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Preparation of lorazepam-loaded microemulsions for intranasal delivery and its pharmacokinetics

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The purpose of this study was to develop a microemulsion system for intranasal delivery of lorazepam. The phase behavior and properties of microemulsions were characterized in a pseudo-ternary system composed of Cremophor EL 35/Transcutol P/Lauroglycol FCC or Labrafil M 1944CS/water, and intranasal absorption of lorazepam from microemulsions was investigated in rabbit. The microemulsions, comprising of FCC, Cremophor EL 35/Transcutol P (1.5 : 1) and water, were optimal for intranasal delivery of lorazepam. These systems had a higher solubilization capacity with the particle size of <150 nm, and were stable at ambient conditions for at least six months. *In vivo* absorption studies showed that intranasal absorption of lorazepam from microemulsions at 0.38 mg/kg had the larger AUC_{0-t}, the longer half-life and the prolonged circulation time with the mean bioavailability of 80.84% for ME2 and 63.48% for ME8 as compared to the intramuscular injection at 0.16 mg/kg. These results indicate that microemulsions may be a promising approach for the intranasal delivery of lorazepam.

1. Introduction

Lorazepam, 7-chloro-5(2-chlorophenyl)-1,3-dihydro-3-hydroxy-2*H*-1,4-benzodiazepin-2-one (Papini et al. 2006), has several advantages over diazepam including a longer duration of action (Walker et al. 1979; Crawford et al. 1987), potent anticonvulsant activity, possibly less respiratory depression (Appleton et al. 1995; Treiman et al. 1998), less requirement of repeated doses, and higher effectiveness in management of status epilepticus in both adults (Walker et al. 1979) and children (Lacey et al. 1986). Besides, it has been reported that intranasal lorazepam is effective, safe, and provides a less invasive alternative to intramuscular paraldehyde in children with protracted convulsions.

Lorazepam is a nearly white powder, and almost insoluble in water. Presently, lorazepam is available in tablet and injection dosage forms. These formulations release lorazepam into the peripheral circulation, which results in limited drug uptake across the blood-brain-barrier and drug distribution to nontargeted sites. In emergency conditions especially when the patient cannot autonomously administrated and i.v. administration is not immediately available, intranasal administration is an alternative, because it is a simple, effective and convenient administration manner with the advantages of rapid absorption, fast onset of therapeutic action, avoidance of liver first pass effect and metabolism by the gastrointestinal membrane and so on (Behl et al. 1998). Moreover, intranasal administration offers a practical, noninvasive, and an alternative route of administration for rapid drug delivery to the brain (Zhang et al., 2004; Zaki et al. 2006; Kaur and Kim 2008; Kumar et al. 2008). The direct nose-to-brain transport of drugs may occur after nasal administration as suggested in the study by Zhang et al. (2004), where about 26–89% of nimodipine content at 6 h was transported to the brain via the olfactory pathway after intranasal administration. Vyas et al. (2006) have reported that higher brain/blood ratios of clonazepam and lower T_{max} for the brain were observed after intranasal administration, which further confirms the direct nose-to-brain transport. Therefore, the intranasal delivery of lorazepam was investigated in this study.

A few issues that should be carefully considered in formulation development are the solubilization of poorly water-soluble lorazepam and the improvement of drug uptake across mucosa. Microemulsions, which have a droplet size in the range of 10–100 nm, are thermodynamically stable, isotropically clear products consisting of oil phase, aqueous phase, surfactant/s and co-surfactant. Oil-in-water microemulsions are suitable for the incorporation of poorly water-soluble drugs and may increase the permeability for the oral (Ke et al. 2005; Gui et al. 2008), percutaneous (Sintov and Shapiro 2004; Hua et al. 2004) and intravenous delivery (Corswant et al. 1998) of the drugs. It has been reported that the microemulsions might enhance the brain uptake of drugs (Tong et al. 2002; Yao et al. 2005; Yao et al. 2006; Yao et al. 2008). Recently, microemulsions have been used for the intranasal drug delivery to improve the solubility of insoluble drugs and enhance their uptake across nasal mucosa (Li et al. 2002; Zhang et al. 2004; Vyas et al. 2005).

In the present work, a number of microemulsions were evaluated for their potential as the intranasal delivery of

lorazepam. The lorazepam-loaded microemulsions were prepared with Cremophor EL 35 as the surfactant, Transcutol P as the co-surfactant and Lauroglycol FCC (FCC) as the oil phase.

2. Investigations, results and discussion

2.1. Solubility of lorazepam in various vehicles

It is essential for the drug to have the higher solubility in the components of the microemulsions to reach the required dose. The solubility of lorazepam in various solvents is presented in Table 1. The highest solubility of lorazepam (180 mg/g) was observed in Transcutol P. Moreover, compared to PEG 400, Transcutol P has a reasonable affinity for both the oil and aqueous phases, which may facilitate the formation of stable microemulsions. Moreover, it causes less mucosa irritation compared with benzyl alcohol. Therefore, Transcutol P was selected as the co-surfactant for the microemulsion development.

According to previous studies, the higher solubility of drugs in the oil phase was helpful for inhibiting the precipitation of lipophilic drugs from the o/w microemulsions in storage. Based on the results of lorazepam solubility in vesicles, Labrafil M 1944CS (1944CS) and FCC were selected as the oil for the study of pseudo-ternary phase diagram. Moreover, they could both produce successful microemulsion formulations.

Compared with Tween-80, some advantages of Cremophor EL 35 as the surfactant were considered as follows. More stable microemulsions with smaller particle size could be obtained. Furthermore, less water was required to form the clear microemulsion, so its o/w microemulsion region area and the maximum oil-loaded content were much higher. A maximum of only 5% 1944CS was incorporated in Tween 80-based microemulsions at Km of 2 : 1, and that was 21% for FCC, which was much less than those for the Cremophor EL 35-based systems (44.5% for FCC and 39.5% for 1944CS in Table 2). A broader microemulsion region and larger oil-loaded capacity were helpful for freely performing the formulation design to suffice the various requirements and assuring the stability of microemulsions both in storage and under physiological conditions. The systemic pathway by which some of the drug is absorbed into the systemic circulation and subsequently reaches the brain by crossing blood brain barrier (BBB) is one of the main pathways of drug uptake into the brain from the nasal mucosa (Thorne et al. 2004), suggesting that a fraction of drug would have to be transported through BBB into the brain. Cremophor EL was shown to inhibit the multi-drug resistance (MDR) phenotype in cultured cells at concentrations likely to be achieved clinically (Lo 2003). Thus, Cremophor EL in the microemulsion formulations may enhance brain uptake of drugs by inhibiting efflux drug transporters. Moreover, Cremophor EL can inhibit the cytochrome P450 3A biotransformation activity (Ren 2009), which may contribute to increasing the absorption of lorazepam by avoiding the metabolism of drugs in the nasal cavity. Therefore, Cremophor EL 35 was selected as the surfactant in this study.

Table 1: Solubility of lorazepam in various vesicles at 25 °C

Components	Vehicles	Solubility, mg/g
Surfactants	Tween-80	67.863 ± 0.379
	Cremophor EL 35	58.506 ± 6.119
Co-surfactants	Benzyl alcohol	150.867 ± 7.012
	PEG-400	140.581 ± 3.271
	Transutol P	180.926 ± 5.354
	Alcohol	10.139 ± 0.077
Oils	Cradamol GTCC	3.440 ± 0.103
	Labrafil M 1944CS	17.907 ± 0.221
	Lauroglycol FCC	14.770 ± 1.533
	Tea oil	0.821 ± 0.183
	Oleic acid	2.518 ± 0.020

Values are mean ± S.D. for n = 3

Table 2: Area of o/w miroemuslion region ($Area_{o/w}$) and maximum oil-loaded content in Labrafil M 1944CS-based microemulsions and Lauroglycol FCC-based microemulsions at various weight ratios of surfactant/co-surfactant (Km)

Microemulsions	Km	$Area_{o/w}$, %	Maximum oil-loaded content, %
Labrafil M 1944CS	2 : 1	9.71	39.5
	1.5 : 1	8.04	28.4
	1 : 1	6.88	10.8
Lauroglycol FCC	2 : 1	13.90	44.5
	1.5 : 1	20.34	36.9
	1 : 1	10.96	29.1

2.2. Phase behavior

The pseudo-ternary phase diagrams of the microemulsions with various Km are displayed in Fig. 1, which shows the effect of Km on the phase behavior of the microemulsion systems with 1944CS or FCC as oil phase. The area of o/w miroemuslion region and maximum oil-loaded content at different Km are listed in Table 2.

When FCC or 1944CS were used as the oil phase, the o/w microemulsions were formed at well-suited Km. The maximum oil-loaded content was both increased with the higher Km in two microemulsion systems, which indicated that the maximum of oil that could be solubilized into the microemulsion increased by increasing the surfactant concentration. Moreover, higher area of o/w microemuslion region (9.71%) for 1944CS system at Km 2 : 1 was obtained, and 20.34% for FCC system at Km 1.5 : 1. As seen from the phase diagram, the maximum oil-loaded content and area of o/w miroemuslion region in the FCC systems were all higher than those in the 1944CS systems at the same Km. A broad microemulsion region is preferred considering the requirement of the optimum design of the formulation.

2.3. Characterization of microemulsions

The incorporation of lorazepam in various microemulsions at different Km is reflected in Table 3. From a formulation point of view, Km of 1.5 : 1 was selected because more lorazepam was solubilized, compared with other ratios, in both FCC-based and 1944CS-based microemulsions with the fixed water content of 60%. On the other hand, the adequate content of Transcutol P in microemulsions may provide a greater opportunity for the solubility of lorazepam and decrease the surfactant content to avoid the potential nasal mucosal ciliotoxicity. Moreover, the amount of drug solubilized in microemulsions was increased by properly decreasing the water content. Unfortunately, the 1944CS-based microemulsions (ME4-6) stored at ambient

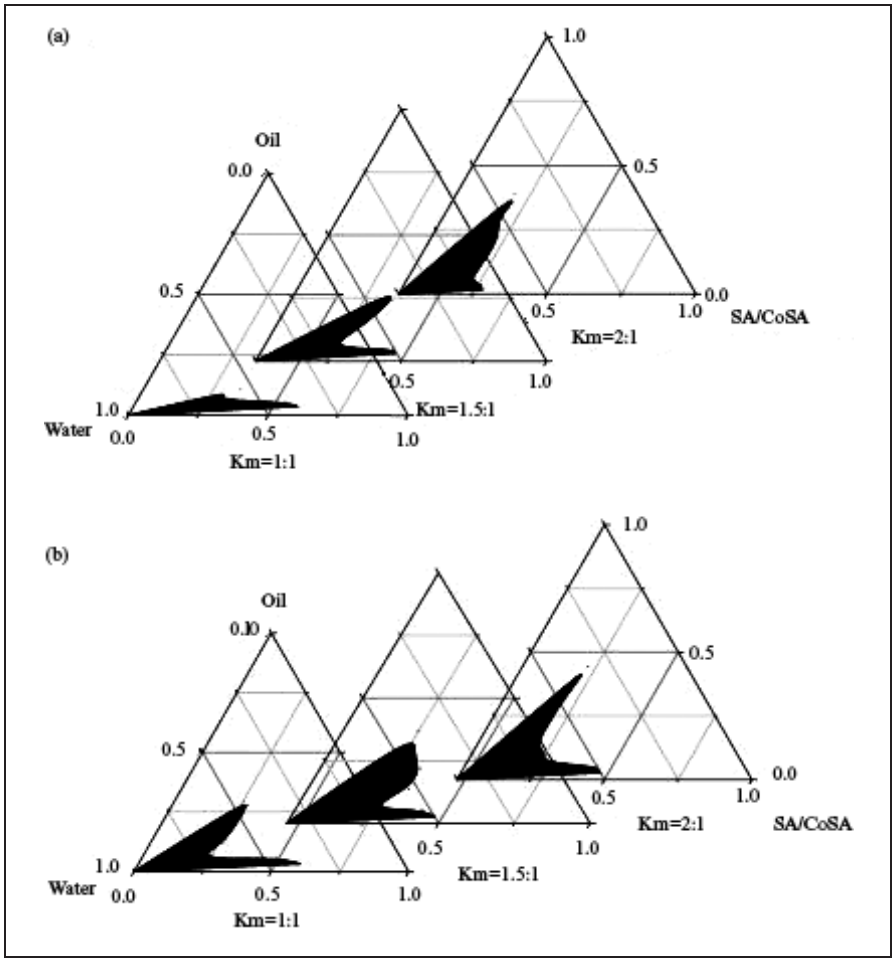

Fig. 1:
Pseudo-ternary phase diagrams for (a) Labrafil M 1944CS-based microemulsions system, and (b) Lauroglycol FCC-based microemulsions system at three different weight ratio of surfactant/co-surfactant (SA/CoSA) (Km) of 2 : 1, 1.5 : 1 and 1 : 1. The shade regions represent the o/w microemulsion regions

temperature turned cloudy in the third week although no drug was separated. In addition, the drug was separated from ME 3 and ME7 after the long-term storage.

Taken together, considering the solubilization capacity, stability and potential nasal ciliotocixity, the formulations 2 (ME2) and 8 (ME8) of the FCC-based microemulsions seem to be optimal formulations for nasal delivery of lorazepam. Two microemulsions with lorazepam were stable for at least six months.

The morphology and particle size of the selected microemulsions were investigated. The particle size of the microemulsions was not significantly affected by the presence or absence of lorazepam. The mean particle sizes of ME2 and ME8 without or with lorazepam were 109.9 ± 1.77 nm, 137.1 ± 7.3 nm, 39.0 ± 1.56 nm, 37.5 ± 0.71 nm, respectively. Besides, the size distribution of ME8 (PI: 0.202 ± 0.011) was narrower than that of ME2 (PI: 0.617 ± 0.081). The same results were shown from the morphology of the microemulsions by TEM analysis.

Figure 2 demonstrates the morphology of the microemulsions observed through a transmission electron microscope. The two vesicles had similar geometries, and differed only in size and distribution. The ME8 had more round and order appearance, smaller size and more uniform distribution of size.

The mucosa irritation from the microemulsions was studied. Optical microscopic results showed that a number of cilia were still beating on the edge of the mucosa that was treated with ME2 for 30 min, which indicated that ME2 had little effect on the cilia movement while ME8 caused moderate irritation. It was observed that some cilia fell off and the remaining were still beating, which resulted from the lower water content in ME8. The irritation may be attributed to the surfactant in formulations. The cilia movement was in part inhibited after the mucosa was treated with Cremophor EL while the effect of Transcutol P and FCC was slight, or even absent.

Table 3: Incorporation of lorazepam in various microemulsions at three different surfactant/co-surfactant ratios (Km)

Formulations	Km	Oils	Water %	Solubility, mg/g
ME1	2 : 1	Lauroglycol FCC	60	4.165
ME2	1.5 : 1	Lauroglycol FCC	60	5.780
ME3	1 : 1	Lauroglycol FCC	60	4.015
ME4	2 : 1	Labrafil M 1944CS	60	4.940
ME5	1.5 : 1	Labrafil M 1944CS	60	5.595
ME6	1 : 1	Labrafil M 1944CS	60	4.290
ME7	1.5 : 1	Lauroglycol FCC	50	8.560
ME8	1.5 : 1	Lauroglycol FCC	45	7.470

2.4. Nasal delivery of lorazepam-loaded microemulsions

Figure 3 represents the mean plasma concentration-time profiles of lorazepam following i.m. administration of lorazepam injection (0.16 mg/kg) and intranasal administration of two selected microemulsions in rabbits at 0.38 mg/kg dose. The non-compartment pharmacokinetic parameters are listed in Table 4.

Compared with the control group, plasma concentrations of lorazepam after intranasal administration of the ME2

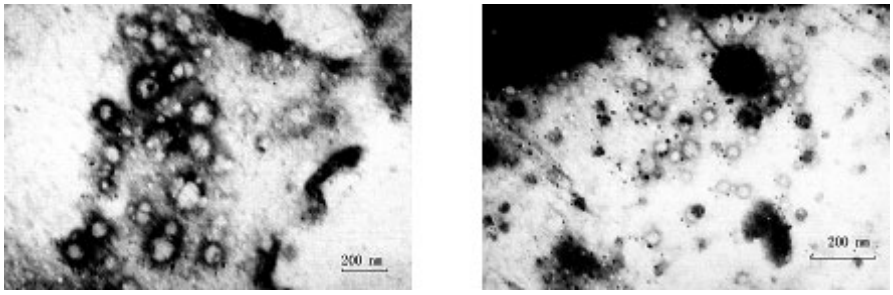

Fig. 2:
Transmission election micrograph of lorazepam-loaded microemulsions (A, ME2; B, ME8)

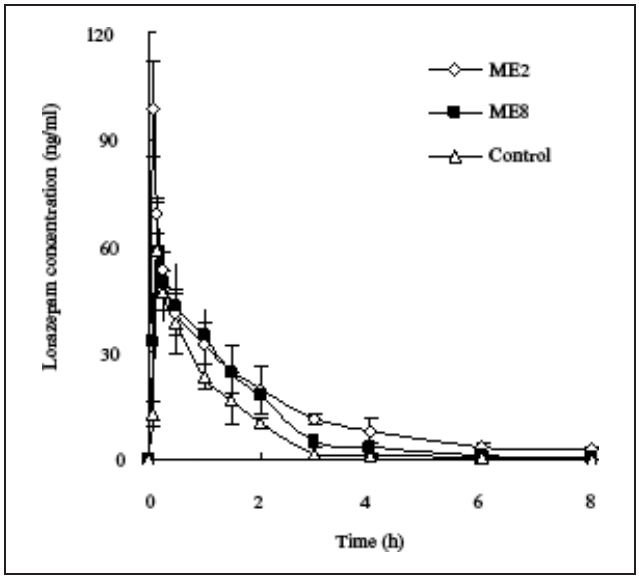

Fig. 3: Mean plasma concentration-time curvers of lorazepam after intranasal and i.m. administration of lorazepam-loaded microemulsions in rabbits

were increased when the dose level increased about twice. The mean AUC_{0-t} value of ME2 was 1.92 times (115.284 ng · h/mL versus 60.044 ng · min/mL) greater than that of the control group ($p < 0.05$). This value was also considerably higher than that obtained after intranasal administration of ME8, but no significant variation was observed ($p > 0.05$). Moreover, the C_{max} value of lorazepam was far greater with the ME2 group in comparison to the other two groups ($p < 0.01$). The peak time (T_{max}) of ME2 was 5 min, more rapid than that of ME8 and the control group with 10 min. As is known that the T_{max} offers an independent criterion to evaluate the *in vivo* drug absorption rate, the lorazepam in ME2 was more rapidly absorbed into systemic circulation, which demonstrated the superiority of intranasal over i.m. administration for achieving faster drug absorption, with a potential of clinical application in acute situations such as severe nausea and vomiting. Similar results were obtained with other drugs when administered nasally. The T_{max} of diazepam from the microemulsions at 1 or 2 mg/mL dose in rabbit, now available as an intranasal spray, was 6.7 ± 2.9 min and 3.0 ± 1.7 min respectively as reported by Li et al. (2002).

As to the ME8 group, based on the AUC and C_{max}, the absorption of lorazepam was similar in comparison to the control group ($p > 0.05$), although its mean AUC_{0-t} was higher. The results might in part be explained by a slower release of drug from the ME8 in comparison to ME2 (data not shown). Besides, it also suggested that the size span of the microemulsions from 39 nm to 137 nm might not affect the absorption of drug via nasal mucosa significantly.

In addition, compared with the control group, there was a slower elimination phase ($T_{1/2} = 2.187 \pm 0.590$ h for ME2 and 1.210 ± 0.203 h for ME8) in lorazepam profiles from ME2 and ME8, followed by a smaller clearance (CL).

Calculated on AUC_{0-8h}, the mean bioavailabilities (F %) of ME2 and ME8 were 80.84% and 63.48%, respectively. In the previous report, the mean bioavailability of lorazepam in human via intranasal administration with Cremophor EL as a vehicle was 51% of that following i.v. administration with half dose of intranasal dose (Lau and Slattery 1989). However, the amount of Cremophor EL in ME2 and ME8 was much less than that above, which should contribute to decreasing the irritation of nasal mucosa.

Based on the advantages in solubilizing capacity and enhancing absorption of drugs, microemulsion systems have been widely used as an effective carrier for the intranasal (Li et al. 2002; Kumar et al. 2008), dermal (Sintov and Shapiro 2004; Zhu et al. 2008) and oral delivery of drugs. In these studies, the microemulsions showed the exerted advantage for enhancing the absorption of drugs. The nasal absorption of diazepam from an ethyl laurate-based microemulsion was found to be fairly rapid and the bioavailability was about 50% (Li et al. 2002). Microemulsion with Cremophor EL35 as surfactant shows more excellent characteristics than the micelle and ternary solvent system for the transdermal delivery of ivermectin (Chen and Zhou 2006). The cu-

Table 4: Pharmacokinetic parameters of lorazepam in rabbit plasma following intranasal administration of microemulsions and i.m. administration of control group

Parameters	Control	ME2	ME8
C_{max} (ng/mL)	59.256 ± 10.312	$98.612 \pm 9.588^{**}$	$58.634 \pm 4.681^{\square\square}$
T_{max} (min)	10	5	10
AUC_{0-t} (ng · h/mL)	60.044 ± 16.616	$115.284 \pm 15.853^{*}$	90.528 ± 31.880
$AUC_{0-\infty}$ (ng · h/mL)	60.137 ± 17.032	123.241 ± 21.237	91.359 ± 32.400
MRT_{0-t} (h)	1.080 ± 0.187	$1.948 \pm 0.101^{**}$	$1.456 \pm 0.194^{\square}$
$T_{1/2}$ (h)	0.609 ± 0.105	$2.187 \pm 0.590^{*}$	$1.210 \pm 0.203^{*}$
CL (L/h/kg)	0.006 ± 0.001	$0.003 \pm 0.001^{*}$	$0.005 \pm 0.002^{\square}$
F %	100	80.84	63.48

Results are given as mean ± S.D. (n = 4). * $p > 0.05$ and ** $p > 0.01$ vs. control group. □ $p > 0.05$ and □□ $p > 0.01$ vs. ME2 group

mulative amount of penciclovir permeated through excised mouse skins from microemulsion composed of oleic acid, Cremorphor EL and ethanol was about 3.5 times that of the commercial cream (Zhu et al. 2008).

In conclusion, the pharmacokinetic parameters of lorazepam were significantly altered in microemulsions via intranasal administration with 0.38 mg/kg. Lorazepam in ME2 and ME8 had a prolonged half-life (3.591 and 1.987 times, respectively) and larger AUC_{0-t} (1.907 and 1.498 times, respectively) as compared to that in the control group under i.m. administration with 0.16 mg/kg.

Overall, the results of the *in vivo* absorption study indicated that a more than twofold dose of intranasal administration of lorazepam in microemulsion formulation (0.38 mg/kg) provided a comparable or even higher plasma drug concentration-time profile than that of i.m. administration of a 0.16 mg/kg dose. Considering the factors such as the administration convenience and potential for targeting to brain, the intranasal delivery with microemulsions as a carrier might be a feasible alternative for lorazepam. These results indicated that microemulsions might be a promising approach for the intranasal delivery of lorazepam. Moreover, considering the rate and degree of *in vivo* absorption, ME2 may be a better formulation than ME8 for intranasal delivery of lorazepam.

3. Experimental

3.1. Materials

Lorazepam (99.4% purity) was purchased from Xuzhou RYEN Pharma. Co., Ltd. (Xuzhou, China). Lorazepam injection was prepared using propylene glycol – PEG400 (5 : 1) mixture with 2% benzyl alcohol. Polyoxyethylene 35 castor oil USP24/NF19 (PEG-35 castor oil, Cremophor EL 35) was purchased from BASF (Ludwigshafen, Germany). Diethylene glycol monoethyl ether (Transcutol P®), propylene glycol monolaurate (Lauroglycol FCC®) and oleoyl macrogolglycerides (Labrafil M 1944CS®) were kindly gifted by Coloncon (U.K.). Medium-chain triglyceride (Caprylic/capric triglyceride, Cradamol GTCC®) was a kind gift from CRODA (Singapore). Diazepam (internal standard, IS) was purchased from the National Institute for the Control of Pharmaceutical and Biological Products (NICPBP). Other chemicals were of HPLC or analytic grade.

3.2. Solubility determination

The solubility of lorazepam was determined in various oils, surfactants and co-surfactants. An excess of lorazepam was placed in 2 g of the vehicle and the mixture was heated at 50 °C to facilitate the solubilization using a vortex mixer. The resulting mixtures were equilibrated at 25 °C for 48 h in a water bath and then centrifuged to separate undissolved drug. Aliquots of supernatant were diluted with methanol and quantified by HPLC.

3.3. Construction of pseudo-ternary phase diagram

In order to find the appropriate components and concentration range of components in the formulation of o/w microemulsions, pseudo-ternary phase diagrams were constructed using a H_2O titration method at room temperature (Yao et al. 2008).

The phase diagrams were prepared with the 2 : 1, 1.5 : 1 and 2 : 1 weight ratios of surfactant/co-surfactant (Km), respectively. For each phase diagram at a specific Km, the ratios of oil to the mixture of surfactant and co-surfactant 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. The mixtures of oil, surfactant and co-surfactant at certain weight ratios were diluted with H_2O in a drop-wise manner, under moderate stirring. After being equilibrated, the mixtures were assessed visually and determined as being microemulsions, crude emulsions or gels.

After the identification of o/w microemulsion region in the phase diagrams, the microemulsion formulations at desired component ratios were selected according to the area of o/w microemulsions region and the maximum oil-loaded content as the major indexes.

3.4. Characterization of microemulsions

In order to confirm the microemulsion formation, the solubility in several selected microemulsion formulations and their characteristics were investigated. A photo correlation spectrometer (Zetasizer 3000 HS, Malvern Instruments Corp., U.K.) was used to measure the particle size of the microemulsions following dilution with double-distilled water. The shape and surface morphology of the microemulsions were observed with a Hitachi H-700 transmission electron microscope (TEM). Negative staining with 1% phosphotungstic acid solution was performed to enhance image quality.

Nasal ciliotoxicity studies were carried out using in situ toad palate model (Zhang et al. 2004). In brief, the upper palate of toad (30–40 g, Center Animal Laboratory of China Pharmaceutical University, China) was exposed and treated with 0.5 mL test microemulsions containing lorazepam at 2.5 mg/mL for 30 min, then rinsed with saline. The palate was dissected out and the mucocilia was examined with an optical microscope (Leica DM LP, Leica Microsystems, Germany). Saline was used as a negative control.

3.5. Nasal absorption studies

New Zealand white rabbits (2.0–3.0 kg) were used for lorazepam nasal and intramuscular (i.m.) administration with a washout period of 2 weeks.

Rabbits were weighed and restrained in rabbit restrainers before the experiment. For i.m. administration, the lorazepam injection (2 mg/mL) was delivered through the upper leg muscle of rabbit at 0.16 mg/kg dose. For intranasal administration, 100 µl of the nasal formulation was added into each nostril of rabbit. Blood samples were collected into heparinized tubes through a marginal ear vein at 0 (predose), 5, 10, 15 min, 0.5, 1.0, 1.5, 2.0, 3.0, 4.0, 6.0 and 8.0 h after administration. Plasma was obtained by centrifugation at 4000 × g for 10 min and samples were stored at −20 °C until analysis. Measurements were made using 4 rabbits at each time point.

A 20 µL aliquot of IS working solution (120 ng/mL in methanol), 100 µL of 1 M sodium hydroxide and 4 ml of acetoacetate/n-hexane (1 : 1) were added to 200 µl of rabbit plasma samples. The mixtures were vortex-mixed for 5 min and centrifuged at 2000 × g for 10 min. The organic phase was transferred to a clean test tube and evaporated to dryness under a gentle nitrogen stream at 35 °C. The residue was reconstituted in 100 µL of the mobile phase as the sample solution for pre-emergency.

3.6. LC-MS analysis of plasma samples

Aliquots of 20 µL were injected into the LCMS-2010EV (Shimadzu Biotech, Japan) system. Electrospray parameters were as follows: The source block and desolvation temperatures were set at 200 and 250 °C, respectively, capillary voltage at 4.5 KV, nebulizer pressure at 0.1 Mpa and the nebulizing gas (nitrogen) flow at 1.5 L/min. The detector voltage is 1.5 KV. The LC-ESI-MS was performed in the selected ion-monitoring (SIM) mode. The ESI in positive ion mode was adopted for the analytes quantitation with the following parameters: $[M + H]^+$ m/z at 284.9 for diazepam, $[M + Na]^+$ m/z at 342.9 for lorazepam. Chromatographic separation was achieved on a Shim-pack C_{18} column (150 × 2.0 mm, 5 µm, Shimadzu). The mobile phase consisted of a mixture of methanol and 150 mM sodium acetate (62.5 : 37.5, v/v). The flow rate was 0.2 mL/min and the column temperature was maintained at 35 °C.

The standard curves of peak area ratio (y) as a function of lorazepam concentration (x) over a range of 1.2–150 ng/mL were linear with regression coefficients (r^2) of 0.9968 for plasma. The calibration equation was as follows: $y = 17.771x - 7.3128$. The extraction recoveries of lorazepam from rabbit plasma were greater than 95%. The intra-day and inter-day precision of lorazepam in rabbit plasma were $<5\%$ and $<11\%$, respectively. The lowest limit of quantitation (LLOQ) was 1.2 ng/mL. So the precision and accuracy of the assay for plasma were acceptable as defined in the State Food and Drug Administration (SFDA) guidelines.

3.7. Data and statistical analysis

All plasma concentration data were analyzed using a statistical moment algorithm by the DAS 2.1.1 software. The areas under the concentration-time curve (AUC_{0-t}) and the area under the first moment curve ($AUMC_{0-t}$) were calculated using the linear trapezoidal rule for infinite time. The mean residence time (MRT) was determined by dividing $AUMC_{0-t}$ by AUC_{0-t} (Yao et al. 2006). The statistical differences among group means were assessed using the one-way unweighted means analysis of variance (ANOVA) test and a value of $p < 0.05$ was considered statistically significance.

The mean bioavailability (F %) of nasal administration from microemulsions was calculated using the following Equation:

$$F(\%) = (AUC_{0-t,\,nasal} \times Dose_{im})/(AUC_{0-t,\,im} \times Dose_{nasal}) \times 100\,. \quad (1)$$

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