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Formulation and Characterization of Nanoemulsion-Based Drug Delivery System of Risperidone

Mukesh Kumar and Kamla Pathak
Department of Pharmaceutics Rajiv Academy for Pharmacy, Mathura, Uttar Pradesh, India

Ambikanandan Misra
Pharmacy Department, Faculty of Technology and Engineering, The Maharaja Siyajirao University of Baroda, Vadodara, Gujarat, India

Risperidone nanoemulsion (NE) and mucoadhesive NE formulations were successfully prepared by the spontaneous emulsification method (titration method) using Capmul MCM as the oily phase on the basis of solubility studies. The NE formulation containing 8% oil, 44% S_{mix}, 48% (wt/wt) aqueous phase that displayed an optical transparency of 99.82%, globule size of 15.5 ± 2.12 nm, and polydispersity of 0.172 ± 0.02 was selected for the incorporation of mucoadhesive components. The mucoadhesive formulation that contained 0.5% by weight of chitosan displayed highest diffusion coefficient that followed Higuchi model was free from nasal ciliotoxicity and stable for 3 months.

Keywords risperidone; formulation considerations; brain targeting; transmucosal delivery; nasal ciliotoxicity

INTRODUCTION

The site-specific targeted drug delivery negotiates an exclusive delivery to specific preidentified compartment with maximum intrinsic activity of drugs and concomitantly reduced access of the drug to irrelevant nontarget cells. A number of strategies are followed to target various body tissue/organs. The brain is a delicate organ with many vital functions, and formidable mechanisms isolate and protect it from the outside world. Unfortunately, the same mechanisms that prevent intrusive environmental chemicals accessing the brain also prevent the access of therapeutic chemicals. Hence, a number of strategies such as invasive approach blood-brain barrier (BBB disruption and intracerebral implants), physiological approach (pseudonutrients, ligand-binding proteins, and chimeric peptides), and pharmacological approach (liposome, nanoparticles, nanoconjugates, and chemical drug delivery) are used for targeting drug molecules to brain (Soni et al., 2004). The olfactory region of nasal mucosa that provides a direct connection between nose and brain can be exploited for targeting central nervous system (CNS)-acting drug molecules used in conditions such as Alzheimer's disease, depression, migraine, schizophrenia, and so on (Astic, Saucier, & Coulon, 1993).

Risperidone (RSP), 3-[2-[4-(6-fluoro-1,2-benzisoxazol-3-yl)-1-piperidinyl]ethyl]-6,7,8,9-tetrahydro-2-methyl-4H-pyrido[1,2-a] pyrimidin-4-one, is an approved antipsychotic drug belonging to the chemical class of benzisoxazole derivative and is available as tablet, oral liquid (Risperidal®), and orally disintegrating tablet (Risperidal® M-TAB). These dosage forms exhibit low bioavailability due to extensive first-pass metabolism, and the nontargeted delivery results in numerous side effects. As the target site of the RSP is brain, thereby a strategy is desirable that not only improves the bioavailability by preventing first-pass metabolism but also provides targeting to the receptor site and bypasses the blood–brain barrier so as to achieve desired drug concentration at the site of action, hence preventing the availability of drug at nontargeting sites and reducing the side effects. Earlier studies (Behl, Pimplaskar, Sileno, De Meireles, & Romeo, 1998; Chow, Chen, & Matsuura, 1999; Candace & Pollock, 2005; Illum, 2002; Vyas, Babber, Sharma, Singh, & Misra, 2006a, b) have demonstrated direct transport of drugs to brain, circumventing the brain barriers following intranasal (i.n.) administration that provides a unique feature and better option for targeting drugs to brain (Li, Nandi, & Kim, 2002; Mathison, Nagilla, & Kompella, 1998; Pietrowky, Thieman, Kern, Fehm, & Bern, 1996; Qizhi et al., 2004). However, few formulation factors need to be addressed while designing the drug delivery system for i.n. administration (Illum, 2003). The formulation designed should be such that it provides a rapid transport of drug across nasal mucosa and a longer residence time in nasal cavity to overcome the nasal mucociliary clearance (Chien, Su, & Chang, 1989; Ugwoke, Verbeke, & Kinget, 2001). In a report by Jug and Lacan (2007), RSP and its inclusion complex with hydroxypropyl-beta-cyclodextrin

Address correspondence to Kamla Pathak, Department of Pharmaceutics Rajiv Academy for Pharmacy, NH #2, Mathura 281001, Uttar Pradesh, India. E-mail: kamla_rap@yahoo.co.in; kpathakrap@rediffmail.com

were loaded into microparticles by spray drying using Hydroxyl propyl methyl cellulose (HPMC), carbomer, and HPMC/ carbomer interpolymer complex (IPC) as mucoadhesive components, and the mucoadhesive properties of the microparticles were studied. Carbomer and IPC-based microparticles revealed superior mucoadhesive microparticles compared with HPMC-based microparticles. Drug incorporation into microparticles reduced their mucoadhesive properties, whereas incorporation of the cyclodextrin complex caused no additional reduction in mucoadhesion. This study aims at using chitosan and polycarbophil as mucoadhesive components in designing nanoemulsions (NEs) of RSP.

NEs have a higher solubilization capacity than simple micellar solutions, and their thermodynamic stability offers advantages over unstable dispersions, such as emulsions and suspensions, because they can be manufactured with little energy input (heat or mixing) and have a long shelf life. NEs by virtue of their lipophilic nature and low globule size are widely explored as a delivery system to enhance uptake across nasal mucosa (Lawrence & Rees, 2000). Addition of mucoadhesive agents such as polyelectrolyte polymer(s) helps in the retention of the formulation on the nasal mucosa (Gavini et al., 2006; Gavini, Rassu, Muzzarelli, Cossu, & Giunchedi, 2008; Sinswat & Tengamuay, 2003), thereby providing an extended delivery of drug to the olfactory region and henceforth to the brain.

Direct nose to brain delivery of formulated risperidone nanoemulsion (RNE) has been reported from our laboratory (Kumar et al., 2008). Biodistribution of RNE, risperidone mucoadhesive nanoemulsion (RMNE), and risperidone solution (RS) in the brain and blood of Swiss albino rats following i.n. and intravenous (i.v.) administration was examined using optimized technetium-labeled (99mTc-labeled) RSP formulations. The brain–blood uptake ratio of 0.617, 0.754, 0.948, and 0.054 for RS (i.n.), RNE (i.n.), RMNE (i.n.), and RNE (i.v.), respectively, at 0.5 h is indicative of direct nose to brain transport by passing the blood–brain barrier. Higher drug transport efficiency (DTP) and direct nose to brain drug transport percentage for mucoadhesive NEs indicated more effective and brain targeting of RSP among the prepared NEs and gamma scintigraphy imaging of rat brain following i.v. and i.n. administration of the prepared NEs, which ascertained the localization of drug in brain. This report describes the formulative considerations, characterization, mucosal diffusion, stability, and acute mucosal toxicity of the formulated RNEs.

MATERIALS AND METHODS

Drugs and Reagents

RSP was a gift from Sun Pharmaceuticals (Mumbai, India). Capmul MCM and Capmul GMO were received as gift samples from Abitech Corporation Limited (Columbus, OH, USA). Labrafac CC, Labrafil 1944 MS, and transcutol were gift samples received from Colorcon Asia Ltd. (New Delhi, India). Tween 80 and ethyl oleate were purchased from S.D. Fine Chemicals (Mumbai, India). Polycarbophil (AA-1, pharmagrade, molecular weight approximately 3.5 million) and Chitosan (medium viscosity, mol. wt 400 kDa; degree of deacetylation: 83–85%) were purchased from Noveon Polymers (Cleveland, USA).

Spectrophotometric Studies

RSs of 5, 10, 15, 20, 25, 30, 35, and 40 μg/mL were prepared in phosphate buffer (pH 5) and λ_{max} values were determined by scanning these solutions against the reagent blank (phosphate buffer, pH 7.4) in the range of 200–400 nm (UV-Vis spectrophotometer, Shimadzu 1700, Kyoto, Japan). The interference of oily phases, surfactants, and cosurfactants in the estimation of RSP was determined by measuring the absorbance at λ_{max} of respective drugs against the reagent blank. All the excipients were scanned over the UV range to determine their contribution at λ_{max} of drugs. For reliability, additional spectrophotometric study was carried out where maximum percentages of all the excipients projected to be used in the formulation were added to the sample solutions of drug separately and the absorbance maxima recorded.

Solubility Determination

Solubility of RSP was determined in oily phase (Capmul MCM, Capmul GMO, Labrafac CC, Labrafil 1944, and ethyl oleate), in surfactant (Tween 80), and in cosurfactants (propylene glycol and transcutol). Drug was added in excess to different oils, surfactants, and cosurfactants separately and stirred for 24 h on mechanical shaker (Hicon Enterprises, New Delhi, India). The samples were centrifuged (REMI Pvt. Ltd., Mumbai, India) at $2{,}862 \times g$ for 10 min and the drug in the supernatant, after proper dilution with methanol, was analyzed at 300 nm. The drug solubilities were calculated in various excipients.

Pseudoternary Phase Diagrams

The pseudoternary phase diagrams containing oily phase, surfactant, cosurfactants, and water were developed by the spontaneous emulsification method (titration method) (Prapaporn, Karen, Anja, Thomas, & Varaporn, 2006). The surfactant was blended with a mixture of cosurfactant (1:1) in fixed weight ratios (1:1:1, 2:1:1, 3:1:1, and 4:1:1), and aliquots of each surfactant and cosurfactant mixture (S_{mix}) were then mixed with oil at ambient temperature. For each phase diagram, the ratio of oil to the S_{mix} was varied as 9:1, 8:2, 7:3, 6:4, 5:5, 4:6, 3:7, 2:8, and 1:9 (wt/wt). Water was added dropwise to each oil–S_{mix} mixture under vigorous stirring. After equilibrium, the samples were visually checked and determined as being clear NEs or emulsions. No heating is conducted during the preparation. Phase diagrams were prepared using CHEMIX School Ver. 3.50 software (MN, USA). The mixture compositions at different points in the phase diagrams were defined by the expression %*A*

(Capmul MCM) + %*B* (Tween 80 + propylene glycol + transcutol) + %*C* (water) = 100.

Optimization of NE

Considering the amount and solubility of drug to be incorporated in the NE, certain oil–S_{mix}–water mixtures within the NE region were prepared and the final composition of NE was optimized based on transparency, dilution characteristics, and globule size. For the optimization process, the NEs were prepared by varying the stirring speed and stirring time and the globule size was taken as response.

Preparation of NE

The NEs for RSP were prepared by the spontaneous emulsification method (titration method). The calculated amount of drug (3.5 mg/mL of RSP) was added to the oily phase of NEs and magnetically stirred (Hicon Enterprises) until dissolved followed by the addition of S_{mix} in a fixed proportion to produce a clear mixture. Then a definite proportion of water (Table 1) was added and stirred to produce clear NEs (RNE) of RSP. The mucoadhesive NEs of RSP were prepared by initially preparing NE of the drug using minimum volume of external phase and then adding the required volume of polymer solution (1%, wt/vol) so that the final concentration of polymer in the mucoadhesive NEs was 0.5% (wt/wt). The mucoadhesive NE containing 0.5% (wt/wt) chitosan was labeled as RMNE, whereas the one formulated with 0.5% (wt/wt) polycarbophil was labeled as RMNE1. After the addition of polymer solution(s) the NEs were allowed to homogenize for 10 min.

Preparation of Drug Solution

The RS meant for comparative evaluation of NE-based systems was prepared by dissolving RSP (35 mg) in a mixture of 1 mL ethanol (95%, vol/vol) and 2 mL propylene glycol, finally volume was made to 10 mL with distilled water resulting in a solution of 3.5 mg/mL.

TABLE 1
Composition of Risperidone Nanoemulsion (RNE) and Risperidone Mucoadhesive Nanoemulsions (RMNE and RMNE1)

Ingredient (by wt)	RNE	RMNE	RMNE1
Risperidone (mg/mL)	3.50	3.50	3.50
Capmul MCM	8.00	8.00	8.00
Tween 80	29.33	29.33	29.33
Propylene glycol	7.33	7.33	7.33
Transcutol	7.33	7.33	7.33
Water	48.00	48.00	48.00
Chitosan	—	0.50	—
Polycarbophil	—	—	0.50

Characterization of NE

Qualitative Tests

The dilution test was performed by diluting 1 mL of prepared NE(s) to 100 mL and observed for clarity/turbidity. In another test, to identify the type of NE, methyl orange, a water-soluble dye, was sprinkled over the NEs and observed microscopically (10 X, Olympus, Watford WD24 4JL, UK). For centrifugation test, the prepared NE(s) were centrifuged (REMI Pvt. Ltd.) at $402 \times g$ for 15 min and examined for whether the system was monophasic or biphasic (Rolan, Piel, Delattre, & Evrard, 2003). All determinations were performed in triplicate.

Quantitative Tests

- *pH Measurement*: The pH of the NEs was measured using pH meter (Systronics 335, Naroda, Gujarat, India) using 5-mL sample in a 10-mL beaker.
- *Conductivity Measurement*: The conductivity of the NEs was measured with Conductometer CM 180 (Elico, Hyderabad, AP, India) by inserting the probe in 10 mL of prepared NE sample in a beaker.
- *Viscosity Measurement*: The viscosity of 5 mL of the sample was determined using a rotational viscometer coupled with a concentric cylinder RV No. 3 at 20 rpm at 30°C.
- *Transmittance (%T)*: The percentage transmittance of 2 mL NE(s) was checked against distilled water using UV-VIS spectrophotometer at 650 nm.
- *Globule size and zeta potential determination*: The globule size determination was performed using photon correlation spectroscopy with in-built zetasizer (model: Nano ZS, Malvern Instruments, Westborough, MA, USA) at 633 nm. Helium–neon gas laser having an intensity of 4 mW was the light source. The equipment was programmed to provide 18 mm laser width. Electrophoretic mobility (μm/s) was measured using small volume disposable zeta cell and converted to zeta potential (Rolan et al., 2003) by in-built software using Helmholtz–Smoluchowski equation. All quantitative test(s) determinations were made in triplicate.
- *Osmolarity Determination*: The osmolarity of the formulations was determined by the following expression (Lieberman & Lachman, 1993):

$$\text{mOsm/liter} = \frac{\text{concentration in gram per liter}}{\text{molecular weight in grams}} \times 10{,}000.$$

Ex Vivo Diffusion Study of RSP Formulations

The freshly excised sheep nasal mucosa (Gavini, Rassu, Sanna, Cossu, & Giunchedi, 2005), except the septum part, was collected from the 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 cell. The tissue was stabilized using phosphate buffer (pH 6.4) in both the compartments and allowed to stir for 15 min on a magnetic stirrer. After 15 min, solution from both the compartments was removed and fresh phosphate buffer (pH. 6.4) 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 crossing over the cell.

Franz diffusion cell used for ex vivo 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) 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.

A volume of 0.30 mL of each RS, RNE, RMNE, and RMNE1 was placed in the donor compartment of Franz diffusion cell along with 1.7 mL of phosphate buffer (pH 5) added additionally. Samples from the receptor compartment were withdrawn at predetermined time intervals and analyzed. 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 and the results were subjected to one-way ANOVA.

Test for Nasal Cilio Toxicity of NEs

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 (P1, P2, and P3) with uniform thickness were selected and mounted on Franz diffusion cells. P1 was treated with 0.5 mL of PBS (pH 6.4, negative control), P2 with 0.5 mL of isopropyl alcohol (positive control), and P3 was treated with NE (RNE) 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 NEs and photographed by microscope (Olympus).

Stability Studies

The formulations, RNE and RMNEs, were subjected to stability studies for a period of 3 months at room temperature and refrigerated conditions (4°C). After 3 months of storage, the NEs were subjected to test for physical stability (creaming, phase separation, or flocculation), accelerated centrifugation cycle (2,862 × g for 15 min), drug content, particle size, and zeta potential determinations (Rolan et al., 2003).

RESULTS AND DISCUSSION

Solutions of different concentrations (5–40 μg/mL) of RSP were scanned in overlay mode and the spectra showed peaks at 277 and 279 nm in methanol and phosphate buffer, respectively. The oily phases (Capmul MCM, Labrafac CC, Labrafil MS, and ethyl oleate) and Tween 80 when scanned over 200–400 nm exhibited interference at these wavelengths. Hence, second-order derivatization of zero-order spectra of RSP was done and the λ of 300 nm (smoothing factor 8 and scale of 100) was selected as the λ for the RSP estimation in methanol, as the excipients showed negligible interference at this wavelength, whereas λ of 305 nm (smoothing factor 8 and scale of 100) was selected for the estimation of RSP in diffusion media. Hence, calibration curves for RSP were made at 300 and 305 nm in methanol and phosphate buffer, respectively. The calibration curve with r^2 of .9998 was found to be linear in the range of 5–40 μg/mL.

The solubility of practically insoluble RSP was increased in the selected oily phases (Figure 1) and was highest in Capmul MCM and least in ethyl oleate. Hence, Capmul MCM was selected as the oily phase for the preparation of NE. The type of NE formed depends on the properties of the oil, surfactant, and the cosurfactant. Most single-chain surfactants do not lower the oil–water interfacial tension sufficiently to form NEs and short-to-medium-chain length alcohols are necessary as cosurfactants. The cosurfactant also ensures that the interfacial film is flexible enough to deform readily around each droplet as their intercalation between the primary surfactant molecules decreases both the polar head group interactions. In this study, Tween 80 and transcutol were selected as the surfactant–cosurfactant system and propylene glycol as a solvent. An important criterion for selection of the surfactants is that the required hydrophilic lipophilic balance (HLB) value to form the oil–water NE be greater than 10. The right blend of low and high HLB surfactants leads to the formation of a stable NE formulation (Craig, Barker, Banning, & Booth, 1995). In this study, we selected Tween 80 as a surfactant with an HLB value

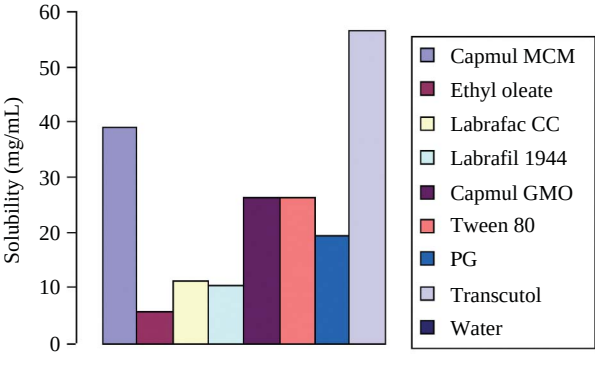

FIGURE 1. Graphical representation of solubility of RSP in selected oily phases (Capmul MCM, Capmul GMO, Labrafac CC, Labrafil 1944, and ethyl oleate), surfactant (Tween 80) and cosurfactants (propylene glycol and transcutol), and water.

of 15. Transient negative interfacial tension and fluid interfacial film are rarely achieved by the use of single surfactant; usually, addition of a cosurfactant is necessary. The presence of cosurfactant decreases the bending stress of interface and allows the interfacial film sufficient flexibility to take up different curvatures required to form NEs over a wide range of composition (Kawakami, Yoshikawa, & Moroto, 2002). Thus, the cosurfactant selected for the study was transcutol, which has an HLB value of 4.2.

A ternary phase diagram explains the selection of the formulations from the phase diagrams to avoid metastable formulations having minimum surfactant concentration, in the least possible time. Ternary phase diagrams were constructed by varying Tween 80 : propylene glycol : transcutol (T:PG:t) ratios as 1:1:1, 2:1:1, 3:1:1, and 4:1:1 (Figure 2). The shaded areas of phase diagrams show the NE regions, whereas the nonshaded area displays the emulsion region. Thus, the ternary phase system of T:PG:t (4:1:1) that exhibited maximum area for NE formation was selected for the optimization of NE batches. 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 4:1:1. It was clearly evident that an increase in the concentration of Tween 80 resulted in a decrease in globule size. Thus, at the lowest concentration of surfactant, the globule size was 32.31 ± 0.27 nm, whereas at the highest concentration of Tween 80 it reduced to 16.32 ± 0.09 nm, and hence the ratio of 4:1:1 (Tween 80:propylene glycol:transcutol) was selected for optimization studies. The optimization of NEs was carried out on the basis of percentage transmittance (%*T*), globule size, and zeta potential, and the results are tabulated in Table 2. According to the solubility study of RSP in Capmul MCM, a minimum of 8% by the weight of oily phase was required to fulfill the dose requirement, and with T:PG:t maintained at 4:1:1 and water at 48% by weight, eight batches of RSP-loaded microemulsions were formulated and evaluated.

The globule size decreased with the increase in the concentration of T:PG:t in the formulations (Table 2). The globule size of batch B1, containing 24% of surfactant–cosurfactant mixture, was highest (121 ± 6.21 nm) and was least (14.8 nm ± 2.32) for highest concentration (52%) of the T:PG:t. All the formulations had droplets in the nano range, which is very well evident from the low polydispersity values. Polydispersity is the ratio of standard deviation to mean droplet size; hence, it indicates the uniformity of droplet size within the formulation. The higher the polydispersity, the lower the uniformity of the

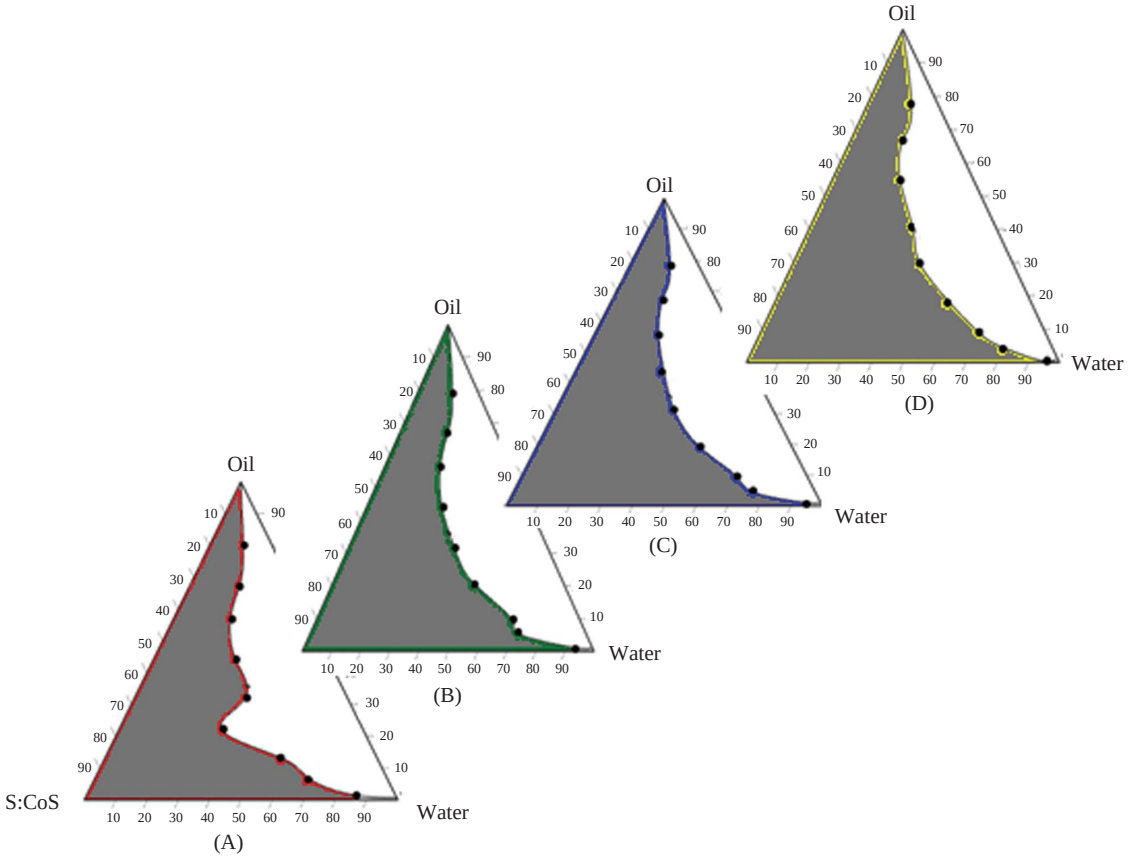

FIGURE 2. Pseudoternary phase diagrams of Capmul MCM, S_{mix} (surfactant–cosurfactant, 1:1) and water in a ratio of (A) 1:1:1, (B) 2:1:1, (C) 3:1:1, and (D) 4:1:1.

TABLE 2
Various Ternary Phase Compositions and Characterization Parameters of Blank Nanoemulsions

Batch	Capmul MCM (%,wt/wt)	T:PG:t (4:1:1) (%,wt/wt)	Water (%,wt/wt)	%*T* (*DF* = 10)	Zeta potential (mV) ± *SD*	Globule size (nm) ± *SD*	PDI ± *SD*
B1	8	24	68	67.3	−9.15 ± 1.23	121.0 ± 6.21	0.205 ± 0.02
B2	8	28	64	94.9	−8.41 ± 1.12	114.0 ± 3.43	0.195 ± 0.04
B3	8	32	60	97.3	−5.50 ± 0.08	78.0 ± 2.01	0.263 ± 0.06
B4	8	36	56	99.6	−3.78 ± 0.06	46.0 ± 3.35	0.223 ± 0.04
B5	8	40	52	99.86	−12.0 ± 2.24	25.3 ± 2.63	0.166 ± 0.05
B6	8	44	48	99.82	−6.0 ± 0.81	15.5 ± 2.12	0.172 ± 0.02
B7	8	48	44	99.69	−11.0 ± 1.43	15.0 ± 1.61	0.165 ± 0.01
B8	8	52	40	99.87	−8.7 ± 1.04	14.8 ± 2.32	0.156 ± 0.03

T is denoted for Tween 80; PG for propylene glycol; t for transcutol; %*T* for percentage transmittance; *DF* for dilution factor and PDI for polydispersibility index (n = 3).

droplet size in the formulation. Although the polydispersity values of all formulations were very low, indicating uniformity of droplet size within each formulation, it was least for B8 (0.156 ± 0.03).

The batch B6 (oil : surfactant–cosurfactant : water, 8:44:48) was selected as the optimized batch as it displayed optimum response variables of 99.82% optical transparency, low globule size (15.5 ± 2.12 nm), polydispersity of 0.172 ± 0.02, and zeta potential to the tune of −6.0 ± 0.81. Although batches B7 and B8 showed lower values for globule size and PDI that may be attributed to higher S_{mix} concentrations, the difference was insignificant ($p < .05$) when compared with B6. Moreover, higher concentrations of S_{mix} may cause damage to nasal mucosa; hence, B6 was selected for further processing.

The process for the preparation of NE was optimized by varying the stirring speed (365, 550, and 825 rpm) and time (5, 10, and 20 min for each stirring speed) followed by the globule size determination. Based on the minimum globule size of 15.6 ± 0.16 nm, a stirring speed of 825 rpm and stirring time of 10 min were selected as the optimized process parameters to obtain drug-loaded NE. NEs form spontaneously without the aid of high shear equipment or significant heat input (heat and gentle mixing are required only if it is necessary to melt any of the ingredients), and their microstructures are independent of the order of addition of the excipients.

Characterization of RSP-loaded NEs was performed based on both qualitative and quantitative tests. In general, an emulsion exhibits the characteristics of its external phase. Several methods are available for identifying the emulsion type. Dilution tests are based on the fact that the emulsion is only miscible with the liquid that forms the continuous phase. Upon dilution, no change in the globule size of NE was observed and the emulsion retained its clarity indicating to be an oil–water type of NE. Staining tests in which a water-soluble dye is sprinkled onto the surface of the emulsion also indicate the nature of continuous phase. With an oil–water emulsion, there is rapid incorporation of the dye into the system, whereas with water–oil emulsion, the dye forms microscopically visible lumps. Methyl orange, a water-soluble dye, could be readily incorporated in the NE system without clumping, hence proving the system to be of oil–water type. Neither phase separation nor creaming on centrifugation of the RNEs indicated stability of the prepared system.

The data for quantitative tests of RNEs are tabulated in Table 3. The pH of all the NEs ranged between 4.32 and 5.15, approximating the normal pH range of nasal fluids (Illum, 2000), which is one of the formulation consideration that may help reducing the irritation produced upon instillation. A percentage transmittance of 99.32 for RNE indicated clear dispersion, whereas RMNE and RMNE1 with no transmittance were hazy due to the presence of respective mucoadhesive component(s) in

TABLE 3
Characterization Parameters of Optimized RSP Nanoemulsion (RNE) and RSP Mucoadhesive Nanoemulsions (RMNE and RMNE1)

Formulation Code	% Assay	pH	%*T* (*DF* = 10)	Size (nm)	PDI*	Zeta Potential (mV)	Conductivity (mS)	Viscosity (cp)
RNE	98.86 ± 1.10	5.15 ± 0.11	99.32	15.5 ± 1.2	0.124 ± 0.03	−12.0 ± 2.21	0.156 ± 0.06	231 ± 5.34
RMNE	99.12 ± 0.96	4.62 ± 0.15	—	16.7 ± 1.4	0.163 ± 0.02	−9.15 ± 0.04	0.163 ± 0.03	244 ± 4.52
RMNE1	99.04 ± 1.02	4.32 ± 0.21	—	16.2 ± 2.1	0.207 ± 0.05	−2.66 ± 0.09	0.168 ± 0.07	246 ± 2.86

*Polydispersity index.

the formulations. The narrow globule size range of 15.5–16.7 nm and polydispersibility indices of 0.210, 0.191, and 0.207 for RNE, RMNE, and RMNE1, respectively, indicated that the NEs 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 –12.0, –9.15, and –2.66 mV on the globules of RNE, RMNE, and RMNE1, respectively, conferred physical stability to the system. Zeta potential (ξ) is the main indicator of emulsion stability, and for ξ values higher than –10 mV, the emulsion tends to destabilize.

As all the excipients used were nonionic in nature, low zeta potential values could be attributed to the drug molecules. Conductivity measurements rely on the poor conductivity of oil compared with water and give low values in water–oil emulsions where oil is the continuous phase. The reverse happens for oil–water emulsion. The conductivity measurements (0.156–0.168 mS) indicate the NEs to be of oil-in-water type, and it was observed that the viscosity of the NE formulations generally was very low. This was expected, because one of the characteristics of NE formulations is of lower viscosity (Lawrence & Rees, 2000). Low viscosity values ensure easy handling, packing, and hassle-free administration of formulations.

Although the nasal passages can tolerate a wide range of tonicity without pain, isotonicity is significantly important. Nasal solutions with osmolarity comparable to aqueous 0.5–2.0% (equivalent to 85.47–341.88 mOsmol/L) sodium chloride solution are relatively comfortable and do not harm nasal cilia. The osmolarity of RNE, RMNE, and RMNE1 were calculated as 270.16, 270.17, and 270.18 mOsmol/L, respectively, which is within the suggested limits and the preparations are unlikely to cause potential discomfort upon instillation. All the formulations were of an osmolarity of almost similar magnitude as the presence of high molecular weight (~300,000) mucoadhesive agents (0.5% by weight of both chitosan and polycarbophil) contributed negligibly to the osmolarity.

In ex vivo diffusion studies of RSP formulations, the recorded successful diffusion through sheep nasal mucosa and the results obtained are presented in Figure 3, and the calculated diffusion coefficients are tabulated in Table 4 along with the regression coefficients (r^2) for first-order, Higuchi, and

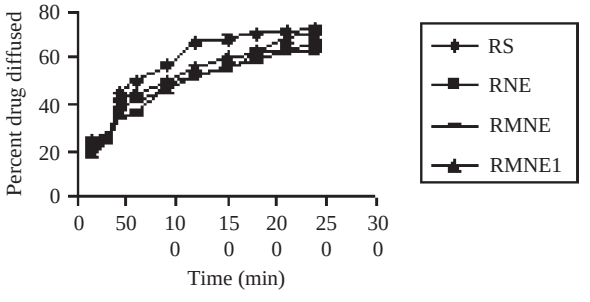

FIGURE 3. Percent cumulative drug diffused versus time profiles of RSP solution (RS), nanoemulsion (RNE), and mucoadhesive nanoemulsions (RMNE and RMNE1).

TABLE 4
Diffusion Coefficients and Modeling Parameters of RSP Nanoemulsion, Mucoadhesive Nanoemulsions and Solution

Formulation Code	Diffusion Coefficient (cm^2/min)	Zero Order	First Order	Higuchi
		r^2	r^2	r^2
RS	0.2134 ± 0.02	.8054	.8886	.9051
RNE	0.1873 ± 0.04	.8716	.9003	.9219
RMNE	0.1965 ± 0.01	.9274	.9730	.9784
RMNE1	0.1943 ± 0.02	.8902	.9531	.9552

zero-order modeling of the diffusion profiles for each formulation. The decreasing order of diffusion coefficient for the tested formulations was RNE < RMNE1 < RMNE < RS (although not significantly different at $p < .05$).

For RS, the drug exhibited highest diffusion coefficient, whereas it was least for RNE. The mucoadhesive NEs 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 (Sinswat & Tengamuay, 2003). Among the two mucoadhesive microemulsions (RMNE and RMNE1), the mucoadhesive microemulsion containing 0.5% (wt/wt) chitosan (RMNE) showed higher diffusion coefficient compared with the mucoadhesive microemulsion containing 0.5% (wt/wt) polycarbophil (RMNE1) indicative of better mucoadhesive performance of chitosan than polycarbophil. This can be related to the penetration-enhancing properties of chitosan (Gavini et al., 2005). These results have been supported by in vivo biodistribution studies conducted in rats (Kumar et al., 2008).

Biodistribution studies of 99mTc-RSP formulations following i.v. administration (RNE) and i.n. administrations (RS, RNE, and RMNE) on Swiss albino rats were performed and the radioactivity was estimated at different intervals of up to 8 h. The brain–blood ratio of the drug at all sampling time points for different formulations was also calculated. The RSP concentrations in brain following the i.n. of RMNE (Figure 1) were found to be significantly higher at all the time points compared with both RNE (i.n.) and RNE (i.v.), whereas the brain concentration of RSP after i.n. administration of RNE was comparable with that of i.v. administration of RNE at all the time points. The brain–blood ratios of 0.617, 0.754, 0.948, and 0.054 of RS (i.n), RME (i.n), RMNE (i.n), and RME (i.v.), respectively, at 0.5 h were indicative of direct nose to brain transport bypassing the blood–brain barrier, thus proving the superiority of nose to brain delivery of RSP by microemulsion. The pharmacokinetic parameters calculated for the RSP formulations displayed lower T_{max} values for brain (1 h) when compared with blood (2 h) that was attributed to preferential nose to brain transport following i.n. administration. When the C_{max} and AUC of brain concentration of RS (i.n.), RNE (i.n.), and RMNE (i.n.) were compared, the C_{max}

(0.11%/g) and AUC (0.48 h %/g) of RMNE were found to be higher because of the addition of mucoadhesive agent that decreased the mucociliary clearance, which under normal circumstances rapidly clears the instilled formulation.

RMNE showed the highest DTP% and direct nose to brain transport % among the three tested formulations, followed by RS and then by RNE. Nearly twofold higher DTP% of RMNE compared with RS and RNE showed the benefit of mucoadhesive NE formulation (Sakane, Yamashita, Yata, & Sezaki, 1999; Ugwoka, Exaud, Mooter, Verbeke, & Kinget, 1999; Vyas, Shahiwala, Marathe, Misra, 2005). The gamma scintigrams in rats, 0.5 h post-i.v. administration of RNE and i.n. administration of RMNE, clearly demonstrated the accumulation of formulations in brain administered via respective routes. Major radioactivity accumulation was seen in brain following i.n. administration of RMNE as compared with i.v. administration of RNE.

On modeling, the diffusion of drug from RSP 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 (Garti, Spernath, Abraham, & Lutz, 2005).

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 nasociliary damage (Figure 4A) and the nasal membrane remained intact, whereas an extensive damage to nasal mucosa coupled with loss of nasal cilia (Figure 4B) could be observed with positive control. However, with NE, no damage to nasal mucosa could

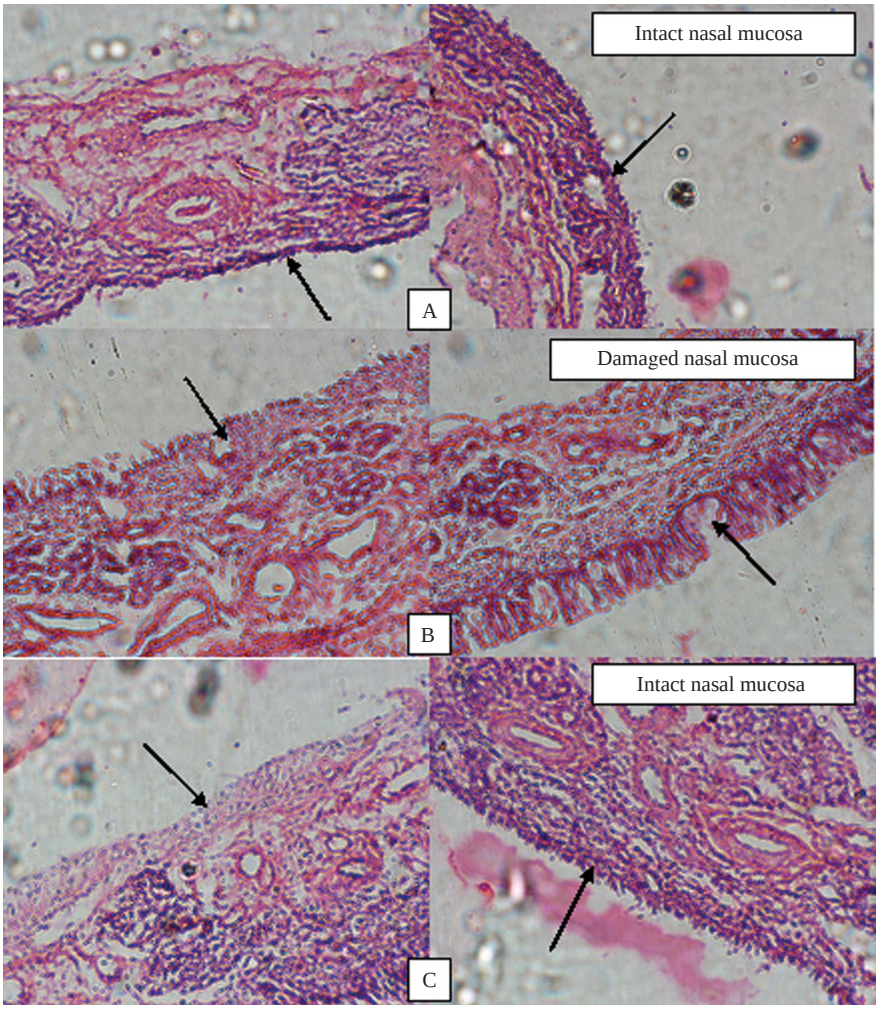

FIGURE 4. Photographs of sheep nasal mucosa demonstrating histological characteristics when treated with (A) phosphate buffer saline (pH 6.4), (B) isopropyl alcohol, and (C) blank nanoemulsion B6.

TABLE 5
Results of Stability Testing of the RSP Nanoemulsion and Mucoadhesive Nanoemulsion Containing 0.5% (wt/wt) Chitosan (RMNE)

Test	RNE	RMNE
% Assay	97.64	98.32
% Transmittance	98.06	—
Globule size (nm)	18.6	20.34
Polydispersibility index	0.124	0.163
Zeta potential (mV)	–6.25	–8.25

be observed (Figure 4C), thus substantiating the safety of the excipients used in the formulation.

In stability studies, the NEs exhibited no precipitation of drug, creaming, phase separation, and flocculation on visual observation and were found to be stable after centrifugation ($1{,}118 \times g$ 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 in the quantitative parameters of RNE and RMNE after 3 months of storage, thus substantiating the stability of NEs for 3 months.

CONCLUSION

On the basis of low droplet size and polydispersity, optimum surfactant and cosurfactant concentrations, the mucoadhesive formulation RMNE of RSP that contained 0.5% by weight of chitosan as the mucoadhesive component displayed highest diffusion coefficient. The formulation was free from nasal ciliotoxicity and was found to be stable for 3 months. The in vitro studies supported by in vivo studies demonstrated the potential of developed NEs for direct nose to brain delivery of RSP.

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