



RESEARCH ARTICLE

CAPMUL MCM BASED NANOEMULSION FOR INTRANASAL DELIVERY OF AN ANTIDEPRESSANT

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The rationale of this study acquaint with improvement of sertraline hydrochloride (STH) solubility, formulation of STH nanoemulsion (NE) for intranasal delivery to achieve rapid onset of action and to omit first pass effect with enhanced bioavailability. STH nanoemulsion (NE) system was formulated consisting of capmul MCM as oil phase, tween 80 as surfactant and propylene glycol as co-surfactant. The developed system was characterized for phase behaviour and solubilization capacity and water titration method was utilized for the preparation of STH nanoemulsions (SNEs). All formulations were evaluated for globule size, drug content, nasal ciliotoxicity, pH and viscosity. A high STH solubility of 94.28 mg/ml was observed with the NE system containing 20.0% capmul MCM, 33.3% surfactant/co-surfactant (Labrasol:Transcutol P at 2:1) and 46.7% water. *In vitro* diffusion studies for nasal absorption explanation were executed on goat nasal mucosa. *In vitro* nasal absorption through goat nasal mucosa was found to be $62.85 \pm 0.56\%$. These results suggested that intranasal delivery of STH may be beneficial over the available oral delivery for the treatment of depression.

Key words: Sertraline hydrochloride, First-pass effect, Solubilization, Intranasal, Depression.

INTRODUCTION

Recent investigations on nasal delivery recognize the nasal crater as a surrogate route for the drugs with deprived aqueous solubility, vulnerable to acidic or enzymatic devastation and hepatic metabolism. Nasal crater is a well-tolerated and non-invasive route with ease of administration. Nasal delivery provides self administration and dosage control when required which facilitate home treatment and a cost-effective substitute. Systemic delivery of drugs acting on central nervous system (CNS), such as antidepressants, is considerably complicated due to the discriminatory physiological barriers that selectively seize the CNS from the circulatory system. Brain drug levels following nasal administration are the

results of double absorption pathway *i.e.* direct transfer through olfactory region and absorption into the systemic circulation then transport across the blood brain barrier (BBB) (Pardridge, 1999). Absorption of therapeutics via BBB is significantly affected by the properties like lipophilicity, molecular size and specificity of drug for a variety of ATP-dependent transport systems (Graff and Pollack, 2004; Pardridge, 1999). Blood and the cerebrospinal fluid are also discriminated by blood-CSF barrier, which is made up of a single uninterrupted layer of polarized epithelial cells with rigid junctions that line the choroid plexus. This barrier has a wider range of enzymes with 1000 times lower surface area but less restrictive than BBB (Graff and Pollack, 2004; Loscher and Potschka, 2005). The

transfer of drug molecules to CNS after nasal administration is evident by the sniffing of illegal drug cocaine which results euphoria within 3-5 min and behavioral and physiological effects are initiated before the rise in plasma levels of cocaine following a single nasal dose (Perez-Reyes and Jeffcoat, 1992; Farre *et al* 1993; McCance-Katz *et al* 1993). The results of an animal study showed three times higher levels of cocaine in olfactory bulbs one minute after nasal administration in comparison to *i.v.* administration (Chow *et al* 1999), which indicates a direct pathway *via* olfactory region.

NEs exhibit remarkable potential for the future of cosmetics, diagnostics, drug delivery systems and biopharmaceuticals (Bielinska *et al* 2008). Many researchers have reported nanoemulsion for intranasal or transdermal administration (Shakeel *et al* 2007; Kumar *et al* 2009; Zheng *et al* 2010; Talegaonkar *et al* 2011; Choksey *et al* 2011; Parmar and Lumbhani, 2012; Borges *et al* 2013).

NEs have fascinated the pharmaceutical arena as drug delivery systems owing to their cut above drug solubilization properties, amplified shelf-life and spontaneous method of preparation. They characteristically composed of an aqueous phase, an oil phase, a surfactant and a co-surfactant. Amalgamation of a surface active agent to a formulation can significantly upgrade drug stability and clinical potency with augmented drug absorption. The optimized concentration of surfactant in NE or lyotropic liquid crystalline system put forward the additional advantages in terms of superior solubilization capacity and thermodynamic stability (Lieberman *et al* 1998; Lachman *et al* 1986; Attwood, 1994).

NEs can be characterized as emulsions with mean globule size stretch of 50 nm to 1000 nm. Exploitation of NEs for the delivery of therapeutics has experiencing an enthusiastic intensification in the area of controlled delivery and targeting.

To accomplish the therapeutic dose of STH in the effective nasal delivery within volume $\leq 300 \mu\text{l}$ (150 μl /nostril), the extreme solubilization capacity is essential which is presented by the NE systems. The other challenge is to accomplish rapid onset of action to meet the emergency therapeutic purpose of this formulation.

The aim of this work was to develop a NE system using GRAS (generally regarded as safe) materials for the solubilization and rapid-onset intranasal delivery of STH.

MATERIALS AND METHODS

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 was purchased from Across chemicals, Mumbai, India. Isopropyl myristate was purchased from Central drug house (P) Ltd., New Delhi, India. Labrasol and Transcutol P were received as gift samples from Gattefosse, France. Tween 80 was purchased from Merck Ltd., Mumbai, India. Propylene glycol was purchased from Qualigens fine chemicals, Mumbai, India. The freshly excised goat nasal mucosa was collected from the local slaughter house.

Solubility studies

Solubility of the STH was ascertained in a variety of oil phases such as capmul MCM, ethyl laurate, isopropyl myristate and surfactants such as tween 80, labrasol and co-surfactants such as propylene glycol and transcutol P. STH was taken in excess with 2 ml of different oils, surfactants and co-surfactants and stirred for 24 h at 25°C on wrist action shaker (Remi Equipment Ltd., Mumbai, India). After standing for 24 h at room temperature, the undissolved STH was removed by centrifugation at 8000 rpm for 10 min and the content of STH was quantified in the supernatant after appropriate dilution with methanolic 0.01 N HCl using UV-Visible spectrophotometer (Shimadzu, Kyoto, Japan) at 274 nm (Kumar *et al* 2009).

Phase diagram studies and NE formulation

The GRAS listed surfactant tween 80 in combination with co-surfactant propylene glycol was used for phase behavior study of selected components for the formulation of nasal NEs. Capmul MCM was preferred as the oil phase for the present study. The water titration technique (Vyas *et al* 2006) was employed for the construction of pseudoternary phase diagram to get the concentration ranges of oil, surfactant, co-surfactant and water that can produce the maximum NE region.

Phase diagram for NE system was constructed by varying the ratio of oil to the surfactant/co-surfactant mixture (Smix) from 1:9-9:1 (w/w) (Vyas *et al* 2008). The oil-Smix mixture was titrated with water under vigorous stirring to form a transparent NE. Surfactants were blended with co-surfactants in fixed weight ratios (1:1

and 2:1) to analyze the effect of surfactant concentration on globule size.

After the recognition of NE region from the phase diagram the desired component ratios were selected for the preparation of NEs. The STH loaded NEs were prepared by dissolving the drug in oil-Smix mixture, adding the required amount of water and stirring to form the transparent NE.

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 NE for 1 h, then rinsed with saline. Saline and isopropyl alcohol were used as a negative and positive control, respectively (Kumar *et al* 2009). All treated nasal mucosa were examined with an optical microscope.

Globule size analysis

The globule size analysis of NEs with or without STH was done using photon correlation spectroscopy with in-built zetasizer (Malvern Instruments, UK). The influence of surfactant to co-surfactant ratio on the globule size was also evaluated.

Characterization of optimized NEs

The optimized NE was evaluated for parameters like pH, globule size, solubilization capacity, conductivity, clarity and physical stability. STH content in optimized formulation was determined using UV-Visible spectrophotometer at 274 nm. pH was determined using digital pH meter (Systronics, India). Ten ml of sample NE was taken in a beaker and glass electrode was dipped in NE for pH measurement.

For solubilization capacity, STH was taken in excess with 2 ml of different formulations stirred for 24 h at 25°C on Wrist Action Shaker. After standing for 24 h at room temperature, the undissolved STH was removed by centrifugation at 8000 rpm for 10 min and the content of STH was quantified in the supernatant after appropriate dilution with methanolic 0.01 N HCl using UV-Visible spectrophotometer at 274 nm. Globule size, conductivity and clarity were analyzed by photon correlation spectroscopy with in-built zetasizer.

In vitro diffusion studies

Optimized NE was screened for *in vitro* release study through goat nasal mucosa in phosphate

buffer solution pH 5 (PBS) for a period of 4 h using Franz diffusion cell. The freshly excised goat nasal mucosa, except the septum part, was collected from the slaughter house in PBS. The membrane was kept in PBS for 15 min to equilibrate. The superior nasal conche was identified and separated from the nasal membrane (Vyas *et al* 2006a). The excised superior nasal membrane was then mounted on Franz diffusion cell. The tissue was stabilized using PBS in both the compartments and allowed to stir for 15 min on a magnetic stirrer (Remi Equipment Ltd., Mumbai, India). After 15 min, solution from both the compartment was removed and fresh PBS was filled in the acceptor compartment.

The mounting of the nasal membrane was done using glue at the brim of the donor compartment to avoid leakage of the test sample and supported with thread crossover the cell. The temperature of the receiver chamber, containing 14 ml of diffusion media was controlled at 37°C±1 under continuous stirring with teflon coated magnetic bar at a constant rate, in a way that the nasal membrane surface just flushes the diffusion fluid.

RESULTS AND DISCUSSION

STH solubility in NE components

The solubility of STH in individual components of NE system was studied and represented in **Table 1**.

Table 1. Solubility of STH in various nanoemulsion components

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

It showed that 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 NE development.

The solubility of STH in tween 80 and propylene glycol was also promising to solubilize the therapeutic dose of STH within a single

nasal administration.

Phase behavior studies

The pseudoternary phase diagram of both surfactant/co-surfactant weight ratios (1:1 and 2:1) are represented in **Figure 1**.

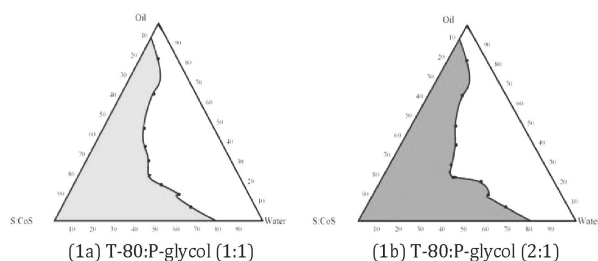


Fig. 1. Phase diagrams of capmul MCM

The NE 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: co-surfactant concentrations on the NE isotropic region can be evidently seen. In both systems the NE region increased in size with the higher surfactant concentration. This increase was toward the oil-water axis, indicating that by increasing the tween 80 concentration, the maximum amount of water and capmul MCM that could be solubilized into the NE increased. On the basis of phase behavior data it is concluded that the globule size depends on the concentration of the surfactant. Owing to their nano range globules NEs encompass stability against sedimentation or creaming with Ostwald ripening forming the

main mechanism of NE breakdown. The concentration of surfactant also affects the viscosity of NE. The formulations with higher weight percentage of tween 80 showed greater viscosities than the formulations with lower weight percentage.

NE composition and characterization

From the developed phase diagrams the NE formulations were selected on the basis of viscosity and solubility of STH in the selected NE formulations. One NE was selected from the tween-80/propylene glycol (2:1) system as optimized formulation for further evaluation and defined as SME 1. The composition of optimized formulation is shown in **Table 2**.

Table 2. Composition of optimized NE formulation

Ingredients	SME 1
Capmul MCM (% w/w)	20.0
Tween-80 (% w/w)	22.2
Propylene glycol (% w/w)	11.1
Water (% w/w)	46.7

Table 3 shows the characterization of SME 1 in terms of solubilization capacity, particle size distribution, and conductivity. The solubility of STH, a low solubility compound (3.4 mg/ml), was improved dramatically by the NE system and SME 1 (94.28 mg/ml) produced a high solubilizing capacity of STH.

The globule size of SME 1 fell into the size range of NE (10-150 nm) as shown in **Table 3**. Although, the mean globule size varies within the NE system with a mean diameter of 78.1 nm.

Table 3. Characterization of NE formulations

Formulation	Solubilization capacity (mg/ml)	Globule size (nm)	pH	Conductivity (mS/cm)	%Transmittance
SME 1	94.28	79.7	5.17	0.119	99.39

The formulation was clear and transparent with the % transmittance of 99.39. The optimized NE was 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 SME 1 was 5.17 which fall well within the nasal pH range and hence the formulation will not produce irritation upon nasal instillation. The electric conductivity value for SME 1 was 0.119. The type of the NE

could not be distinctly defined as *w/o* or *o/w* NE according to the conductivity value; however, the rise in conductivity indicates the aqueous nature of continuous phase of the formulation.

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 NE to evaluate the toxic effects of excipients used in the formulation. The

nasal mucosa treated with PBS (pH 6.4) (negative control) showed no nasociliary damage and the nasal membrane remained intact. The nasal mucosa treated with isopropyl alcohol (positive control) showed an extensive damage to nasal mucosa coupled with loss of nasal cilia. However, with blank NE, no damage to nasal mucosa could be observed, 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 SME 1 was evaluated (**Table 4**).

Table 4. *In vitro* diffusion studies across goat nasal mucosa

Time (min)	% Drug diffused
	SME 1
15	7.65±0.93
30	12.31±0.32
45	19.87±0.67
60	22.68±0.77
90	35.31±0.81
120	42.20±0.73
150	47.56±0.48
180	53.68±0.63
210	59.29±0.84
240	62.85±0.56

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SME 1 showed 62.85±0.56% diffusion of STH through the goat nasal mucosa in the 4 h study. Considering the solubilization property, particle size analysis, and *in vitro* absorption findings, SME 1 is believed to be a better formulation for the rapid-onset intranasal delivery of STH.

CONCLUSION

The NE system comprising capmul MCM, tween 80, propylene glycol and water showed high solubilization capacity of STH. *In vitro* absorption studies revealed that STH diffusion from a NE containing 20.0% capmul MCM, 33.3% surfactant/co-surfactant (Tween 80: Propylene glycol at 2:1) and 46.7% water was 62.85±0.56% over a period of 4 h. In conclusion, the NE system of STH might be a promising approach for the rapid-onset intranasal delivery of STH for the treatment of depression. The optimized nasal formulation showed effective absorption in terms of *in-vitro* release through excised goat nasal mucosa. Further animal studies are required to prove the therapeutic potential of dosage form and to establish the *in-vitro-in-vivo* correlation.

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