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PREPARATION AND EVALUATION OF INTRANASAL NANOEMULSION OF ACE-INHIBITOR

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ABSTRACT

Purpose: This study aimed to prepare and evaluate intranasal nanoemulsion of Ace-Inhibitor.

Methods: The mucoadherent Nanoemulsions were developed by first creating a drug nanomulsion with the least amount of external phase and then adding the needed amount of concentrated polymer solution to it to get the desired final concentration. Ace-Inhibitor Mucoadhesive nanoemulsion was made as stated in Ace-Inhibitor-Captropril nanoemulsion preparation, and chitosan was added for 30 minutes while continuously stirring. Droplet size and size distribution analysis, as well as polydispersity index (PDI), were performed on the prepared mucuadhesive nano emulsion.

Results and Conclusion: The F7 batch had the highest% drug concentration of 96.12 0.052% when the% drug content was calculated. The optimal concentration of oil, surfactant, and co-surfactant was required for maximal drug loading in the formulation in order to achieve maximum drug content in the optimised batch (F7). The cumulative% drug release profile of STS from trial batches of formulation was determined to be 83.433.25% for F7 batch using dialysis membrane at PBS (pH 6.4). Batch F7 had the highest percentage of cumulative drug release from a mucoadhesive nano-emulsion.

Keywords: mucoadhesive nano-emulsion, Migrane, intranasal, NDDS, Ace-Inhibitor.

Introduction

Migraine is a common disorder characterized by a unilateral headache, which is often associated with nausea, vomiting, gastrointestinal disturbance, and extreme sensitivity to light and sound. [1] Ace-Inhibitor is the first member of a new class of anti-migraine compounds that act as a specific and selective 5-hydroxytryptamine-1 receptor agonist. Ace-Inhibitor has low bioavailability after oral administration (about 15%), with a large inter- individual variation, although not affected by concomitant food intake. The dose is 50-100 mg orally. *T*max is reached at approximately 2 h and is slightly delayed by the presence of food and during an acute migraine attack.

The pharmacokinetics of Ace-Inhibitor-Captropril is linear over the dose range 25-200 mg, with the exception of rate of absorption. Ace-Inhibitor is extensively metabolized in the liver predominantly by monoamine oxidase type A and is excreted mainly in the urine as the inactive indole acetic acid

derivative and its glucuronide. Total plasma clearance is 1160 ml/min, of which 20% is renal. The elimination half-life is about 2 h. [2]

Nanoemulsions are isotropic mixture of oil, surfactant and water with droplet diameter approximately in the range of 10–100 nm . They are thermodynamically stable and have various advantages as drug carriers, e.g. rapid onset of action, ease of preparation and scale up, drug protection against hydrolysis and oxidation, improvement of drug efficacy and minimizing total dose required as well as the side effects [3,4]

The delivery of a drug to the brain via the oral route can be limited by the blood-brain barrier (BBB), resulting in unsatisfying bioavailability [5]. Thus, an alternative route via the nasal administration has emerged, since the nose-tobrain path can provide a direct brain-targeted delivery of drugs [6]. Moreover, nasal brain transport of nanocomposites has been reported to be an available strategy [7]. Therefore, the development of drug-loaded nanoemulsions via the nose-to-brain path may enhance the brain targeting of a drug and improve the bioavailability. These studies suggest that nanotechnology is a potential approach to enhance the nose-to-brain delivery of drugs.

In recent years, nanoemulsion systems have received increasing attention as an appropriate carrier system for insoluble active compounds to increase their bioavailability and modify drug release characteristic.[8]

In order to enhance bioavailability of Ace-Inhibitor-Captropril, in this study, we attempted to develop and optimizea novel mucoadhesive nanoemulsion formulation.

Materials and methods

Chemicals and reagents:

Ace-Inhibitor-Captropril was purchased from MSN Laboratories Pvt, Ltd. Hyderabad. Oleic acid and Polyethylene sorbitan monolaurate was supplied by S. D. Fine Chemicals, India. All other ingredients were used analytical grade.

Methods:

Preformulation Study

Characterization and confirmation of drug

The drug (Ace-Inhibitor-Captropril) was characterized and confirmed by determination of melting point, Fourier transforms infrared spectroscopy (FT-IR spectroscopy), Differential scanning calorimetry (DSC) and X-raydiffraction (XRD) [9]

METHODS:

Preparation of nanoemulsion:

Captropril nanoemulsion were prepared by titration method using Oleic acid as as oil, carbitol as cosurfactant and tween 20 as surfactant and purified water as continuous phase. Oil phase were mixed with Smix of a particular ratio, Oil and Smix ratio (0-3:3-0) were taken in various ratios (1-9:9-1) and finally titrated with purified water. Water was added to drug loaded internal phase in dropwise manner

under continuous stirring. The compositions which are optically clear have been evaluated further by constructing pseudo ternary phase diagrams.

Preparation of mucoadhesive nanoemulsion:

Mucoadhesive nanoemulsions of Captropril were prepared by addition of mucoadhesive polymer (showing maximum strength, ref section of selection of mucoadhesive agent) such as chitosan optically clear nanoemulsion. Themucoadhesive Nanoemulsions were prepared by first preparing a nanomulsion of the drug using minimum volume of external phase and then adding the required volume of concentrated polymer solution to it such that the required final concentration. Captropril Mucoadhesive nanoemulsion were prepared as described under Captropril nanoemulsion preparation and chitosan was added in a continuous stirring for 30 minutes.

Table 1: Composition of mucuadhesive nanoemusion of Captropril

Batch	**Oils (mL) Cap+IPP**		**Surfactant (mL)**	**Cosurfactant (mL)**	**Drug (mg)**	**Chitosan (mmw) (15mg/2mL)**	**Water (mL)**	**Final Volume (mL)**
F1	0.5	0.5	1	1	20	2	25	30
F2	0.5	0.5	3	1	20	2	23	30
F3	0.5	0.5	4	2	20	2	21	30
F4	1	1	1	1	20	2	24	30
F5	1	1	4	2	20	2	20	30
F6	2	2	1	1	20	2	22	30
F7	**1**	**1**	**3**	**1**	**20**	**2**	**22**	**30**
F8	2	2	3	1	20	2	20	30
F9	2	2	4	2	20	2	18	30

Characterization and evaluation of the mucoadhesive nanoemulsion

Identification Test for Nanoemulsion

- Staining Test
- Dilution Test

Evaluation of mucoadhesive nanoemulsion

Droplet size and size distribution analysis and polydispersity index (PDI)

The average Droplet size of prepared nanoemulsion (MNE F7) was determined in which analyses the fluctuations in light scattering due to the Brownian motion of the particles using a Zetasizer ZS 90, (Malvern Instruments Ltd., UK). The formulation was diluted with double distilled water and light scattering was monitored at 25˚C at a 90˚cangle. All measurements were made in triplicate

Determination of surface Morphology of MNE

The morphology of MNE was visualized using high-resolution Transmission Electron Microscope (HR-TEM, JEM-2100®, Joel Datum Ltd., Boston, USA). An extremely small amount of material is suspended in water/ethanol (just enough to obtain a slightly turbid solution). The solution is homogenized using ultrasonicatorto disperse the particles, A dropof the solution is then pippeted out and cast the drop on carbon-coated grids of 200 mesh the grid is dried and fixed in the specimen holder and Samples were viewed under HR-TEMand photographed at 200kV at different magnification

Zeta potential Determination

The zeta potential parameter is used to characterize the charge on the surface of the oil droplets that plays a vitalrole in determining the stability of the formed nanoemulsion. The formulation (0.1 ml) was diluted 100 times using double distilled water and analyzed using Zetasizer ZS 90, (Malvern Instruments Ltd. UK), which calculatesthe zeta potentialby determining the electrophoretic mobility of the batch F7.

Refractive Index

Refractive index of selected formulations was determined in triplicate using an Abbe-type Refractometer. and compare this value was compared with the standard solution (water).

Transmittance (%T)

The percentage transmittance of 2 mL MNE(s) was checked against distilled water usingUV-VIS spectrophotometer at 227 nm.

pH Determination

The pH of the formulation is an important to factor for compatibility of formulation with nasal mucosa (pH range
4.5 to 6.5). The apparent pH of the formulations was measured bya pH meter (Systronic 362 µ pH system, India)in triplicate at 25° C

Drug Content by using UV spectroscopy

Sumatriptan succinate from MNEs formulations was extracted by dissolving 1 ml of MNE in methanol, 6.4pH phosphate buffer. then 1mL solution was diluted up to 10mL by using methanol and STS content in the methanolic extract was analyzed spectrophotometrically (UV 1700, Shimadzu, Japan) at 227 nm. against the standard methanolic solution of STS

Ex-vivo Mucoadhesive study

Mucoadhesion studies were carried out to ensure the adhesion, or retention time of formulation to the mucosa fora prolonged period of time at the site of absorption. Stronger the bio adhesive force more is the nasal residence time and so increased the bioavailability of the drug because mucoadhesive nanoemulsion adequately adheres on the nasal mucosa. The ratio of the adhered MNEs is expressed as percent mucoadhesion. But if the mucoadhesionis too strong the formulation can damage to the mucosal membrane Mucoadhesion of the prepared mucoadhesive nano-emulsions was measured by previously method with slight modification as here we used goat nasal mucosa instead of using agar. Sheep nasal mucosa was obtained from local slaughterhouse immediately after slaughtering. The mucosa was cut

into longitudinal sections (1× 2 cm) and 100 mg of each formulation was centered on the mucosa and left for 2 min to assure attachment. Mucosal sections were attached to USP disintegration test apparatus (USA) and moved up and down in PB pH 6.4 at 37 1
○C. The time taken by the formulations to separate completely from the mucosa was recorded as residence time (RT).

In-Vitro Drug Release study from MNEs

In vitro diffusion study of mucoadhesive nano-emulsion (MNEs) of optimized batch and PDS, NE is carried out by using Franz diffusion cell apparatus (Fig;1) Franz diffusion cell having 2.0 cm diameter and 20 ml capacity. Dialysis membrane (Himedia) having molecular weight cut off range 12000 – 14000 kDa was used as diffusion membrane. Pieces of dialysis membrane were soaked in phosphate buffer saline (PBS) pH 6.4 for 24 h prior to experiment. Acceptor compartment of diffusion cell was filled with pH 6.4 phosphate buffer saline and dialysis membrane was mounted on cell. The temperature was maintained at 37˚C. The mucoadhesive nano-emulsion equivalent to 2 mg of STS is dispersed in the donor chamber. One mL Samples were periodically withdrawn from the receptor compartment for 8 hours after 15,30, 60, 120,180,240,300,360,420,480 min of the time interval and dilute up to 10 mL with PBS solution for analysis and replaced with the same amount of fresh pH 6.4 phosphate buffer saline to maintaining the sink condition and drug concentration was analyzed by spectrophotometrically.

Model fitting to drug release profile

To study the release kinetics of optimized formulation, data obtained from in- vitro drug release studies were plotted in various kinetic models: zero order (“equation 2”) as the cumulative amount of drug released Vs. time, first order (“equation 3”) as

Ex-vivo permeation study

The ex-vivo permeation from optimized mucoadhesive nano-emulsion batch (F7), Plain drug solution (PDS) and Nano-emulsion (NE) containing drug was comparatively studied.

Isolation of goat nasal mucosa

The freshly excised goat nasal mucosa except for the septum part was collected from the slaughter house in phosphate buffer saline (PBS), pH 6.4. It was cleaned properly by rinsing with PBS to remove adhered tissues onthe mucosal surface and allowed to equilibrate in freshly prepared PBS for 15–20 min. The superior nasal conchawas identified and separated from the nasal membrane.

Ex-Vivo diffusion studies were performed using Franz diffusion cell with a receptor volume capacity of 20 ml through goat nasal mucosa on similar formulations used for in- vitro study (PDS, NE, MNEs). The freshly excisedgoat nasal mucosa, with a thickness of0.2mm and skin 1.77cm2 (measured using Vernier caliper, CD-6’’ CSX digital, Mitutoyo Corp., Kanagawa, Japan) was sandwiched between the receptor and donor compartment. Each donor compartment was filled with formulation equivalent to 5 mg of STS. Receptor compartment was filled withPBS (pH 6.4), and maintain the temperature 37oC with stirring rate 50rpm. Then 1mL sample of each formulationwas withdrawn at 15,30,60,120,180,240,300,360,420,480 min interval, and diluted up to 10 ml with Phosphate buffer and the drug concentration was analyzed Spectro photo metrically at 227nm.

The mean cumulative values for percentage drug diffused versus time were plotted against time (h) individually for PDS, NE, MNEs Amount of drug permeated/unit area of nasal mucosa (mg/cm2)

versus time were plotted against time (h) for PDS, NEs, MNEs and from the slope of individual plot, flux and diffusion coefficients were calculated The data obtained from ex-vivo study was fitted to various mathematical equations of different kineticmodels viz zero order (cumulative percentage of drug release versus time), first order (log cumulative of drug remaining versus time) and Higuchi model (cumulative percentage of drug release versus square root of time).

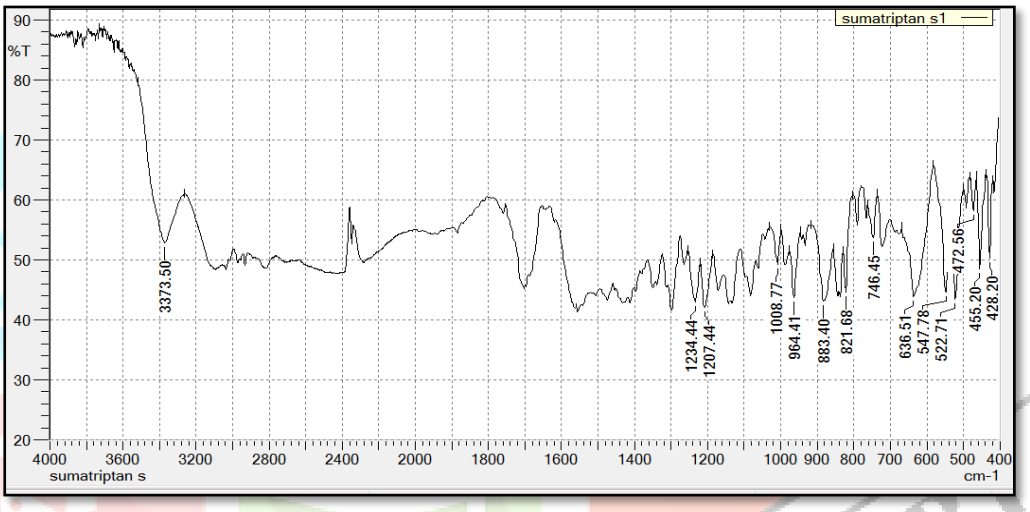
Results and discussion:

Determination of melting point:

The average melting point of the drug was found to be 169.56 ± 0.71 ℃ by using the digital melting point apparatus.

Fourier transform infrared spectroscopy (FT-IR spectroscopy):

Fig 1: FT-IR spectrum of drug



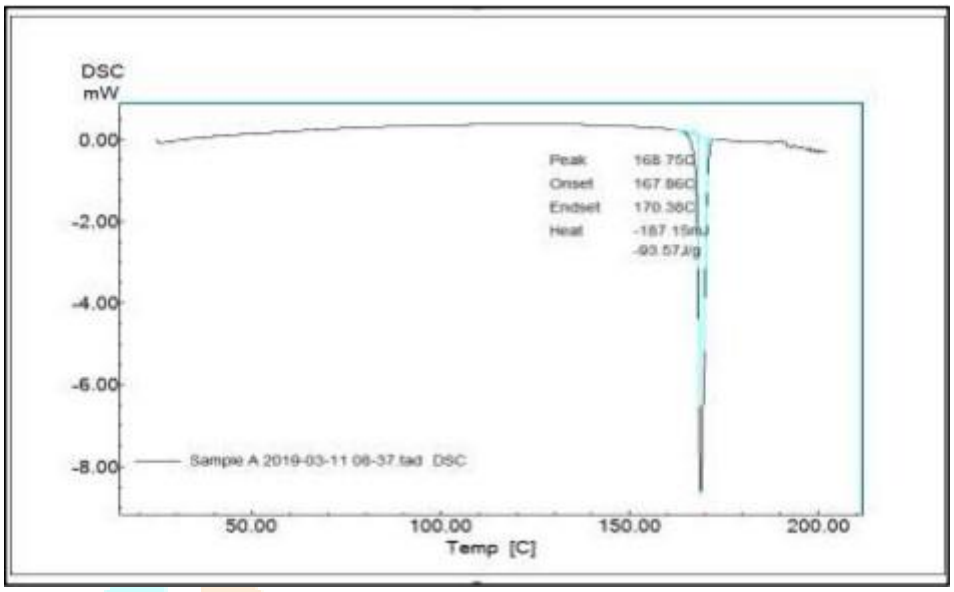
The characteristic peaks of the drug included O-H stretching, C-H stretching, aromatic C=C bending, ester and aromatic C-H bending as shown in table

Table 2: FT-IR spectrum of drug sample

Functional Group	**Vibration**	**Wave number (cm^{-1})(Observed value)**	**Wave number (cm^{-1})(Reported value)**
Alcohol –OH	Stretching	3373.64	3271.10
Alkyl C-H	Stretching	2934.82	2927.37
Aromatic C=C	Bending	1554.69	1562.69
Ester	Stretching/Bending	1300.08	1300.49
Aromatic C-H	Bending	879.58	881.73

Differential scanning calorimetry (DSC):

Fig 2: Differential scanning calorimetry of Sumatriptan succinate



As shown in figure 2 the thermogram of the drug showed a sharp endothermic peak at 168.75℃ with enthalpy
141.48 (J/g). The endotherm signifies the process of melting of thedrug at the temperature since the reported valuefor the melting point of the drug is between 169 – 171 ℃. The DSC result also confirmed the presence of Sumatriptan succinate.

X-ray diffraction

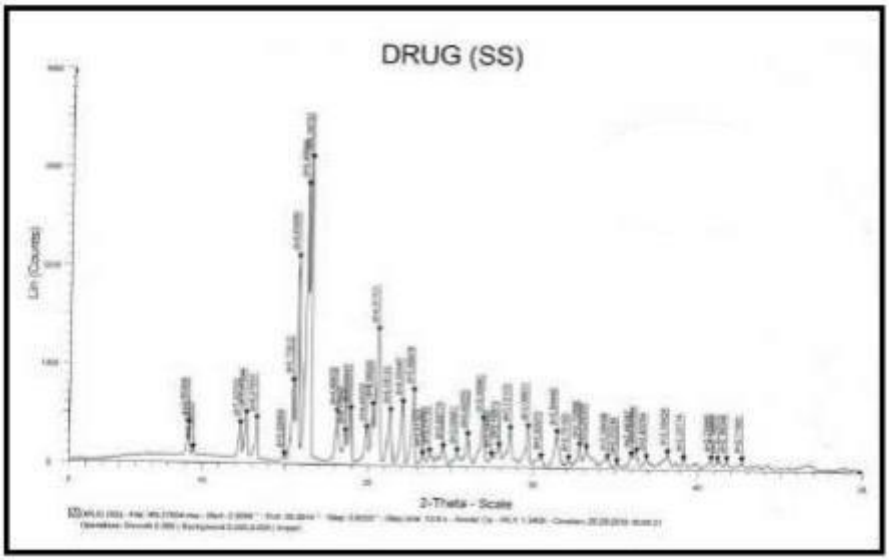
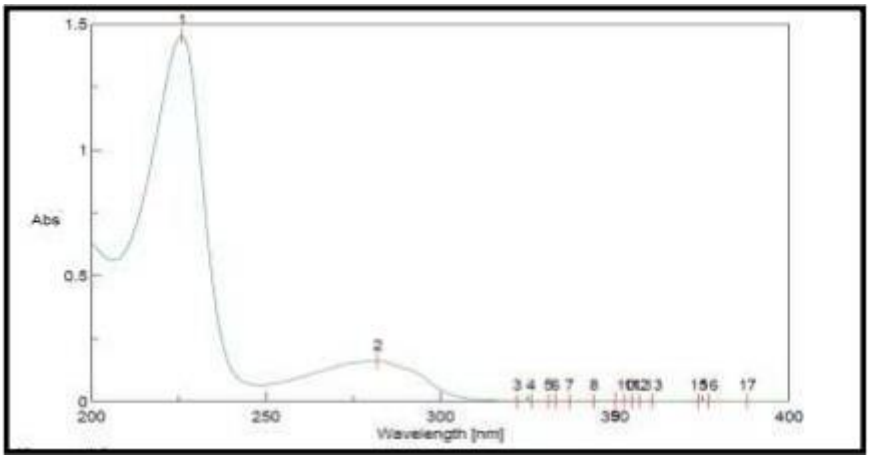


Fig 3: X-ray diffraction spectrum of drug

Ultra-violet visible spectroscopy (UV-Visible Spectroscopy)

Fig 4: Ultra-violet visible spectroscopy of drug



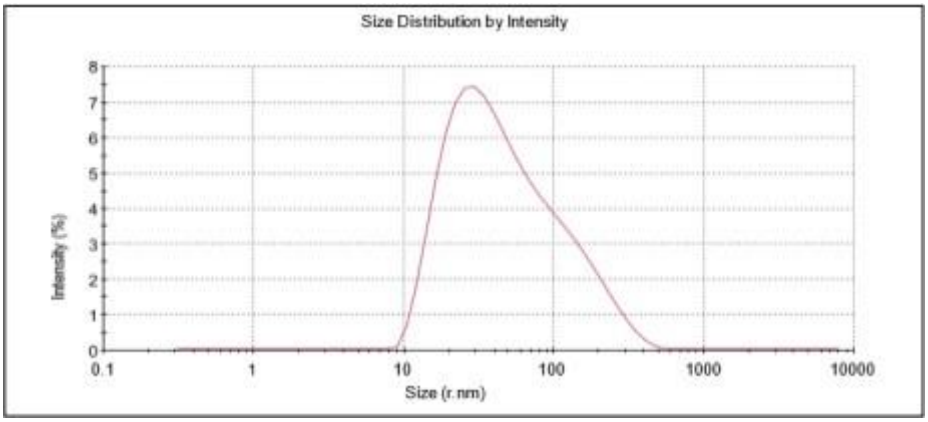
Construction of calibration curve at λmax of the drug in Methanol, Water, Phosphate buffer pH 6.4. The calibration curve of the drug was plotted in water, methanol, and phosphate buffer pH 6.4 at 227 nm in the concentration range of [1-16 (µg/mL)].

Table 3: calibration curve of at λmax

Sr. No.	**Concentration (µg/mL)**	**Absorbance in the water at 227 nm**	**Absorbance in phosphate buffer pH 6.4at 227 nm**	**Absorbance in methanol at 227 nm**
1	2	0.2523	0.0278	0.1421
2	4	0.4278	0.0787	0.3465
3	6	0.642	0.2314	0.4652
4	8	0.8413	0.4155	0.5619
5	10	1.064	0.5965	0.7249
6	12	1.2361	0.7621	0.9164
7	14	1.510	0.9396	1.0429
8	16			1.2978

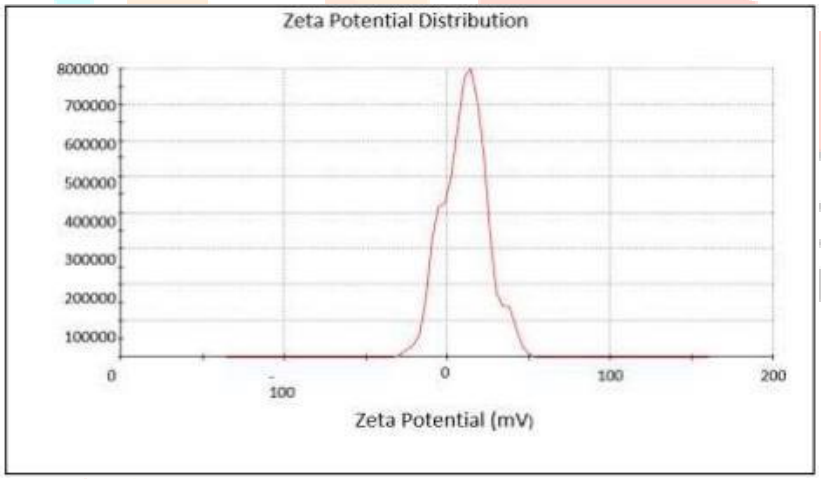
Particle size distribution:

Fig 5: Particle size distribution of Captropril



Zeta potential

Fig 6: zeta potential of Captropril



Percent of Drug Content

The % Drug content was carried out for that F7 batch showed highest % drug content of 96.12 ± 0.052 %. The optimum concentration of oil, surfactant, and co-surfactant was essential for maximum drug loading in the formulation to give maximum drug content of optimized batch (F7). All formulation variables showed a significant effect on drug content.

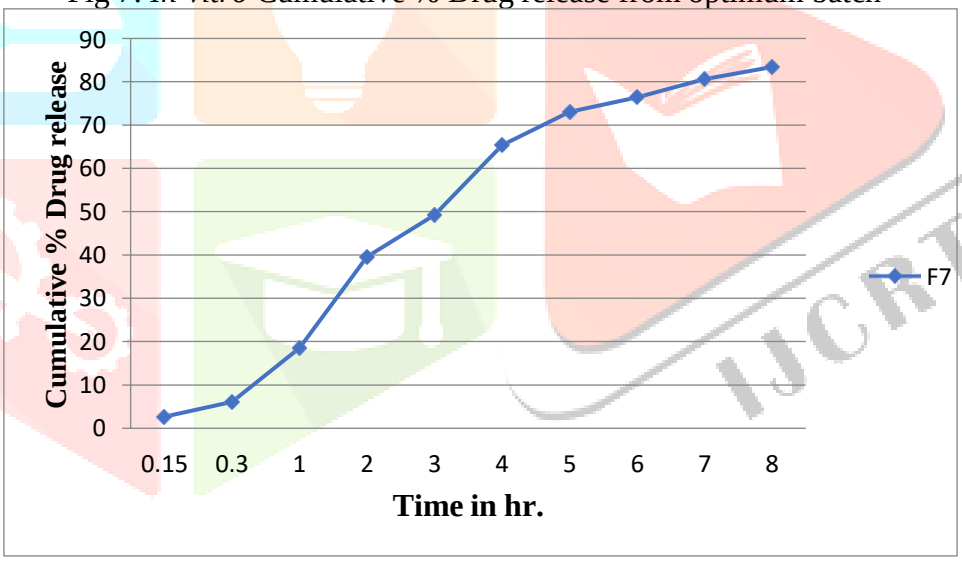
***In-vitro* drug release from optimum batch**

The cumulative % drug release profile of STS from trial batches of formulation was carried out and it was found to 83.43± 3.25% for F7 batch through the dialysis membrane at PBS (pH 6.4). Batch F7 showed the highest % cumulative drug release from mucoadhesive nano-emulsion. It followed first release kinetics models with negligible burst effect.

Table 4: *In-vitro* Cumulative % Drug release

Cumulative % Drug release	
Time in hr.	**F7 (%)**
0.15	2.779± 3.22
0.3	6.178± 3.56
1	18.541± 4.21
2	39.608± 5.69
3	49.246± 4.13
4	65.449± 2.31
5	73.106± 3.65
6	76.427± 3.48
7	80.625± 4.21
8	83.432± 3.25

Fig 7: *In-vitro* Cumulative % Drug release from optimum batch



Ex-vivo drug permeation from trial batches

Results of *ex-vivo* drug permeation from the prepared trial MNEs formulation was studied on goat nasal mucosa for 8 hr. by using 6.4 pH phosphate buffer solution. Formulation F7 exhibited good drug permeate profile due to the smallest droplet size (22 nm) of nano-emulsion with favorable evaluation parameters. Hence, the formulation F7 was chosen as an optimized formulation to see the permeation through the nasal mucosa and it was compared with the plain drug solution and nano-emulsion. It was observed that permeation of drug from optimized MNEs F7 was 89.121± 4.26% at the end of 8 hr.

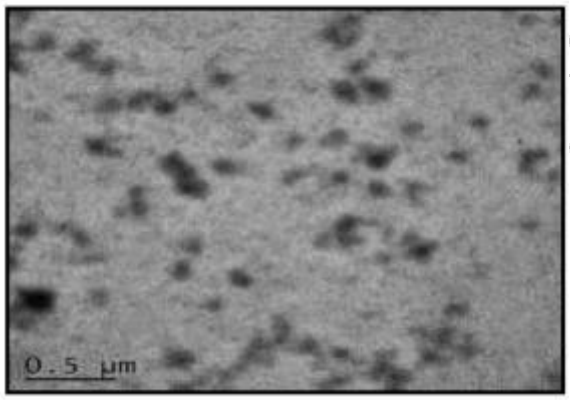
Table 5: *Ex-vivo* Cumulative % Drug release

Cumulative % Drug release	
Time in hr.	**F7 (%)**
0.15	4.360± 2.38
0.3	8.089± 3.22
1	18.099± 4.19
2	41.910± 2.63
3	57.592± 2.89
4	68.482± 3.63
5	79.660± 4.03
6	84.371± 3.59
7	87.316± 2.67
8	89.121± 4.26

Determination of surface Morphology of MNEs

The morphology of the droplet was studied using TEM (HR-TEM, JEM-2100®, Joel Datum Ltd., Boston, USA 200kv), which showed the presence of distinct spherical droplets,. A good correlation was obtained in droplet sizein the range of (140 nm) as observed by zeta sizer.

Fig 8: Surface Morphology of MNEs



***Ex-vivo* Mucoadhesive study**

The detachment stress (an indicator of ex vivo mucoadhesive strength or retention time) for optimized F7 batch of NEs and MNEs formulations was performed using USP disintegration test apparatus (USA) in phosphate buffer pH 6.4 at 37±2 °C and it was found to be 12±6 sec and 48±3 sec respectively. Results revealed that the retention time of MNEs was 4.4-folds greater compared to NEs. The stronger mucoadhesive strength of MNEs over NEs may be attributed to the strong electrostatic interactions between cationic amino groups of chitosan, orienting outside from the MNEs globules with anionic sialic and sulfonic acid moieties contained in the mucin on nasal mucus layer. The high mucoadhesive strengths and retention of MNEs would be beneficial for a high residence time of the formulation over the nasal mucosal membrane

Table 6: Retention time of F7 MNEs and NEs formulation

Formulation	Residence Time in (Sec)
NEs	12±6
MNEs	43±3

Data is expressed as mean ± S.D., (n = 3)

MNEs: Mucoadhesive nano-emulsion NEs: Nano-emulsion without Chitosan

In-Vitro Drug Release study

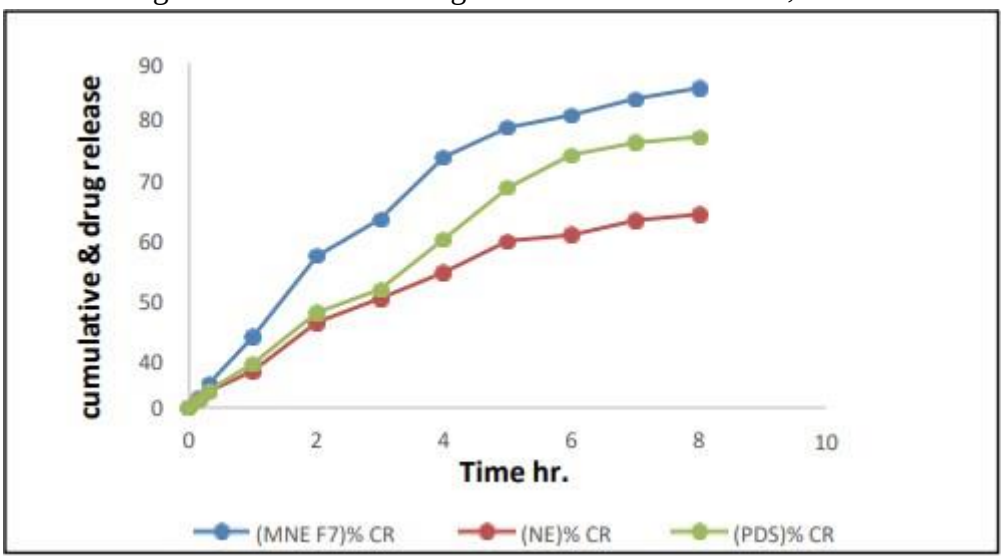
The comparative release profile of STS from optimized batch (F7) of mucoadhesive nano-emulsion (MNEs) nano-emulsion (NEs) and plain drug solution (PDS) through the dialysis membrane in phosphate buffer solution (pH 6.4) is shown in Fig 57 and Table 41. The release pattern of optimized nano-emulsion appears to be fast release with negligible burst effect. The cumulative % drug release through dialysis membrane of MNEs, NEs, PDS formulation was found to be in the range of 50-83% after 8 hr. as shown in table 41 and the values are 83.432± 2.13 %, 50.482±3.21%, & 70.701±6.03% respectively. The R^2 value 0.9895 shows first-order kinetic for F7 batch. Hence, we can conclude that the mucoadhesive nano-emulsion gives higher & drug release from the dialysis membrane than NEs and PDS.

Table 7: Cumulative Percent drug release of MNEs NEs, PDS

Time in hr.	Cumulative % Drug release		
	MNEs F7 (%)	NEs (%)	PDS (%)
0.15	2.779±0.23	2.039±0.53	2.029±0.23
0.3	6.178±0.63	4.261±0.34	4.456±0.28
1	18.541±2.65	9.562±2.61	11.402±2.13
2	39.608±3.01	22.340±4.10	24.887±2.69
3	49.246±0.41	28.460±2.03	30.888±4.50
4	65.449±0.63	35.361±2.01	44.071±4.23
5	73.106±5.01	43.536±4.01	57.467±3.25
6	76.427±1.02	45.117±0.61	65.975±3.61
7	80.625±0.56	48.863±0.36	69.246±2.31
8	83.432±2.13	50.482±3.21	70.701±6.03

Data is expressed as mean ± S.D., (n = 3)

Fig 9: Cumulative % Drug release from MNEs NEs, PDS



Stability study

A sample of MNEs (F7) was subjected to Stability studies for a period of 1 month at RT and at refrigerated temperature (4°C). At every 10 days, samples were withdrawn and analyzed for physicochemical parameters. At the end of the month, the comparative study was tabulated and any change in macroscopic appearance like % Transparency, homogeneity, phase separation, turbidity, pH, viscosity, drug content % drug released, etc. was evaluated. The result is shown in Table 44. No significant differences between the initial and respective value during the study and at the end of 1 month. Changes were negligible enough to conclude that the prepared MNEs formulation remained stable throughout the stability period. It can be concluded that with the value of zeta potential of optimized batch (17.7mV), the formulation could remain stable.

Table : Stability study of optimized batch

Stability parameter	**Temp**	**Test period**			
		Initial	**10 Days**	**20 Days**	**30 Days**
Phase separation	RT	No	No	No	No
	4 °C	No	No	No	No
% T	RT	93.17±0.127	93.14±0.213	93.01±0.136	92.24±0.125
	4 °C	93.17±0.127	93.10±0.121	92.23±0.312	92.01±0.213
pH	RT	5.62±0.04	5.58±0.01	5.52±0.23	5.50±0.41
	4 °C	5.62±0.04	5.60±0.369	5.55±0.401	5.49±0.89
Drug Content	RT	96.12±0.052	96.01±0.089	95.89±0.56	95.21±0.01
	4 °C	96.12±0.052	96.08±0.0367	94.69±0.245	94.21±0.369
Viscosity	RT	51.7±2.82	51.6±1.23	52.23±2.01	52.89±2.68
	4 °C	51.7±2.82	50.1±1.08	51.98±3.02	51.7±3.46
% Drug release	RT	83.43±3.25	83.01±2.46	82.23±2.69	81.63±2.13
	4 °C	83.43±3.25	83.27±1.20	82.14±3.89	83.16±2.10

Data is expressed as mean ± S.D., (n = 3)

Conclusion:

Nano-emulsions and mucoadhesive nano-emulsions were successfully prepared and evaluated. All parameters are shows optimum results and the optimum concentration of oil, surfactant, and co-surfactant was essential for maximum drug loading in the formulation to give maximum drug content of optimized batch (F7). Results of *ex-vivo* drug permeation from the prepared trial MNEs formulation was studied on goat nasal mucosa for 8 hr. by using 6.4 pH phosphate buffer solution. Formulation F7 exhibited good drug permeate profile due to the smallest droplet size (22 nm) of nano-emulsion with favorable evaluation parameters. The detachment stress (an indicator of ex vivo mucoadhesive strength or retention time) for optimized F7 batch of NEs and MNEs formulations was performed using USP disintegration test apparatus (USA) in phosphate buffer pH 6.4 at 37±2 °C and it was found to be 12±6 sec and 48±3 sec respectively. A sample of MNEs (F7) was subjected to Stability studies for a period of 1 month at RT and at refrigerated temperature (4°C).

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