



Development And Evaluation Of Peppermint Oil Containing Microemulsions For Intranasal Delivery

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Abstract

Objective: To develop and formulate a non toxic, user-friendly, and comfortable intranasal spray formulation of peppermint oil for alleviating migraine.

Materials and Methods: Peppermint oil, Tween80, Span80, PEG400, nasal spray pumps, reagents and solvents of analytical grade were used. Microemulsions were prepared by water titration method. Effect of 1% glycerin on formulation viscosity was studied. VP7/100A, VP7D/100A and VP6/100A spray pumps were evaluated for spray pattern, shot weight and spray content. *In vitro* and *Ex vivo* diffusion studies were carried through cellulose acetate membrane and sheep nasal mucosa respectively. *In vivo* efficacy was assessed with Digital Actophotometer.

Results: Formulations were developed using (Smix) Tween 80: Span 80 in the ratios of 1:0.25 and 1:0.5, PEG 400 (0.5 gm) and glycerin (1%). These were stable, clear with pH of 4.5 to 6.5 and viscosity was 304.5cps. Vp6/100A exhibited the most consistent spray and uniform delivery amongst the tested pumps. The amount of drug diffused was 80% through cellulose acetate membrane and 75% through sheep nasal mucosa within 24hrs. Histopathological studies revealed no significant irritation and damage to nasal mucosa. 74% inhibition of motility was seen with the developed formulations.

Conclusion: Developed intranasal spray formulations containing peppermint oil showed potential to alleviate migraine.

Keywords

Migraine, Peppermint oil, Microemulsions, Blood Brain barrier, Intranasal.

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Introduction

Intranasal drug delivery is non invasive, rapid and comfortable route of administration [1- 3]. It bypasses the BBB and thus has systemic exposure reducing the systemic side effects [2, 3]. The rate and extent of absorption and plasma concentration versus time profiles obtained through intranasal delivery are relatively comparable to those obtained by intravenous medication [4]. The nasal mucosa has respiratory and olfactory mucosa, principally responsible for transport of drug across the nasal mucosa [3, 5]. Low molecular weight, lack of ionisation at physiological pH and lipophilicity of an agent favours penetration [3, 6]. Lipophilic agents after absorption have bioavailability comparable to those observed after IV administration. Hence, lipophilic agents like peppermint oil will easily cross the barrier and show their potential effects. Peppermint oil can be used for complementary treatment of migraine. Since the sense of smell is altered during migraine, essential oils can be used for treatment. Neuronal connections between the nasal mucosa and the brain provide a unique pathway for the non invasive delivery of therapeutic agents to the brain [7]. The deposition of drug in the area is mainly dependent on the delivery system and formulation characteristics because nasal cavity has peculiar anatomy and physiology. Hence microemulsions are preferred for intranasal delivery as they are thermodynamically stable, transparent or translucent dispersions of oil and water stabilized by an interfacial film of surfactant molecule [8-10].

Microemulsions provide ultra low interfacial tensions and large o/w interfacial areas as well as ability to concentrate and localize significant amount of both oil and water soluble materials within the same isotropic medium [11, 12]. Schulman et al have postulated that interfacial tension is a key process in microemulsion formation and that the main role of the surfactant is to reduce the interfacial tension to very low values [13, 14]. Microemulsions exist in equilibrium with excess water, oil or both. These equilibrium systems are referred as TYPE I, TYPE II, TYPE III [15]. When oil, water and surfactant are mixed, microemulsions are only one of the number of association structures that can form, depending on the nature and concentration of each component as well as

prevailing temperatures and pressure. A useful approach to illustrate the complex series of interactions that occur when different ratios of components are mixed is by construction of phase diagram. The phase diagrams help in determining the ratios of oil: water: surfactant- cosurfactant at the boundary of microemulsion region [16]. It also depicts microemulsion, micellar and crystalline regions [15].

Migraine headaches are a common medical problem that physicians frequently encounter in their practice. A migraine headache is felt as a throbbing or pulsating headache that is often one sided (unilateral) and associated with nausea; vomiting; sensitivity to light, sound, and smells; sleep disruption; and depression. Attacks are often recurrent and tend to become less severe as the migraine sufferer ages. It is the name given to severe headaches, normally lasting from 4 to 72 hours. During migraine, there is contraction or dilation of the blood vessels. Triggers of a migraine attack include anxiety, stress, lack of food or sleep, exposure to light and hormonal changes (in women). Migraine headaches are sometimes called vascular headaches (having to do with the blood vessels). Studies suggest that a migraine is caused by swelling of the blood vessels in the scalp and tissues around the brain, causing more blood to pump through the brain. Most primary headaches slowly develop over minutes to hours. The pain experienced in headache is transmitted by the slowest of all unmyelinated nerves.

Migraines can be disabling, leading to the individual's suffering if not treated appropriately and quickly. There is a variety of medications and treatment approaches that can be used to relieve pain and any associated symptoms. Since the sense of smell is altered and often heightened during a migraine, aromatherapy is definitely best used between attacks use at the use at the earliest stage of migraine [17-19]

Microemulsions can cross the blood brain barrier due to their stable nature. Thus headaches may be alleviated because of microemulsion administration through nasal route by crossing the BBB. Hence attempts were made to develop microemulsion formulations of peppermint oil for intranasal delivery. In the present work we aim to develop intranasally administered microemulsion formulations of peppermint oil. Inhalation of these formulations will help in complementary treatment of migraine headaches. Efficacy of the developed formulations has been compared with the marketed formulation containing sumatriptan using motility model in rats as animal.

Material and Methods

Peppermint oil (Vedic Life Sciences, Mumbai), Tween80, Span80 and PEG400 (S. D. Fine Chemicals Ltd. and Merck Ltd.), nasal spray pumps (Valois Pvt. Ltd.) All the other reagents and solvents were of analytical grade.

Experimental methods:

For microemulsion formulation, assessment of solubility of active in various solvents is essential. Solubility data gives an idea about lipophilicity of compound.

I) Solubility analysis of Peppermint oil:

For determination of solubility, 25 mg of active oil was taken in 2ml of the solvent system. Solvents like water, ethanol, ether, methanol, chloroform, glacial acetic acid were tried out. The system was allowed to saturate with the oil. The amount of the

active soluble in the system was estimated using developed HPTLC method.

II) Formulation of Microemulsions using Pseudo ternary Phase Diagram:

In the present work an attempt was made to develop microemulsion formulations of the Winsor type III where, the oil phase and the aqueous phase exist in equilibrium and surfactants are concentrated in surfactant rich bicontinuous middle phase. In the present study combination of surfactants Tween 80: Span 80 in two ratios (1:0.25) and (1:0.5) was used and formulations with or without cosurfactant PEG 400 (23%) were also prepared.

Following optimization steps were involved in preparation of microemulsions:

- 1) Varying the ratio of surfactant to one another.
- 2) Assessing the effect of cosurfactant in formulations
- 3) Varying the ratio of surfactant mixture to oil.

In order to optimize surfactant and cosurfactant concentration, phase diagrams surfactant and cosurfactant combinations were constructed. Phase diagrams were constructed by using aqueous titration method. The ratio of selected surfactant to cosurfactant (Smix) was kept constant while oil to Smix ratio was varied. Five different combinations of oil and Smix (1:9, 2:8, 3:7, 4:6, 5:5) were made so that maximum range of concentrations was covered for the study to delineate the boundaries of phases precisely formed in the phase diagrams. Aqueous phase was titrated slowly with each weight ratio of oil and Smix till turbidity is observed. Transparency, flowability and physical state of developed microemulsions were observed visually noted. Amount of water required was noted down and was converted into %. Ternary phase diagram (fig 1) was plotted using the software TRIDRAW.

III) Optimisation of Glycerin content:

The developed formulations are intended for intranasal administration, so glycerin was added to maintain the physiology of nasal system. Glycerin not only acts as a humectant but also helps in avoiding nasal irritation that may be caused by the excipients in the formulation. Glycerin concentration was varied in the range of 1-5% in the formulation. Effect of glycerin concentration on the appearance of formulation and water addition was also investigated. Based on visual inspection and percent of water uptaken a suitable concentration of glycerin was incorporated into final formulations. Results are given in table 3.

IV) Effect of glycerin (1%) on viscosity of formulation.

Glycerin in 1% concentration was added into final formulation. Effect of 1% glycerin on formulation

viscosity at different shear rates was studied; this was compared with formulations prepared without glycerin. Viscosity of the formulation was measured using Brookfield Model DV III programmable rheometer fitted with SC 4-16 spindle. A sample volume of 1 ml was taken and viscosity was measured in triplicates. To understand the behaviour of developed formulations a plot of shear stress v/s shear rate was drawn.

V) Characterization of microemulsion formulations:

Developed microemulsion formulations were characterized for following parameters:-

- 1) **Globule size and polydispersity index:** Globule size and polydispersity index of the microemulsion formulations was noted using Beckman Coulter Counter N 5. Samples were suitably diluted with water and placed in the cuvette. Particle size of the developed microemulsion was noted.
- 2) **pH:** The pH of gel formulations was determined by digital pH meter. Gel weighing one gram was dissolved in 100 mL of distilled water and stored for two hours. The pH of formulations was measured in triplicate and average value was determined.
- 3) **Stability on Centrifugation:** Formulations were agitated at 3000 rpm for 30 minutes using a centrifuge to see the effect of agitation and stress on stability. Results of characterization studies are depicted in table 3.
- 4) **Drug content:** Drug content of developed formulations was determined using a developed and validated HPTLC method.

Following are the chromatographic conditions maintained during the assay:

- Stationary phase: Silica gel G F254 precoated plates.
- Mobile phase: Benzene
- Saturation time: 40 minutes
- Spraying agent: Vanillin sulphuric acid
- Temperature: 24°C
- Migration distance: 80mm
- Maximum wavelength: 545nm
- Rf value: 0.13-0.21

Standard curve was prepared over a concentration range of 100-500µg for active component (peppermint oil) in methanol. The data of area under peak versus drug concentration was treated by linear least square regression analysis. The Rf value was found to be between 0.13- 0.21. Drug content for developed formulation was determined from standard curve by extrapolating the AUC values.

VI) Selection of intranasal pumps for Intranasal Sprays:

Amongst the pumps Vp7/100ACS20, Vp7D/100 and Vp6/100A, selection was made based upon the spray pattern, shot weight and uniformity of the content sprayed from each pump. Developed microemulsion formulations were filled in canister and pumps were

attached. These were then tested for:

Spray pattern: Nasal spray containing the formulation was held at a distance of approximately 1.5cm from the precoated silica plate. Spray delivered through intranasal pump was impinged on the plate. The spray was delivered and the spots formed were observed under UV chamber. Spray pattern and diameters obtained from each pump were measured. A typical spray pattern obtained on silica gel plate is shown in figure 3

Shot weight: This is the weight of formulation delivered during each actuation. The difference between the initial weight of the container (w1) and after spraying (w2) was determined. This was repeated 5 times; each time noting down the difference in the weight. The spray pattern obtained should be consistent for uniform delivery of the actives. Figure 4 gives the comparison of the shot weights delivered by each pump.

Uniformity of drug content: HPTLC method was used to determine uniformity of content per spray. A single spray of peppermint oil formulation was delivered in a vial containing 1 mL of methanol. This solution was then sonicated for 30 minutes. The resulting solution was centrifuged at 3000 rpm for 15 minutes. The supernatant containing peppermint oil; approximately 5 µL was spotted on precoated silica gel plate to get concentrations of 500 µg. The plate was dipped in the mobile phase benzene and was then sprayed with vanillin sulphuric acid reagent and dried at 105°C for 15 minutes. The plate was scanned at 545nm and the uniformity of the content was determined based on the comparison of the area for the formulation containing peppermint oil with the pure oil.

VII) In vitro drug diffusion study

In vitro permeation study of different formulations was performed through cellulose acetate membrane. Phosphate Buffer Saline (PBS) (pH 6) containing tween 80 (20%) was used as the receptor medium in Franz diffusion cell. Cellulose acetate membrane was sandwiched between the receptor compartment and donor compartment. The receptor fluid was maintained at 37 ± 1°C by circulating water bath. The content of the receptor fluid was stirred continuously using a magnetic stirrer. Samples were withdrawn at different time intervals, replaced with same volume of fresh solution, filtered, and amount of peppermint oil was determined by HPTLC method detected at 540 nm using the developed HPTLC method.

VIII) Ex vivo diffusion studies

Permeation study through pig nasal mucosa of different formulations was investigated using Franz diffusion cell. The PBS (pH 6) containing

tween 80 (20%) was used as the receptor medium in the diffusion cell. Mucosa was placed between the receptor compartment and donor compartment so that the dermal portion was continuously bathed with the receptor fluid maintained at $37 \pm 1^\circ\text{C}$ by circulating water bath. Nasal mucosa side was exposed to ambient temperature. The content of the receptor fluid was stirred continuously using a magnetic stirrer. Samples were withdrawn at different time intervals, replaced with same volume of fresh solution, filtered, and amount of peppermint oil was determined by HPTLC method. Figure 5 is a plot of cumulative drug release vs time through cellulose acetate membrane and sheep nasal mucosa.

IX) Histological studies:

Developed microemulsion formulations were applied for 24 hr on the excised sheep nasal mucosa. After 24 hours formulations were removed; mucosa was wiped off with tissue paper and fixed with neutral carbonate formalin solution in saline for at least 72 hr before routine processing. The tissue was sectioned vertically and each section was dehydrated and embedded in paraffin wax. Tissues were divided into small pieces and stained with hematoxylin and eosin. All sections were then examined by microscope under magnification of 10X. Sheep nasal mucosa untreated with any formulation served as a control.

X) *In vivo* efficacy studies on the developed microemulsion formulations:

Inhalation of essential oils provides calming and comforting effect to the patient thus alleviating pain in migraine sufferers [20]. To collect detailed information on the sedative effect of the oil, motility of the animals was ascertained after inhalation therapy and was compared to caffeine induced agitation [21, 22]. Digital actophotometer was used to study the motility of the animals. Rats were divided in three groups with six animals in each group.

Group 1: Positive control –Caffeine

Group 2: Developed intranasal formulation

Group 3: Conventional formulation containing sumatriptan

Animals in the positive control group were agitated by injecting intraperitoneally, 0.5mL of 0.1% solution of caffeine. Developed formulations and conventional formulations (containing sumatriptan), 100 μL each were administered intranasally and motility was noted for each of the animals using digital actophotometer. The effect on motility was recorded at predetermined time intervals of 30, 60, 120 and 180 mins. Percent reduction in the motility was calculated using the equation

$$\% \text{reduction in motility} = \frac{\text{Caffeine treated grp} - \text{Formulation treated grp}}{\text{Caffeine treated grp}} \times 100$$

Percent reduction in the motility for all the three groups was

compared by plotting percent reduction in motility versus time (figure 7).

Results and Discussion

Solubility studies gave fair idea about lipophilicity of peppermint oil and hence span 80 was used for emulsifying the oil droplets. Span 80 is a hydrophobic surfactant and thus can form emulsions easily. As the concentration of span 80 was increased for formulating the microemulsion, amount of oil incorporated in system was also increased. HLB value of span 80 is low, favouring formation of w/o microemulsions, thus greater incorporation of oil. The amount of water incorporated for formulation with the oil: Smix ratio of (1:0.5) was higher. Co-surfactant helps to further reduce the interfacial tension thus greater addition of water resulting in stable microemulsion formulation. To determine the optimum concentration of PEG-400, PEG-400 in varying concentrations was added to system comprising of oil: Smix ratio of 1:9. These systems were titrated with water. As the concentration of PEG-400 was increased from 0.1gm to 0.5 gm the amount of water incorporated in the system also increased. Approximately, 28% of water was incorporated when the ratio of Smix: oil was adjusted to 9:1. PEG 400 was added in the formulation in the concentration of 23%. This amount helped in decreasing the interfacial tension to very low values as postulated by Schulman *et al* [20]. PEG-400, is composed of hydrophilic polyethylene chains favouring more addition of water. PEG-400 also inhibits lamellar phase formation (rigid layer) of surfactant at the interface of two immiscible systems. It was observed (table 2) that with the addition of glycerin, microemulsion formulation became shiny and transparent. As the concentration was further increased an unusual shine was observed in the formulation. However, the % of water uptake in the formulation diminished. Lower concentration of water in the formulation is undesirable, hence to serve the purpose of humectant, glycerin was added in 1% concentration in the developed formulations.

The plot (Figure 2) indicates the flow pattern of formulations containing 1% glycerin compared with formulations containing no glycerin. Formulations containing glycerin showed thinning behaviour as the viscosity reduced with increasing shear stress. For formulations containing glycerin the rheograms depicted that the formulations follow Newtonian flow pattern, since the shear rate was directly proportional to shear stress. For formulations containing no glycerin shear rate was not proportional to shear stress. Thus the flow behavior of formulations without glycerin was Non Newtonian in nature.

Polydispersity index in the range of 0.0-0.5 is desirable [12]. PI of 0.450 of developed formulations indicates that particles were in desired size range.

The spray pattern observed is depicted in figure 3. As seen in the figure pump vp6/100A nasal spray was able to deliver round uniform droplets. Flow through other two pumps Vp7/100A, Vp7/100ACS20 was in the form of

blotted particles (non-uniform pattern). Vp6/100A is a pump suitable for viscous preparations; the other two pumps are more suitable for aqueous preparations. Developed microemulsions are viscous in nature; hence the flow pattern is uniform from Vp6/100A pump. Thus, Vp6/100A pump was selected due to suitability to deliver the formulation.

Shot weight is the amount of formulation delivered per spray. The dose delivered after each spray was determined and by calculating the difference in the initial weight and weight after one actuation shot weight was noted. Results for the shot weight test are shown in figure 4, indicate that amongst the shot weights for all the three pumps, Vp6/100A exhibited the most consistent spray and uniform delivery through the pump. *In vitro* and *ex vivo* drug diffusion studies were carried out using cellulose acetate and sheep nasal mucosa respectively as membranes. Amount of the active diffused through the membrane and collected in donor compartment was determined by using HPTLC method. As indicated in the figure 5, the amount of active diffused through the membrane and mucosa was almost similar.

A comparative histopathological study for microemulsions was performed on porcine mucosa and further compared with untreated skin as control group. The influence of active on the anatomical structure of the nasal mucosa was elucidated with the aid of light microscope findings. Section of the untreated control group showed normal uniform layered epithelium. The developed formulations are used for cooling and calming effects. Generally, digital actophotometer is used for estimating the locomotor activity of the animals. This model was used for checking the efficacy of the developed formulation. % reduction in the motility was noted as response. A considerable reduction in % inhibition of activity was found. Thus the developed formulation has a potential to reduce the motility and produce calming and comfort in the excited animal. Hence the developed formulations can be used to support antimigraine therapy.

Conclusion

Developed intranasal formulations showed potential in relieving migraine related headache. Essential oils can be used as an effective approach in alleviating the pain of migraine sufferers. Developed intranasal formulations provided comfort and soothing effect. Intranasal administration provides nose to brain targeting of essential oils for antimigraine effect. The effect needs to be further investigated by conducting detailed pharmacokinetics and clinical trials.

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Table 1: Solubility data of Peppermint oil in different solvents

Solvent	Observation
Water	Insoluble
Ethanol	Partly Soluble
Ether	Soluble
Methanol	Soluble
Chloroform	Partly Soluble
Glacial Acetic Acid	Partly Soluble

Table 2: Effect of Glycerin on peppermint microemulsion

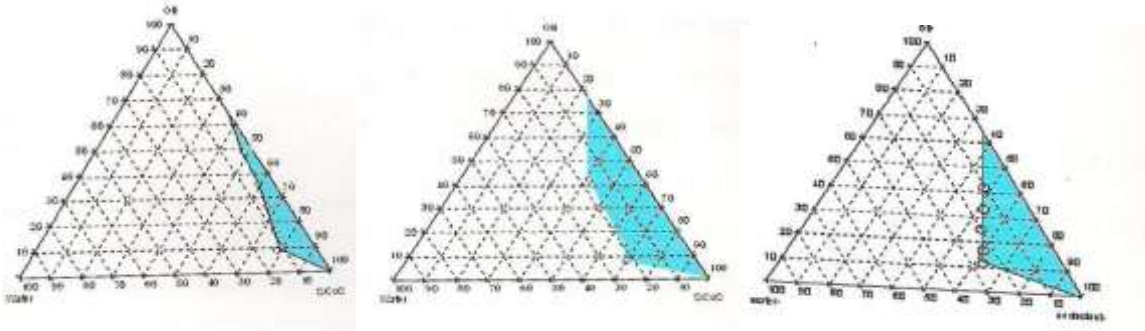
Glycerin (%)	Water (%)	Appearance
1	27	Shiny
2	24	Shiny
3	21	Shiny
4	19	Extremely Shiny
5	18	Extremely Shiny

Table 3: Physicochemical characterization of developed formulation

Characterisation of microemulsion	
Appearance	Clear, shiny
Globule Size	392-400nm
Polydispersity index	0.450
pH	4.5-6.5
Stability	Stable
Oil content	95-105%

Table 4: Spray characteristics of various spray pumps alongwith spray pattern observations for peppermint oil

Nasal Pumps	Suitable for	Spot pattern on TLC plate	Diameter of spot (cm)
Vp7/100ACS20	Aqueous solvent	Blowing	Could not be calculated
Vp7D/100	Aqueous solvent which are preservative free	Slightly blowing	Could not be calculated
Vp6/100A	Viscous solution	Uniform round	1.4



Tween 80: Span80(1:0.25)

Tween 80: Span80(1:0.5)

Tween 80: Span80(1:0.5) and 0.5gm of PEG 400

Figure 1: Ternary phase diagram for peppermint oil microemulsion formulations

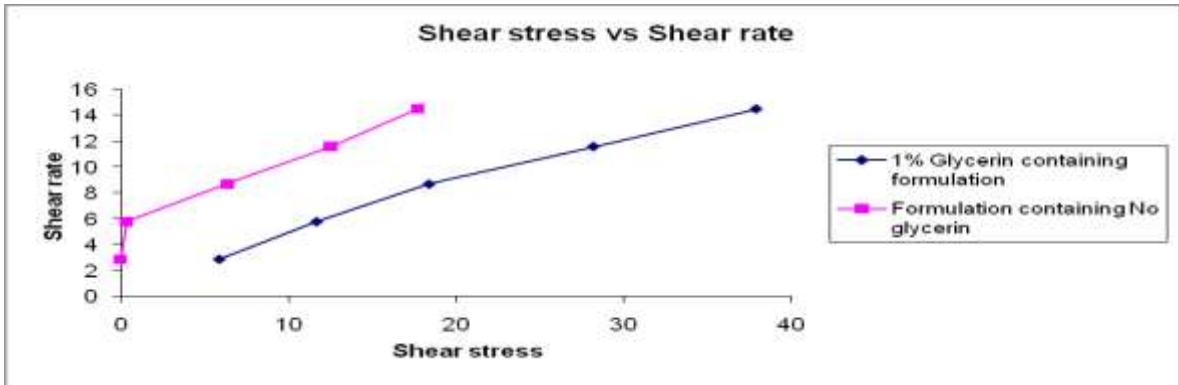


Figure 2: Effect of glycerin on viscosity of the formulation

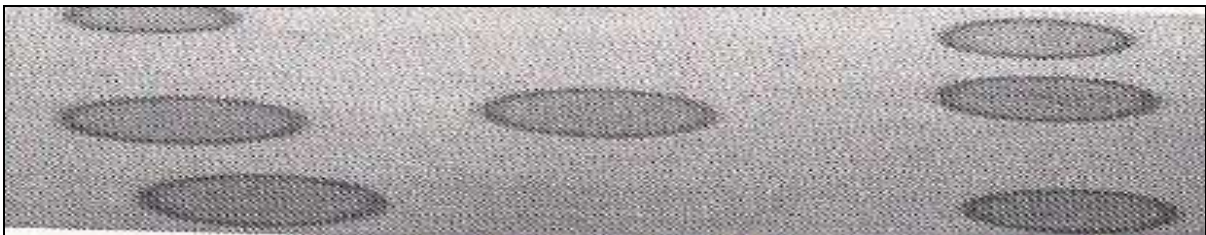


Figure 3 Spray pattern on silica gel plate obtained through the device Vp6/100A for peppermint microemulsion formulation

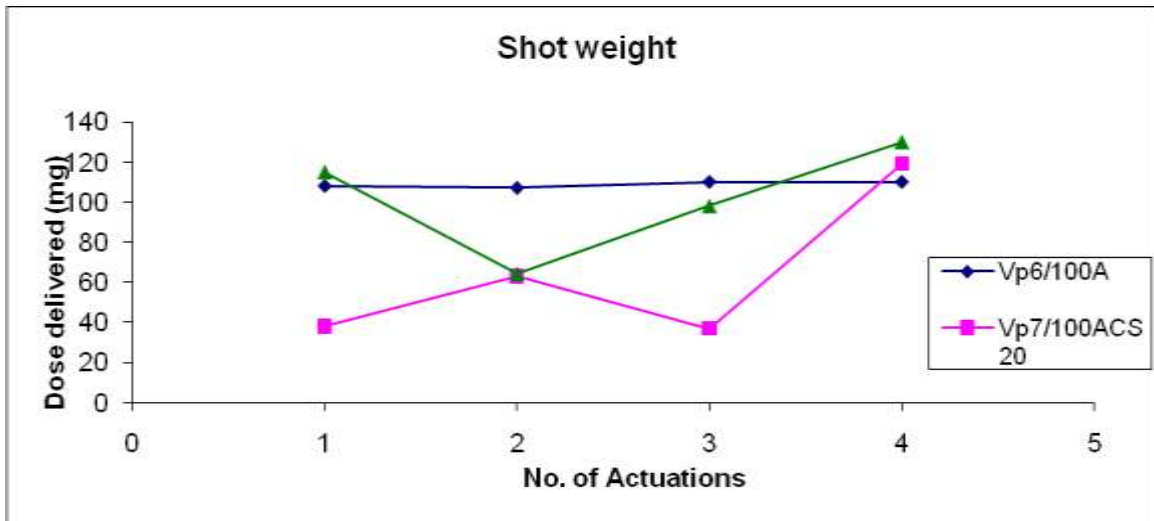


Figure 4: Shot weights obtained from the three pumps for peppermint oil microemulsion

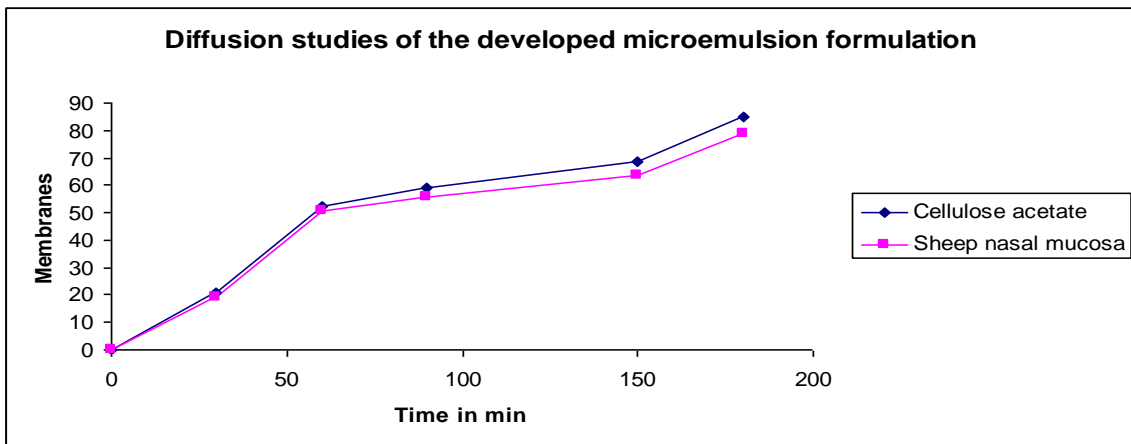
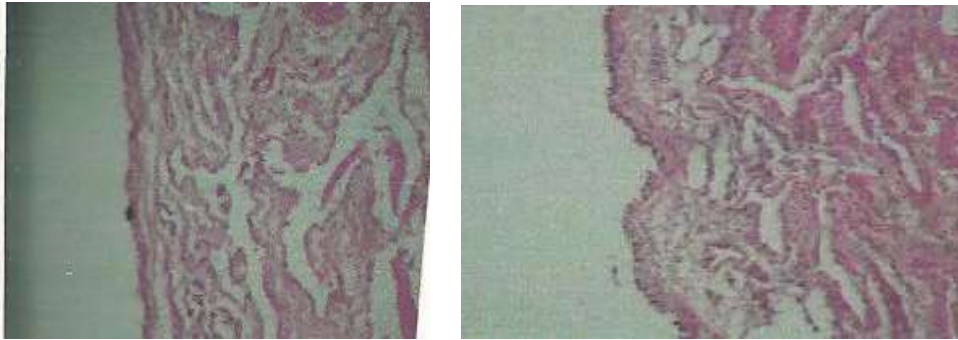


Figure 5: Diffusion studies of the developed formulation through membranes



Control (Untreated nasal mucosa) Formulation treated nasal mucosa

Figure 6: Histopathological studies on untreated and treated nasal

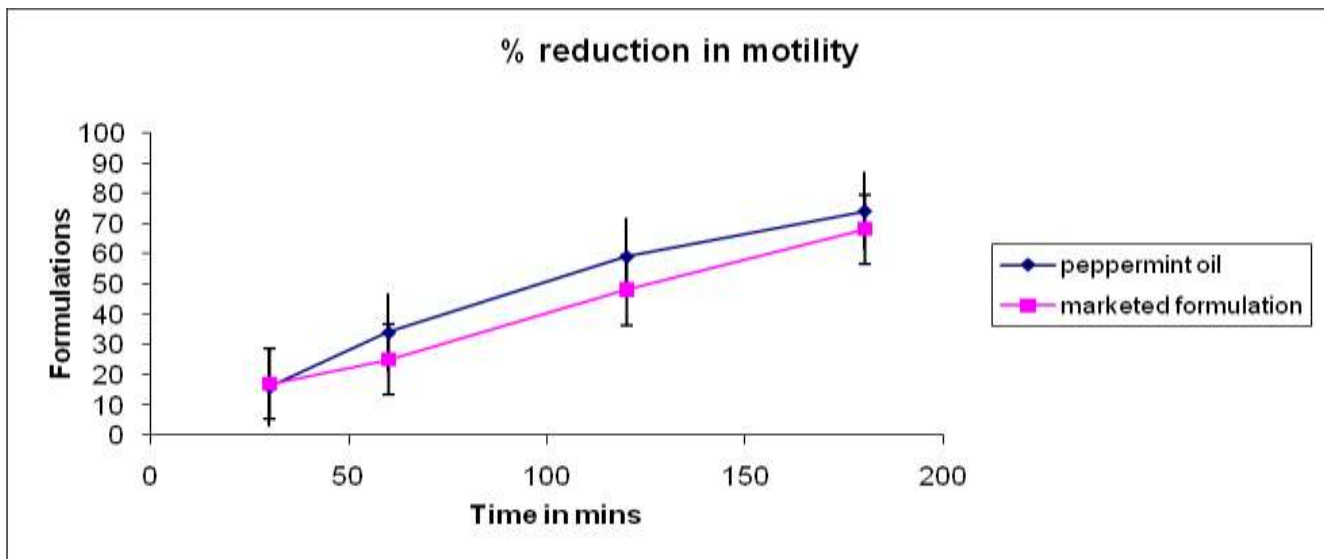


Figure 7: percentage reduction in the motility (locomotor activity) of animals mucosa

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