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FORMULATION AND IN VITRO EVALUATION OF AN ANTIMIGRAINE NANOPARTICULATE SYSTEM IN A MUCOADHESIVE THERMOREVERSIBLE INTRANASAL GEL

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ABSTRACT

Migraine is simply a headache associated with nausea and sensitiveness to light and sound. The present work aims to formulate, optimize and evaluate mucoadhesive thermoreversible in situ gel of antimigraine drug-loaded nanoparticle for intranasal delivery. Characterization studies of drug and excipients were performed and results confirmed drug compatibility with polymers. Ionic gelation method was employed in the preparation of drug-loaded nanoparticles. The drug-loaded nanoparticles were incorporated in the optimized gel formulation. By using the optimized formula, in situ gel with the desired range of gelation temperature and gelling time were obtained. Sumatriptan showed a rapid drug release from the solution. The release of Sumatriptan from the gel-containing nanoparticulate system was very slow and in a controlled manner when compared to the release profile of solution form. *In vitro* drug release of Sumatriptan nanoparticle-loaded gel was best explained by Higuchi's model. The mechanism of release of drug from gel containing nanoparticulate system follows a non-Fickian or anomalous diffusion. From the *Ex vivo* skin permeation study, decrease in flux was observed for Sumatriptan nanoparticle-loaded gel compared with plain Sumatriptan solution. From the result, it was observed that drug permeation became slower when the drug was added to the formulation in its entrapped form rather than in solution form. With this

kind of formulation, the bioavailability of drugs can be increased and undesirable side effects can be avoided. Therefore, an antimigraine drug-loaded nanoparticulate system in a mucoadhesive thermoreversible intranasal gel prepared in this study is more useful than a conventional formulation in therapy.

KEYWORDS: Migraine, Thermoreversible gel, Nanoparticles, Intranasal drug delivery.

INTRODUCTION

Migraine is simply a headache associated with nausea and sensitiveness to light and sound. Migraine is a chronic physiological condition and usually lasts for 4-72 hours. Migraine headaches are most common in persons with high workloads and tension. Migraine headaches are associated with the activation and sensitization of the Trigeminovascular pain pathway.[1] Drug delivery systems are defined as systems or formulations that deliver the drug or active pharmaceutical agent into the body.[2] The major drawback of the conventional nasal dosage form is clearance by the nasal mucosa. Mucociliary clearance negatively affects drug bioavailability. This challenge can be overcome by the use of in situ gelling systems, which on administration will adhere to the nasal mucosa and increase the residence time.[3] The nasal route is an important path to deliver drugs directly into the brain.[4] A novel drug delivery system helps to bypass these barriers and enhances bioavailability.

Sumatriptan is a better choice of drug for antimigraine therapy. Triptans are a group of tryptamine-based drugs considered as abortive medication in antimigraine therapy. Sumatriptan is a sulfonamide triptan that selectively binds to the $5HT_1$ receptor. Agonism of Sumatriptan leads to constriction of cranial blood vessels and inhibits the release of pro-inflammatory neuropeptides.[5] Sumatriptan is structurally similar to Serotonin and activation of these receptors will cause constriction of dilated blood vessels. Formulation of Triptan class of drug in an in situ gelling system has a proven history of enhanced bioavailability and targeting. This resulted in the research of Sumatriptan loaded in situ gelling systems for effective antimigraine therapy.

The blood-brain barrier restricts the entry of most drugs into the brain. Sumatriptan is relatively a hydrophilic drug and various recent animal studies have pointed out the inability to cross blood-brain barrier.[6] The major advantage of the nasal route is to bypass the restriction of the blood-brain barrier.[7] Nose to brain delivery of a drug is mainly facilitated through the olfactory neural pathway.[8] Olfactory neurons originate from upper mucosal part

of nose to the surface of the brain. Olfactory neural pathway will help to deliver the drug directly into the brain. Trigeminal nerve system is another alternative route for a nose to brain delivery.[9]

Nanoparticle-loaded intranasal gel opens a promising approach to drug delivery in antimigraine therapy. Nanoparticles are rapidly uptake in the brain. Biodegradability and biocompatibility of nanoparticles will also reduce side effects. The study was mainly aimed to formulate and evaluate an antimigraine drug-loaded nanoparticulate system in a mucoadhesive thermoreversible intranasal gel.

MATERIALS AND METHODS

Drugs and reagents

Sumatriptan was purchased from Yarrow Chem Products, Mumbai. Poloxamer 407 was purchased from Yarrow Chem Products, Mumbai. HPMC K15M was purchased from Yarrow Chem Products, Mumbai. Chitosan was purchased from Sisco Research Laboratories Pvt Ltd. Tripolyphosphate was purchased from Thermo Fisher Scientific Pvt Ltd. Benzalkonium chloride was purchased from AVA Chemicals, Mumbai. All other chemicals and reagents were of analytical grade during the preparation and evaluation of the formulation.

Preparation of sumatriptan loaded chitosan nanoparticles

0.1% w/v of Chitosan solution was prepared by dissolving chitosan in 1% v/v Acetic acid solution until a clear solution was obtained. 0.1% w/v of Tripolyphosphate solution was prepared by dissolving Tripolyphosphate in deionized water. Sumatriptan was dissolved in Chitosan solution. Tripolyphosphate solution was then added to Chitosan solution dropwise under homogenizer. After 1 hr at room temperature, samples were centrifuged twice for 30 minutes. Settled nanoparticles were resuspended in distilled water for further formulation process. Sumatriptan-loaded Chitosan nanoparticles were prepared by the ionic gelation method as reported by Katas and Alpar.[10]

Preparation of mucoadhesive thermoreversible in situ gel

The cold technique was used in the preparation of mucoadhesive thermoreversible in situ gel. HPMC K15M was dissolved in phosphate buffer solution (p^H 5.5) at different concentrations (0.1-0.6%) and the mixture was stirred until HPMC K15M completely dissolves. Different concentrations (15-20%) of Poloxamer 407 were prepared and added to the above mixture.

Benzalkonium chloride was used as a preservative. The above mixture was stored at 4°C overnight and stirred to get a clear solution.[11]

Preparation of in situ gel containing sumatriptan loaded nanoparticles

Drug-loaded nanoparticles prepared by the previous method were added in an optimized formulation of HPMC-Poloxamer gel solution.[12] The final formulation was stored at 4°C in the refrigerator.

Characterization of sumatriptan loaded chitosan nanoparticles

Particle size, Polydispersity Index, and Zeta potential

The prepared drug-loaded Chitosan nanoparticles were evaluated for particle size, polydispersity index and zeta potential. These characters were evaluated using Photon Dispersity Spectroscopy (PSC) on a Malvern zetasizer 3000 Ver.7.02 at a fixed angle of 90^0 and 25^0C. Samples were placed in clear disposable zeta cells and results were recorded.

Transmission electron microscopy

The morphology, structure, and particle size of drug-loaded Chitosan nanoparticles were examined by JOEL Model JSM-6390LV electronic transmission microscope at 70 kV. In this method, nanoparticles were dispersed onto a surface of a copper grid sample holder and probed with a transmission electron microscope. The image was then photocopied, in which objects were visualized in order of angstroms.

Drug entrapment efficiency

The drug-loaded CS NPs were centrifuged at 15000 rpm for 30 min using Remi CFC free centrifuge CZCI -8899. The supernatant solution was diluted suitably and absorbance was measured using a JASCO V-630 spectrophotometer at 229 nm. The concentration of the drug in the supernatant was calculated using the standard calibration data [13]. The entrapment efficiency was calculated using the formula.

$$\text{Entrapment efficiency (\%)} = \frac{Amount\ of\ total\ drug - Amount\ of\ free\ drug}{amount\ of\ total\ drug} \times 100$$

Characterization of in situ gel containing Sumatriptan loaded nanoparticles

Gelling temperature

Gelation temperature was assessed using a modification of Miller and Donovan technique.[14] 2 ml of gel was transferred to test tubes, immersed in a water bath at 4^0C. The temperature of the water bath was increased by 1^0C. The samples were then examined for gelation.

Gelling time

The solution was kept at its gelation temperature in a water bath. Then the time essential for the conversion of sol to gel was noted.

Mucoadhesive strength

A modified physical balance was used to find mucoadhesive strength. On the upper glass vial, a section of tissue was kept as the mucosal side out. The area of the mucosal membrane was 0.785cm^2. The vial with a section of tissue was connected to the right balance. Another vial was fixed on a height-adjustable pan under the first vial. To the lower vial, gel formulation was applied. A constant force was applied on the upper vial and then it was removed. Then the weight on the left side pan was slowly increased till the two vials just separated from each other. The total weight required to detach two vials were taken as a measure of mucoadhesive strength and was calculated using the below formula.[15]

$$\text{Mucoadhesive strength} = \frac{\text{Force of adhesion}}{\text{area}}$$

***In vitro* release of drug from gel**

Carried out on Franz diffusion cell in which the donor compartment is separated from receptor compartment using a pre-hydrated cellophane membrane. The receptor compartment was comprised of 5 ml of p^H 5.5 phosphate buffer. The temperature of the receptor compartment was maintained at 34^{0}C under a magnetic stirrer at 100 rpm. After a pre-incubation time of 20 minutes, in situ gel formulation equivalent to 5 mg of Sumatriptan and plane Sumatriptan solution was placed in the donor chamber. At predetermined time intervals, 1 ml samples were withdrawn from the receptor compartment, replacing the sampled volume with Phosphate buffer p^H 5.5 after each sampling, for 24 hours. The samples were filtered and analyzed for drug content by UV visible spectrophotometer at 229 nm after suitable dilutions.[16] The cumulative amount of drug released across the cellophane membrane was determined as a function of time. Drug release kinetics were plotted.

***Ex vivo* permeation studies**

Fresh nasal mucosa was carefully removed from the nasal cavity of the goat obtained from the local slaughterhouse. Tissue samples were fitted in Franz diffusion cells showing a permeation area of 0.785 cm^2. The membrane was kept in phosphate buffer p^H 5.5 for 20 min to equilibrate. The receptor compartment was comprised of 5 ml of p^H 5.5 phosphate buffer. The temperature of the receptor compartment was maintained at 34^{0}C under a magnetic

stirrer at 100 rpm. After a pre-incubation time of 20 minutes, in situ gel formulation equivalent to 5 mg of Sumatriptan and plane Sumatriptan solution were placed in the donor chamber. At predetermined time points, 1 ml samples were withdrawn from the receptor compartment, replacing the sampled volume with Phosphate buffer p^H 5.5 after each sampling, for 24 hours. The samples were filtered and analyzed for drug content by UV visible spectrophotometer at 229 nm after suitable dilutions [17]. The cumulative amount of drug permeated across the mucosal membrane was determined.

RESULTS AND DISCUSSION

Preparation and Characterization of sumatriptan loaded chitosan nanoparticles

Sumatriptan-loaded Chitosan nanoparticles were prepared by the ionic gelation method and are shown in Figure 1. Equal ratios of Chitosan and Tripolyphosphate were selected for the preparation of drug-loaded nanoparticles. The average particle sizes of prepared nanoparticles were found to be 205.8nm with a polydispersity index of 0.197 by the ionic gelation method. The zeta potential of drug-loaded Chitosan nanoparticles was found to be 27.1mV. The entrapment efficiency of obtained Chitosan nanoparticles was found to be 77.5%.

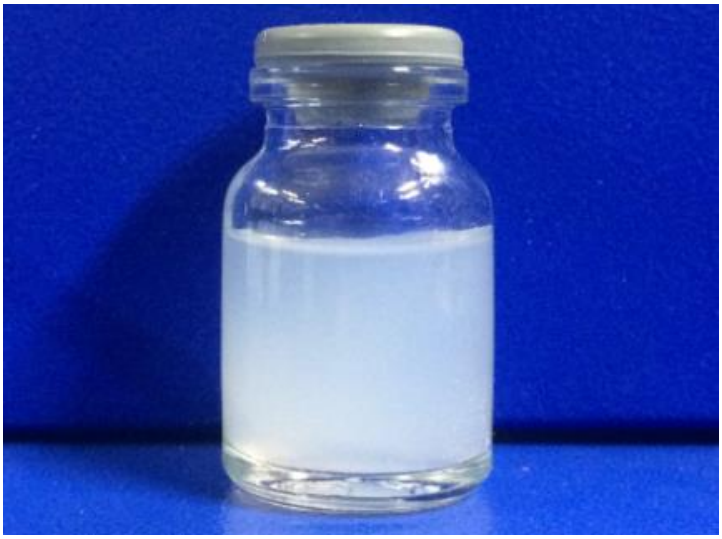

Figure 1: Drug loaded nanoparticles.

Transmission electron microscopy

Transmission electron microscopic image (Figure 2) of Sumatriptan loaded Chitosan nanoparticles showed that the size of formed nanoparticles was small and confirmed the formation of almost spherical-shaped nanoparticles with a smooth surface.

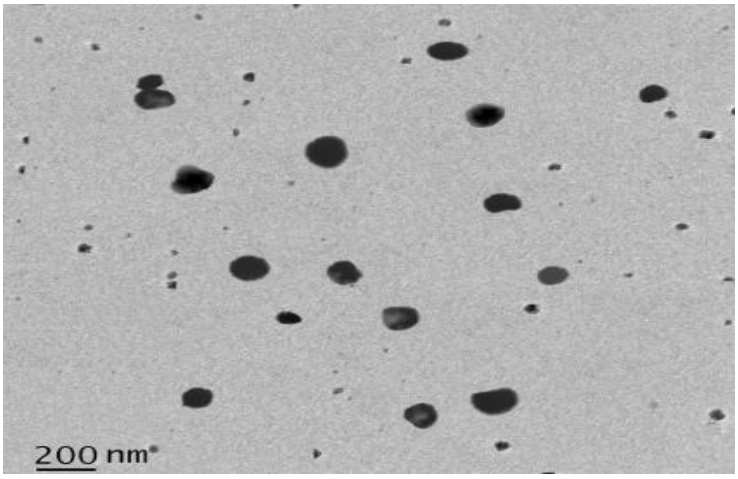

Figure 2: TEM images of drug-loaded nanoparticles.

Preparation and characterization of in situ gel containing Sumatriptan loaded nanoparticles

In situ gel containing Sumatriptan loaded nanoparticles was prepared by cold technique and is shown in Figure 3

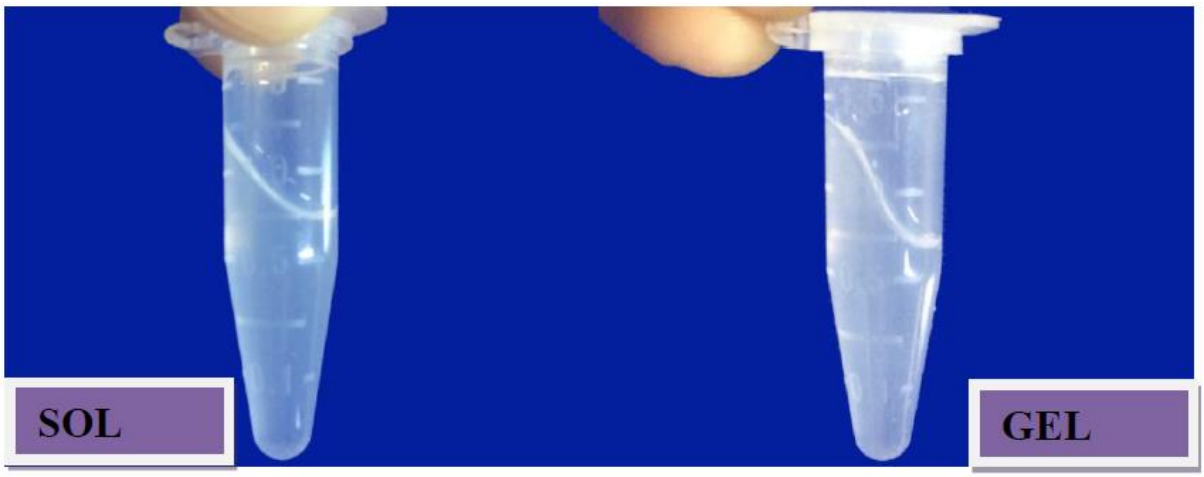

Figure 3: Sol-Gel form of In situ gel.

Gelling temperature

If the gelation temperature of a thermoreversible formulation is lower than 25^0C, a gel might be formed at room temperature leading to difficulty in manufacturing, handling, and administering. If the gelation temperature is higher than 37^0C, a liquid dosage form still exists at the body temperature, resulting in the nasal clearance of the administered drugs at an early stage. It is evident from the data shown in Table 1 that the gelation temperature obtained did not vary more than $\pm 0.3^0$C.

Table 1.

Test no	Gelation temperature (^{0}C)
1	35.1
2	34.8
3	34.5

Gelling time

The time required for sol to gel transition was found to be 58.0±2 s

Table 2.

Test no	Gelling time (s)
1	58
2	56
3	60

Mucoadhesive strength

Mucoadhesive strength of developed in situ gel was determined using goat nasal mucosa and was measured by using a modified physical balance device. The mucoadhesive strength was found to be 1885.09 dynes/cm^2.

***In vitro* drug release study**

Release of Sumatriptan from gel containing nanoparticulate system at p^H 5.5 phosphate buffer solutions was determined. Sumatriptan showed a rapid drug release from a solution which is evidenced by the cumulative percentage drug release profile shown in Figure 4. The release of Sumatriptan from the gel-containing nanoparticulate system was very slow and in a controlled manner when compared to the release profile of solution form. At 24 hours, the cumulative percentage release of Sumatriptan from solution was found to be 96.45% but from the gel formulation, it was only 62.86%.

From Figure 5, it was found that the *in vitro* drug release of Sumatriptan from nanoparticle-loaded In situ gel was best explained by Higuchi's model, as the plot showed the highest linearity with a regression coefficient of 0.985. This explains why the drug diffuses at a comparatively slower rate as the distance for diffusion increases, which is referred to as Higuchi's kinetics.[18]

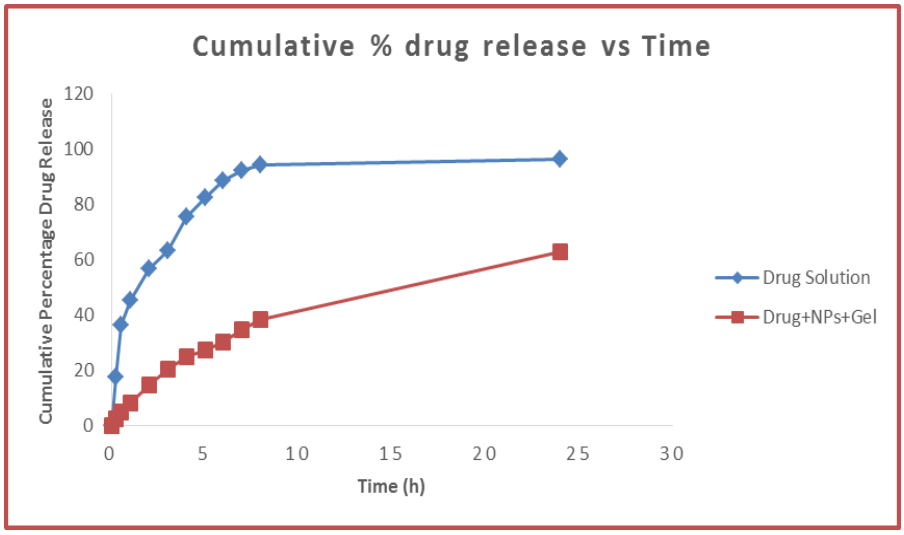

Figure 4: Cumulative percentage drug release profile.

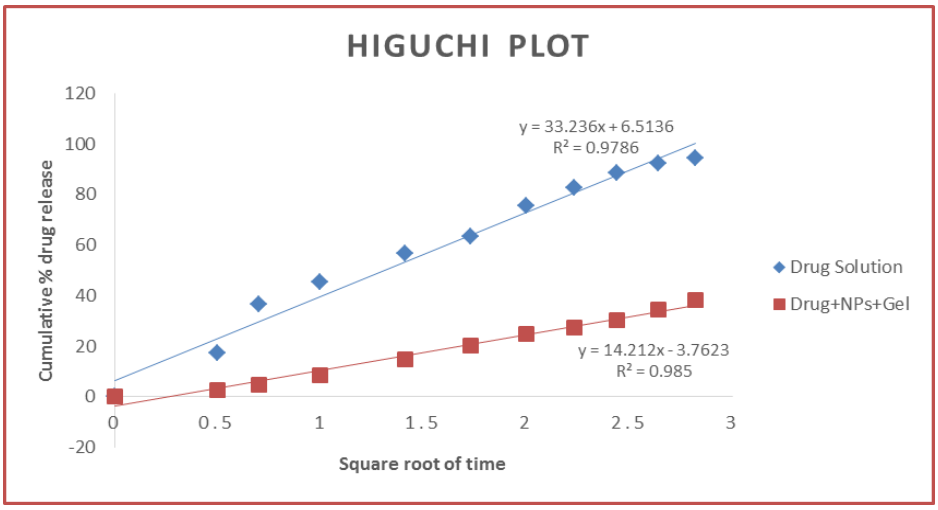

Figure 5: Higuchi plot.

Ex vivo permeation study

Ex-vivo skin permeation study of Sumatriptan nanoparticle loaded gel formulation and Plain Sumatriptan solution were performed by Franz diffusion cell using goat nasal mucosa. A decrease in flux was observed for Sumatriptan nanoparticle-loaded gel compared with plain Sumatriptan solution. From the graph, it was observed that drug permeation became slower when the drug was added to the formulation in its entrapped form rather than in solution form.

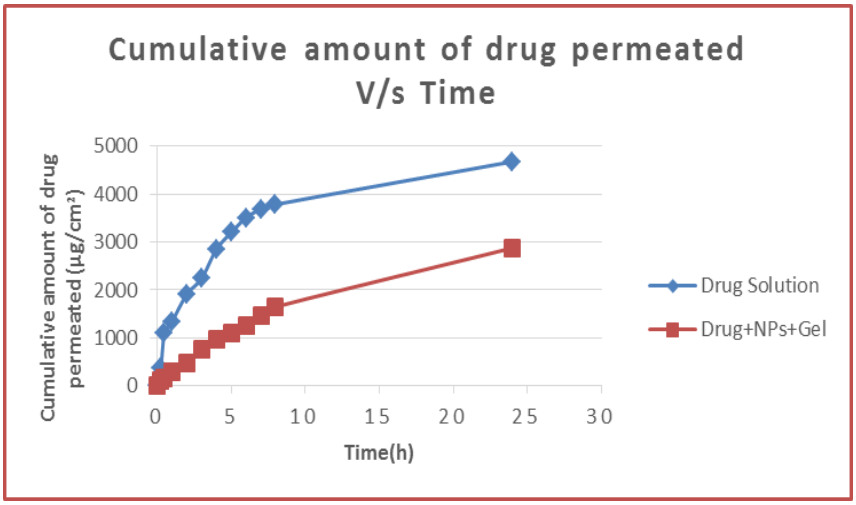

Figure 6: Cumulative amount of drug permeated from drug solution and gel formulation.

CONCLUSION

The development of nanotechnology has opened a huge number of possibilities in the medical field, especially in the area of drug delivery. Various drug carrier systems in the nano-size range have been developed recently. Liposomes, Nanoparticles, Niosomes, Phytosomes are some examples of novel nano-drug delivery systems. The major goal in developing nanoparticles as a delivery system is to minimize particle size and obtain optimum surface properties for sustained release of drug to get site-specific action. The nanoparticle system offers a major improvement in the transport of drugs across the blood-brain barrier through an intranasal route of administration.

The present study aimed to develop and evaluate an antimigraine drug-loaded nanoparticulate system in a mucoadhesive thermoreversible intranasal gel. Drug-loaded Chitosan nanoparticles were prepared by the ionic gelation method. Chitosan nanoparticles prepared by the ionic gelation method resulted in the formation of the desired size of a particle with a good polydispersity index. A good positive zeta potential value confirmed the stability of nanoformulation. Entrapment efficiency was found to be good. The drug-loaded nanoparticles were incorporated in the optimized gel formulation. By using the optimized formula, in situ gel with a gelling temperature in the range of nasal temperature with a gelling time of less than one minute was obtained. The good value of mucoadhesive strength shows the sufficient mucoadhesion of gel. Sumatriptan showed a rapid drug release from the solution which is evidenced by the cumulative percentage drug release profile. The release of Sumatriptan from the gel-containing nanoparticulate system was very slow and in a

controlled manner when compared to the release profile of solution form. *In vitro* drug release of Sumatriptan nanoparticle In situ gel was best explained by Higuchi's model, as the plot showed the highest linearity with a higher regression coefficient. From the *ex-vivo* skin permeation study, a decrease in flux was observed for Sumatriptan nanoparticle-loaded gel compared with plain Sumatriptan solution. From the result, it was observed that drug permeation became slower when the drug was added to the formulation in its entrapped form rather than in solution form.

An antimigraine drug-loaded nanoparticulate system in a mucoadhesive thermoreversible intranasal gel can be developed and modified for more efficient antimigraine therapy. In the future, Chitosan nanoparticles-based carrier systems through intranasal routes can become a breakthrough in the modern medical field. However, more advanced studies are essential to prove *in vivo* efficacy and safety of the product for therapeutic use.

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