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Preparation and evaluation of fexofenadine microemulsions for intranasal delivery

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ABSTRACT

To enhance the solubility and bioavailability of poorly absorbable fexofenadine, microemulsion system composed of oil, surfactant and co-surfactant was developed for intranasal delivery. Phase behavior, particle size, viscosity and solubilization capacity of the microemulsion system were characterized. Histopathology and *in vivo* nasal absorption of the optimized microemulsion formulations were also investigated in rats. A single isotropic region was found in the pseudo-ternary phase diagrams developed at various ratios with Lauroglycol 90 as oil, Labrasol as surfactant and Plurol Oleique CC49 or its mixture with PEG-400 (1:1) as cosurfactant. An increase in the microemulsion region in pseudo-ternary phase systems was observed with increased surfactant concentration. The optimized microemulsion formulations showed higher solubulization of fexofenadine, *i.e.*, F1 (22.64 mg/mL) and F2 (22.98 mg/mL), compared to its intrinsic water solubility (1.51 mg/mL). Nasal absorption of fexofenadine from these microemulsions was found to be fairly rapid. T_{max} was observed within 5 min after intranasal administration at 1.0 mg/kg dose, and the absolute bioavailability (0–4 h) was about 68% compared to the intravenous administration in rats. Our results suggested that these microemulsion formulations could be used as an effective intranasal dosage form for the rapid-onset delivery of fexofenadine.

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1. Introduction

Allergic rhinitis (AR) is an inflammatory disease of the upper airway that is biphasic in its pathophysiology and symptom presentation (Baroody, 2003). Antihistamines, decongestants, intranasal corticosteroids, leukotriene receptor antagonists, topical anticholinergics, mast-cell stabilizers, mucolytics, anti-IgE antibodies are used in the clinical treatment of AR. Though several different mediators are involved in the pathophysiology of allergic diseases, histamine remains the principal one, and plays a fundamental role in the genesis of AR. Thus, antihistamines represent the primary class of medications used for the treatment of allergic rhinitis over the past 60 years (John, 2008).

Fexofenadine, an active metabolite of the second-generation histamine H1 receptor antagonist (antihistamine) terfenadine, is associated with few CNS adverse effects compared to its predecessors (Chen, 2007). Fexofenadine is unique in that it appears to be purely nonsedating, even at higher doses (Pilpot, 2000). In *in vitro* models, efflux transporter P-glycoprotein has been reported to transport fexofenadine and it is considered to be an important determinant of fexofenadine pharmacokinetics (Drescher et al., 2002). Since fexofenadine is the substrate of P-gp and several organic anion transporting polypeptide (OATP), food and co-administration of drugs will have significant effect on its oral bioavailability (Shimizu et al., 2006).

In recent years the nasal route has received a great deal of attention as a convenient and reliable method for the systemic administration of drugs (Costantino et al., 2007). However, polar drugs and some macromolecules are not absorbed in sufficient concentration due to poor membrane permeability, rapid clearance and enzymatic degradation into the nasal cavity (Mainardes et al., 2006). The large surface area of the nasal cavity and the relatively high blood flow, thereby achieving a rapid absorption and avoidance of hepatic first-pass elimination are attractive features of nasal drug administration (Lin et al., 2007a,b). Despite the need for a nasal delivery system (*i.e.*, at the site of action), currently only oral formulations at a dose of 30–180 mg/day are available for fexofenadine HCl. A nasal dose for fexofenadine has not been established. However, based on a daily oral dose of 120 mg and the nasal/oral dose ratio for other antihistamines, a nasal fexofenadine dose in the range 1–5 mg/nostril can be assumed (Illum et al., 2001). Depending on the Biopharmaceutical Classification System (BCS),

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fexofenidine belongs to Class 3 drugs, which means high solubility and low permeability, thus effort to increase the permeation of the drug is needed. It has been demonstrated that low absorption of drugs can be countered using absorption enhancers or increasing the drug residence time in the nasal cavity (Ugwoke et al., 2005; Lin et al., 2007a,b).

A microemulsion is defined as a thermodynamically stable, isotropic dispersion of two relatively immiscible liquids that consists of microdomains of one or both liquids stabilized by an interfacial film or surface-active molecules. Microemulsions offer an interesting and potentially quite powerful alternative carrier system for drug delivery because of their high solubilization capacity, transparency, thermodynamic stability, ease of preparation, and high diffusion and absorption rates when compared to solvent without the surfactant system (Jadhav et al., 2006; Yin et al., 2009). Microemulsions, by virtue of their lipophilic nature and having low globule size, are widely explored as a delivery system for enhancing uptake across mucosa (Li et al., 2002; Zhang et al., 2004; Elshafeey et al., 2009). The aim of the present study is to formulate the o/w microemulsion system of fexofenadine for intranasal delivery using GRAS (generally regarded as safe) materials, thereby achieving rapid onset of action and higher bioavailability with less toxicity. The formulations were characterized by solubility, phase diagram, particle size, viscosity and *in vitro* drug release profile. The optimized formulations were assessed by histopathology and *in vivo* pharmacokinetics studies.

2. Materials and methods

2.1. Materials

Fexofenadine was donated by Handok Co. (Seoul, Korea) and terfenadine was purchased from Sigma–Aldrich (MO, USA). PEG-8 caprylic/capric glycerides (Labrasol), polyglyceryl oleate (Plurol Oleique CC497), propylene glycol monolaurate (Lauroglycol 90) and linoleoyl macrogolglycerides (Labrafil M 2125 CS) were kindly donated by Gattefossé (Toronto, Canada). Polyethylene glycol 400 (PEG 400) was purchased from Duksan pure Chemical Ltd. (Kyungkido, Korea). All other chemicals were of analytical reagent grade and used without further purification.

2.2. Solubility studies

The solubility of fexofenadine in various oils and surfactants was determined by adding excess amount of fexofenadine into 1 mL of each vehicle in a centrifugal tube, followed by mixing (100 rpm) in a shaking incubator (Jeio-Tech, Seoul, Korea) at 25 °C for 24 h. The samples were centrifuged at 13,200 rpm for 10 min to remove the excess fexofenadine, after which the concentration of fexofenadine in the supernatant was measured by HPLC after appropriate dilution with isopropyl alcohol.

2.3. Construction of ternary phase diagram

The existence of microemulsion fields that form microemulsions under dilution and gentle agitation were identified from ternary phase diagrams of systems containing oil–surfactant–cosurfactant. Based on the results of solubility study (Table 1), Lauroglycol 90 was selected as an oil phase. The effect of surfactant (Labrasol) and co-surfactant (*i.e.*, Plurol Oleique CC497 or the mixture of Plurol Oleique CC497 and PEG 400 at 1:1 ratio) on the pseudo ternary phase diagram was systematically observed at room temperature. The surfactant and co-surfactant were weighed at different ratios (2:1 and 3:1, w/w) in each tube, and were vortexed vigorously for 30 s to make the surfactant mixture. Afterwards, the oil phase and the surfactant mixture were mixed, where the ratios of oil to surfactant mixture in the mixtures were varied from 9:1 to 1:9 (w/w). Distilled water was added dropwise to each clear oil and surfactants mixture with gentle stirring to allow equilibration. Following the addition of aliquot of water phase, the mixture was visually examined for transparency. The points from clear to turbid and turbid to clear were designated as emulsion and microemulsion, respectively. The o/w and w/o microemulsions were identified using water-soluble methylene blue and fat-soluble sudan III.

Table 1
The solubility of fexofenadine in various vehicles at 25 °C.

Vehicles	Fexofenadine (mg/mL)
Water	1.51 ± 0.02
Oils	
Castor oil	0.32 ± 0.01
Lauroglycol 90	5.25 ± 0.25
LABRAFIL M 2125 CS	0.20 ± 0.00
Surfactants	
Labrasol	24.59 ± 0.49
Tween 80	11.18 ± 0.04
Cremophore RH 40	9.65 ± 0.27

Based on the results of pseudo ternary phase diagrams, two microemulsions were selected for further experiments (Table 2). In order to determine the maximum drug loading content of microemulsion formulations, excess amount of fexofenadine was added into the microemulsions prepared as mentioned above. It was further mixed in a shaking incubator at 100 rpm for 24 h at 25 °C. Excess fexofenadine was removed by centrifugation at 13,200 rpm for 10 min, after which the content of fexofenadine in the microemulsions was measured by HPLC.

2.4. Characterization of fexofenadine loaded microemulsions

2.4.1. Morphology detection using transmission electron microscopy

The morphology of the microemulsions was examined by an Energy-Filtering Transmission electron microscopy (TEM) (LIBRA 120, Carl Zeiss, Germany) with a 80 kV accelerating voltage. The microemulsions were negatively stained by 2% sodium phosphotungstate (pH 7) and placed on carbon-coated 400 mesh copper grids followed by drying at room temperature before measurements.

2.4.2. Droplet size determination

The droplet size of the microemulsions loaded with fexofenadine was measured by an electrophoretic light-scattering spectrophotometer (ELS-8000, OTSUKA Electronics Co. Ltd., Japan). The microemulsions were transferred to a standard quartz cuvette, and the droplet size of the microemulsions were determined *via* dynamic He–Ne laser (10 mW) light-scattering at an angle of 90° at 25 °C. Data analysis was conducted using a software package (ELS-8000 software) provided by the manufacturer.

2.4.3. Viscosity of microemulsions

The viscosity of microemulsions was measured by DV-E Viscometer (BROOKFIELD, USA) using a 16 spindle at a speed of 100 rpm at room temperature.

Table 2
The composition of fexofenadine microemulsion.

Formulation	Oil (%)	S (%)	CoS 1 (%)	CoS 2 (%)	Water (%)
F1	4.5	30	10	–	55
F2	4.5	30	5	5	55

Oil: oil phase (Lauroglycol 90); S: surfactant (Labrasol); CoS 1: cosurfactant (Plurol Oleique CC497); and CoS 2: cosurfactant (Polyethylene Glycol 400).

2.5. In vitro drug release study

The *in vitro* release profile of fexofenadine from the microemulsions was determined by the bulk equilibrium reverse dialysis bag technique using a USP dissolution apparatus II (ELECTROLAB TDT-08L, India). The paddle was run at a speed of 100 rpm. The medium was 500 mL of phosphate buffer (pH 6.5), and the temperature was kept at 37 ± 0.5 °C. The dialysis bag held 10 mL dissolution medium and was equilibrated for 30 min prior to the experiments. The microemulsions (5 mL) containing the same amount of fexofenadine (10 mg/mL) prepared based on the solubility study were directly introduced into the dissolution medium outside the dialysis bag. At predetermined time intervals (1, 2, 3, 4, 6, 8, 12 h), 0.1 mL of samples were withdrawn from inside the dialysis bag and replaced immediately with the same volume of fresh medium. The fexofenadine concentration in the samples was determined using HPLC.

2.6. HPLC analysis of fexofenadine

For the solubility, the loading content and the *in vitro* release studies, HPLC method was used for the analysis of fexofenadine. Samples were properly diluted by isopropyl alcohol and directly injected (20 μL) into the HPLC system without further treatment. The HPLC system was equipped with a Waters 2487 Dual λ Absorbance Detector, 717 plus Autosampler and 515 HPLC dual pumps. A reverse phase C_{18} column (12.5 cm × 4 mm I.D., 5 μm, LiChroCART, Germany) was used at room temperature. The wavelength of the UV detector was set at 220 nm. Mixture of acetonitrile and pH 2.5 phosphate buffer solution containing 0.5% triethylamine (40:60, v/v) was used as the mobile phase at a flow rate of 1.0 mL/min.

2.7. Pharmacokinetics studies

2.7.1. Experimental procedure

Male Sprague–Dawley rats (250–270 g, Dae-Han Biolink, Daejeon, Korea) were used to perform the *in vivo* pharmacokinetics study. All rats were maintained in a light-controlled room kept at a temperature of 22 ± 2 °C and a relative humidity of 55 ± 5% (Animal Center for Pharmaceutical Research, College of Pharmacy, Seoul National University, Korea). The experimental protocols involving animal study were approved by the Animal Care and Use Committee of the College of Pharmacy, Seoul National University.

Before the experiment, the rats were fasted overnight (12 h) with free access to water and they were randomly divided into four groups. Femoral arteries and veins of the rats were catheterized with polyethylene tubing (PE-50, Clay Adams, Parsippany, NJ, USA) filled with 50 IU/mL of heparin in saline under anesthesia by ketamin (45 mg/kg) and acepromazine (1 mg/kg) at supine position. For the intravenous administration group, fexofenadine aqueous solution (1 mg/mL) was administered *via* the femoral vein at 1 mg/kg dose. For the oral administration group, fexofenadine aqueous solution was administered by oral gavage at 10 mg/kg dose. For the intranasal administration groups, the microemulsions (F1 and F2) were prepared with 10 mg/mL drug loading for the convenient of *in vivo* study at 1 mg/kg dose (for example, 25 μL of the formulation was administered onto the nostrils of 250 g rat) (Illum et al., 2001). Blood samples (~0.25 mL) were withdrawn from the femoral artery at predetermined time intervals for 4 h (intravenous and intranasal administration groups) or 8 h (oral administration group), and 0.2 mL of heparin (50 IU/mL) solution was used to maintain patency of the cannula between sampling. Plasma samples were obtained by immediately centrifuging the blood samples at 7000 × *g* for 5 min, after which 100 μL of plasma samples were transferred to new glass tubes and stored at −20 °C until analyzed by LC–MS/MS. The pharmacokinetic parameters of each formulation were attained using the WinNonlin® program (Version 3.1, Pharsight Co., Mountainview, CA, USA).

2.7.2. Plasma treatment and analysis

Fexofenadine concentration in the plasma samples was analyzed by LC–MS/MS (Huh et al., 2010). Briefly, plasma samples (100 μL) were mixed with 10 μL of internal standard (terfanadine 1 μg/mL) and 200 μL of acetonitrile. The mixture was vortexted for 10 min and centrifuged at 13,200 rpm for 5 min, and the supernatant was evaporated to dryness under nitrogen at 40 °C. The residue was reconstituted with 50 μL of mobile phase and 5 μL was injected onto the LC–MS/MS system consisting of Waters 2695 (Alliance, USA) and LCQ advantage ion-trap mass spectrometer (ThermoFinnigan, USA). Chromatography was performed using Gemini 3 μm C18 column (150 mm × 2 mm I.D., 3 μm, Phenomenex, USA) at a flow-rate of 0.2 mL/min with acetonitrile–0.15% formic acid mixture (4:6, v/v) as a mobile phase. ESI was performed in the positive mode with probe temperature set at 380 °C and MRM mode at unit resolution was employed for the quantification of transition of the protonated molecules of fexofenadine at m/z 502 → m/z 466 and that of terfenadine at m/z 472 → m/z 436. Operational parameters of the MS/MS detector were optimized using built-in automated tune system. Calibration curve was constructed in the range of 5–5000 ng/mL and LLOQ was 5 ng/mL. A mean correlation coefficient (r^2) for the calibration curve was over 0.999. While precision value of LLOQ (20 ng/mL) was under 15% while the other precision (CV) values were under 5%. The accuracy for fexofenadine–HCl was also within acceptable range (−6% to 6%).

2.8. Histopathologic study

Nasal cytotoxicity studies were carried out using male Sprague–Dawley rats weighing 250 ± 50 g. In brief, microemulsion formulations (F1 and F2) containing fexofenadine at 10 mg/mL were administered intranasally at a dose of 1.0 mg/kg after anesthetization of rats with intra-peritoneal injection of ketamin (45 mg/kg) and acepromazine (1 mg/kg). The nasal mucosa from the bottom of inferior meatus was dissected out after 2 or 24 h of intranasal drug treatment by sacrificing the rats. The tissues were immersed in 10% neutral formalin overnight and dehydrated with 100%, 95%, 80%, 70% alcohol and then they were stained with hematoxylin-eosin. The resulting blocks were embedded in paraffin and sectioned anterior to posterior at 5-mm thickness. The sections were examined and photographed using Nikon Eclipse TE2000-S inverted research microscope (Nikon Corp., Japan). The samples were examined for signs of epithelial disorganization, cilia disappearance and slight dysplastic changes.

2.9. Data analysis

All the experiments in the study were performed at least three times and the data were expressed as the mean ± standard deviation (S.D.). A two-tailed unpaired Student's *t*-test was performed at $p < 0.05$.

3. Results and discussion

3.1. Solubility studies

The microemulsion formulations consisted of oil, surfactants, co-surfactants and drug should be a clear and isotropic liquid at ambient temperature and should have good solvent properties to allow presentation of the drug in solution. The solubility of fexofenadine in various vehicles is presented in Table 1. Among the surfactants studied, Labrasol showed the highest solubility for

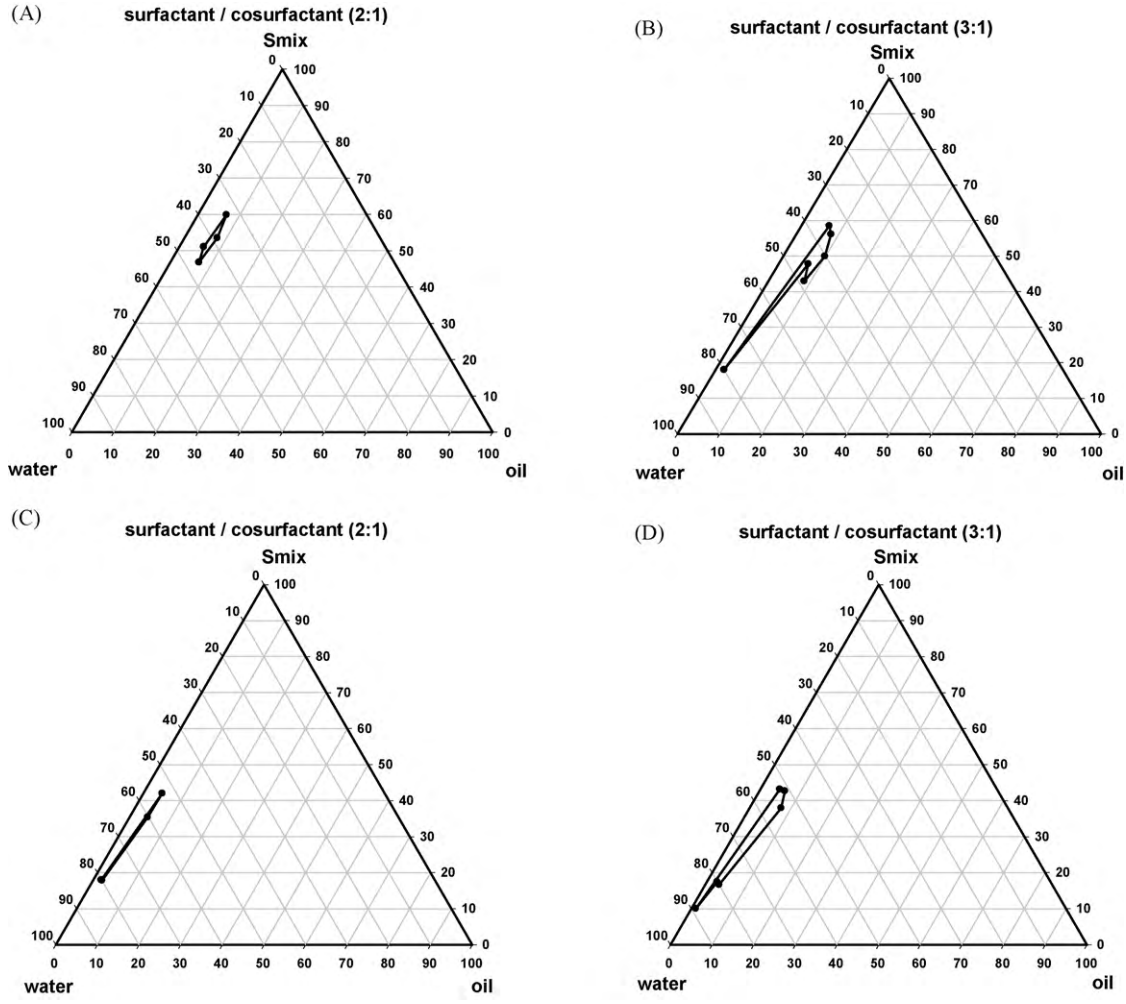

Fig. 1. Pseudo-ternary phase diagrams consisted of Lauroglycol 90 and Labrasol, as oil and surfactant, respectively. Co-surfactant was Plurol Oleique CC 497 (A and B) or mixture of Plurol Oleique CC 497 and PEG 400 at 1:1 (C and D), where the surfactant/cosurfactant ratio was 2:1 (A and C) and 3:1 (B and D).

fexofenadine. Thus, Labrasol, a medium-length alkyl chain surfactant with HLB 14, was selected as a surfactant. Moreover, it has been reported for its enhancing-effect on oral, transdermal and intranasal absorption of drugs (Rhee et al., 2001; Zhang et al., 2004; Balakrishnan et al., 2009a,b). Labrasol had been employed as a pharmaceutical excipient not only for the solubilization of hydrophobic drugs, but also for the poorly absorbable hydrophilic drugs (Koga et al., 2006). Lauroglycol 90 was chosen as an oily phase for its good drug solubility (Table 1) and emulsion forming ability with Labrasol (Balakrishnan et al., 2009a,b). Plurol Oleique CC 497 was selected as a co-surfactant since it was reported that the solubilization of drug was easier in the systems containing its mixture with Labrasol (Djekic and Primorac, 2008).

3.2. Construction of pseudo-ternary phase diagrams

The construction of phase diagram makes it easy to find out the concentration range of components for the existence range of microemulsions. Fig. 1 shows the phase diagrams, constructed to determine the optimum surfactant/cosurfactant, for the formulation of o/w microemulsion consisting of Lauroglycol 90, Labrasol, Plurol Oleique CC 497 and water. Surfactant/cosurfactant was varied as 2:1 and 3:1. As shown in Fig. 1, compared to surfactant/cosurfactant of 2:1 system, the existence area of o/w microemulsion becomes enlarged and higher at surfactant/cosurfactant of 3:1 system. This increase was toward the oil–water axis, indicating that by increasing the Labrasol concentration, the maximum amount of water and fexofenadine that could be solubilized into the microemulsion increased (Zhang et al., 2004). Pseudo-ternary phase diagrams consisted of Lauroglycol 90, Labrasol, Plurol Oleique CC 497 and water were presented in Fig. 1A (2:1) and Fig. 1B (3:1) and the same systems with the mixture of Plurol Oleique CC 497 and PEG 400 (1:1) as co-surfactant presented in Fig. 1C (2:1) and Fig. 1D (3:1). It has been reported that PEG can improve the wettability to optimize the dissolution rate and significantly reduce the particle size (Leuner and Dressman, 2000). Pseudoternary phase diagrams constructed with PEG 400 incorporation showed difference in the microemulsion forming region. Phase behavior investigation of these systems demonstrated the suitable approach to determining the water phase, oil phase, surfactant concentration, and cosurfactant concentration with which the transparent 1-phase microemulsion system was formed (Ghosh et al., 2006). The microemulsion formulations F1 and F2 were selected from the systems showed larger microemulsion region Fig. 1B and 1D, respectively. The exact composition of oil, surfactant, cosurfactant and aqueous phase were showed in Table 2.

3.3. Physicochemical characterization of microemulsions

The physicochemical characteristics of the microemulsions (F1 and F2) appear in Table 3. One of the critical challenges in fexofenadine nasal delivery is the high solubilization of drug to fit

Table 3
The physicochemical properties of fexofenadine microemulsion.

Formulation	Solubility (mg/mL)	Size (nm)	Viscosity (cp)
F1	22.64 ± 0.04	59.9 ± 4.9	103 ± 6
F2	22.98 ± 0.06	30.4 ± 1.0	61 ± 1

the small volume of nasal cavity. The solubility of fexofenadine in F1 (14-fold) and F2 (10-fold) was higher than its aqueous one. The different co-surfactants compositions used in the formulations could have attributed to the difference in solubility as in the case of F1 where Plurol Oleique CC 497 was used while in F2, mixture of Plurol Oleique CC 497 and PEG-400. Moreover, the relative solubility of the drug in various components would contribute to the drug entrapment in a given microemulsion as previously reported (Constantinides, 1995). The particle size analysis showed that the mean droplet size of F1 and F2 were below 100 nm and was consistent with their TEM results (Fig. 2). However, F2 showed significantly smaller droplet size than F1. This could be due to the decreased surface tension by the presence of PEG 400 (Ferri and Stebe, 2000). It was clear from the physicochemical data (Table 3) that F2 had low viscosity compared to F1. It has been reported that the viscosity of nasal application plays commanding role in the pharmacokinetic profile of the drug (Tomoyuki et al., 2007). A drug applied in the nasal cavity is translocated to the nasopharynx and thereafter to the gastrointestinal tract by the coordinated beat of the cilia of respiratory epithelial cells. This is an important non-specific defense mechanism of the respiratory tract and is called mucociliary clearance. To this point, the formulation with high viscosity is preferred in order to increase the mean residence time in the nasal cavity. However, the penetration rate of the formulation into mucus is considered to be decreased with the increase in the viscosity of applied formulation, resulting in a delay of the drug's approach to the cell surface. Furthermore, the viscosity of the formulation can influence the surface area where the drug can spread in the nasal cavity (Furubayashi et al., 2007). Based on these considerations, the moderate viscosity observed for F1 and F2 was thought to be adequate for nasal delivery (Tomoyuki et al., 2007).

3.4. In vitro release kinetics

In order to be an effective and reliable dosage form, nano-sized emulsions should have predictable drug release profiles. The most commonly used technique for assessing *in vitro* transport from submicron emulsions is the side-by-side diffusion cell (barrier) method. However, the major limitation of this method is the potential for violation of sink conditions. The bulk equilibrium reverse dialysis bag technique which was chosen in this dissolution study overcomes the shortcomings of the side-by-side diffusion cell and bulk equilibrium dialysis bag techniques by diluting the submicron-sized emulsion in the donor chamber and by increasing the surface area of the permeating membrane (Chidambaram and Burgess, 1998). In order to mimic the physiology condition in the nasal cavity (the pH of nasal secretions is normally in the region 5.5–6.5), pH 6.5 phosphate buffer was selected in this study. The *in vitro* release profile of fexofenadine formulations was plotted in Fig. 3. A biphasic release profile was obtained using the reverse dialysis bag technique. The faster initial rate may be due to free and micellar solubilized drug release from the donor continuous phase to the receiver chamber. The latter slower rate may be due to drug release from the oil droplets to the receiver chamber through the continuous phase of the donor chamber. The microemulsion formulation (F1 and F2) released rapidly (more than 65% of drug in 4 h) compared to fexofenadine powder (less than 40% in 4 h).

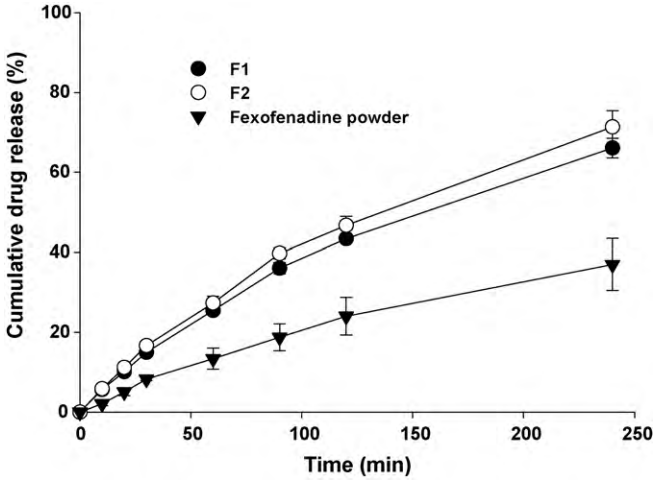

Fig. 3. *In vitro* drug release profiles of F1, F2 and fexofenadine powder.

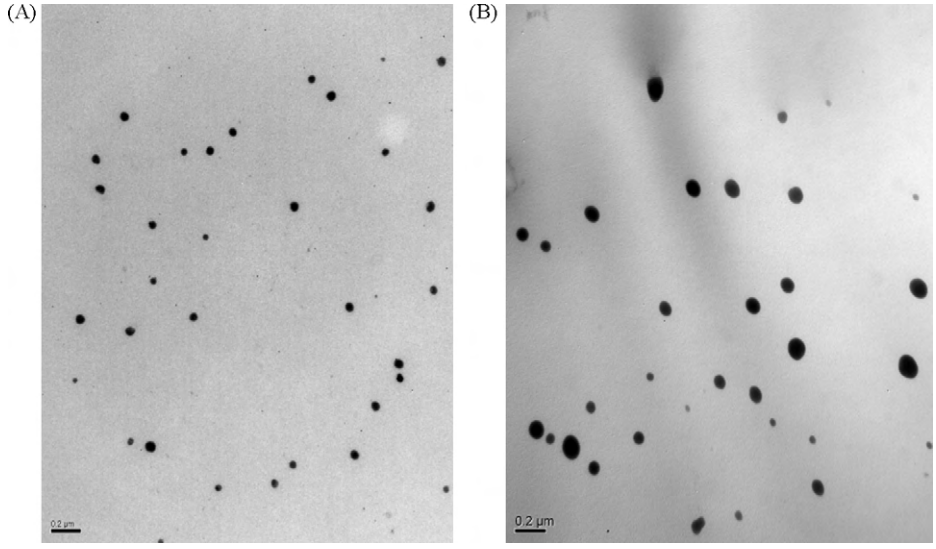

Fig. 2. Transelectron microscopy (TEM) of (A) F1 and (B) F2.

3.5. In vivo pharmacokinetics study

In vivo studies were performed nonsurgically in SD rats. As the rats were remained conscious throughout the experiment, functional mucociliary transport could be supported during the whole procedure. SD rats were selected for the *in vivo* studies as this species has a large nasal cavity volume enough for intranasal application of active substances. The mean volumes measured by fluid-displacement method (FDM), 72 mm^3 (58–86 mm^3), and by acoustic rhinometry (AR), 58 mm^3 (55–61 mm^3), contained in the first 2 cm of the nasal cavity were theoretically large enough for *in vivo* evaluation of nasal formulations (Straszek and Pedersen, 2004). *In vivo* absorption of fexofenadine following nasal administration of F1 and F2 were compared with intravenous administration of fexofenadine aqueous solution (Fig. 4). The plasma concentration profile of fexofenadine after intravenous injection of fexofenadine aqueous solution is shown in Fig. 4(A). The mean plasma concentration profile of fexofenadine from the intranasal delivery of fexofenadine microemulsion formulations and fexofenadine powder is displayed in Fig. 4B. The corresponding bioavailability and pharmacokinetic parameters are shown in Table 4. At 1.0 mg/kg dose, the nasal delivery of F1 and F2 showed similar bioavailability of 62–68% relative to intravenous administration, but oral administration showed only 6.6% relative bioavailability after dose normalization. The T_{max} of drug from oral administration is significantly longer than nasal administration (Table 4). Moreover, despite the higher oral dose of fexofenadine, the C_{max} from oral administration was much lower than that of test formulations nasal administration. The mean maximum concentration ($C_{max)}$ of fexofenadine from F2 was significantly higher ($p < 0.05$) than that of F1. In addition, the plasma level of drug at each sampling time from F2 was slightly higher than that of F1. Since the olfactory and respiratory epithelia are the primary barriers to drug absorption from the nasal cavity, the localization and activity of P-gp within these barriers has a significant impact on the understanding of nasal bioavailability (Kandimalla and Donovan, 2005). It has been reported that efflux transporters such as P-gp and multiresistance associated protein (MRP) 1 and 2 were expressed in the epithelium and nasal glands of the human nasal respiratory mucosa (Wioland et al., 2000). It has also been reported that PEGs could inhibit the function of P-gp by *in vitro* and *in vivo* studies (Lin et al., 2007a,b). Moreover, Labrasol had the most effective inhibition impact with the representative P-gp substrate digoxin (Cornaire et al., 2004). The presence of PEG 400 in addition to Labrasol might be the reason for higher C_{max} and plasma drug profile obtained from F2. The low viscosity of F2 also may have played a role in its higher drug pharmacokinetic profile (Tomoyuki et al., 2007). The microemulsion formulations showed a commendable higher drug plasma profile compared to powder formulation. The elimination half life ($T_{1/2}$) of fexofenadine appears to decline in a manner dependent on the route of drug administration. The $T_{1/2}$ of intranasal administration were similar to that of intravenous, this might be due to the rapid absorption of drug in the intranasal administration

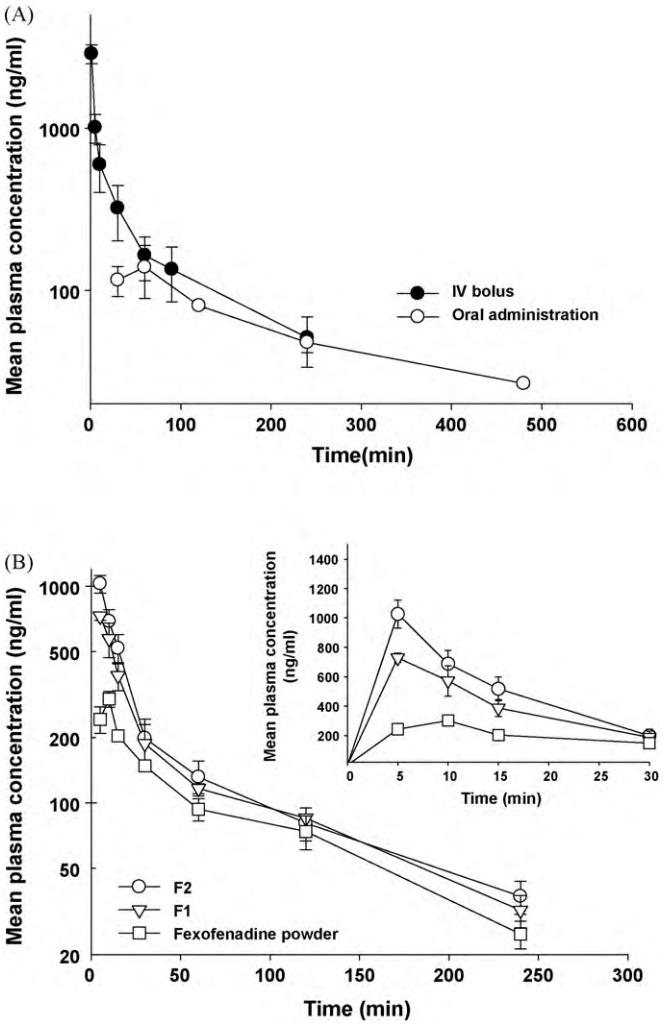

Fig. 4. (A) The plasma concentration profile of fexofenadine after intravenous administration (1.0 mg/kg) and oral (10 mg/kg) of fexofenadine aqueous solution. (B) Intranasal delivery of F1, F2 and fexofenadine powder (inserted inside figure is enlarged part of 0 → 30 min of (B)).

route (Pontiroli et al., 1993; Li et al., 2002). The $T_{1/2}$ of fexofenadine was significantly prolonged compared to intravenous and intranasal administration in oral administration; this might be due to the slower absorption (Fig. 4) of drug in oral administration (Daley-Yates et al., 2001), which needs further investigation. The oral bioavailability of fexofenadine in human is not established and that of other animals is very low such as 4.2% in rats (Strelevitz et al., 2006) which is similar to the result obtained in this study (6.6%) and 2.6% in horses (Olsén et al., 2006). Compared with the oral bioavailability, the bioavailability observed for the nasal fexofenadine formulations (F1 and F2) in this study was significantly

Table 4
Pharmacokinetic parameters of fexofenadine after administration in various routes in rats.

	Dose (mg/kg)	C_{max} (ng/mL)	T_{max} (min)	$T_{1/2}$ (min)	AUC (ng/mL min)	BA (%)
IV bolus	1	–	–	100.46 ± 8.74	56480.91 ± 13358.84	–
PO	10	139.56 ± 50.21	60	219.52 ± 20.16	37297.22 ± 3657.76	6.60
Nasal (F1)	1	728.19 ± 31.73**	5	87.78 ± 21.16	35188.25 ± 303.77**	62.30
Nasal (F2)	1	1026.76 ± 95.25**,***	5	95.69 ± 17.70	38789.87 ± 3164.45*	68.68
Nasal (powder)	1	302.16 ± 25.17	10	91.51 ± 17.44	23887.62 ± 264.30	42.29

All data are expressed the means ± standard deviation ($n = 3$).
* $p < 0.05$ compared with intranasal delivery of fexofenadine HCl powder.
** $p < 0.01$ compared with intranasal delivery of fexofenadine HCl powder.
*** $p < 0.05$ compared with F1.

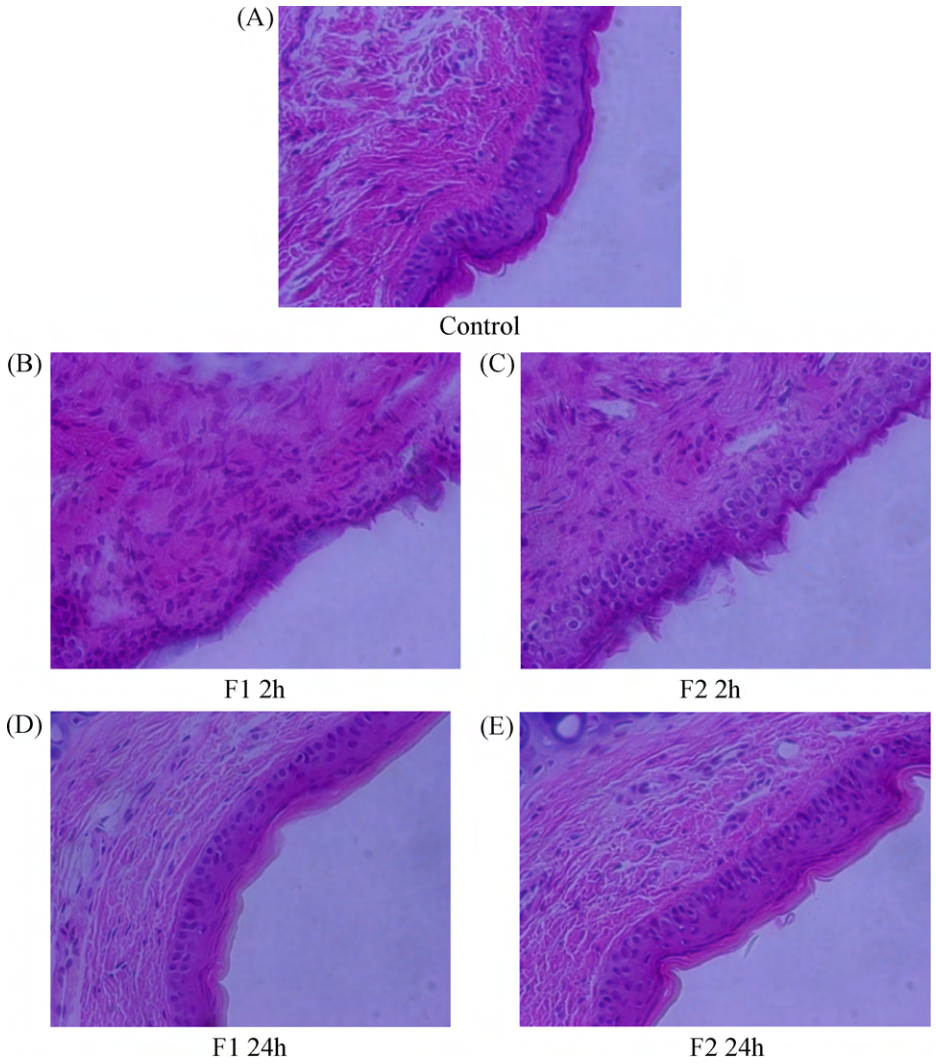

Fig. 5. Optical microscopic picture of nasal mucosae: (A) normal and after 2 h treatment with (B) F1 and (C) F2, and 24 h after treatment with (D) F1 and (E) F2.

higher and rapid (about 10-fold). The enhanced absorption may be explained in terms of (1) the huge specific surface area of the microemulsion droplets, (2) improved permeation of the fexofenadine because of the presence of surfactant, which reduces the interfacial tension to nearly 0, and (3) also may be due to their inhibition function of P-gp and/or permeation enhancing-effect. Considering the solubilization property, particle size analysis, and *in vivo* absorption findings, microemulsion formulation is believed to be a potential nasal formulation for the rapid-onset intranasal delivery of fexofenadine.

3.6. Histopathologic examination

Mucosa ciliary activity of epithelial cells in the upper respiratory tract is important for the proper function of mucociliary clearance and mucus transport. Thus, the histopathological examination was conducted to evaluate the changes of ciliary activity in nasal mucosa induced by fexofenadine microemulsion formulations F1 and F2. The squamous epithelium of the upper respiratory airways is normally in a steady state of cell renewal, but nasal cell turnover could be affected by the concentration and duration of exposure to drugs (Hernández-Escobar et al., 2009). Optical microscopic results revealed abnormal histopathologic findings including ciliary loss, adhesion, or shortening and detachment of the epithelial cells after 2 h of treatment for F1 (Fig. 5B) and F2 (Fig. 5C) compared to normal condition of nasal epithelial cells (Fig. 5A). However, the damaged epithelium was recovered in 24 h and active ciliary movement was observed (Fig. 5D and 5E). This result indicated that the damage was temporary and can be recovered in a short period of time. Nasal irritation is another aspect which needs to be considered as the ingredients used in this study has been reported for possible nasal irritation though they are GRAS materials to use in nasal formulations (Aurora, 2002; Elshafeey et al., 2009). However, it has been reported that the nasal irritation could be lessen by optimizing the nasal liquid formulations that contains more than 10% (w/w) of water, less surfactant and free of alcohol (Li et al., 2002; Aurora, 2002). As the optimized formulations contains 55% of water, free of alcohol and less surfactant, it is assumed to be less nasal irritation (Aurora, 2002). Taken together, considering the solubilization capacity, droplet size and nasal ciliotoxicity, F1 and F2 microemulsions seem to be optimal formulations for nasal delivery of fexofenadine.

4. Conclusions

The microemulsion system comprising oil (Lauroglycol 90), surfactant (Labrasol), and co-surfactant (Plurol Oleique CC497 or Plurol Oleique CC497/PEG 400 mixture) showed high solubilization capacity of fexofenadine. The optimized formulations of microemulsions containing fexofenadine were developed through

the construction of pseudo-ternary phase diagram, viscosity, solubility and particle size analysis. Following intranasal administration in rats, microemulion showed significantly higher C_{max} and AUC, and shorter T_{max}, compared to the powder. Overall, the study has indicated that the feasibility of fexofenadine microemulsion formulations as a promising approach for its intranasal delivery.

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