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Formulation and Evaluation of Microemulsions-Based Drug Delivery System for Intranasal Administration of Olanzapine

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ABSTRACT

This paper describes formulation considerations and *in vitro* evaluation of an oleic acid-based polyelectrolytic polymer-containing microemulsion drug delivery system designed for intranasal administration of a hydrophobic model drug Olanzapine. Drug-loaded microemulsions were successfully prepared by a water titration method. The microemulsion containing 4% oleic acid, 30% surfactant mixture of Labrasol: Cremophor RH 40 (1:1) : Transcutol P (3:1) and 66% (wt/wt) aqueous phase that displayed an optical transparency 99.93%, globule size 25.67 ± 1.17 nm, and polydispersity index of 0.121 ± 0.016 was selected for the incorporation of polyelectrolytic polymer (polycarbophil) as the mucoadhesive component. The mucoadhesive microemulsion formulation of Olanzapine that contains 0.5% polycarbophil (w/w) displayed higher *in vitro* mucoadhesive potential (19.0 ± 2.0 min) and diffusion coefficient ($1.40 \times 10^{-6} \pm 0.019 \times 10^{-6}$) than the microemulsion, followed Higuchi model, was free from nasal ciliotoxicity and stable for six months.

Keywords: diffusion study, *in vitro* mucoadhesion study, microemulsion, nasal ciliotoxicity study, transmission electron microscopy
Abbreviations: BBB, blood brain barrier; **CNS**, central nervous system; **CoS**, cosurfactant; **GI**, gastrointestinal; **GRAS**, Generally Recognized as Safe; **HLB**, hydrophilic lipophilic balance; **HPTLC**, high performance thin layer chromatography; **IM**, Intramuscular; **IR**, infrared; **Km**, surfactant and cosurfactant mixing ratio; **ME**, microemulsion; **MME**, mucoadhesive microemulsion; **OLZ**, Olanzapine; **OME**, Olanzapine microemulsion; **OMME**, Olanzapine mucoadhesive microemulsion; **OS**, Olanzapine solution; **PBS**, phosphate buffer saline; **PCS**, photon correlation spectroscopy; **PDI**, polydispersity index; **S**, surfactant; **Smix**, surfactant and cosurfactant mixture; **%T**, percentage transmittance; **TEM**, Transmission electron microscopy

INTRODUCTION

Schizophrenia is a major psychotic disorder that frequently has devastating effects on various aspects of the patient's life and carries a high risk of suicide and other life-threatening behaviors. In schizophrenia patients experiencing an acute exacerbation of psychotic symptoms, the primary goal of disease management is to achieve optimal control of symptoms. If this can be sustained over an extended period, the resulting stability may enable patients to optimize engagement in personally meaningful activities, such as employment or education. Consequently, improved patient functioning may have a significant impact upon long-term prognosis (Nasrallah *et al.* 2005; Miller 2009; Nordon *et al.* 2012).

Olanzapine (OLZ), {2-methyl-4-(4-methyl-1-piperazinyl)-10H-thieno [2, 3-b] benzodiazepine} belongs to the new generation of neuroleptic drugs used in the treatment of schizophrenia (Hiriyanna *et al.* 2008). The most desirable and convenient method of drug administration is the oral route. However in many instances, oral administration is unsuitable when the drug undergoes significant degradation in the gastrointestinal (GI) tract or is metabolized to a high degree via the first pass effect in liver. After oral administration OLZ shows extensive first-pass metabolism, with approximately 40% of the dose metabolized before reaching the systemic circulation (Kumar *et al.* 2008). Hence, an alternative route of administration should be preferable. Also the treatment of brain disorders is the greatest challenge because of a variety of formidable obstacles in effective drug delivery and maintaining therapeutic concentration in the brain. A practical, noninvasive and alternative route of administration for drug delivery to brain circumventing blood brain barrier (BBB) can be achieved by intranasal administration (Illum 2002). The advantages of nasal route are rapid absorption, higher bioavailability allowing lower doses, fast onset of therapeutic action, avoidance of liver or GI metabolism, avoidance of irritation of the GI membrane, reduced risk of overdose, non-invasive administration, ease of convenience and self-medication, and improved patient compliance. The last two decades have been marked by the recognition of the nasal cavity as a potential route for drug delivery.

Orally disintegrating wafers and intramuscular (IM) injection of OLZ are available to overcome the bioavailability problems because of extensive first-pass metabolism after oral administration (Kumar *et al.* 2008). The approved indications for OLZ IM injection are to rapidly control agitation and disturbed behaviors in patients with schizophrenia or manic episodes when oral therapy is inappropriate. A recent drug safety communication was issued to Eli Lilly (Australia) with the support of the Therapeutic Goods Administration. This communication reported adverse events associated with the administration of OLZ intramuscular (Zyprexa®) (WATAG Advisory Note). These adverse events beside the need of a therapeutic prompt action make OLZ a possible candidate for the development of a nasal formulation. Microemulsions (MEs) have been explored widely as a delivery system by virtue of having considerable potential to enhance transport of a wide range of drug molecules (Patel *et al.* 2009).

A natural defence mechanism, nasal mucociliary clear-

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ance is one of the most important limiting factors for nasal drug delivery. However, addition of a mucoadhesive agent can localize the formulation to a mucosal layer of nasal cavity and thus enhance drug absorption and prevent rapid nasal clearance. Mucoadhesion requires a highly expanded and hydrated polymer network which could promote an intimate contact between microemulsion (ME) and the mucus layer. Thus mucoadhesive microemulsion (MME) using polyelectrolytic polymer has been developed to decrease the effect of mucociliary clearance and increase retention time of ME on olfactory mucosa to enhance direct delivery efficiency (Vyas *et al.* 2006). The feasibility of nasal administration as an alternative route for administering drugs acting on central nervous system (CNS) has been widely investigated (Li *et al.* 2002; Vyas *et al.* 2006; Jogani *et al.* 2008).

Our study aimed to design a new, oleic acid, a lipophilic absorption enhancer (Pierre *et al.* 2006)-based MME drug delivery system for intranasal administration of OLZ which is alcohol free. This paper describes the development, formulation and *in vitro* evaluation of MME in which Generally Recognized As Safe (GRAS) listed ingredients are used to solubilize OLZ and polycarpophil AA-1 as a mucoadhesive component.

MATERIALS AND METHODS

Drugs and reagents

OLZ pure powder was obtained as gratis sample from Torrent Pharmaceutical Ltd. (Ahmedabad, India) with 99.9% purity. Labra-fil M 1944 (oleoyl polyoxylglycerides), labrafac CC (caprylic/capric triglycerides), labrasol (caprylocaproyl polyoxylglycerides), plurol oleique CC (polyglyceryl oleate), lauroglycol 90 (propylene glycol monolaurate), yranscutol P (diethylene glycol monoethyl ether), (Gattefosse Saint-Priest, France) were procured as gratis samples from Gattefosse Asia Ltd. (Mumbai, India). Cremophor RH 40 (polyoxyl 40 hydrogenated castor oil) was procured as a gratis sample from BASF (Mumbai, India). Capmul MCM (glyceryl mono- and dicaprate) was procured as gratis sample from Abitec Corp. (Janesville, USA). Polycarbophil (AA-1, pharmagrade, molecular weight approximately 3.5 million) was procured as gratis sample from Lubrizol Advanced Material India Pvt Ltd. (Mumbai, India). Oleic acid, isopropyl myristate, tween 80, potassium dihydrogen phosphate, methanol and propylene glycol were purchased from SDfine Chemicals (Ahmedabad, India). Ethanol was purchased from Baroda Chemical Ind. Ltd (Dabhoi, India). Double distilled water was used throughout the study. All other chemicals and solvents were of analytical reagent grade and used as received without further purification.

Solubility determination

OLZ drug powder was added in excess to each of the oils, surfactants, cosurfactants and then vortexed for mixing. After vortexing, the samples were kept for 72 h at ambient temperature for attaining equilibrium. The equilibrated samples were then centrifuged at 5000 rpm for 30 min to remove the undissolved drug (Patel *et al.* 2009). The aliquots of supernatant were filtered through 0.45 µm membrane filters and the solubility of OLZ was determined by high performance thin layer chromatography (HPTLC) (Patel *et al.* 2010).

Pseudoternary phase diagram

In order to determine the concentration range of components for the existing range of ME, pseudo-ternary phase diagrams were constructed using water titration method (Li *et al.* 2002; Vyas *et al.* 2006; Jogani *et al.* 2008; Kumar *et al.* 2008; Patel *et al.* 2009) at ambient temperature. Three phase diagrams were prepared with the 1:1, 2:1 and 3:1 weight ratios of a mixture of Labrasol and Cremophor RH 40 (1:1) to Transcutol P, respectively. For each phase diagram at a specific surfactant (S)/cosurfactant (CoS) mixing ratio (Km), the ratios of oil to the mixture of S/CoS (Smix) were varied as 0.5:9.5, 1:9, 1.5:8.5, 2:8, 2.5:7.5, 3:7, 3.5:6.5, 4:6, 4.5:5.5, 5:5, 5.5:4.5, 6:4, 6.5:3.5, 7:3, 7.5:2.5, 8:2, 8.5:1.5, 9:1, 9.5:0.5 (w/w).

Table 1 Composition of Olanzapine microemulsion (OME) and Olanzapine mucoadhesive microemulsion (OMME).

Ingredients (w/w)	CME	CMME
Olanzapine (mg/mL)	8.00	8.00
Oleic acid	4.00	4.00
Labrasol : Cremophor RH 40 (1:1): Transcutol P (3:1)	30.00	30.00
Water	66.00	66.00
Polycarbophil	-	0.50

The mixtures of oil and S/CoS at certain weight ratios were diluted with water drop wise, under moderate magnetic stirring. After being equilibrated at ambient temperature for 24 h, the mixtures were assessed visually and determined as being ME, crude emulsions or ME gels. The stable MEs were also observed under polarizing light to conform their isotropic nature. No attempt was made to distinguish between oil-in-water, water-in-oil or bicontinuous type ME. Gels were claimed for those clear and highly viscous mixtures that did not show a change in the meniscus after tilted to an angle of 90º.

Preparation of microemulsions and mucoadhesive microemulsions

The ME for OLZ was prepared by the water titration method. The calculated amount of drug (8 mg/mL of OLZ) was added to the oily phase of ME and magnetically stirred until dissolved followed by addition of Smix in a fixed proportion to produce clear mixture. Then a defined proportion of water was added and stirred to produce clear ME of OLZ (OME). The MME of OLZ (OMME) was prepared by initially preparing ME of the drug using minimum volume of external phase and then adding the required volume of polymer solution (1%, w/v) so that the final concentration of polymer in the MME was 0.5% (w/w). After the addition of polymer solution the MME was allowed to homogenize for 10 min. The composition of OME and OMME is shown in **Table 1**.

Preparation of drug solution

The OLZ solution (OS) meant for comparative evaluation of MME-based systems was prepared by dissolving OLZ (80 mg) in 10 mL of propylene glycol resulting in a solution of 8 mg/mL (Kumar *et al.* 2008).

Characterization of microemulsion

1. Particle size and zeta potential measurements

The average droplet size and polydispersity index (PDI) of ME was measured by photon correlation spectroscopy (PCS) with in-built Zetasizer (Nano ZS, Malvern Instruments, UK) at 633 nm. A Helium-neon gas laser with a 4 mW intensity served as the light source. Droplet size was calculated using the Stokes-Einstein relationship by Zetasizer Software (Vyas *et al.* 2006). Electrophoretic mobility (µm/s) was measured using small volume disposable zeta cell and converted to zeta potential by in-built software using Helmholtz–Smoluchowski equation (Li *et al.* 2002; Vyas *et al.* 2006; Jogani *et al.* 2008; Kumar *et al.* 2008; Patel *et al.* 2009). Particle size and zeta potential determinations were made in triplicate.

2. Transmission electron microscopy (TEM)

TEM was used to characterize the microstructure of OLZ loaded ME. OME was placed on a carbon-coated copper grid (Taab Laboratories Equipment Ltd, UK) and then a drop of 1% phosphotungstic acid covered on ME. The superfluous phosphotungstic acid on ME was wiped off by filter paper. The TEM images were obtained using a Tecnai G2 20 TEM (Philips, Holland) (Patel *et al.* 2009).

3. Determination of drug content

For determination of drug content, about 1 g of the ME was weighed in a 10-ml volumetric flask and dissolved in methanol; it was diluted appropriately and analyzed by HPTLC (Patel *et al.* 2010). Drug content determinations were made in triplicate.

4. Percent transmittance measurement

The percent transmittance (%T) of the system was measured using a colorimeter (Digital Colorimeter, D-801, Photocon) at 570-590 nm (Li *et al.* 2002; Vyas *et al.* 2006; Jogani *et al.* 2008; Kumar *et al.* 2008; Patel *et al.* 2009). %T determinations were made in triplicate.

5. Polarizing microscopy

In order to verify the isotropic nature of ME, samples were examined using cross-polarized light microscopy (Polarizing Microscope RPL-55 Series, Radical Instuments, India). A drop of ME was placed between a cover slip and a glass slide and then observed under cross-polarized light (Patel *et al.* 2009).

6. pH measurement

The pH value of ME was determined using digital pH meter (Orion pH meter 420A, Allometric Ltd., Baton Rouge, LA), standardized using pH 4 and 7 buffers before use (Vyas *et al.* 2006; Jogani *et al.* 2008; Kumar *et al.* 2008; Patel *et al.* 2009). pH determinations were made in triplicate.

7. Viscosity measurement

The viscosity of ME was measured using a Brookfield Viscometer LVDV – IIIU (Brookfield Engineering LABS, Stoughton, MA) with spindle SC 18 at 100 rpm using interval of 30 seconds. All aspects of testing were controlled using Rheocalc Software. Viscosity determinations were made in triplicate.

8. Conductivity measurement

The electric conductivity of ME was measured with a conductivity meter (Equip-Tronics, EQ-664, Mumbai, India) equipped with inbuilt magnetic stirrer. This was done by using conductivity cell (with a cell constant of 1.0) consisting of two platinum plates separated by desired distance and having liquid between the platinum plate acting as a conductor (Patel *et al.* 2009). Conductivity determinations were made in triplicate.

Infrared study

The infrared (IR) spectra of OLZ, plain ME, optimized OME and OMME were taken using an IR spectrophotometer (Spectrum GX FT-IR, Perkin Elmer, Norwalk, CT). The plain ME, OME and OMME were spread as a thin layer on potassium bromide cell and then scanned between 4000-400 cm^{-1}. The resulting IR spectra of OLZ and plain ME were then compared with OME and OMME to detect any possible interaction between the drug and different components used.

In vitro diffusion study of Olanzapine formulations

The freshly excised sheep nasal mucosa, except for the septum part, was collected from a slaughter house in phosphate buffer saline (PBS), pH 6.4. The membrane was kept in PBS pH 6.4 for 15 min to equilibrate. The superior nasal conche was identified and separated from the nasal membrane. The excised superior nasal membrane was then mounted on Franz diffusion cells (Orchid Scientific, Nashik, India). The tissue was stabilized using phosphate buffer pH 5.0 in both the compartments and allowed to stir for 15 min on a magnetic stirrer (Multimagnetic stirrer, DBK, India). After 15 min, solution from both the compartments was removed and fresh phosphate buffer (pH. 5.0) was filled in the receptor 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 crossing over the cell.

The Franz diffusion cells used for *in vitro* diffusion studies had a diameter of 10 mm and mucosa thickness 0.2 ± 0.1 mm. The temperature of the receiver chamber containing 25 mL of diffusion media (phosphate buffer, pH 5.0) and was controlled at 37°C ± 1 using a circulating equibath (Model 8506, Medica Instrument Mfg. CO, Mumbai, India). Diffusion media was continuously stirred with a Teflon-coated magnetic bar at a constant rate, in a way that the nasal membrane surface just flushes the diffusion fluid.

A volume of 1 mL of each OS, OME, and OMME was placed in the donor compartment of the Franz diffusion cell. Samples from the receptor compartment were withdrawn at predetermined time intervals and analyzed using HPTLC (Patel *et al.* 2010). Each sample removed was replaced by an equal volume of diffusion media. Each study was carried for a period of 4.0 h, during which the drug in receiver chamber (μg/mL) across the sheep nasal membrane was calculated at each sampling point. The formulations were studied in triplicate for diffusion studies and the mean cumulative values for % drug diffused versus time were plotted against time. The slopes of the graphs were used to calculate the diffusion coefficients.

Test for nasal cilio toxicity

Freshly excised sheep nasal mucosa, except for the septum, was collected from the slaughter house in saline phosphate buffer pH 6.4. Three sheep nasal mucosa pieces (S1, S2, and S3) with uniform thickness were selected and mounted on Franz diffusion cells. S1 was treated with 0.5 mL of PBS pH 6.4 (negative control), S2 with 0.5 mL of isopropyl alcohol (positive control), and S3 was treated with OMME for 1 h. After 1 h, the mucosa were rinsed with PBS pH 6.4 and subjected to histological studies to evaluate the toxicities of ME and photographed by microscope (Li *et al.* 2002; Vyas *et al.* 2006; Jogani *et al.* 2008).

In vitro mucoadhesion study

The mucoadhesive potential of the OME and OMME was evaluated by reported *in vitro* method (Bachhav and Patravale 2009). Briefly, an agar plate (1%, w/w) was prepared in pH 5.0 phosphate buffer, OME and OMME formulations, each 50 mg was placed at the center of plate. After 5 min, the agar plate was attached to a USP disintegration test apparatus and moved up and down in pH 5.0 phosphate buffer at 37 ± 1°C. The OMME formulation on the plate was immersed into the solution at the lowest point and was out of the solution at the highest point. The residence time of the OMME on the plate was noted visually.

Stability studies

The formulations, OME and OMME, were subjected to stability studies for a period of 6 months at room temperature and refrigerated conditions (4°C). After 6 months of storage, the formulations were subjected to test for physical stability (creaming, phase separation, or flocculation), accelerated centrifugation cycle (3000 × *g* for 15 min), drug content, particle size, and zeta potential determinations (Patel *et al.* 2009).

Statistical analysis

All data are reported as mean ± SEM and the groups were compared using ANOVA, with $P < 0.05$ considered statistically significant.

RESULTS AND DISCUSSION

Preparation and optimization of microemulsion formulations

The solubility of practically insoluble OLZ was determined in different oily phases and was found highest in oleic acid (203.27 ± 5.69 mg/mL). Also, oleic acid is a lipophilic permeation enhancer and can be useful to improve the membrane permeability (Pierre *et al.* 2006). Hence, Oleic acid

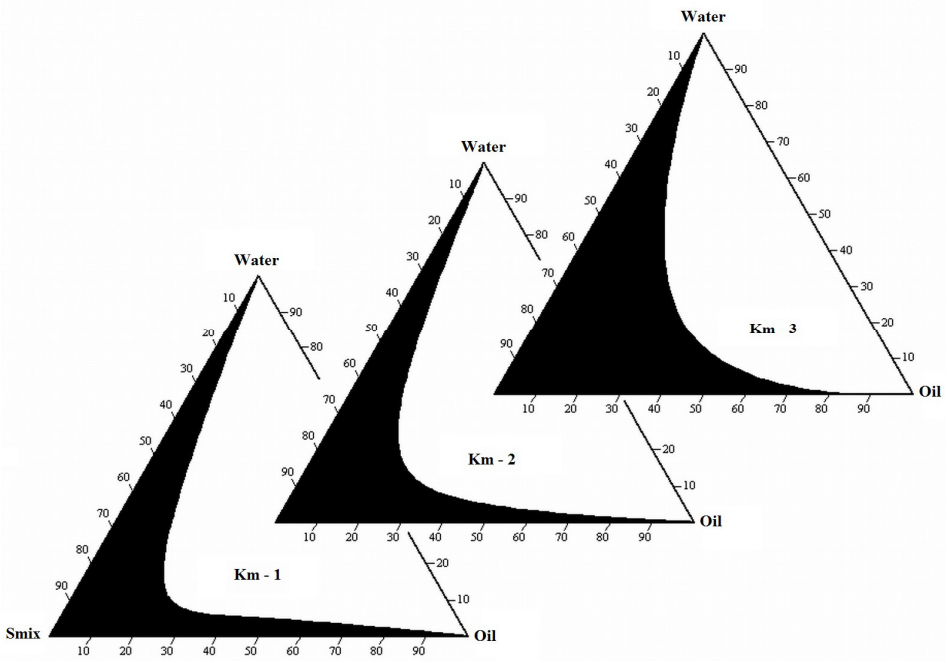

Fig. 1 The pseudoternary phase diagrams of the oil-surfactant mixture-water system at the 1:1, 2:1 and 3:1 weight ratio of mixture of labrasol: cremophor RH 40 (1:1) to transcutol P at ambient temperature, dark area represent microemulsion region.

Table 2 Various ternary phase compositions and characterization parameters of plain microemulsions. (n = 3)

Batch	Km	Oil (% wt/wt)	Smix (%wt/wt)	Water (%wt/wt)	Globule size (nm) ± SD	PDI ± SD	Zeta potential (mV) ± SD	% T
O1	3	4	20	76	126.60 ± 3.55	0.382 ± 0.023	–21.37 ± 2.18	97.44
O2	3	4	22	74	100.00 ± 2.39	0.381 ± 0.049	–17.31 ± 2.14	97.32
O3	3	4	24	72	88.01 ± 2.18	0.349 ± 0.036	–18.47 ± 2.34	99.70
O4	3	4	26	70	86.10 ± 2.46	0.281 ± 0.021	–24.11 ± 1.58	99.37
O5	3	4	28	68	41.07 ± 1.44	0.148 ± 0.017	–26.67 ± 2.45	99.76
O6	3	4	30	66	25.67 ± 1.17	0.121 ± 0.016	–35.14 ± 2.12	99.93
O7	3	4	32	64	23.87 ± 1.07	0.167 ± 0.021	–31.88 ± 2.63	99.88
O8	3	4	34	62	23.45 ± 1.24	0.134 ± 0.027	–32.45 ± 2.36	99.81

was selected as the oily phase for the preparation of ME. The type of ME formed depends on the properties of the oil, S, and CoS. An important criterion for selection of the surfactants is that the required hydrophilic lipophilic balance (HLB) value to form the oil–water ME be greater than 10. Both Labrasol and Cremophor RH 40 are non-ionic, GRAS listed excipients and widely used in pharmaceutical preparations. The combined use of surfactant showed apparent advantages over the single use of surfactant; the ME region was greatly increased in the phase diagram (data not shown). In this study, we selected blend of surfactants containing Labrasol as less hydrophilic but more capable of solubilizing hydrophobic drug components and Cremophor RH 40 as more hydrophilic compared to Labrasol. The combined use of surfactants might have provided a better HLB. As a result it enhanced the flexibility of S layer that was formed and also S ability to partition at higher levels into the oil-water interface must have increased. These both phenomena in turn stabilized oil-water ME formed.

Transient negative interfacial tension and fluid interfacial film are rarely achieved by the use of only S; usually, addition of a CoS is necessary. The presence of CoS decreases the bending stress of interface and allows the interfacial film sufficient flexibility to take up different curvatures required to form ME over a wide range of composition. Thus, the CoS selected for the study was transcutol P. In this study, mixture of Labrasol and cremophor RH 40 with transcutol P were selected as the S–CoS system.

A ternary phase diagram explains the selection of the formulations from the phase diagrams to avoid metastable formulations having minimum S concentration, in the least possible time. Ternary phase diagrams were constructed by varying Smix ratios as 1:1, 2:1 and 3:1 (**Fig. 1**). The shaded areas of phase diagrams show the ME regions, whereas the non-shaded area displays the turbid region based on visual observation. No liquid crystalline structure was observed using cross polarizer. The area of ME isoptropic region changed slightly in size with increasing ration of S to CoS.

Various MEs were selected from the 3:1 phase diagram**.** Apart from the ternary phase diagrams, globule size determinations were also performed as it could provide supportive evidence for the selection of phase diagram of ratio 3:1. Also, globule size of ME containing lowest concentration of Smix (1:1) was found to be 130.16 ± 4.21 nm, whereas at the highest concentration of Smix (3:1) it reduced to 25.13 ± 2.55 nm, and hence the ratio of Smix 3:1 (Km 3) was selected for optimization studies. The optimization of ME was carried out on the basis of percentage transmittance (%*T*), globule size, and zeta potential. According to the solubility study of OLZ in oleic acid, a minimum of 4% by the weight of oily phase was required to fulfill the dose requirement, and with Smix maintained at 3:1 and water as aqueous phase, eight batches of OLZ-loaded ME formulations were prepared and characterized (**Table 2**).

The globule size decreased with the increase in the concentration of Smix in the formulations (**Table 2**). The globule size of batch O1, containing 20% of Smix, was highest (126.60 ± 3.55 nm) and was least (23.45 ± 1.24 nm) for highest concentration (34% w/w) of the Smix. All the formulations had droplets in the nano-range, which is very well evident from the low PDI values. PDI is the ratio of standard deviation to mean droplet size; hence, it indicates the uniformity of droplet size within the formulation. The higher the PDI, the lower the uniformity of the droplet size

Table 3 Characterization parameters of optimized OLZ microemulsions (OME) and OLZ mucoadhesive microemulsions (OMME). (n = 3)

Formulation	pH	Drug content %	% T	Globule size (nm) ± SD	PDI ± SD	Zeta potential (mV) ± SD	Conductivity (mS)	Viscosity (cp)
OS	5.65 ± 0.09	99.78 ± 1.22	-	-	-	-	-	-
OME	5.87 ± 0.11	101.03 ± 0.77	99.93	22.41 ± 1.31	0.121 ± 0.016	–35.44 ± 2.17	0.108 ± 0.08	75 ± 5.00
OMME	5.95 ± 0.13	102.18 ± 1.56	-	31.66 ± 1.14	0.241 ± 0.032	–42.15 ± 3.08	0.104 ± 0.03	93 ± 6.21

in the formulation (Patel *et al.* 2009). Although the PDI values of all formulations were very low, indicating uniformity of droplet size within each formulation, it was least for O6 (0.121 ± 0.016). The globule size of the ME containing OLZ was not significantly affected by incorporation of drug when compared to the globule size of ME prepared without drug.

Batch O6 (oil: S–CoS: water, 4:30:66) was selected as the optimized batch as it displayed optimum response variables of 99.93% optical transparency, low globule size (25.67 ± 1.17 nm), polydispersity of 0.121 ± 0.016, and zeta potential to the tune of –35.14 ± 2.12. Although batches O7 and O8 showed lower values for globule size and PDI that may be attributed to higher Smix concentrations, the difference was insignificant ($p < 0.05$) when compared with O6. Moreover, higher concentrations of Smix may cause damage to nasal mucosa; hence, O6 was selected for further study.

Polycarbophil has been studied extensively as mucoadhesive platforms for drug delivery to the nasal mucosa in a concentration of 0.5% w/w (Vyas *et al.* 2006). This anionic polymer is the most widely employed mucoadhesive polymer within pharmaceutical formulation due to its high mucoadhesive functionality and low toxicity. Such polymer is characterised by the presence of carboxyl functional groups that give rise to a net overall negative charge at pH values exceeding the pKa of the polymer. The non-ionized carboxylic acid groups bind to the mucosal surfaces via hydrogen bonding interactions. Polycarbophil form easily modified networks, is non-irritant, non-toxic and considered safe (GRAS status) for use by the FDA (Vyas *et al.* 2006). Thus, polycarbophil (0.5%, w/w) was selected as mucoadhesive polymer and incorporated in optimized ME (O6) formulation to obtained OLZ-loaded MME.

Characterization of microemulsion

Characterization data of OME and OMME are tabulated in **Table 3**. The narrow globule size of 22.41 ± 1.31 and 31.66 ± 1.14 nm and PDI of 0.121 and 0.241 for OME and OMME, respectively, indicated that the ME approached a monodispersed stable system and could deliver the drug effectively owing to larger surface area. The presence of zeta potential to the tune of –35.44 ± 2.17 and –42.15 ± 3.08 mV on the globules of OME, and OMME, respectively, conferred physical stability to the system. OME showed net negative charge and addition of mucoadhesive agent further contributed negatively to the system. This may be attributed to the fact that the increase in surfactant level resulted in a decrease in surface tension and surface free energy of the formed micelles. Therefore, net negative charge (anionic) of the ME increased (Illum 2002). The MEs were expected to have good physical stability (phase separation) as zeta potential is less than –30mV (Vyas *et al.* 2006; Jogani *et al.* 2008). Moreover, addition of mucoadhesive polymer (Polycarbophil) may further stabilize the system since it increased negative charge of the system (Vyas *et al.* 2006; Jogani *et al.* 2008). The TEM imaging of OME is shown in **Fig. 2**. The TEM images revealed that particle size was in nanometric range and that the particles had nearly spherical morphology. The globule size of OME from TEM images accords with that from PCS. A percentage transmittance of 99.93% for OME indicated clear dispersion, whereas OMME was hazy due to the presence of mucoadhesive component in the formulation. The samples were examined by ocular inspection in a cross polarizer for sample homogeneity and birefringence. The OME appeared completely dark when observed under cross polarizer which confirmed its optically isotropic nature (Patel *et al.* 2009). The pH of all the OS, OME, and OMME ranged between 5.5 to 5.9, approximating the normal pH range of nasal fluids (Karasulu *et al.* 2008), which is one of the formulation considerations that may help reducing the irritation produced upon instillation. The OLZ content (%) of OS, OME, and OMME was found to be 99.67 ± 1.08, 99.91 ± 1.21 and 99.87 ± 1.76, respectively of the theoretical value (8 mg/mL). It was observed that the viscosity of the ME formulations generally was very low. This was expected, because one of the characteristics of ME formulations is of lower viscosity (Patel *et al.* 2009). Low viscosity values of OME (75 ± 5.0 cp) and OMME (93 ± 6.21 cp), ensure easy handling, packing, and hassle-free nasal administration of formulations. Conductivity measurements rely on the poor conductivity of oil compared with water and give low values for water in oil ME where oil is the continuous phase. The reverse happens for oil in water ME (Patel *et al.* 2009). The conductivity measurements of OME (0.108 ± 0.08 mS) and OMME (0.104 ± 0.03 mS) indicated their oil-in-water type nature.

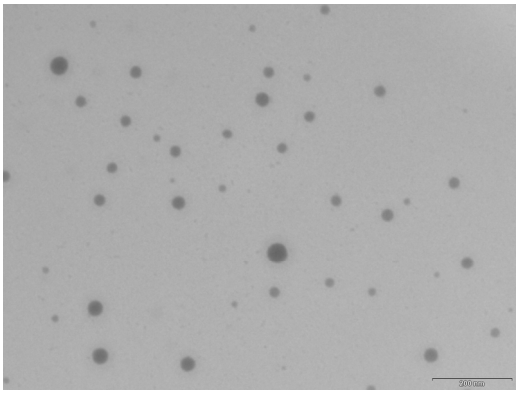

Fig. 2 Transmission electron microscopy image of Olanzapine-loaded microemulsion.

IR study

The infrared spectra of OLZ pure powder, plain ME, OME and OMME are as shown in the **Fig. 3**. Three different degradation products of OLZ which show IR absorption at 1695, 1637, and 1702 are reported in the literature (Hiriyanna *et al.* 2008). These data revealed that all degraded products contain –C=O group. In our study, IR spectra of pure OLZ and OLZ loaded formulation as shown in the **Fig. 3** revealed that such peak due to –C=O stretching was not observed around 1700 cm^{-1} which emphasized the absence of any possible degradation of OLZ and interaction between the OLZ and formulation components used.

In vitro diffusion and nasal cilio toxicity study

In vitro diffusion studies of OLZ formulations shows successful diffusion through sheep nasal mucosa and the results obtained are presented in **Fig. 4**, and the calculated diffusion coefficients are tabulated in **Table 4** along with the regression coefficients (r^2) for first-order, Higuchi, and zero-order modeling of the diffusion profiles for each formulation.

Drugs cross the nasal mucosal membrane using two different pathways: transcellularly (across the cells) and

Table 4 Diffusion coefficients and modeling parameters of OLZ solution (OS), OLZ microemulsion (OME) and OLZ mucoadhesive microemulsion (OMME). (n = 3)

Formulation	Diffusion coefficient (cm^2/min)	Zero order	First order	Higuchi
		r^2	r^2	r^2
OME	$1.11 \times 10^{-6} \pm 0.021 \times 10^{-6}$	0.8701	0.9643	0.9724
OMME	$1.40 \times 10^{-6} \pm 0.019 \times 10^{-6}$	0.9187	0.9817	0.9845
OS	$0.92 \times 10^{-6} \pm 0.013 \times 10^{-6}$	0.9101	0.9611	0.9717

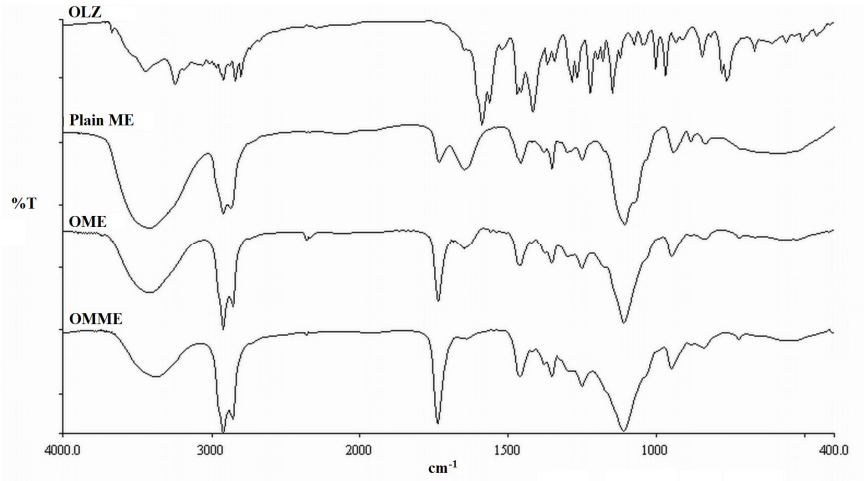

Fig. 3 Infra red spectra of Olanzapine (OLZ), plain microemulsion (plain ME) drug-loaded microemulsion (OME) and drug-loaded mucoadhesive microemulsion (OMME)

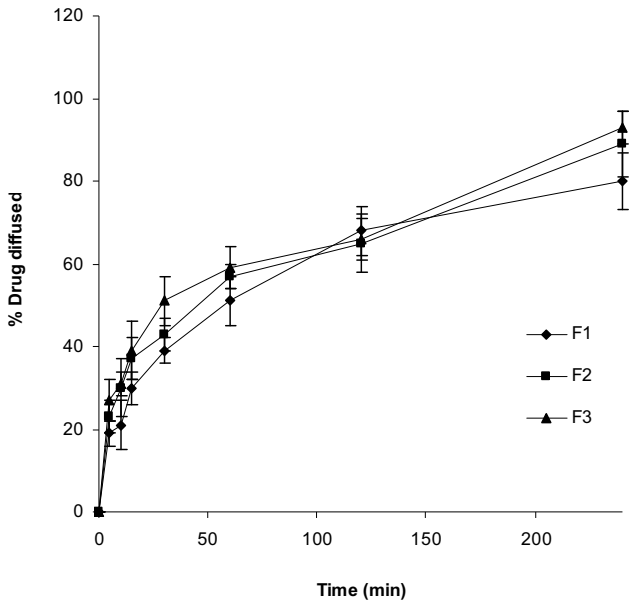

Fig. 4 Percent cumulative drug diffused versus time profiles of Olanzapine solution (OS, F1), microoemulsion (OME, F2), and mucoadhesive microemulsions (OMME, F3).

paracellularly (between the cells). Lipophilic drugs are transported transcellularly by a concentration dependent passive diffusion process, by facilitated diffusion using a receptor or carrier molecule, or by vesicular transport mechanisms. It is possible to improve nasal absorption of drugs by administering them in combination with a penetration enhancer that promotes the transport of the drug across the nasal membrane. Although the precise mechanism of action of the penetration enhancers is not known, it is speculated that these agents promote drug absorption by (a) increasing membrane fluidity, (b) expanding the dimension of paracellular pathway to solute transport, or (c) creating transient pores by reverse micelle formation in the cell membrane. However, many penetration enhancers irritate to mucous membranes (Jogani *et al.* 2008).

The presence of oil droplets containing OLZ along with external aqueous phase appeared in favor of OLZ permeability. It might be stated that ME could act as drug reservoirs where loaded-drug is released from the internal phase to the external phase and finally onto the skin or mucosa. On the other hand, when MEs are used as nasal formulations, the interaction between the oil droplets and the nasal mucosal membrane is likely to play an important role in the enhancement mechanism. The effect of penetration enhancers depends on the vehicle and frequently these substances are applied at relatively large doses (Karasulu *et al.* 2008). Our experiments were performed with low doses of formulation containing a low concentration of oleic acid.

OLZ showed better diffusion from OMME ($1.40 \times 10^{-6} \pm 0.019 \times 10^{-6}$) than OS ($0.92 \times 10^{-6} \pm 0.013 \times 10^{-6}$) through sheep nasal mucosa after 4 h. The decreasing order of diffusion coefficient for the tested formulations was OS < OME < OMME (although not significantly different at $p < 0.05$). For OMME, the drug exhibited highest diffusion coefficient, whereas it was least for OS. The results of our study are in accordance with similar reports available in the literature (Jogani *et al.* 2008). It is known that usually polymers may increase the permeability by altering and /or damaging the tight junction of nasal epithelium (Karasulu *et al.* 2008). The OMME exhibited higher diffusion due to the presence of mucoadhesive agent that probably due to its intrinsic character tends to adhere to mucosa thereby causing increased contact and hence increased diffusion (Karasulu *et al.* 2008). The present results suggest that polyelectrolytic polymer-polycarbophil enhances the action of the lipophilic permeation enhancer oleic acid and that the combination of oleic acid and polycarbophil as a co-enhancer can be a useful tool to improve the membrane permeability in the nasal delivery of lipophilic drugs using ME as drug delivery system.

On modeling, the diffusion of drug from OLZ formulations exhibited higher r^2 values for the Higuchi model compared with zero- and first-order model(s). This may be due to the fact that the diffusion system used has a reservoir compartment (donor compartment) and sheep mucosa acts as a barrier or controlling membrane; hence, the diffusion process will mimic and shall be closer to reservoir system than zero-order (concentration independent) or first-order (concentration gradient) diffusion.

The chemical enhancers may be harmful especially in

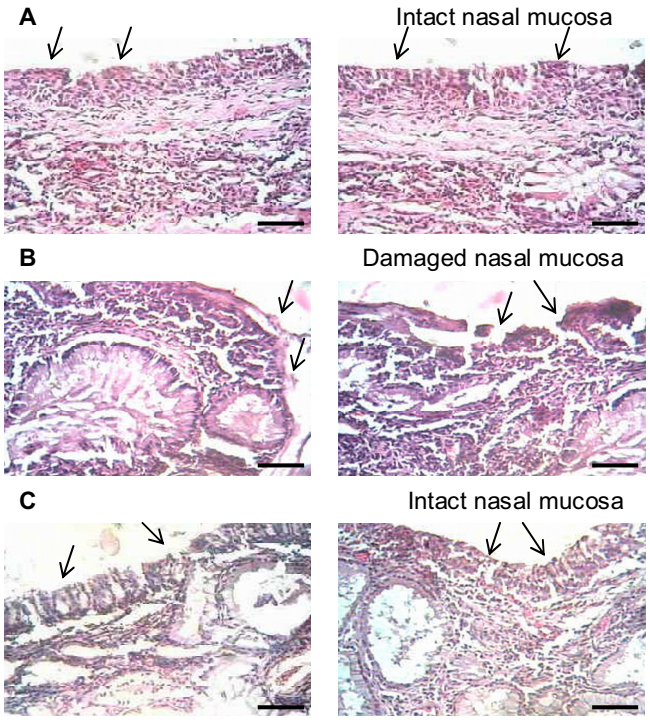

Fig. 5 Photographs of sheep nasal mucosa demonstrating histological characteristics when treated with (**A**) phosphate buffer saline pH 6.4 (**B**) isopropyl alcohol and (**C**) Olanzapine-loaded mucoadhesive microemulsion (OMME). Bars = 0.1 mm.

chronic applications, since many of them are usually irritants. Because of the potential for structural damage to the mucosal membrane, the safety of any oil, surfactant, cosurfactant and polymer being considered for use as a nasal permeation enhancer must be carefully evaluated. The acceptance of a permeation enhancer is dependent not only on its ability to enhance absorption, but also on its overall safety profile with regard to both local and systemic effects (Karasulu *et al.* 2008). Thus, nasal cilio-toxicity studies were carried out in an attempt to evaluate any potential toxic effects of excipients used in the formulation on the nasal mucosa. The nasal mucosa treated with PBS (pH 6.4, negative control) showed no sign of inflammation, erosion and nasociliary damage (**Fig. 5A**) and the nasal membrane remained intact, whereas an extensive damage to nasal mucosa coupled with loss of nasal cilia (**Fig. 5B**) could be observed with positive control. However, with OMME, no damage to nasal mucosa could be observed (**Fig. 5C**), which substantiating the safety of the excipients used in the formulation. Thus, formulation components can be considered to be biocompatible and do not induce serious histological changes in the nasal mucosa. Further experiments are required to study the influence of these particular excipients/permeation enhancers *in vivo* focusing on the production of pro-inflammatory cytokines and enzymes indicative of cell damage (Karasulu *et al.* 2008).

In vitro mucoadhesion study

The mucoadhesive potential of OMME was evaluated by *in vitro* method. The retention times showed by OME and OMME were 4.0 ± 2.0 and 19.0 ± 2.0 min, respectively (n = 3). The retention time on agar plate showed by OMME was significantly higher than OME ($P < 0.05$). Thus it was hypothesized that the develop mucoadhesive preparation, OMME, was able to increase the residence time of the formulation on the nasal mucosa which can be attributed due to presence of polycarbophil (0.5%, w/w). Polycarbophil is indeed a well recognized and widely used bioadhesive polymer, known for its peculiar mucoadhesion properties. Polycarbophil interact with mucus and biological surfaces through hydrogen bonding of the ionized carbonyl functionalities. Because of their mucoadhesive properties, the polycarbophil has been investigated in nasal dosage forms for the enhancement of intranasal bioavailability (Vyas *et al.* 2002).

Table 5 Results of stability testing of the OLZ microemulsions (OME) and OLZ mucoadhesive microemulsions containing 0.5% (w/w) polycarbophil (OMME). (n = 3)

Test	OME	OMME
% Assay	99.21	99.03
% Transmittance	99.78	-
Globule size (nm)	25.18	38.61
Polydispersibility index	0.174	0.287
Zeta potential (mV)	−32.44	−39.28

Stability studies

In stability studies, the ME exhibited no precipitation of drug, creaming, phase separation, and flocculation on visual observation and was found to be stable after centrifugation (3000 × g for 15 min) both at room temperature and at 2–8°C. The results of stability studies (**Table 5**) showed that there are negligible changes ($P > 0.05$) in the parameters such as drug content, % transmittance, globule size and zeta potential of OME and OMME after 6 months of storage, thus substantiating the stability of ME for 6 months.

CONCLUSION

On the basis of low droplet size and PDI, optimum S and CoS concentrations, the mucoadhesive formulation OMME that contained 0.5% by weight of polycarbophil as the mucoadhesive component displayed highest mucoadhesive potential and diffusion coefficient. The formulation was free from nasal ciliotoxicity and was found to be stable for six months. The *in vitro* studies demonstrated the potential of developed OMME for intranasal delivery of OLZ. Given the results of this study, it is clear that OMME formulation is potentially useful for enhancing permeation of OLZ through its nasal delivery. It is possible to conclude that, intranasal administration of OLZ may be considered as an alternative to intravenous and oral administration of OLZ to overcome its disadvantages.

Further *in vivo* studies are necessary to demonstrate the potential of MME based drug delivery system and to confirm the existence of a transport pathway for a drug (OLZ) to the brain directly from the nasal cavity. Thus authors are currently working on brain targeting study of OMME formulation.

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