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Research Article

Development and Characterization of Nasal Mucoadhesive Microemulsion of Sumatriptan Succinate

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ABSTRACT

The purpose of this study was to enhance the brain uptake of sumatriptan succinate (SS) in o/w microemulsion, which was suitable for intranasal delivery. Microemulsion system with Tween 80, Span 80 as surfactants and n-butanol as cosolvent and iso propyl myristate as oil was developed for intranasal delivery of sumatriptan succinate. Single isotropic region, which is considered as o/w microemulsion was found in the pseudo-ternary phase diagrams developed at various tween 80, span 80 and n butanol ratios. The optimal microemulsion formulation consisted of 5% iso propyl myristate, 25% water, and 70% (w/w) surfactant/cosurfactant [surfactants are 52.5% (tween 80 36.75% span 80 15.75%) cosurfactant (n butanol) 17.5% at 3: 1 weight ratio] With the increase of tween 80 concentration, the microemulsion region area, microemulsion viscosity, and the amount of water into the microemulsion system increased. Mucoadhesive microemulsion was prepared by using HPMCK4M as a mucoadhesive polymer to increase the residence time inside the nasal cavity for prolonged action and direct targeting release of drug to the brain. Nasal absorption of sumatriptan succinate from this mucoadhesive microemulsion was found to be fairly rapid, as it converts into gel inside the nasal cavity and increase the residence time and could improve bioavailability of the drug. The result suggests that this mucoadhesive microemulsion may be a useful approach for the rapid onset delivery of sumatriptan succinate during the emergency treatment of acute attack of migraine.

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INTRODUCTION

Migraine attack is troublesome physiological condition associated with throbbing intense headache in one half of the head. During an attack the blood vessels in the brain dilates and then draw together with stimulation of nerve endings near with the affected blood vessels. These changes to the blood vessels and stimulation of verve what cause the pain [1]. Although the migraine is still a poorly understood conditions or phenomenon. Sumatriptan succinate, triptan derivatives are serotonin agonist ($5HT_{D1}$) used in the treatment of migraine. It is administered orally, in the doses of 25, 50 or 100 mg as a single dose, nasally in doses of 10 or 20 mg and also subcutaneously, as 2, 6 mg doses within 24 hours [2]. Migraine patients not only suffer from gastric stasis but also have severe nausea and vomiting, which results in erratic absorption of SS from GIT. Low oral bioavailability (15%) due to high first pass metabolism justifies a need of nasal drug delivery[2]. Nasal delivery is an alternative route of drug delivery that can selectively target drug directly into the various regions of the brain, including vasculature which is needed for the treatment of acute attack of migraine[3].

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Major advantages offered by the nasal route such as rapid drug absorption and quick onset of action, avoidance of first pass effect and enzymatic degradation in GIT, highly permeable structure of nasal mucosa results in higher bioavailability, thus require lower doses of drug. The intranasal administration offers the practical non-invasive alternative route of administration for the drug delivery to the brain[3]. Intranasal administrations allow transport of drugs to the brain circumventing BBB. Thus providing unique features and better option to target the drugs to the brain[1]. However one of the problem associated with nasal delivery of SS solution is lower retention time in the nasal cavity, because of high mucociliary clearance resulting in lower bioavailability. Hence objective of this study was to develop the intranasal drop formulation of SS i.e. mucoadhesive microemulsion which convert into gel inside the nasal cavity for prolong action and direct targeting release of the drug to the brain.

MATERIALS AND METHODS

Materials

Sumatriptan succinate (ss) was received as a kind gift from Dr. Reddy's Laboratory, Hyderabad, India. Hydroxyl propyl methyl cellulose (HPMC K4M) was obtained as a gift sample from Colorcon Limited, Goa, India. Tween 80,

span 80, N butanol, iso propyle myristate and methanol were procured from Loba Chemie, Mumbai, India and used as received. All other reagents used were of analytical grade.

Methods

Phase Diagram Preparation and Microemulsion Formulations

To find out the appropriate component in the formulation of o/w and w/o microemulsions, two safe and non compatible non ionic surfactants were used, namely Tween 80 and Span 80 which are hydrophilic and lipophilic in nature respectively. N- butanol is used as cosolvent. The oil employed in the present study was iso propyl myristate (IPM), the double distilled water as an aqueous phase. The pseudoternary phase diagram of oil, surfactant, cosurfactant and water were constructed using water titration method to obtain the components and their concentration ranges that can results in large existence area of microemulsion. Surfactant was mixed with cosurfactant in fixed weight ratios (1:1, 2:1, 3:1) in which Tween 80 and Span 80 are used in 7:3 proportions respectively. Aliquots of each surfactant and cosurfactant mixture (Smix) were then mixed with oil at room temperature. (25^0c). For each phase diagram, the ratio of oil to Smix was varied as 9:1, 8:2, 7:3, 6:4, 5:5, 4:6, 3:7, 8:2, and 9:1 (w/w). Water was added dropwise to each mixture under vigorous stirring for 20-30 minutes by using magnetic stirrer. Then each mixture visually observed for clarity and flowability. No heating was conducted during the preparation, however well covered magnetic stirring was performed through out the titration process for a thorough mixing.

After the identification of microemulsion region in the phase diagram, the microemulsion formations were selected at desired component ratios. The preparation of selected microemulsion was simply performed by adding the weighed components (IPM, tween 80, span 80, N butanol, and water) together and stirring to form a clear microemulsion[4, 5].

Preparation of Microemulsion

Formulation was further optimized with surfactant /cosurfactant 3:1. The liquid microemulsion was prepared by dissolving drug (SS) and HPMCK4M (0.05%) in double distilled water (25%). The resultant solution was added dropwise into the mixture of Tween 80 (36.75%), Span80 (15.75%), n-butanol (17.25%) and IPM (5%) with continuous stirring on magnetic stirrer. No heat is required during formulation. Final concentration of SS in the microemulsion was 2%. The resulting microemulsion were tightly sealed and stored at ambient temperature and their physical stability was measured by observing periodically for the occurrence of phase separation. The selected microemulsions were characterized. Surfactant like cremophor RH 40 and EL, PEG 400, triacetin brij 35 was tried. Soyabean oil as an oil and Carbopol 934P, sodium alginate as mucoadhesive agents were also tried out.

Formulation and Development

ME1, ME2 are w/o and ME3, ME4 are o/w type of microemulsions. MME3A, MME3B are mucoadhesive microemulsions, in which 0.05% and 0.1% mucoadhesive polymer was used respectively.

Table 1: Formulation composition of microemulsions

Sl. No.	Ingredients	Formulations composition (% w/v)					
		ME1	ME2	ME3	ME4	MME 3A	MME 3B
1	IPM	40	50	5	10	5	5
2	Tween 80	11.25	10	36.75	34.12	36.75	36.75
3	Span 80	26.25	9.0	15.75	14.62	15.75	15.75
4	N butanol	12.5	21.0	17.5	16.25	17.5	17.5
5	Double/ Distilled Water	10	10	25	25	25	25
6	HPMCK4M	-	-	-	-	0.05	0.1
7	Drug (SS)	2	2	2	2	2	2

Identification test for type of microemulsions

Dilution test

If the continuous phase is added in microemulsion, it will not crack or separate into phases. if water is added in o/w type of microemulsion it will remain stable.

Staining test

Water soluble dye such as methylene blue/amaranth was added in water and microemulsion was prepared with oil and surfactant. A drop of microemulsion was observed under microscope. Background was found to be blue / red and globule will appear colorless respectively.

Characterization of Mucoadhesive Microemulsion

Clarity test

It observed visually, because microemulsions are clear and transparent.

Dilutability test

The microemulsions formed were diluted in 1:10, and 1:100, ratios with double distilled water to check if the system shows any signs of separation.

pH measurement

The pH of microemulsion for nasal delivery should be 4.5 to 6.5, in acidic pH, lysozyme found in nasal secretion which is responsible for destroying certain bacteria. Under alkaline condition lysozyme is inactivated and the nasal tissue susceptible to microbial infection therefore advisable to keep pH 4.5 to 6.5. The pH measurement was carried out using pH meter (μ pH system 362)

Particle size measurement

Particle size analysis is mainly carried out by photon correlation spectroscopy with a Beckman N5 submicron particle size counter which can measure the size range from 5nm to approximately 3μm. For size analysis approximately 0.1 ml microemulsion is added to 10 ml double distilled water in order to obtain the optimum scattering intensity. Another instrument is dynamic light scattering method employing a zeta potential / particle sizer[1, 3, 5]. The particle size of microemulsion should be less than 150 nm. Particles are nanosize loaded with drugs show drug release at appropriate rate and dose at specific sites in the brain for a certain time to realize the accurate nasal delivery, which enhances the therapeutic effect, reduces the toxicity and side effect, decreases the dose and frequency of dosing, and perhaps even the cost of the therapy[6]. Nanodroplets are extremely stable will not separate into different phases[7]. The particle size is below 0.22 μm, can be sterilized by filtration[8].

Zeta potential measurement

It found to be or must be negative or neutral. Which indicate that droplets of micro emulsion having no charge that is system is stable. Zeta potential is determined by using Zetasizer. Zeta potential is essentially useful for assessing flocculation since electrical charges on particles influence the rate of flocculation[9].

Viscosity measurement

Low viscosity is required to make them good in appearance and easy to handle and packed. Also provide good spray ability. Viscosity measured by using Brookfield Viscometer (DV-E). Spindle number 3 was used.

Centrifugation

The microemulsion system was centrifuged at 3000 rpm for 15 minutes to determine whether the system shows signs of creaming or phase separation. The system was observed microscopically for appearance

Measurement of mucoadhesive strength

The mucoadhesive potential of each formulation was determined by measuring the force required to detach the formulation from nasal mucosal tissue using a modified method described in literature[10]. In brief, nasal tissues were carefully removed from the nasal cavity of sheep obtained from the local slaughterhouse. Tissues were immediately used after separation. At the time of testing, a section of nasal tissue was secured (keeping the mucosal side out) to the upper probe using a cyanoacrylate adhesive. The upper probe was attached to precalibrated force displacement transducer SS12LA, (BIOPAC Systems Inc, Santa Barbara, CA) connected to the Biopac MP-30 data acquisition system (BIOPAC Systems). The surface area of each exposed mucosal membrane was 0.785cm^2. At room temperature, fixed amount of samples of each formulation were placed on the lower probe. The probes were equilibrated and maintained at 34 ^{0}c. Probe with nasal tissue was lowered until the tissue contacted the surface of the sample. Immediately, a force of 0.1 N was applied for 2 minutes to ensure intimate contact between the tissues and the samples. The probe was then moved upwards at a constant speed of 0.15 mm/s. The bioadhesive force, expressed as the detachment stress in dyne/cm^2, was determined from the minimal weights that detached the tissues from the surface of each formulation using the following equation;

$$\text{Detachment Stress (dyne/cm}^2) = mg/A$$

where m is the weight added to the balance in grams; g is the acceleration due to gravity taken as 980 cm/s^2 and A is the area of tissue exposed. Measurements were repeated thrice for each of the microemulsion preparations, but before each measurement a fresh smooth gel surface was created[10].

Drug content of microemulsion

10 mg equivalent of SS dissolved in 100ml of methanol. The concentration of solution was found to be 100 µg/ml. The drug content was estimated at 228nm. The drug content of formulation MME3A was determined by UV spectrophotometric method as described in literature.

***In-vitro* drug diffusion study**

In-vitro diffusion study of microemulsion was carried out by Franz diffusion cell having 2.0 cm diameter and 16 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 (PB) pH 6.6 for 24 hrs prior to experiment. Diffusion cell was filled with phosphate buffer pH 6.6 and dialysis membrane was mounted on cell. The temperature was maintained at 34^{o}C. After a pre-incubation time of 20 minutes, the microemulsion equivalent to 10 mg of sumatriptan succinate was placed in the donor chamber. Samples were periodically withdrawn from the receptor compartment for 4 hours and replaced with the same amount of fresh phosphate buffer solution, and assayed by a spectrophotometer at 228 nm.

***Ex- Vivo* Permeation Studies**

Fresh nasal tissues were carefully removed from the nasal cavity of sheep obtained from the local slaughterhouse. Tissue samples were inserted in Franz diffusion cells displaying a permeation area of 0.785 cm2. 16 ml of phosphate buffer saline (PBS) pH 6.6 at 34 ^{0}c was added to the acceptor chamber. To ensure oxygenation and agitation, a mixture of 95% O2 and 5% CO2 was bubbled through the system. The temperature within the chambers was maintained at 34 ^{0}c. After a pre-incubation time of 20 minutes, pure drug solution and formulation equivalent to 10 mg of sumatriptan was placed in the donor chamber. At predetermined time points, 1-ml samples were withdrawn from the acceptor compartment, replacing the sampled volume with PBS pH 6.6 after each sampling, for a period of 4 hours. The samples withdrawn were filtered and used for analysis. Blank samples (without sumatriptan) were run simultaneously throughout the experiment to check for any interference. The amount of permeated drug was determined using a UV-visible spectrophotometer at 228 nm.

Accelerated Stability Studies

The microemulsion batches of optimized formulation were stored in stability chamber (Remi CHM-10S®) at 40^{0}C and 75% RH for 3 month and samples were evaluated for physicochemical parameters like particle size, microscopic appearance at one month interval.

Histological study

It is necessary to examine histological changes in nasal mucosa caused by formulations, if it is to be considering for practical use. Histological studies shows control mucosa (phosphate buffer treated nasal mucosa) stained with hematoxylin-eosin and the effect of formulation on sheep nasal mucosa is observed with help of photomicrographs. Mucosal structure is seen when treated with formulation as compared to the control.

RESULTS AND DISCUSSION

Phase behavior

The following figures represent the pseudoternary phase diagrams for microemulsions systems along with the ratios of surfactant and cosurfactant, as 1:1 and 3:1. Each of the vertices of triangle represents 100% of each of oil, water and surfactant and cosurfactant mixture (Smix). The change in the area of microemulsion region can be

very well seen in the ternary phase diagram (Fig 1 and Fig 2) as the ratio of surfactant to cosurfactant was changed from 1:1 to 3:1.

The area of microemulsion did not show significant change when the surfactant to cosurfactant ratio was changed from 1:1 to 2:1. But when ratio of surfactant to cosurfactant was 3:1, there was increased in microemulsion region, because of high concentration of surfactant. Also the presence of low molecular weight alcohols (n- butanol) can influence the formation of microemulsions by both interfacial and bulk effects. Their short hydrophobic chain and terminal OH group, enable them to interact with surfactant monolayer at the interface thereby affecting their packing which is in turn can influence the curvature of the interface and interfacial energy. The low molecular weight cosurfactant also enables them to distribute between the aqueous and oil phase thereby altering the relative hydro/ lipophilicity. These two properties render short chain alcohols useful for the preparation of microemulsions.

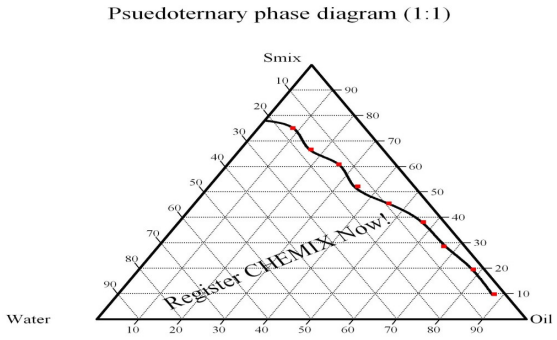


Figure 1: Phase diagrams of surfactant-co surfactant- at different Tween 80: Span 80: n-butanol ratios of 1:1

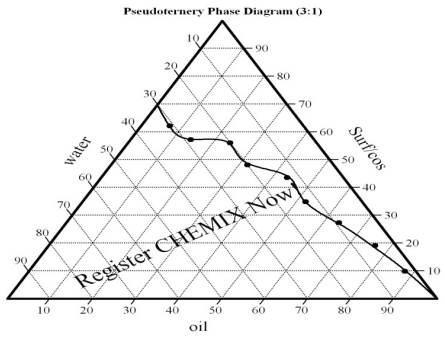


Figure 2: Phase diagrams of surfactant-cosurfactant- at different Tween 80: Span 80: n-butanol ratios of 3:1

Identification of type microemulsion

From the image the type microemulsion was found to be o/w type. As amaranth water soluble dye was used for test, hence background was found to be red and globule appears colorless (Fig 3).

Physicochemical characterization of microemulsion

The physicochemical characteristics of the developed microemulsion appear in Table 2.

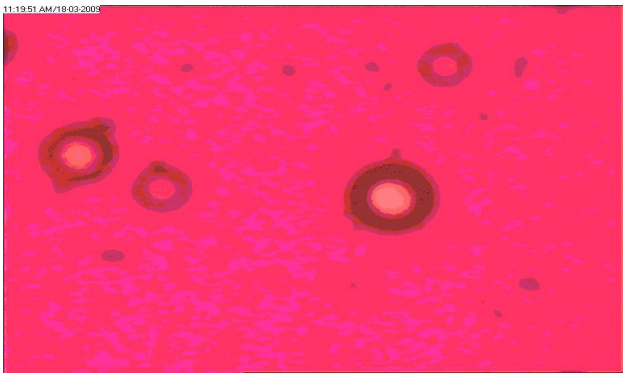


Figure 3: Photomicrograph of o/w type of microemulsion (MME3A)

Table 2: Characteristics of microemulsions

Formulation code	pH	Drug Content (%)	Globule size (nm)	Zeta potential (mv)
ME2	5.12	94.5	30.56	-14.23
ME2	5.04	95.0	28.46	-15.75
ME3	5.11	97.5	35.65	-18.43
M34	5.12	98.33	32.49	-26.23
MME3A	5.3	99.1	38.78	-17.2
MME3B	5.6	100.83	79.25.	-25.12

Microemulsion developed was clear and transparent, with pH 5.3 and particle size of optimized formulation (MME3A) was found to be 38.78 nm (Fig 4). The zeta potential of MME3A was -17.2 mv (Fig 5).

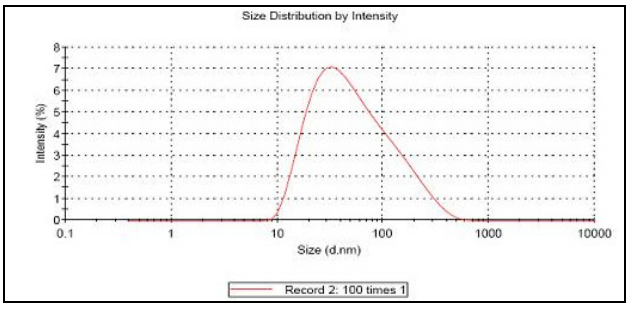


Figure 4: Particle size distribution of MME3A

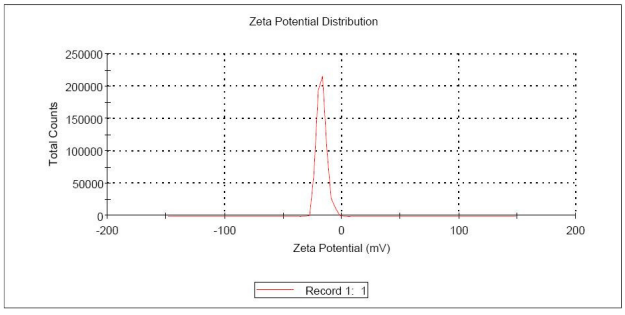


Figure 5: Zeta potential of MME3A

The microemulsion was found to be stable on centrifugation at 3000 rpm for 15min. The viscosity of microemulsion at room temperature was 303.4cP. The drug content of MME3A was found to be 99.1%. In vitro diffusion studies of developed formulation gave a control release with HPMCK4M was 95.52 % in 4h. From dilution and staining test it can be concluded that the system was o/w type. The nanometric size range of particle was retained even after 100 times dilution with water, which proves systems compatibility with excess water. The enhanced absorption may be explained in terms of the huge specific surface area of the microemulsion droplets. Improved the permeation of the sumatriptan succinate because of presence of surfactant, which reduces the interfacial tension to nearly zero. Stable intranasal formulations of sumatriptan succinate have been developed and evaluated. The microemulsion based intranasal delivery might be a promising approach for the rapid onset and controlled delivery of sumatriptan succinate.

Viscosity and mucoadhesion study

Viscosity of ME1 and ME2 (w/o) was less than ME3 and ME4 (o/w).Because the surfactant concentration in o/w was more than w/o. Surfactant used i.e. Tween 80 and Span 80 were highly viscous in nature. Viscosity of Tween 80 and Span 80 was 970-1080 cP and 425 cP respectively (Table 3). The detachment stress of o/w was also more because of high viscosity. In case of w/o concentration of oil was more and viscosity of oil (IPM) was very less i.e. 5-6 cP, therefore detachment stress was less. In case of MME3A and MME3B concentration of mucoadhsive polymer in MME3A was 0.05% and MME3B was 0.1%. Therefore viscosity and detachment stress of MME3A was less than MME3B. But because of high concentration of mucoadhsive polymer MME3B formed the stiff gel which may cause damage to mucosa (Table 3).

Table 3: Viscosity and detachment stress of microemulsions

Formulation code	Viscosity (cP)*	Detachment stress (dyne/cm^2)*
ME1	130.2±0.16	-
ME2	147.6±0.55	115.6±0.3
ME3	202.1± 0.19	332.27±0.25
ME 4	191.3±0.12	-
MME3A	303.4±0.30	346.484±0.23
MME3B	385.2±0.18	455.643±0.3

*Values expressed as Mean ± SD, n =3

***In vitro* drug diffusion studies**

Figure 6 shows highest diffusion in case of MME3A microemulsion because it contained mucoadhesive polymer HPMCK4M. Because of mucoadhesion, the residence time inside the nasal cavity was increased and also increased the drug absorption.

***In-vitro* permeation study**

In vitro nasal permeability data shown in figure 9. The drug diffused at faster rate from microemulsion. The total percentage diffusion was much higher from the microemulsion system. After 4hrs of diffusion 97.74 % of drug was diffused from microemulsion.

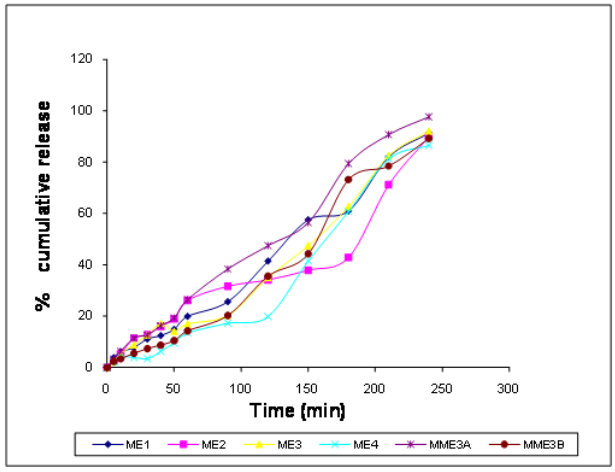
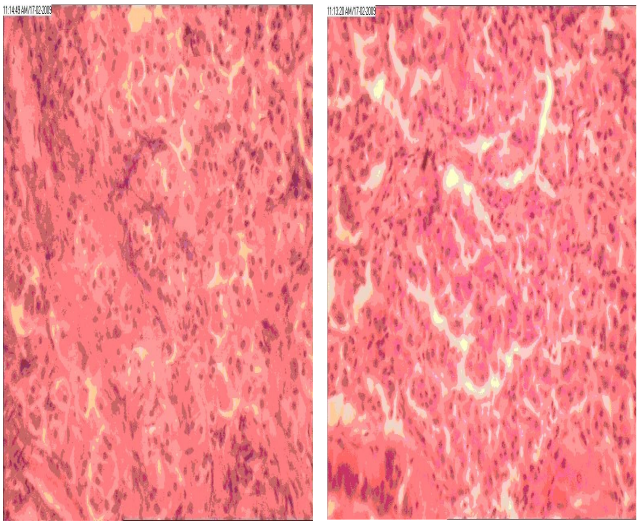


Figure 6: In vitro drug release profile of microemulsions

Histological Evaluation of Mucosa

The microscopic observations indicate that the optimized formulation has no significant effect on the microscopic structure of mucosa. As shown in Figure 7, neither cell necrosis nor removal of the epithelium from the nasal mucosa was observed after permeation of MME3A. The epithelium layer was intact and there were no alterations in basal membrane and superficial part of submucosa as compared with PBS-treated mucosa. Thus, microemulsion formulations seem to be safe with respect to nasal administration.



A. control mucosa B. MME3A treated mucosa

Figure 7: Histological examination of nasal mucosa.

Stability of the microemulsion

Formulations showing optimum particle size which are selected for stability studies. According to ICH guidelines, selected formulation MME3A was stored at 40^{0}C temperature and 75% relative humidity (RH) for a period of 3 months. Formulation was evaluated at periodical intervals of one month for particle size and microscopic appearance. The average particle size was remained relatively unchanged. Microemulsion remained clear and transparent.

CONCLUSION

The study demonstrated that the microemulsion formulation can be employed to improve the bioavailability of a poorly absorbed drug. The optimum microemulsion formation contained iso propyl myristate (5%), Tween 80 (36.75%), Span 80(15.75%), n-butanol (17.5%) and double distilled water (25%), which was a transparent and less viscous system. Enhanced rate and extent of transport of SS following intranasal administration of MME3A may help decreasing the dose and frequency of dosing and possibly maximize the therapeutic index. In conclusion microemulsion system is a promising approach for the intranasal delivery of sumatriptan succinates for the therapeutic effect improvement.

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