DESIGN, CHARACTERIZATION AND EVALUATION OF NOVEL MUCOADHESIVE NASAL INSERTS FOR THE TREATMENT OF MIGRAINE

Bindiya Amin[a], Jobin Jose[a]* and Lalit Kumar[b, c]

(Received 21 January 2021) (Accepted 22 August 2023)

ABSTRACT

Recently, nasal administration has gained more attention as a safe way for delivering the active ingredients used to treat migraines. Strategies can be implemented by changing aspects like the medicines, delivery vehicle, and other components to get around the obstacles associated with conventional therapies. This research work aimed to explore the potential of lyophilized nasal inserts of eletriptan hydrobromide for the treatment of migraine attack. Here, the lyophilization technique is used for the nasal inserts preparation. The developed inserts were tested for various characterization studies,such as mucoadhesive examinations, water uptake, *in vitro* drug release, *in vitro* cytotoxicity, *ex vivo* investigations and stability. The inserts showed acceptable pH values, satisfactory mucoadhesion potential and excellent water uptake activity. *In vitro* release data for the formulations followed first-order kinetics. The cytotoxicity studies showed that there was a reduction in cell toxicity of drug embedded in the inserts when compared to the pure drug. When the prepared nasal inserts were used for treatment, there was no significant impact on the epithelium of nasal mucosa. The novel lyophilized nasal inserts of eletriptan hydrobromide could be a better alternative for the treatment of migraine.

Keywords: Lyophilization, inserts, mucoadhesion, eletriptan hydrobromide, cytotoxicity

INTRODUCTION

Nasal delivery has drawn a lot of attention recently, as a crucial alternative method for delivering active molecules to the desired site. The applications of nasal cavity in both topical and systemic therapy are enabled because of its greater permeability and less enzymatic environment[1]. As it is a painless procedure, easy for self-administration, and doesn't require high sterility conditions, it has become a perfect alternative to the parenteral route[2,3]. To reach a substantial concentration in the cerebrospinal fluid, these intranasal medications are carried along the olfactory sensory nerve cell[4]. Nasal inserts are small devices and light in weight. Nasal inserts are novel, bioadhesive, unit dosage forms which can be prepared by various methods to ensure the extended release of the medicament. The mechanism of action of nasal inserts is that after administration, it can interact with highly vascularized nasal mucosal membrane, and it will absorb the nasal fluid to form a gel[5].

The nasal inserts were prepared by lyophilization technique. Through one or more holes punctured in the impermeable membrane covering the container, the substance inside is lyophilized completely in this procedure. The holes are large enough to allow water vapor from the compound contained in the container to escape[5]. The lyophilization process involves creating nasal inserts with a drug-embedded polymeric matrix that resembles spongy material. The main active component is released in a controlled manner from the transformed gel formed after absorbing fluid from the nasal mucosal membrane. The release of the drug occurs only when nasal insert comes in contact with vascularized nasal mucous tissue layer. As these nasal inserts are not rigid in structure, they have specific advantages such as easy to use, pain-free administration and better patient compliance[6].

A migraine is a chronic, excruciating headache that usually affects one side of the brain, which is pulsating in

[a] NITTE (Deemed to be University), NGSM Institute of Pharmaceutical Sciences, Department of Pharmaceutics, Mangalore- 575 018, Karnataka, India

[b] Department of Pharmaceutics, Manipal College of Pharmaceutical Sciences, Manipal Academy of Higher Education, Manipal - 576 104, Karnataka, India

[c] Department of Pharmaceutics, National Institute of Pharmaceutical Education and Research, Hajipur, Vaishali - 844 102, Bihar, India

*For Correspondence: E-mail: jjmattam07@gmail.com

https://doi.org/10.53879/id.60.10.12854

nature which may last even upto 72 h. An anti-migraine drug is a medication intended to minimize the intensity and cure a migraine headache[4]. The treatment of a migraine headache may vary from over the counter medicines to prescription medications. Triptan classes of drugs like sumatriptan, almotriptan over-the-counter, eletriptan, and zolmitriptan are most widely used in the treatment of migraine. Eletriptan reduces swelling of the blood vessels surrounding the brain which is associated with a migraine, and it also acts as 5-HT1 receptor agonist[3]. There are several different nasal formulations available, including nasal spray, nasal gel and nasal drops. However, these formulations are unfavourable for many drugs due to their poor absorption and limited bioavailability. Lyophilized nasal inserts have better water uptake capacity as well as better mucoadhesion, which prolongs the release of the drug[7]. This research work was aimed to formulate lyophilized nasal inserts incorporated with eletriptan hydrobromide, as the therapeutic agent and to evaluate its potential as a cutting-edge delivery method for the treatment of migraine.

MATERIALS AND METHODS

Eletriptan hydrobromide, was a gift sample received from USV Private Ltd., Ahmedabad, India. Gum xanthan and guar gum were bought from Loba Chemie Pvt. Ltd., Mumbai, India. Analytical-grade substances were employed for all other compounds and reagents.

Preparation of nasal inserts

By precisely weighing appropriate amounts of xanthan gum and guar gum, various polymeric solution ratios were prepared as shown in Table I. The polymers were dissolved in distilled water in one-third of required volume. Eletriptan hydrobromide was dissolved in a solvent and was combined with the polymer solution[8]. The resulting solution was continuously mixed with the desired volume of distilled water to obtain a uniform dispersion. The prepared dispersion was then kept for 24 h under stirring. The dispersion was filled into blister moulds shaped like bullets, frozen at -25°C for 4 h, and then lyophilized in lyophilizer (Esquire biotech freeze dryer; sample temperature -59.4^{0}C, condenser temperature -20.4^{0}C the pressure 40mbar) for 24 h. The inserts were lyophilized and then put in desiccators[9].

Nasal inserts characterization

To assess the general characteristics, visual inspection of each implant was performed. The size of the inserts was measured by the use of digital vernier calliper. The weight of the inserts was measured by using electronic balance[10].

Table I: Formulation of nasal inserts

Formulation code	Drug (mg)	Xanthan gum: Guar gum	Xanthan gum (mg)	Guar gum (mg)
F1	100	1:1	1000	1000
F2	100	1:2	666.66	1333.33
F3	100	1:3	500	1500
F4	100	2:1	1333.33	666.66
F5	100	3:1	1500	500
F6	100	4:1	1600	400

Drug content estimation

The determination of drug content of nasal inserts was done by solubilizing the required quantity of formulations in simulated nasal fluid. Simulated nasal fluid was prepared as follows : by accurately weighed $CaCl_2$ (0.59g), NaCl (8.77g), and 2.98 g of KCl were dissolved in small volume of distilled water in volumetric flask of 1000 mL and volume was adjusted. Finally, by utilizing phosphoric acid, the pH was adjusted to 5.8, and it was continuously stirred until the inserts were completely dissolved[10]. After filtering the mixture, the amount of eletriptan hydrobromide was calculated using ultraviolet spectrophotometer at 216 nm after validation. The results are given in Table II.

Surface pH

Agar was dissolved in simulated nasal fluid to prepare an agar solution (2% w/V). It was then transferred into a petri dish and kept for some time to form a gel. The agar medium's surface was covered with pre-weighed nasal inserts. By using pH meter, the pH of all formulations was estimated. The surface was in contact with the electrode of the pH meter which was left for one minute to equilibrate before the pH of the formulations was measured at least three times, with the average being calculated[11].

Hydrophilicity of nasal inserts

This study was performed to measure their, capacity for moisture absorption. For this, inserts were weighed and kept on the surface of saturated ammonium chloride solution for 48 h at room temperature. Then, the inserts were weighed again and again, to give an idea about hydrophilicity of inserts[12].

Water uptake studies

In a simulated nasal fluid medium, an appropriate household sponge was soaked. The soaked sponge (7× 3.5× 3 cm) was transferred to a petri plate containing

Table II: Characterization of nasal inserts

Formulation code	pH± SD	Thickness (mm) ± SD	Drug content (%) ± SD	Hydrophilicity of nasal inserts (%) ± SD	Water uptake (%)
F1	6.1±0.24	2.1±0.24	79.5±0.23	20±0.54	780±3.53
F2	6.2±0.23	2.5±0.33	75±0.38	18±0.24	619±4.42
F3	6.2±0.36	2.3±0.78	69±0.43	14±0.18	565±4.38
F4	6.4±0.38	2.5±0.52	90±0.64	25±0.26	950±2.59
F5	6.2±0.21	2.2±0.51	82.5±0.28	22±0.58	785±3.01
F6	5.8±0.57	2.2±0.54	76.5±0.35	19±0.36	621±2.69

simulated nasal fluid as a medium. The soaked sponge was half dipped in the medium and was continued throughout the experiment. A previously soaked square filter paper of size (3cm×3cm) was kept above the sponge for half an hour, and then the nasal inserts which were previously weighed were kept on top of the filter paper. At a predetermined interval of 1 h, the water uptake of nasal inserts was noted and continued till 8 h. By using the equation given below, the percentage of water uptake was determined[12].

$$\% \text{ Water uptake (mg/mg)} = \frac{W_w - W_d}{W_d} \times 100$$

where,

W_w= Weight of wet inserts; W_d = Weight of insert before uptake of water.

Mucoadhesion of nasal inserts

Accurately weighed 100g of hot agar/mucin solution(1:2) was prepared by using pH 6.6 phosphate buffer solution and then transferred to a petri dish. This solution was kept at 4-8 °C for 3 h to form a gel. Once the gel was formed, the gel in the petri dish was maintained at 22 °C in the test condition for 1 h to achieve the equilibrium conditions. The pre-weighed inserts were kept on top of the prepared gel. The downward displacement of inserts was measured in cm when the petri dish was positioned vertically for 6 h. It was discovered that the displacement of inserts and adhesive potential were inversely connected[13].

Differential scanning calorimetry (DSC)

Whether the drug and excipients were compatible was determined using differential scanning calorimetry. After calibrating the instrument with indium, DSC thermograms of pure drug, physical mixture and prepared nasal inserts were collected. 2 mg of different samples were weighed and sealed. Then it was placed in pin holed aluminium pan under standard conditions[14].

X-ray diffraction studies (XRD)

Using a Bruker AXS D8 Advance Germany under predetermined conditions, the XRD patterns of the samples and lyophilized nasal inserts were recorded. 30 mA current was applied at 2θ angle range with a 3°/min scanning rate[15].

Scanning electron microscopy (SEM)

The surface morphology and particle size of lyophilized inserts were examined using SEM (Zeiss, Sigma). Minute quantities of lyophilized inserts were slit open using a blade to reveal the inner anatomy of the insert, and it was firmly placed on standard specimen stub, dried for 24 h, and sputter coating was done with gold before evaluation[15].

Drug release study and kinetic analysis

In vitro release studies were done in USP type 1 dissolution apparatus which was loaded with nasal inserts of eletriptan hydrobromide. The simulated nasal fluid was transferred into the dissolution vessel and kept at 37±0.5 °C. The inserts which were weighed previously were placed in the basket and dipped into the medium, and the shaft was rotated at 75 rpm. About 3 mL of sample were taken out for 8 h at specified intervals and the equal volume of fresh sample was added to sustain sink conditions[16].The sample analysis was performed using UV spectrophotometer set at 216 nm[17].The experiment was performed in triplicate[18].The obtained results were fitted to different kinetic models and contrasted to understand the drug release pattern.

Histological investigation

The histological examination was conducted by using isolated nasal mucosa of a goat. The freshly separated nasal mucosa was cut into three pieces and was kept in contact for 2h with isopropyl alcohol as positive control, 0.9% w/V NaCl as negative control, and lyophilized nasal inserts respectively. Hematoxylin and eosin staining of

isolated tissues was carried out after cleaning with distilled water. Then, the nasal mucociliary was isolated and checked by the pathologist using an optical microscope[17].

Ex vivo permeation study

An isolated nasal mucosa was utilized for this study. Goat nasal mucosa was obtained from the butcher shop. For better results, permeation studies were carried out soon after dissection of the mucosal layer. Lyophilized nasal inserts loaded with eletriptan hydrobromide, which was in contact with the mucous membrane, were kept in the donor compartment. Simulated nasal fluid was taken as receiver medium and maintained at 37 °C with 50 rpm stirring speed. The perfect sink condition was kept throughout the trial, and samples were taken out at predetermined intervals up to 8 h. With the help of UV spectrophotometer, the total permeated drug amount from nasal mucosa was determined[18].

In vitro cytotoxicity studies

Utilizing the MTT test, *in vitro* cytotoxicity investigations were conducted. The toxicity of nasal inserts was checked on human nasal septum cell line; RPMI2650. Dulbecco's Modified Eagle's medium (DMEM) was provided to the cells and 1.0 x 10^5 cells were found to be present per mL of the medium. The cells were kept overnight for incubation at 37 °C. Triplicate wells were made for each of the samples like control (medium), pure drug, and nasal inserts. The samples were then added to the respective wells. MTS reagent (Promega, USA) of about 20 µL was added to each of these wells. After the addition of MTS reagent, the incubation of plate was done for 4h at 37 °C. Optical densities of formazan crystals' were measured at 570 nm after dissolving in 200 µL DMSO solution[17].

STABILITY STUDIES

Stability studies were carried out for 3 months at both ambient temperature (25 ± 2°C; RH 65 ± 5%) and high temperature (40 ± 2°C; RH 75 ± 5%). Various parameters like appearance, pH and drug content were examined by withdrawing samples of the formulations in each month[15] and testing.

STATISTICAL METHODS

Standard deviation (SD) was used to express all data as mean values. Using SPSS 16.0, one-way analysis of variance (ANOVA) was done to analyse the data. The cell viability percentage mean in relation to the control (100%) plus the standard deviation was used to express the cytotoxicity data.

RESULTS AND DISCUSSIONS

Formulation of nasal inserts

Nasal inserts were successfully formulated by lyophilization technique as mentioned previously. These inserts were off-white (Fig. 1), and they were further evaluated for various parameters.

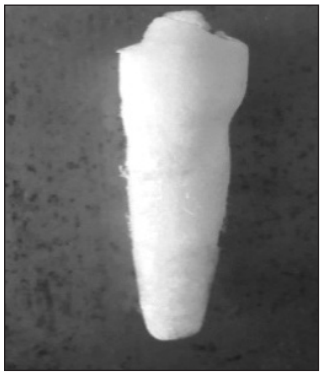


Fig. 1: Formulated nasal insert

Evaluation of nasal inserts

Thickness and weight of the inserts

All the nasal inserts that were developed had a thickness of about 2.5 cm (as shown in Table II). The weight of the formulated inserts was found to be in the range of 20-25 mg. The varying ratios of polymers utilized could be the reason for the variance in thickness and weight.

Drug content estimation

The determination of drug content was performed for the formulated nasal inserts, and the values are mentioned in Table II. The drug content in the formulated inserts was found to be in the range of 75-90%.

Surface pH

The developed nasal inserts were found to have a surface pH between 5.8 and 6.4. The pH was within the acceptable limits and results are shown in Table II. The combination of two different polymers, xanthan gum and guar gum, showed acceptable pH values, hence there must be less chance to cause any irritation to the nasal mucosa[18].

Hydrophilicity of nasal inserts

The hydrophilicity of nasal inserts increases along with the polymer's capacity to absorb moisture. By performing this study, the formulation was found to be sustainable in the nasal cavity at humid environment. The insert's ability

to absorb moisture determines its hydrophilicity, and the results are shown in Table II.

Water uptake study

This study was conducted to determine the level of hydration and the gel transformation of the nasal inserts when inserted into the nasal cavity.To make sure that inserts adhere to the nasal mucosa, the gel must be formed. The water uptake was found to be 565-950% and is shown in Table II. The numerous functional groups included in the hydrogels affect how well they can absorb water. All the formulations showed a similar pattern of water uptake in the initial period, and this may be due to slow hydration initially or may be due to the structure of nasal inserts and lack of void space. After the initial hydration process, there was an increase in the uptake of water by nasal inserts, due to its spongy nature at higher hydration levels[19].

Mucoadhesion of nasal inserts

The lyophilized nasal inserts must attach to the mucosal layer after being inserted into the nasal cavity to absorb water and undergo gelation. A critical stage in the development of nasal inserts is the polymerization due to water molecules. This process will result in the expansion and creation of a macromolecular mesh of the desired size. It also influences the mobility of the polymer chains and the penetration process between the mucus layer. The values of mucoadhesion of inserts were ranging from 0.28 - 0.55 cm (as shown in Fig. 2) and the formulation F4 showed maximum mucoadhesion value compared to the other formulations. The improved mucoadhesion of these inserts may be due to the physical entanglement between negatively charged polymer and mucus[15].

DSC studies

Eletriptan hydrobromide, a pure drug, displayed a noticeable peak on the DSC thermogram at 130.5ºC (Fig. 3a), and the thermogram of the physical mixture

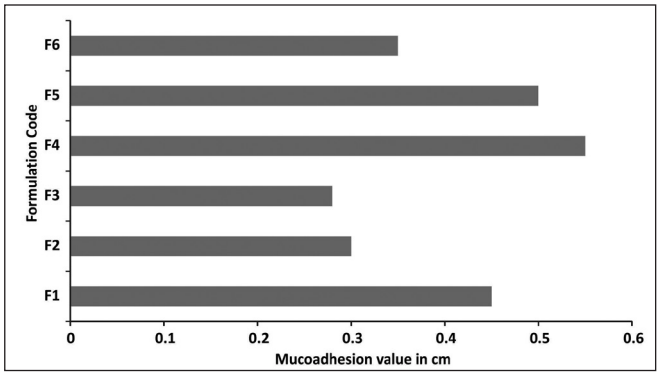


Fig. 2: Mucoadhesion of nasal inserts

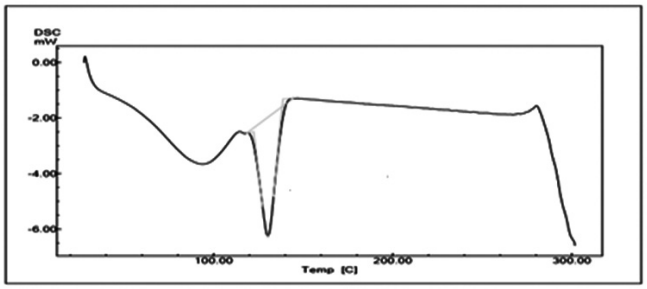


Fig: 3a: DSC thermogram of eletriptan hydrobromide

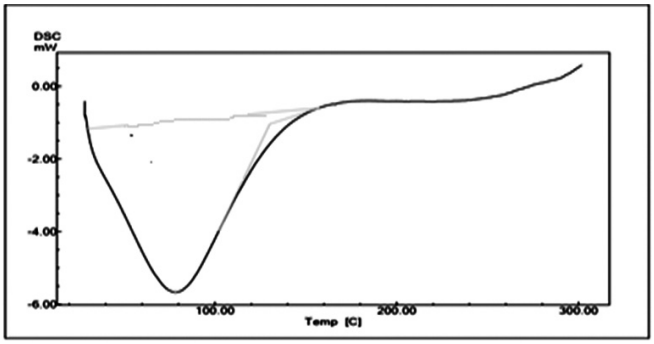


Fig. 3b: DSC thermogram of the physical mixture

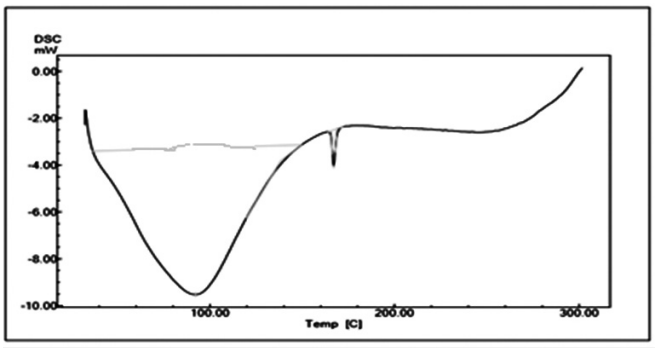


Fig. 3c: DSC thermogram of nasal inserts

(Fig. 3b) revealed a broadening and reduction in the sharp endothermic peak. The reduced crystalline character of the pure drug is shown by the broadening and reduction of the endothermic peak of drug-loaded nasal inserts (Fig. 3c), which may be caused by a partial conversion of the drug to its amorphous form[16].

XRD Studies

Using an X-ray diffractometer, the XRD patterns of eletriptan hydrobromide, physical mixture, and lyophilized nasal insert were captured. The XRD spectra of eletriptan hydrobromide(Fig 4a.) confirmed its crystalline nature. The reduction in the intensity of the prominent peak for the crystalline nature of the drug in eletriptan hydrobromide loaded nasal inserts and physical mixture (Figs. 4c and 4b) indicated that after lyophilization, the drug has been embedded in the polymer matrix[15].

SEM studies

SEM was performed to assess the internal structure and spongy nature of the developed nasal implants. The spongy structure is crucial as it provides a larger surface area and also helps in transformation of the inserts to gel, by absorption of moisture when placed in the nasal cavity. The spongy structure is essential because it provides a larger surface area and help the inserts turn into gel when they are inserted in the nasal cavity by absorbing moisture. As the surface area of the inserts increases, the water uptake capacity of the inserts will increase due to the capillary forces[16].The spongy structure of the lyophilized nasal inserts is shown Fig. 5.

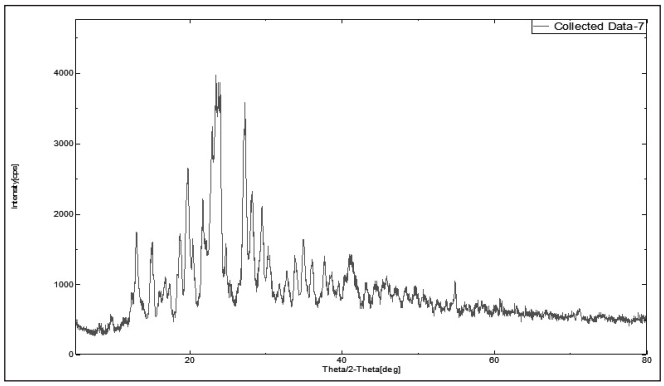


Fig. 4a: X-ray diffractogram of eletriptan hydrobromide

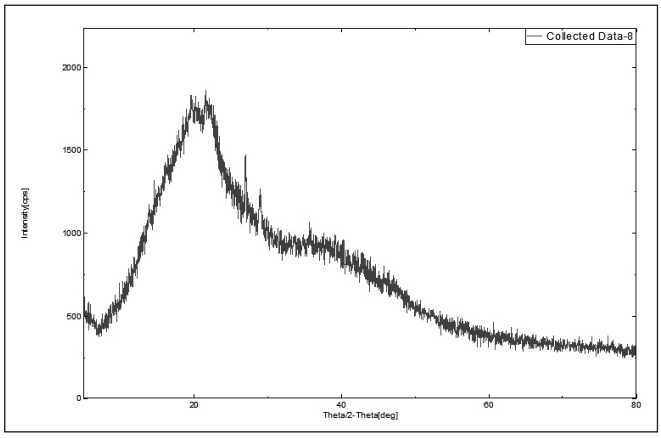


Fig. 4b: X-ray diffractogram of the physical mixture

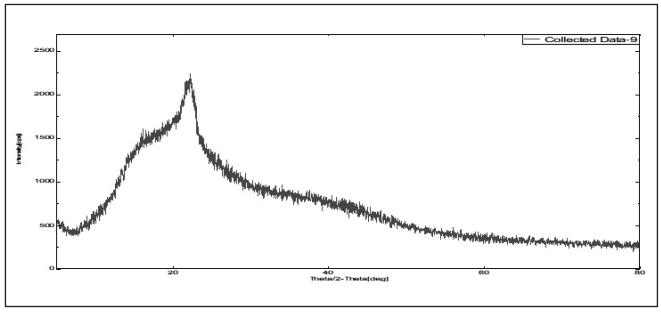


Fig. 4c: X-ray diffractogram of the lyophilized nasal insert

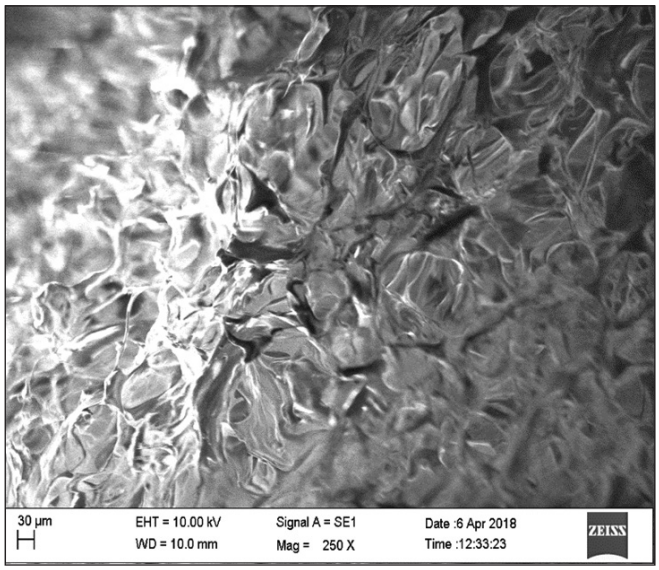


Fig. 5: SEM of optimised formulation F4

Drug release study and kinetic analysis

The release of drug from the nasal inserts in varying amounts depends on drug-polymer interaction, physical state of the drug, and viscosity after spreading.

The release of eletriptan hydrobromide from various formulations is shown in Fig. 6. The changing concentrations of the polymers employed in the formulation of inserts are the cause of the variations in drug release from the formulations. The formulation F4 showed 83% drug release. The swelling due to absorption of water forms a thick, viscous gel and the drug will slowly diffuse from the gel and get absorbed[19].The obtained results were fed to following models: zero order, first order, Higuchi

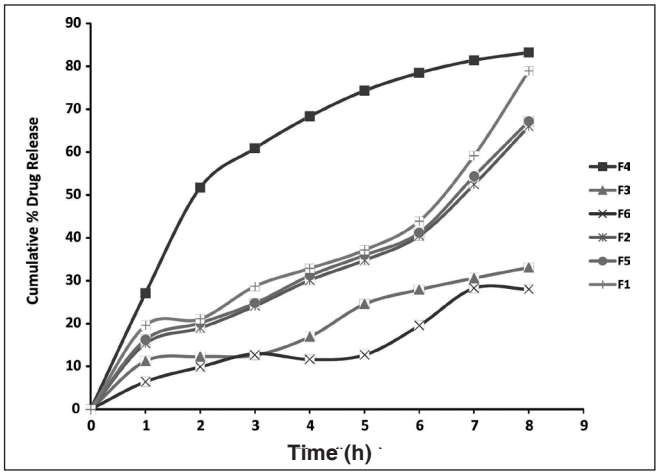
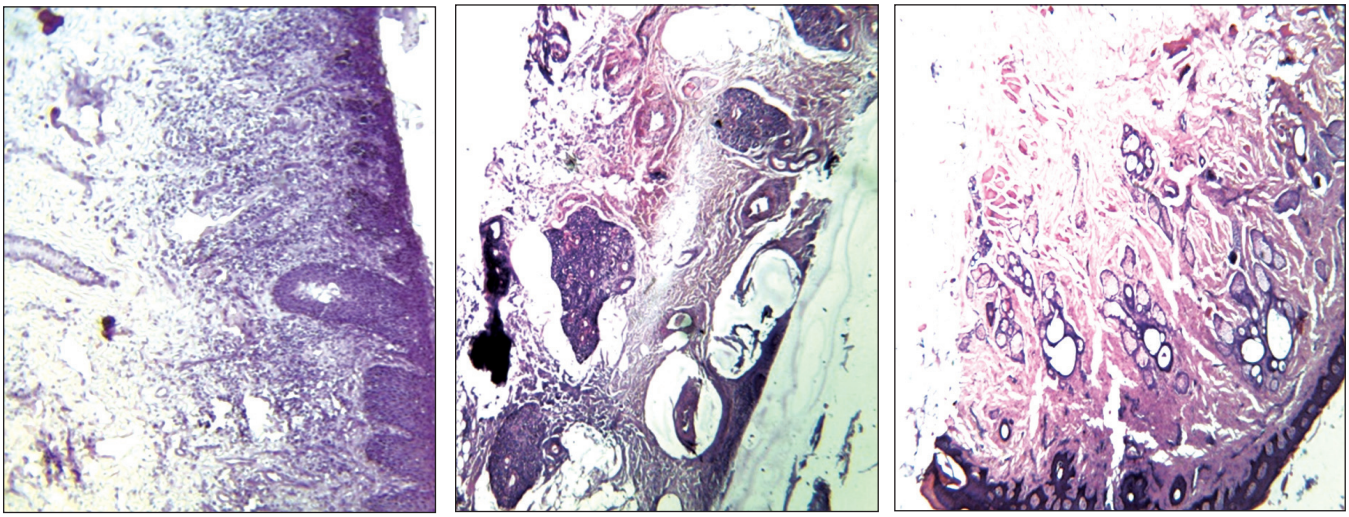


Fig. 6: *In vitro* drug release profile for nasal inserts



7a. Normal saline | 7b. Isopropyl alcohol | 7c. Nasal inserts

Fig. 7: Histological investigation

model and Korsemeyer-Peppas model. With an R2 value of 0.9758 and 0.48 a release exponent value ("n"), the significant model for the release study of the formulations followed first-order kinetics and showed that the drug release is of the Fickian diffusion type II[20, 21].

Histological investigation

The isolated goat nasal mucosa was used for the histological analysis of nasal inserts. The nasal mucosa with normal saline treatment revealed no significant change in the mucosal layer (Fig 7a). The isopropyl alcohol treatment showed significant damage to the mucosa which served as the positive control (Fig. 7 b). The epithelium of nasal mucosa showed no visible damage in case of the formulated nasal inserts (Fig. 7c).

Permeation study

This is a crucial method for determining the degree of drug penetration, so that it is available at the site of action[22]. Drugs can pass through biological membranes depending on their physicochemical properties and formulation variables[23]. The goat nasal mucosa was employed for this study on the optimized F4 formulation. In Fig. 8, it was demonstrated that a goat's isolated nasal membrane released about 61.25% of eletriptan hydrobromide over the course of 8 h.

Cytotoxicity studies

In order to assess and analyze if there is any reduction in cell toxicity shown by the nasal inserts when compared to the pure drug, RPMI 2650 nasal epithelia cell lines were

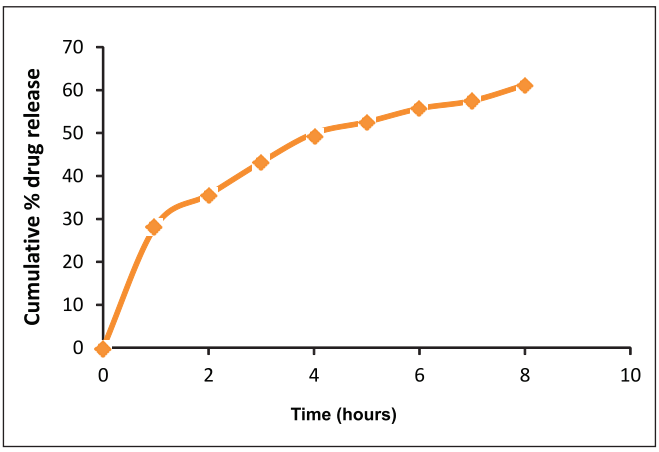


Fig. 8: *Ex vivo* release profile of optimized formulation (F4)

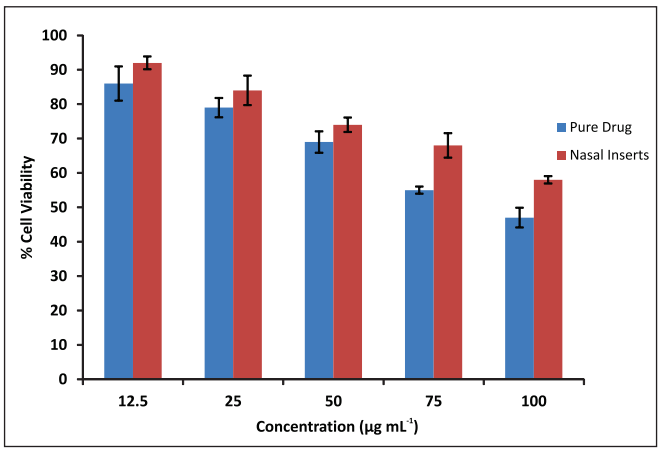


Fig. 9: *In vitro* assessment of cytotoxicity studies

chosen, followed by MTT assay with various fixations (12.5 – 100 µg mL^{-1}) of the drug (Fig. 9). There were significant differences in the reduction of cytotoxicity of drug embedded in the inserts when compared to the pure drug. The drugs sustained release from the polymer matrix in the nasal inserts may account for the higher percentage of cell survival compared to the free drug at the same concentration[24].

Stability studies

For the chosen formulation (F4), stability tests were conducted over the course of 3 months. Numerous parameters, including visual appearance, pH, drug content and drug release were determined. The study findings support the assertion that the formulation was found to be stable throughout the 90 days of study[25].

CONCLUSION

Lyophilized nasal inserts of eletriptan hydrobromide were prepared by using polymers in different ratios. Lyophilized nasal inserts have a high advantage over various other formulations due to their high-water uptake and mucoadhesion properties. Solid porous structures were shown by lyophilized nasal inserts accomplishing the requirements for easy administration. Based on the release and permeation studies, formulation F4 was selected as the optimized formulation which was stable throughout the experiment. In conclusion, the novel nasal inserts of eletriptan hydrobromide could be a better alternative for the management of migraine.

REFERENCES

1. Bertram U., Bernard M.C., HaenslerJ., Maincent P. and Bodmeier R.: *In situ* gelling nasal inserts for influenza vaccine delivery, **Drug Dev. Ind. Pharm**., 2010, 36(5), 581–593.
2. Salade L., Wauthoz N., Google J. and Amighi K.: How to characterize a nasal product. The state of the art of *in vitro* and *ex vivo* specific methods, **Int. J. Pharm**., 2019,561, 47-65.
3. Joana G., Bicker J., Filipa G., Joana L., Oliveira R. C., Alves G., Falcão A. and Fortuna A.:Nose-to-brain delivery of levetiracetam after intranasal administration to mice, **Int. J. Pharm.**, 2019,564, 329-339.
4. Costantino H.R., Illum L., Brandt G., Johnson P.H. and Quay S. C.: Intranasal delivery: physicochemical and therapeutic aspects, **Int. J. Pharm.**, 2007,337, 1–24.
5. Santos C. and Weaver D. F.: Topically applied linoleic/linolenic acid for chronic migraine, **J. Clin. Neurosci.,** 2018, 58, 200-208.
6. Ikeda K., Aoyagi J. and Hanashiro S.: Preventive Treatment with Lomerizine increases Cerebral Blood Flows during the Interictal Phase of Migraine, **J. Stroke Cerebrovasc. Dis.**,2018, 27,998-1002.
7. McInnes F. J., Thapa P. and Baillie A.J.: *In vivo* evaluation of nicotine lyophilised nasal insert in sheep, **Int. J. Pharm.,** 2005, 304, 72–82.
8. Falconer J. L., Alt J. A. and Grainger D. W.: Comparing *ex vivo* and *in vitro* translocation of silver nanoparticles and ions through human nasal epithelium, **Int. J. Pharm.,** 2018, 171, 97-106**.**
9. Garmise R. J., Mar K., Crowder T. M., Hwanq C.R., Ferriter M., Huanq J., Mikszta J. A., Sullivan V.J. and Hickey A.J.: Formulation of a dry powder influenza vaccine for nasal delivery, **AAPS Pharm. Sci. Tech.,** 2006, 7E19.
10. Pardeshi C.V., Rajput P.V., Belgamwar V.S. and Tekade A.R.: Formulation, optimization and evaluation of spray-dried mucoadhesive microspheres as intranasal carriers for valsartan**, J. Microencapsul.**, 2012,29, 103–114.
11. Shetty S., Jose J., Kumar L. and Charyulu R.N.: Novel ethosomal gel of clove oil for the treatment of cutaneous candidiasis, **J. Cosmet. Dermatol.,** 2019 ,18(3), 862-869.
12. Thapa P., Baillie A. J. and Stevens H. N.: Lyophilization of unit dose pharmaceutical dosage forms, **Drug Dev. Ind. Pharm.**, 2003, 29, 595–602.
13. Garmise R. J., Staats H.F. and Hickey A.J.: Novel dry powder preparations of whole inactivated influenza virus for nasal vaccination, **AAPS Pharm. Sci.Tech.,** 2007, 88E81.
14. Phaechamud T. and Ritthidej G.C.: Sustained-release from layered matrix system comprising chitosan and xanthan gum**, Drug Dev. Ind. Pharm.**, 2007, 33, 595–605.
15. Bertram U. and Bodmeier R.: *In situ* gelling, bioadhesive nasal inserts for extended drug delivery: *in vitro* characterization of a new nasal dosage form, **Eur. J. Pharm**. **Sci.**, 2006, 27, 62–71.
16. McInnes F.J., O'Mahony B., Lindsay B., Band J., Wilson C. G., Hodges L.A. and Stevens H.N.: Nasal residence of insulin containing lyophilized nasal insert formulations, using gamma scintigraphy, **Eur. J. Pharm. Sci.,** 2007, 31, 25–31.
17. Wolfe T.R. and Bernstone T.: Intranasal drug delivery: an alternative to intravenous administration in selected emergency cases, **J. Emergency Nursing**, 2004, 30, 141–147.
18. Cho H. J., Balakrishnan P., Shim W.S., Chung S.J., Shim C.K. and Kim D. D.: Characterization and *in vitro* evaluation of freeze-dried microparticles composed of granisetron–cyclodextrin complex and carboxymethylcellulose for intranasal delivery, **Int. J. Pharm.**, 2010, 400, 59–65.
19. Lee J.W., Park J.H. and Robinson J. R.:Bioadhesive-based dosage forms: the next generation, **J. Pharm. Sci.,** 2000,89, 850–866.
20. Tafaghodi M. and Rastegar S.: Preparation and *in vivo* study of dry powder microspheres for nasal immunization, **J. Drug Target,** 2010,18, 235–242.
21. Bertram U. and Bodmeier R.: Parameters affecting the drug release from *in situ* gelling nasal inserts, **Eur. J. Pharm. Biopharm.**, 2006,63, 310–319.
22. Mishra D.N. and Gilhotra R.M.: Design and characterization of bioadhesive *in situ* gelling ocular inserts of gatifloxacin sesquihydrate, **DARU,** 2008,16, 1–8.
23. Prabhu A., Jose J., Kumar L., Salwa S., Vijay Kumar M. and Nabavi S.M.: Transdermal delivery of curcumin-loaded solid lipid nanoparticles as microneedle patch: An *in vitro* and *in vivo* Study, **AAPS Pharm. Sci. Tech.,** 2022, 23(1),1-2.
24. Rodrigues L.R. and Jose J.: Exploring the photo protective potential of solid lipid nanoparticle-based sunscreen cream containing Aloe vera, **Environ. Sci. Pollut. Res. Int.**, 2020,27, 20876-20888.
25. Nafee N.A., Boraie M.A., Ismail F.A. and Mortada L.M.: Design and characterization of mucoadhesive buccal patches containing cetylpyridiniumchloride, **Acta Pharm.,** 2003, 53, 199–212.