	0.035	0.035
Water q.s (ml)	10 ml	10 ml	10 ml	10 ml

Table 3: Composition of Optimized Nasal Solution

S. No.	Ingredients	MNS5
1	Nanoparticles (ml)	10 ml
2	Sodium Cholate	0.10 gm
3	PEG400	1.0 ml
4	Sodium CMC	0.01 gm
5	Glycerin	0.25 ml
6	Methyl Paraben	0.035 g
7	Purified Water	qs to 10 ml

Evaluation of Nasal Spray Formulation [95-98]: The prepared nasal spray formulation was evaluated for several critical quality parameters to ensure its safety, efficacy, and stability. The pH of the formulation was measured using a digital pH meter, and the mean of three readings was considered. Spray content uniformity was assessed by analyzing multiple sprays from individual containers to determine the amount of drug delivered per actuation, expressed as a percentage of the label claim. [15] According to acceptance criteria, no more than 1 of 10 containers should fall outside 80–120% of the label claim, none should exceed 75–125%, and the mean should be within 85–115%. Weight loss of the formulation was monitored at initial, 3 months, and 6 months, with samples stored in various orientations to evaluate the effect of storage conditions. Priming and repriming studies were conducted to determine the number of actuations required before uniform dosing was achieved, considering different storage orientations. Sterility testing was carried out according to USP guidelines using fluid thioglycollate medium, prepared with L-cysteine, sodium chloride, dextrose monohydrate, agar, yeast extract, pancreatic digest of casein, sodium thioglycollate, and resazurin solution; the medium was sterilized, cooled, inoculated with the sample, and incubated at 35 ± 2°C for 14 days, followed by observation for microbial growth. [16, 17] Finally, stability studies were conducted at 25 ± 2°C/60 ± 5% RH for 3, 6, 9, and 12 months, and at 40 ± 2°C/75 ± 5% RH for 3 and 6 months, with evaluation of clarity, pH, viscosity, drug content, sterility, and percentage drug diffusion after specified storage periods.

RESULTS & DISCUSSION

The crude ethanol extract were screened the present alkaloids, flavonoids, glycoside, steroid, tannins and saponins using the simple chemical test as reported in a standard reference book. The pre-phytochemical screening of the ethanol extract of the aerial parts of the plant revealed the presence of several classes of bioactive constituents. Alkaloids tested positive in the Dragendorff, Hager, and Mayer tests, while the Wagner test showed a negative result. Glycosides were confirmed to be present through a positive Keller–Killiani test. For saponins, the foam test was negative, whereas the hemolytic test was positive. Steroids showed positive reactions

in both the Salkowski and Liebermann–Burchard tests. Tests for tannins and phenolic compounds, including the lead acetate and ferric chloride ($FeCl_3$) assays, were negative. Flavonoids were detected, as indicated by a positive Shinoda test.

Figure 1: Qualitative analysis of ethanol extract

Column Chromatography: The column developed with n hexane as solvent system with increasing polarity. First approximately 200ml n-hexane solvent then Ethyl acetate was prepared for eluting column, which is collected in 25 ml volumetric flask and TLC was performed for each volumetric flask. The samples showing the same TLC pattern were mixed. The entire fractions were subjected to TLC using solvent system Ethyl acetate: methanol. Fraction showed single spot was in sufficient quantity chosen for analysis, hence selected for further characterization.

The column chromatography of the ethanol extract of the aerial parts of the plant was carried out using various solvent systems, and the corresponding eluates were analyzed for the number of spots and their Rf values. Successive elution with hexane (50 ml × 4) at a 100% ratio produced no detectable spots. Elution with hexane and ethyl acetate (90:10) yielded two spots with Rf values of 0.213 and 0.45. The hexane–ethyl acetate system at 70:30 produced three spots with Rf values of 0.45, 0.58, and 0.90, while the 50:50 mixture gave three spots with Rf values of 0.54, 0.58, and 0.67. Pure ethyl acetate (100 ml) produced two spots with tailing at Rf 0.54 and 0.67. Ethyl acetate–methanol (95:5) showed two spots without reported Rf values, whereas the 90:10 mixture produced two spots with Rf values of 0.54 and 0.67, followed by another fraction of the same ratio yielding a single spot at Rf 0.45. Further elution with ethyl acetate–methanol (85:15) resulted in two spots at Rf 0.45 and 0.67, and the 80:20 system produced three spots with tailing at Rf values of 0.45, 0.67, and 0.90.

The ethanol extract of *M. ferrea* was purified by CC on silica gel. The isolated compounds were identified as: Friedelin. The identifications of all isolated compounds were achieved by spectroscopic analysis on U.V., IR, ^{1}H-NMR, ^{13}C-NMR, and MS data. These data were also confirmed by comparison with previously reported spectral data. Compound A (Friedelin) was obtained as a white solid, MP. 261.5-263.0°C.

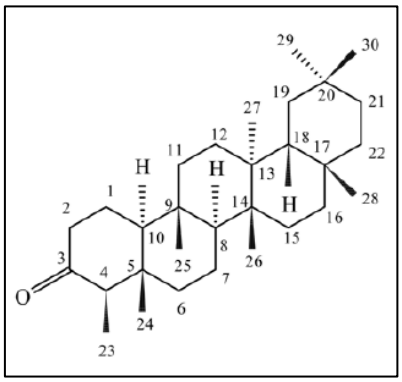

Figure 2: Structure of isolated compound friedelin

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Characterization of the Nanoparticles: The Isolated compound (Friedelin) nanoparticles were prepared by ionic gelation, a gentle technique that involves the mixing of two aqueous solutions at ambient temperature without any harsh chemicals or production stress such as sonication or use of organic solvents. Isolated compound (Friedelin) nanoparticles were prepared at different concentration of drug, polymer and TPP and it was optimized based on its particle size, polydispersity index and zeta potential and the results were depicted in Table 4.

At the concentration of chitosan: STPP ratio of 4:1 it was found that the mean particle size, polydispersity index and zeta potential were 153.1±2.42 nm, 0.121±0.081, +52.1±3.11 mV respectively optimized formulations (Fig. 3 & 4). The NP prepared using optimized parameters had an entrapment efficiency and drug loading of 76.65 ± 3.12% and 28.56± 2.26. High positive values of the zeta potential indicate due to presence of positive charge of amine group of chitosan and it improves the stability of nanoparticles dispersion. Nanosuspension particle size and shape was determined by SEM photomicrographs for the optimized Isolated compound (Friedelin) loaded nanoformulation shown in Fig. 5. The SEM photographs reveals that the particle size were in the range of 170nm to 200nm with spherical shape morphology.

Table 4: Characterization of optimized Isolated compound (Friedelin) nano-formulation.

CS (mg)	STPP (mg)	Drug: Polymer ratio	Particle Size (nm)	PDI	Zeta Potential (mV)	Entrapment Efficiency (%)	Loading Capacity (%)
40	10	1 : 2	153.1 ± 2.42	0.121 ± 0.081	+52.1 ± 3.11	76.65 ± 3.12	28.56 ± 2.26

All values represent mean ± SEM, n=3.

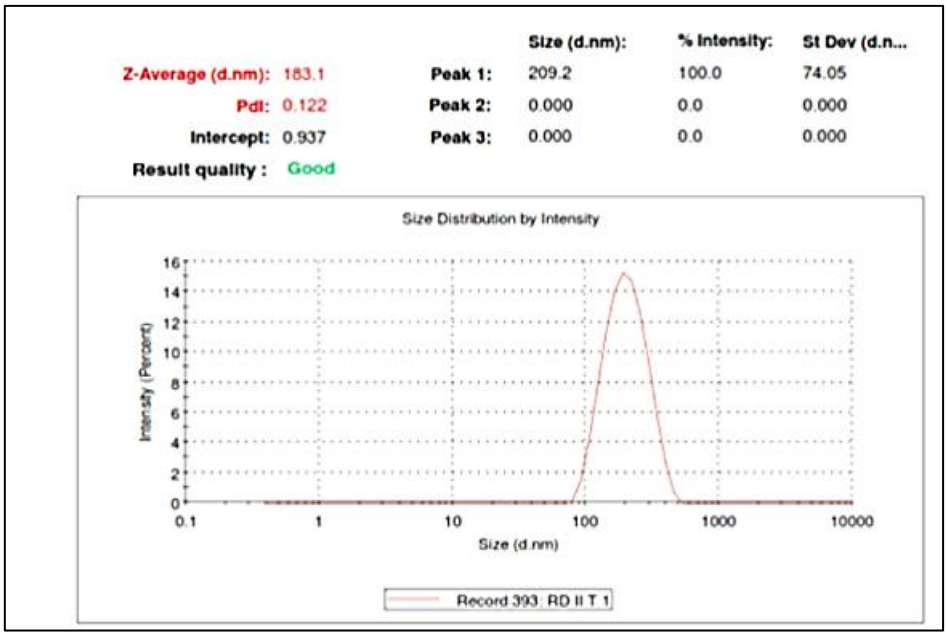

Figure 3: Particle Size and polydispersity index of Friedelin-NP

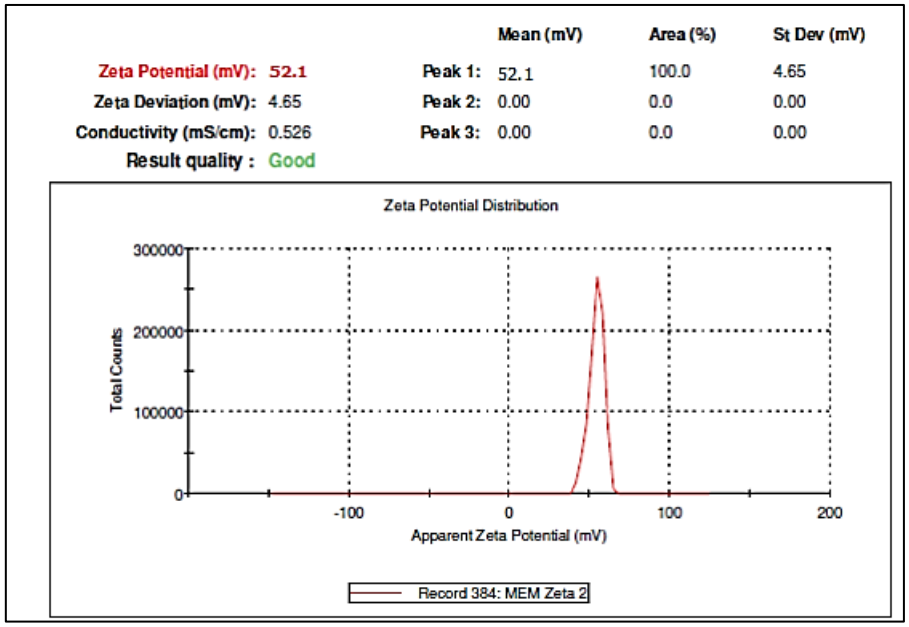

Figure 4: Zetapotential of Isolated compound (Friedelin) NP.

In vitro drug release studies: The in vitro drug release showed a cumulative percentage release of 21.02% to 42.23% for 300 min for Isolated compound (Friedelin) nanoparticles suspension. Isolated compound (Friedelin) loaded chitosan nanoparticles showed a biphasic release pattern. Initial burst release effect occurred within 30min and the remaining amount of drug was released in a sustained manner for a period of 300 min (Fig. 5.12). The initial burst release from nanoparticles is attributed to drug molecules adsorbed on the surface of the nanoparticles which instantaneously dissolve when the particles come in contact with the release medium.

From the release kinetics it can be observed that the release of Isolated compound (Friedelin) from the Isolated compound (Friedelin) NP exhibit Anomalous (non-Fickian) diffusion, and closely follows Korsmeyer-Peppas Model and also highly correlated with Higuchi Model.

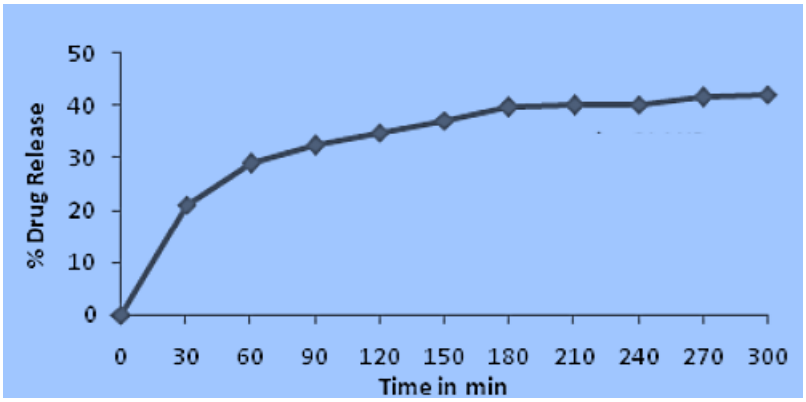

Figure 5: In vitro drug release of Isolated compound (Friedelin) loaded chitosan nanoparticles.

PRELIMINARY STUDY

Surfactant sodium cholate concentration selected: The preliminary formulations (MNS1 to MNS4) were evaluated for clarity, pH, drug content, % diffusion at 10 minutes and viscosity. The four formulations were clear solution. The viscosity of the formulations was obtained as 19 cp, 21 cp, 17 cp and 19 cp for MNS1 to MNS4 batches respectively. The pH of the formulations was observed as 6.45, 6.64, 6.87 and 6.04, while the drug content was obtained as 100.04%, 96.26%, 99.32%and 97.52%

respectively. The % diffusion at 10 minutes was found to be 82.64%, 73.45%, 85.43% and 79.98% for the respective batches. The results for % diffusion for batches MNS1 to MNS4 were show in figure 6.

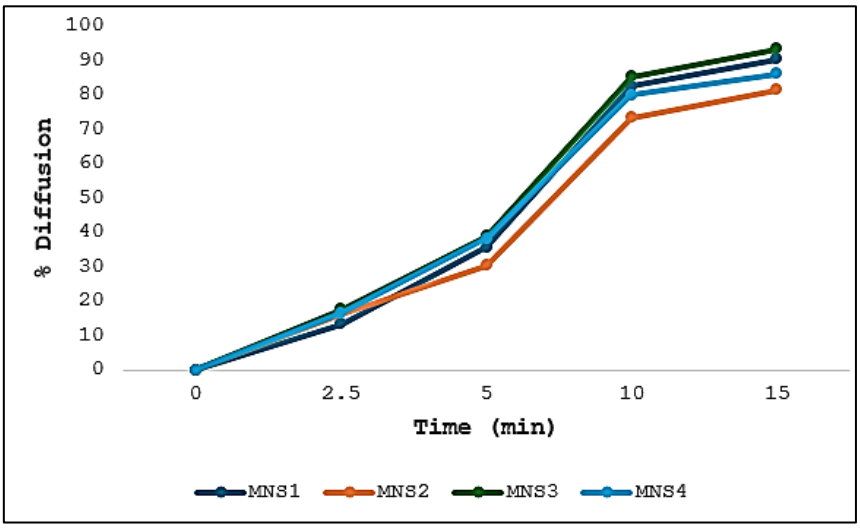

Figure 6: % Drug diffusion of formulation MNS1-MNS4

Increasing the concentration of sodium cholate, the increase in diffusion at 10 minutes was observed upto certain level. Based upon the results, the concentration range 0.02 gm to 0.12 gm of sodium cholate as surfactant was selected for further study.

Co-Surfactant Polyethylene glycol 400 concentration selected: Based on the results for selection of concentration of surfactant, batches were prepared and similar parameters were evaluated for selection of cosurfactant. Polyethylene glycol 400 was used as good candidate for cosurfactant. The batches (MNS5, MNS6, MNS7 and MNS8) displayed clear nasal solution with observed viscosity as 21 cp, 17 cp, 19 cp and 24 cp respectively. The pH of the formulations was 6.11, 6.27, 6.39 and 6.56, while drug content was 99.65%, 100.11%, 97.58 and 98.94% respectively. The % diffusion at 10 minutes was 82.98%, 87.45%, 80.11% and 79.64% respectively. The % diffusion curve for cosurfactant concentration was as per Fig. 7.

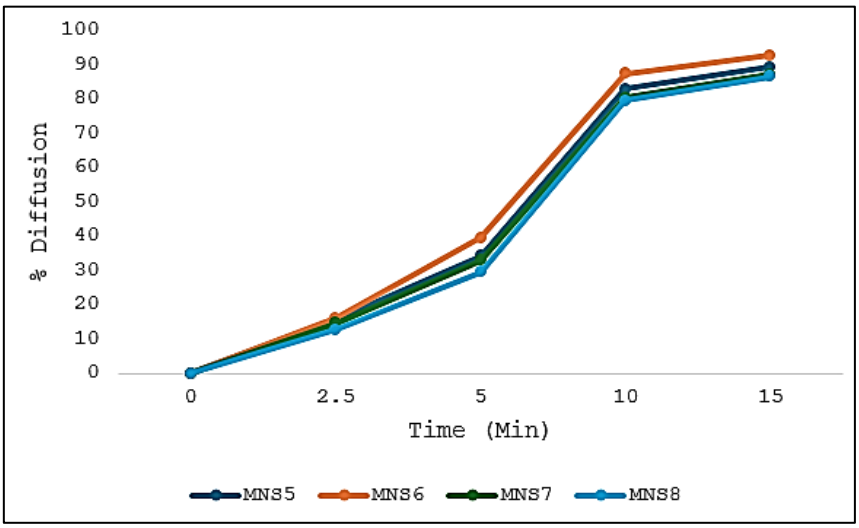

Figure 7: % drug diffusion of formulation MNS5-MNS8

The satisfactory results were obtained for the concentration range of 0.50 ml to 2.00 ml of polyethylene glycol 400. Hence, the concentration range of 0.50 ml to 2.50 ml of polyethylene glycol 400 as cosurfactant was selected for further study.

Evaluation Of Nasal Spray Formulation: The results of optimized batch MNS5 were displayed in Table 5. The optimized formulation of nasal solution for adult use (MNS5) was filled in container closure system and then evaluated for following attributes:

Table 5: Results for batch MNS5

S. No.	Parameter	Result
1	Clarity	Clear solution
2	pH	6.11
3	Assay	99.65
4	% Diffusion (10 min)	82.98 ±0.65
5	Viscosity	21

pH: The higher or lower pH of formulation induces irritation at the application site, hence the pH of the formulation is necessity to be controlled for effective delivery. The pH evaluation of the formulations showed that ONS1 exhibited a pH of 6.80 ± 0.00, while ONS2 and ONS4 both recorded a pH of 6.56 ± 0.05. The pH of ONS3 was measured at 6.50 ± 0.00, whereas ONS5 had a pH of 6.70 ± 0.00. ONS6 displayed a pH value of 6.53 ± 0.05.

Spray Content Uniformity: The formulation needs to be assessed in expressions of secreted dose content uniformity. The control of content uniformity is observed as the global enactment estimation of a system by assessing the spray formulation, valve, manufacturing process and the actuator. Nasal spray formulations are comprised of therapeutically active ingredient dissolved in solutions or blends of excipients in non-pressurized vending machine (dispenser) which transport a spray enclosing a measured dose of the active ingredient. The drug content analysis among different containers showed that formulation ONS1 contained 96% drug content, while ONS2 and ONS3 exhibited drug contents of 94% and 93%, respectively. The drug content evaluation among the same container types revealed that ONS4 had a drug content of 92%, ONS5 showed the highest value at 95%, and ONS6 contained 91% drug content.

Weight Loss: The nasal spray formulation stability was evaluated according to ICH guidelines for conducting stability study of drug products, where the formulation was stored in inverted and horizontal position and then evaluated for weight loss. The containers labelled ONS5, were used for weight loss study Net weight in the product container closure was checked initially and 3 months and 6 months. The results of weight at initial, 3 months and 6 months were presented in Table 6. The results revealed that any change was not observed with respect to horizontal and inverted position for weight loss indicating the integrity of container closure system.

Priming and Repriming study: The priming study was performed on container closure labelled as ONS5 to ONS6 and the results obtained were 98.96 % and 97.32% respectively for first actuation as presented in Table 7. The results of priming study revealed that first actuation itself delivered greater than 95 % of drug content which indicated that only one actuation requirement as priming. The minimum results found for priming study was 97.6 %. Repriming study was implemented on formulation container ONS5 and ONS6 at interval of 5 days, 10 days and 30 days and the results were shown in Table 8. The minimum result for repriming was found to be 96.64% while the maximum repriming result was 100.12 %. It was observed that only one actuation was sufficient for priming as well as repriming to meet the drug product requirement.

Sterility test: The sterility of the optimized formulation (batch MNS5) was carried out according to USP criteria. Sterility test was performed using container ONS5. The sterility test showed no microbial growth, which indicated that the formulation was sterile.

Table 6: Inverted position for weight loss evaluation

Sr. No	Container	Initial Wt	Wt after 3 months	Wt after 6 months
1	ONS5	15 gms	15 gms	15 gms
2	ONS5	15 gms	15 gms	15 gms
3	ONS5	15 gms	15 gms	15 gms
4	ONS5	15 gms	15 gms	15 gms
5	ONS5	15 gms	15 gms	15 gms
6	ONS5	15 gms	15 gms	15 gms

Table 7: Priming study for nasal spray formulation

Sr. No.	Container Number	Priming Result Actuation 1	Priming Result Actuation 2
1	ONS5	98.96 %	98.16 %
2	ONS6	97.32%	97.97 %

Table 8: Repriming study

Repriming study for 5 days				
Cont. No.	**Duration**	**Repriming**	**No of Actuations**	**Drug Content**
ONS5	5 d	Yes	1	97.23%
ONS6	5 d	Yes	1	96.74%
Repriming study for 10 days				
ONS5	10 d	Yes	1	100.12%
ONS6	10 d	Yes	1	97.82%
Repriming study for 30 days				
ONS5	30 d	Yes	1	98.76%
ONS6	30 d	Yes	1	98.64%

Stability study of Optimized Nasal Solution: The stability of optimized nasal formulation (Batch MNS5) was conceded at 25 ± 2 ºC / 60 ± 5% RH for 6 months. The results were displayed in Table 9. No significant change was observed in context of initial results. During and at the completion of the stability study, the Batch MNS5 disclosed drug content comparable to original results. Batch MNS5 also demonstrated the satisfactory appearance, pH, in vitro diffusion, viscosity and sterility at the completion of the stability study.

Table 9: Results of Stability Study (Batch MNS5) at 25 ± 2 °C/ 60 ± 5% RH

Specification	Initial (0 m)	At 3 months	At 6 months
Appearance	Clear solution	Clear solution	Clear solution
pH	6.11 ± 0.0	6.13 ± 0.0	6.15 ± 0.0
Drug content (%)	99.65 ±0.38	99.67± 0.56	99.40 ± 0.62
% diffusion (10 min)	89.32 ±2.11	88.32 ± 1.12	88.02 ± 1.34
Viscosity (cp)	21.00 ± 0.22	22.04 ± 0.11	22.54 ± 0.51

CONCLUSION

The present study successfully demonstrated the development of a novel friedelin-loaded chitosan nanoparticle nasal spray for the management of asthma, integrating natural-product-based therapy with advanced drug-delivery technology. Overall, the findings highlight the potential of friedelin-loaded nanoparticles delivered via a nasal spray as a promising, patient-friendly therapeutic option for asthma, offering improved bioavailability, targeted delivery, and enhanced stability compared with conventional treatments.

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