

FORMULATION DEVELOPMENT OF SERTRALINE HYDROCHLORIDE MICROEMULSION FOR INTRANASAL DELIVERY

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ABSTRACT: Nasal cavity has used as alternative route for the drugs with poor water solubility, susceptible to acidic or enzymatic degradation and hepatic metabolism. The objective of the present study was to improve solubility of sertraline hydrochloride (STH), formulate microemulsions containing STH to accomplish rapid onset of action and to bypass the first-pass metabolism. A microemulsion system with Capmul MCM as oil phase, Labrasol as surfactant and Transcutol P as cosurfactant was developed for intranasal delivery of STH. Phase behaviour and solubilization capacity of the developed microemulsion system were characterized and STH microemulsions (SME) were prepared by titration method and evaluated for globule size, drug content, nasal ciliotoxicity, percentage transmittance, pH and viscosity. *invitro* studies for nasal absorption were carried out on goat nasal mucosa. The drug shows a high solubility of 117 mg/ml in a microemulsion containing 22.2% Capmul MCM, 44.5% (w/w) surfactant/cosurfactant (Labrasol: Transcutol P at 2:1) and 33.3% H₂O. In *invitro* studies the nasal absorption was found to be 66.07±0.78% from SME2. These results indicate that intranasal microemulsion of STH may be beneficial for the treatment of depression.

Keywords: Sertraline hydrochloride, First-pass metabolism, Solubilization, Intranasal, Mucoadhesive, Depression.

INTRODUCTION AND EXPERIMENTAL

Microemulsions have attracted large interest in the pharmaceutical industry as drug delivery systems due to their improved drug solubilization properties, increased shelf life and ease of preparation. They normally consist of an aqueous phase, an oil phase, a surfactant and a cosurfactant. When the concentrations of these components are favorable, they spontaneously emulsify to form a monodisperse, thermodynamically stable, transparent microemulsion. Many drugs that are insoluble in aqueous media are prepared as preconcentrates consisting of oil, surfactant and alcohol. This is then diluted with water to form the microemulsion prior to administration to the patient.

The addition of a surface active agent to a drug delivery vehicle can result in improved drug stability and clinical potency and increased drug absorption. If the drug vehicle containing a surfactant is further optimized to form microemulsion or lyotropic liquid crystalline system, the additional advantages of potentially increased solubilization of poorly soluble drug and thermodynamic stability are realized. From the structural point of view the systems containing oil, water and surfactant can create various colloidal structures from liquid microemulsions to lamellar liquid crystals depending on the ratio of the components^[1].

Nasal drug delivery is easy, well-tolerated and noninvasive. The patient can self administer the drug and

control the dosage when appropriate, which enables home treatment and a cheaper alternative. Nasal cavity has used as alternative route for the drugs with poor water solubility, susceptible to acidic or enzymatic degradation and hepatic metabolism. Systemic treatment of many central nervous system (CNS) diseases, such as depression, is considerably impaired by limited delivery of therapeutics. Poor CNS access is mainly related to selective barriers that sequester the CNS from the circulatory system. Drug concentrations in the brain after nasal administration are usually the result of absorption into the systemic blood circulation and subsequent transport across the BBB, i.e. the drug is transported to the CNS systemically. However, delivery of drugs from the systemic circulation to the CNS is heavily limited by the BBB. The BBB is an endothelium of capillaries with epithelial-like high resistance tight junctions that perfuses the mammalian brain ^[2]. Penetration of a drug through the BBB depends on characteristics such as the lipophilicity and size of the molecule and its specificity for a variety of ATP-dependent transport systems ^[3]. Small lipophilic drugs are transported across the BBB via free diffusion, but other drugs require the active carrier- or receptor-mediated transport mechanisms that exist for transfer of endogenous substances such as nutrients or vitamins ^[2]. Efflux proteins such as P-glycoprotein (P-gp) in the BBB protect the brain from potentially harmful substances. There is also a barrier between the blood and the cerebrospinal fluid (CSF), the blood-CSF barrier (BCSFB), which consists of a single continuous layer of polarized epithelial cells with tight junctions that line the choroid plexus. This barrier is not as restrictive as the BBB and has a 1000- fold smaller surface area, but has a wider range of enzymes than the BBB ^[3, 4].

Individuals have reported euphoria as soon as 3-5 min after sniffing the illegal drug cocaine and the initiation of the behavioral or physiological effects of cocaine precedes the rise in plasma cocaine concentrations after a single nasal dose ^[5-7]. Thus, early cocaine effects are not all due to nasal or oral absorption into the systemic blood circulation and subsequent transport across the BBB; some cocaine has been directly transferred to the CNS, presumably via the olfactory pathways, bypassing the BBB. An animal study demonstrated three times higher levels of cocaine in the olfactory bulbs one minute after a nasal dose compared with i.v. administration ^[8], which indicates that cocaine can be transferred to the CNS via olfactory pathways.

To achieve the therapeutic dose of STH in the effective nasal delivery volume $\leq 300\mu\text{l}$ (150 μl /nostril) the high solubilization capacity is required which is offered by the microemulsion systems. The other challenge is the achievement of rapid-onset nasal absorption of STH to meet the emergency therapeutic purpose of this formulation. The aim of this current project was to develop a microemulsion system using GRAS (generally regarded as safe) materials for the solubilization and rapid-onset intranasal delivery of sertraline hydrochloride. The solution-like feature of

microemulsion could provide advantages over regular emulsion in terms of the dose uniformity and formulation physical stability.

MATERIALS

Sertraline hydrochloride (STH) was received as a gift sample from Sun Pharmaceuticals Ltd., India. Capmul MCM was received as a gift sample from Abitech Corporation Limited, Columbus, Ohio. Ethyl laurate and Chitosan were purchased from Across chemicals, Mumbai. Isopropyl myristate was purchased from Central drug house (P) Ltd., New Delhi. Labrasol and Transcutol P were received as gift samples from Colorcon Asia Ltd., India. Tween 80 was purchased from Merck Ltd., Mumbai. Propylene glycol was purchased from Qualigens fine chemicals, Mumbai. The freshly excised goat nasal mucosa was collected from the local slaughter house.

METHOD ^[9,10]

Solubility Determination in Microemulsion Components:

Solubility of the STH was determined in different oil phases such as Capmul MCM, Ethyl laurate, Isopropyl myristate and in surfactants such as Tween-80, Labrasol and cosurfactants such as Propylene glycol and Transcutol P. STH was added in excess to different oils, surfactants and cosurfactants and stirred for 24 hrs on Wrist Action Shaker. After stirring for 24 hr samples were centrifuged at 8000 rpm for 10 min and the drug was analyzed in the supernatant after proper dilution with methanolic 0.01N HCl by UV-Visible spectrophotometer at 274nm to calculate the solubility of drug .

Phase Diagram Development and Microemulsion Formulation:

To select the different components in the formulation of nasal microemulsions, two compatible surfactants Labrasol and Tween-80 in combination with Transcutol P and Propylene glycol respectively as cosurfactants were used. Capmul MCM was selected as the oil phase for the present study. The water titration method was used for the construction of pseudoternary phase diagram of oil, surfactant, cosurfactant and water to obtain the concentration ranges of different components that explores the microemulsion region.

For the phase diagram construction of each microemulsion system the ratio of oil to the surfactant/cosurfactant mixture (S_{mix}) was varied from 1:9-9:1 (w/w). The oil- S_{mix} mixture was titrated with water in dropwise manner under vigorous stirring to obtain a transparent microemulsion. Surfactants were blended with cosurfactants in fixed weight ratios (1:1 and 2:1) to study the effect of surfactant concentration on globule size.

After the identification of microemulsion region from the phase diagram the desired component ratio were selected for the microemulsion preparations. The microemulsions were simply prepared by adding the weighed amount of

components together and stirring to form a transparent microemulsion. The STH loaded microemulsions were prepared by dissolving the drug in oil-S_{mix} mixture, adding the required amount of water and stirring to form the transparent microemulsion. Mucoadhesive formulations were also prepared by adding chitosan to the aqueous phase.

Nasal Ciliotoxicity Studies:

For nasal ciliotoxicity studies freshly excised goat nasal mucosa, except for the septum were collected from the slaughter house in saline and treated with 0.5 ml of microemulsions for 1 h, then rinsed with saline. The treated nasal mucosa was examined with an optical microscope. Saline and isopropyl alcohol were used as a negative and positive control, respectively.

Globule Size Analysis:

The globule size analysis of microemulsions with or without STH was conducted using photon correlation spectroscopy with in-built Zetasizer (Malvern Instruments, UK). The influences of the ratio of surfactant to cosurfactant on the globule size were evaluated.

Characterization of Optimized Microemulsions:

The optimized microemulsion and mucoadhesive microemulsion were evaluated for parameters like pH, globule size, solubilization, conductivity, clarity and physical stability. STH content in optimized formulations were determined by UV spectroscopy method.

in Vitro Diffusion Studies:

in vitro release kinetic studies through goat nasal mucosa in phosphate buffer were carried out for the optimized microemulsion and mucoadhesive microemulsion for a period of 4 h using Franz diffusion cell.

RESULTS AND DISCUSSION

STH Solubility in Microemulsion Components:

The solubility of STH in individual components of microemulsion system was studied and represented in Table 1. According to the table the solubility of STH in Capmul MCM was about 66 mg/ml, much higher than its solubility in other oils. Therefore Capmul MCM was selected as the oil phase for the microemulsion development.

Phase Behaviour:

The pseudoternary phase diagrams of both surfactant/cosurfactant systems (Labrasol/Transcutol P and Tween-80/Propylene glycol) with two surfactant/cosurfactant weight ratios (1:1 and 2:1) are represented in figure 1. The microemulsion area is presented in the phase diagrams as shaded region. The rest of the region on the phase diagram represents the turbid and conventional emulsions based on visual identification. From these phase diagrams, the influence of relative surfactant: cosurfactant concentrations on the microemulsion isotropic region can be evidently seen. Fig. 1 shows the effect of Tween 80: Propylene glycol ratio and Fig. 2 shows the effect of Labrasol: Transcutol P ratio on the phase behavior of these pseudo-ternary

systems. In both systems the microemulsion region increased in size with the higher surfactant concentration. This increase was toward the oil–water axis, indicating that by increasing the Tween 80 or Labrasol concentration, the maximum amount of water and Capmul MCM that could be solubilized into the microemulsion increased.

The concentration of surfactant also affects the thickness of microemulsion. The formulations with higher weight percentage of Tween 80 and Labrasol show greater thickness than the formulations with lower weight percentage.

Microemulsion Composition and Characterization:

From the developed phase diagrams the microemulsion formulations were selected on the bases of viscosity and solubility of STH in the selected microemulsion formulations. One microemulsion was selected from the Tween-80/Propylene glycol (2:1) system and one microemulsion was selected from the Labrasol/Transcutol P (2:1) system and defined as SME1 and SME2 respectively. The composition of these two formulations is shown in Table 2.

Table 3 shows the characterization of these two microemulsions in terms of solubilization capacity, particle size distribution, and conductivity. The solubility of STH, a low solubility compound (3.4mg/ml), was improved dramatically by the microemulsions, and SME2 (117 mg/ml) produced a higher solubilizing capacity of STH than SME1 (94 mg/ml).

The particle size of SME1 and SME2 fell into the size range of microemulsion (10–150 nm) as shown in Table 3. Although the mean particle sizes of these two MEs were about the same, with a mean diameter of 78.1 nm for SME1 and 76.2 nm for SME2.

Both of the formulations were clear and transparent with the %transmittance of 99.39 and 99.87 respectively. These two microemulsion formulations were physically stable at room temperature with the presence or absence of STH for a period of 2 months, without the occurrence of phase separation and significant particle size change. No degradation of STH was detected during the study period.

The pH value for SME1 was 5.13 and for SME2 was 5.21 which fall well within the nasal pH range and hence the formulations will not produce irritation upon instillation.

The electric conductivity values for SME1 and SME2 were 0.119 and 0.107 mS/cm, respectively. The type of these microemulsions could not be distinctly defined as w/o or o/w microemulsion according to these conductivity values; however, the conductivity appears to rise.

Nasal Ciliotoxicity:

Nasal ciliotoxicity studies were carried out in an attempt to evaluate any potential toxic effects of excipients used in the formulation on the nasal mucosa. Thus the nasal mucosa of goat was treated with blank microemulsion to evaluate the toxic effects of excipients used in the formulation.

The figure 2 shows the nasal mucosa treated with PBS pH 6.4 (negative control) no nasociliary damage was observed and the nasal membrane remained intact. The Figure 3 show the nasal mucosa treated with isopropyl alcohol (positive control), and an extensive damage to nasal mucosa was observed coupled with loss of nasal cilia. However, with blank microemulsion, no damage to nasal mucosa could be observed (Figure 4), thus substantiating the safety of the excipients used in the formulations.

***in Vitro* Diffusion Studies:**

in-vitro absorption of STH through the goat nasal mucosa from SME1 and SME2 were evaluated and compared with each other. (Fig.5) SME1 shows $62.85\% \pm 0.56$ and SME2 shows $66.07\% \pm 0.78$ diffusion of STH through the goat nasal mucosa in the 4 hour study. Considering the solubilization property, particle size analysis, and *in vivo* absorption findings, SME2 is believed to be a better formulation than SME1 for the rapid-onset intranasal delivery of STH.

The microemulsion system comprising Capmul MCM, Labrasol, Transcutol P and H₂O showed high solubilization capacity of STH. *invitro* absorption studies revealed that STH diffusion from a microemulsion containing 22.2% Capmul MCM, 44.5% (w/w) surfactant/cosurfactant (Labrasol: Transcutol P at 2:1) and 33.3% was $66.07 \pm 0.78\%$ over a period of 4h. In conclusion, the microemulsion system of STH might be a promising approach for the rapid-onset intranasal delivery of STH for the treatment of depression. The optimized nasal formulations show effective absorption in terms of *invitro* release through excised goat nasal mucosa. Further animal studies are required to prove the therapeutic potential of dosage form and to establish the *invitro- invivo* correlation.

Table 1: Solubility of STH in various microemulsion components

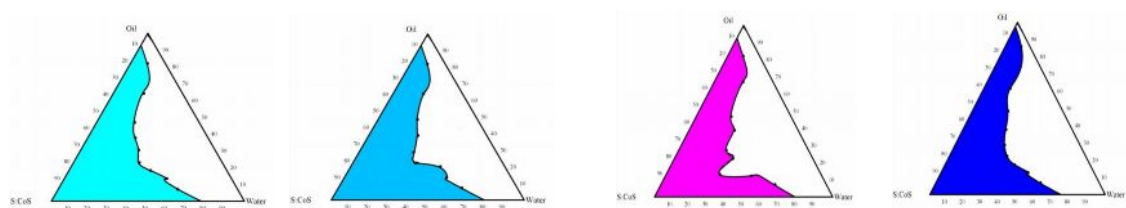
| Components | Solubility (mg/ml) |
|---------------------|--------------------|
| Capmul MCM | 65.71 \pm 4.1 |
| Ethyl laurate | 1.1 \pm 0.3 |
| Isopropyl myristate | 1.97 \pm 0.3 |
| Tween-80 | 57.14 \pm 3.9 |
| Labrasol | 13.31 \pm 1.7 |
| Propylene glycol | 94.28 \pm 4.3 |
| Transcutol P | 21.94 \pm 1.1 |
| Water | 3.2 \pm 0.2 |

Table 2: Composition of optimized microemulsion formulations

| Ingredients | SME 1 | SME 2 |
|-------------------------|-------|-------|
| Capmul MCM (%w/w) | 20 | 22.2 |
| Tween-80 (%w/w) | 22.2 | - |
| Propylene glycol (%w/w) | 11.1 | - |
| Labrasol (%w/w) | - | 29.67 |
| Transcutol P (%w/w) | - | 14.83 |
| Water (%w/w) | 46.7 | 33.3 |

Table 3: Characterization of microemulsion formulations

| Formulation | Solubilization capacity(mg/ml) | Globule size(nm) | pH | %Transmittance | Conductivity (mS/cm) |
|-------------|--------------------------------|------------------|------|----------------|----------------------|
| SME1 | 94.28 | 78.1 | 5.13 | 99.39 | 0.119 |
| SME2 | 117.14 | 76.2 | 5.21 | 99.87 | 0.107 |



T-80:P-glycol (1:1) T-80:P-glycol (2:1) L-sol:T-cutol P (1:1) L-sol:T-cutol P (2:1) Figure 1: Phase diagrams of Capmul MCM

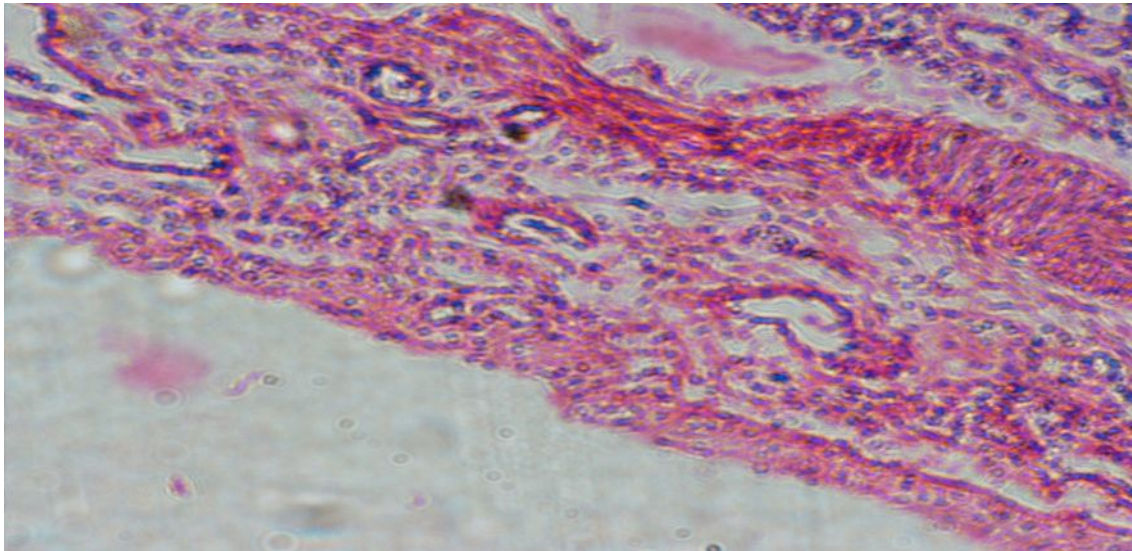


Figure 2: Nasal mucosa treated with phosphate buffer saline (pH 6.4)

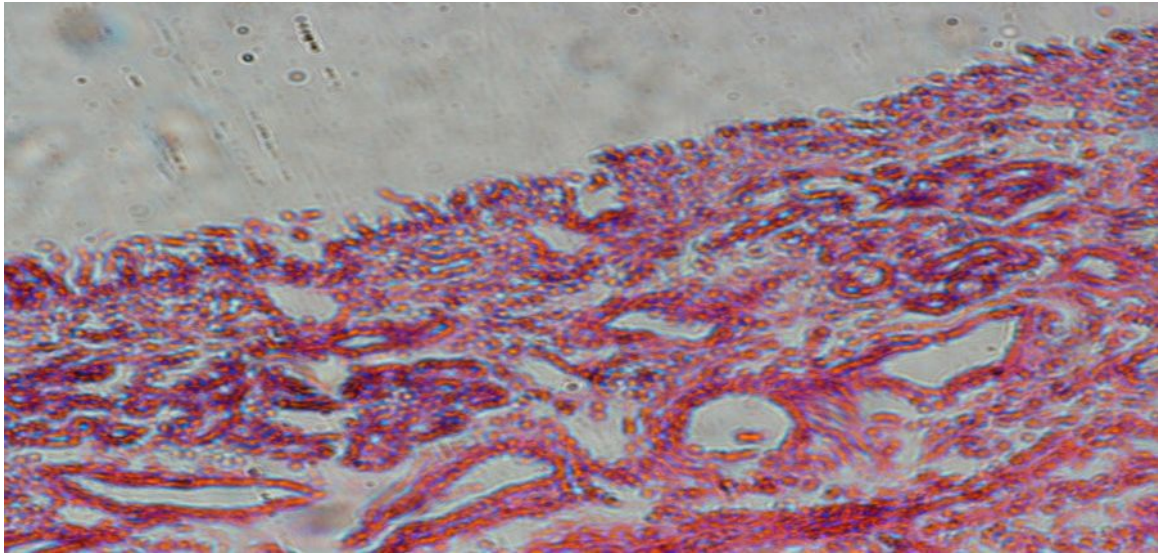


Figure 3: Nasal mucosa treated with Isopropyl alcohol

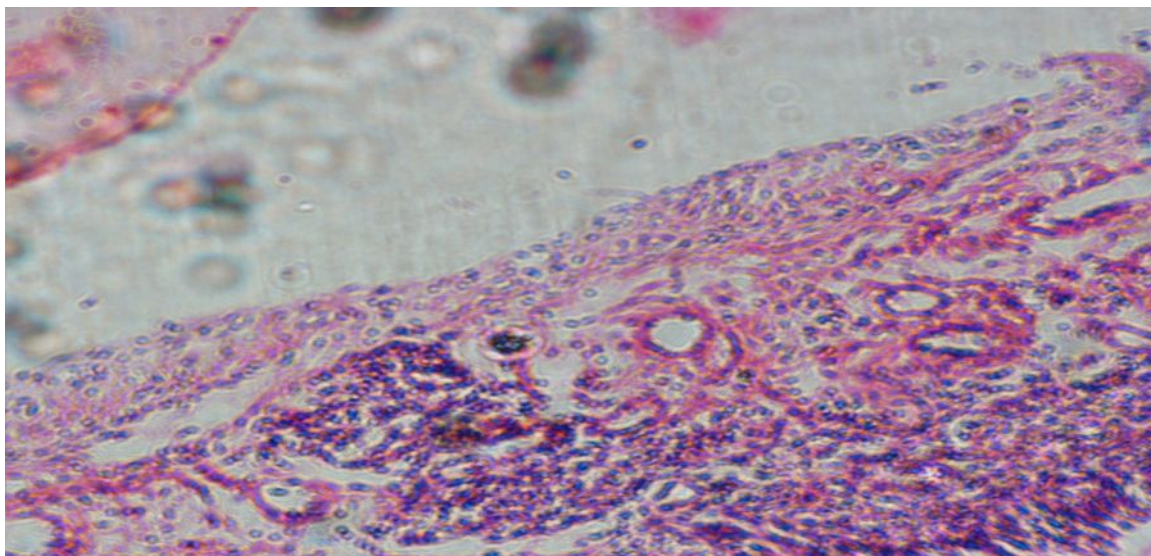


Figure 4: Nasal mucosa treated with blank microemulsions

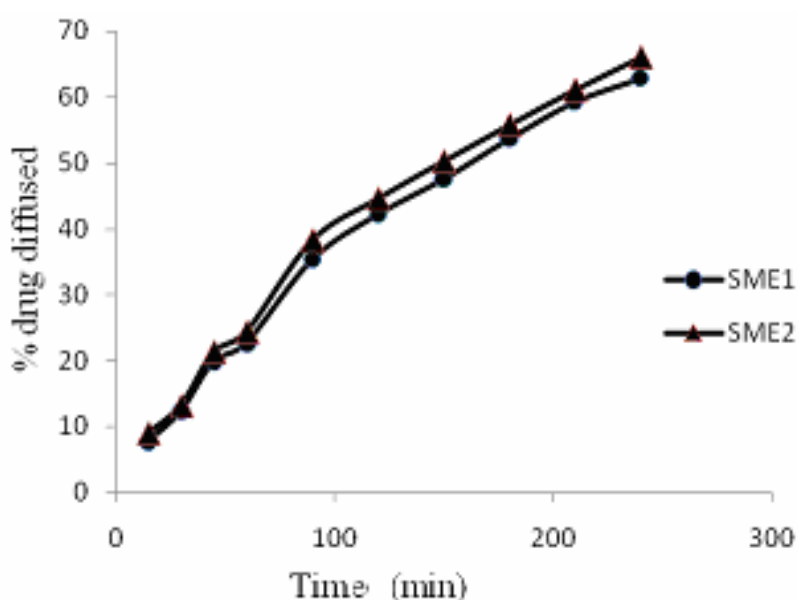


Figure 5: Percent drug diffused v/s time profile of SME1 and SME2

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