Regular Article

Formulation and Evaluation of a Self-microemulsifying Drug Delivery System Containing Bortezomib

Eon-Pyo Hong,[a,b,#] Ju-Young Kim,[c,#] Su-Hyeon Kim,[d] Kyu-Mok Hwang,[d] Chun-Woong Park,[e] Hyo-Jung Lee,[e] Dong-Wook Kim,[f] Kwon-Yeon Weon,[g] Seo Young Jeong,*[,a] and Eun-Seok Park*[,d]

[a] *College of Pharmacy, Kyung Hee University; Seoul 02447, Republic of Korea:* [b] *R&D Center, Handok Inc.; Seoul 02108, Republic of Korea:* [c] *College of Pharmacy, Woosuk University; Wanju-gun 55338, Republic of Korea:* [d] *School of Pharmacy, Sungkyunkwan University; Suwon 16419, Republic of Korea:* [e] *College of Pharmacy, Chungbuk National University; Cheongju 28644, Republic of Korea:* [f] *Department of Pharmaceutical Engineering, Cheongju University; Cheongju 28503, Republic of Korea: and* [g] *College of Pharmacy, Catholic University of Daegu; Gyeongsan 38430, Republic of Korea.*

Received January 13, 2016; accepted April 6, 2016

The purposes of the present study were to develop a self-microemulsifying drug delivery system (SMEDDS) containing bortezomib, a proteasome inhibitor. The solubility of the drug was evaluated in 15 pharmaceutical excipients. Combinations of oils, surfactants and cosurfactants were screened by drawing pseudo-ternary phase diagrams. The system exhibiting the largest region of microemulsion was considered optimal. Bortezomib SMEDDS spontaneously formed a microemulsion when diluted with an aqueous medium with a median droplet size of approximately 20–30 nm. *In vitro* release studies showed that the SMEDDS had higher initial release rates for the drug when compared with the raw drug material alone. Measurement of the viscosity, size, and ion conductivity indicated that a phase inversion from water in an oil system to oil in a water system occurred when the weight ratio of the water exceeded 30% of the entire microemulsion system. In a pharmacokinetics study using rats, the bortezomib microemulsion failed to improve the bioavailability of the drug. The reason was assumed to be degradation of the drug in the microemulsion in the gastrointestinal tract. However, bortezomib in Labrasol® solution (an aqueous solution containing 0.025% Labrasol®) showed significantly increased area under the curve from 0–24 h (AUC_{0-24h}) and maximum plasma concentration (C_{max}) values compared to the drug suspension. The findings of this study imply that oral delivery of a bortezomib and colloidal system containing Labrasol® could be an effective strategy for the delivery of bortezomib.

Key words bortezomib; labrasol; microemulsion; self-microemulsifying drug delivery system (SMEDDS); pharmacokinetics

Bortezomib, marketed as Velcade® in the United States, is a proteasome inhibitor for the treatment of relapsed multiple myeloma and mantle cell lymphoma. The mechanisms of the drug associated with its anticancer activity are clear that proteasome inhibition could promote degradation of anti-apoptotic proteins and prevent degradation of pro-apoptotic proteins, resulting in programmed cell death in malignant cells.[1] Bortezomib is administered to the patients as intravenous or subcutaneous injections. It was reported that the drug could be orally available in the early stage of development. However, oral delivery of the drug has been not investigated and there has been no pharmacokinetic parameter related to oral absorption.[2] It was suspected that oral absorption of bortezomib was limited due to low aqueous solubility of the drug and efflux by P-glycoproteins (P-gp).[3,4]

Microemulsion is a colloidal system which consists of oil, water, surfactants and cosurfactants. They can offer the advantages including simple manufacturing processes, thermodynamic stability, nano-sized droplets and improved solubilization of poorly soluble drug.[5] Therefore, they could be potentially excellent carriers for the drug with low solubility and have been applied in various administrations such as oral, intranasal and vaginal routes.[6–10] Pre-concentrates of microemulsions are homogeneous liquids, which contain oil, surfactants and the drug without aqueous phase. They are known as self-microemulsifying drug delivery system (SMEDDS). When SMEDDS is in contact with medium such as water or gastrointestinal fluid, it spontaneously forms microemulsion with gentle agitation.

Labrasol is a pale-yellow liquid and composed of mixtures of monoesters, diesters, and triesters of glycerol and monoesters and diesters of polyethylene glycols with mean relative molecular weight between 200 and 400.[11] It is used as self-emulsifying and solubilizing agents in oral and topical pharmaceutical formulations[11] in addition to increasing the intestinal absorption of hydrophilic macromolecular drug.[12] It is also known that Labrasol may inhibit the function of P-gp in rat ileum and colon, thereby increasing intestinal absorption and bioavailability of P-gp substrates.[13] In addition, results of our preliminary study indicated that Labrasol might enhance the permeation of the drug across Caco2 cell (unpublished data).

Therefore, our study put emphasis on the enhancement of oral absorption of bortezomib by formulation of SMEDDS of the drug. Labrasol was chosen as the vehicle for the SMEDDS considering its promising effect on P-gp inhibition. The objectives of this study were to develop SMEDDS formulation containing bortezomib and to evaluate its physicochemical characterizations including solubility, droplet size, and release

[#] These authors contributed equally to this work.

* To whom correspondence should be addressed. e-mail: syjeong@khu.ac.kr; espark@skku.edu

behaviors. Pharmacokinetic study was also performed in rats.

Experimental

Bortezomib was donated by Merck Millipore (Darmstadt, Germany). Propylene Glycol Caprylate (Capryol 90), linoleoyl polyoxyl-6 glycerides (Labrafil M2125CS), oleoyl polyoxyl-6 glycerides (Labrafil M1944CS), propylene glycol dicaprylate/dicaprate (Labrafac PG), medium-chain triglycerides (Labrafac WL1349), caprylocaproyl polyoxyl-8 glycerides (Labrasol), and highly purified diethylene glycol monoethyl ether (Transcutol HP) were donated by Gattefossé (Nanterre, France). Polysorbate 20 (Tween 20), polysorbate 80 (Tween 80), and castor oil were purchased from Samchun pure chemical (Seoul, Korea). Polyoxyl 40 hydrogenated castor oil (Cremophor RH 40) and polyoxyl 35 castor oil (Cremophor EL) were obtained from BASF chemical Co. (Limburgerhof, Germany). Antipyrine, an internal standard for analysis, was purchased from Sigma-Aldrich (St. Louis, U.S.A.). All other ingredients, reagents and solvents were of analytical grade.

Solubility Studies The solubility of bortezomib in various vehicles was determined. Fifty milligrams of the drug were added into conical tubes which contained 1 mL of each vehicle. Samples were placed in a shaking incubator (100 rpm) for 24 h at 25°C prior to centrifugation at 3000 rpm for 10 min to remove undissolved drug. The supernatant was filtered through a syringe membrane filter (0.45 μm, Whatman, GE Healthcare Life Science, U.S.A.) and was diluted with mobile phase. Concentration of the drug was analyzed by a validated HPLC-UV method. Agilent 1100 HPLC system equipped with Gemini-NX C18 column (250×4.60 mm, 5 μm) was used. The mobile phase contained HPLC grade water (pH was adjusted to 3.0 with formic acid) and acetonitrile in the volume ratio of 4 : 6. The analytical conditions were as follows; injection volume—100 μL, flow rate of the mobile phase—1.0 mL/min, column oven temperature—25°C and UV wavelength for detection—270 nm.

Construction of Pseudo-ternary Phase Diagrams Pseudo-ternary phase diagrams were constructed using water titration method. Briefly, mixtures of surfactants and cosurfactants (S/CoS mixture) were prepared in the volume ratio of 1 : 3 to 5 : 1 and then S/CoS mixture was added to oil to form blank SMEDDS. The weight ratio of S/CoS mixture and oil ranged from 1 : 9 to 9 : 1. Distilled water was added drop-wise to blank SMEDDS containing oil, surfactant and cosurfactant. After vortexing, the mixtures were visually examined for transparency. The points at which the mixtures became transparent or turbid were designated as microemulsion or emulsion region, respectively. The area of microemulsion region was calculated using Image-pro Express software (ver. 4.5, Mediacybernetics, Silver Spring, U.S.A.).

Preparation of SMEDDS Containing Bortezomib SMEDDS was prepared by mixing specific amounts of bortezomib, oil, surfactant and cosurfactant. For Capryol 90-Labrasol-Transcutol HP mixture (CLT-Blank-SMEDDS), Capryol 90, Labrasol and Transcutol HP were considered as oil, surfactant and cosurfactant, respectively. For Labrafil M1944CS-Cremophor EL-Labrasol mixture (L'CL-Blank-SMEDDS), Labrafil M1944CS, Cremophor EL, Labrasol mixture was considered as oil, surfactant and cosurfactant, respectively. The excipients with larger non-polar parts was designated as surfactants. Surfactant and cosurfactant were mixed with magnetic stirrer. Oil was then added and mixed. Total amount of blank SMEDDS was maintained at 3 g. L'CL-Blank-SMEDDS with the weight ratio of Labrafil M1944CS : Cremophor EL : Labrasol=2 : 6 : 2 was considered an optimized composition and was employed for further investigations. Finally, Bortezomib SMEDDS was prepared by adding 40 mg of the drug into 3 g of optimized L'CL-Blank-SMEDDS and by mixing until transparent mixture was obtained.

Dispersibility Studies Bortezomib SMEDDS was prepared by adding 4 mg of the drug into 300 mg of blank SMEDDS. Blank SMEDDS or bortezomib SMEDDS were diluted 10, 20, 50 and 100 times with distilled water, simulated gastric fluid without enzyme (pH 1.2 medium), simulated intestinal fluid without enzyme (pH 6.8 medium), fasted simulated intestinal fluid (fasted SIF) or fed simulated intestinal fluid (fed SIF). The droplet size of diluted SMEDDS was analyzed using Zetasizer 3000HS (Malvern Instruments Inc., U.K.) with a scattering angle of 90° at 25°C.

Drug Release Behavior The encapsulation of the drug in SMEDDS was evaluated using dialysis bag (MWCO 100000, Cole-Parmer International, Vernon Hills, U.S.A.) and it was confirmed that more than 90% of the drug was in the droplets of SMEDDS by HPLC-UV method. Bortezomib SMEDDS which contained 4 mg of the drug in 300 mg of blank SMEDDS was manually instilled into the hard gelatin capsules for *in vitro* release study. Release behavior of the drug from SMEDDS was determined in U.S. Pharmacopeia (USP) dissolution apparatus II with a rotating speed of 100 rpm in 500 mL of simulated gastric fluid (pH 1.2, 37°C). Release behavior of raw drug powder in hard gelatin capsule was also evaluated. Five milliliters of samples were withdrawn at specific time intervals (5, 10, 15, 30, 45, 60, 90, 120 min) and were filtered through a syringe membrane filter (0.45 μm, Whatman, GE Healthcare Life Science, U.S.A.). The drug concentration in the filtrate was properly diluted to be analyzed using HPLC method described in Solubility Studies section.

Viscosity, Size, Ion Conductivity and Morphology of Bortezomib Microemulsion Bortezomib SMEDDS was diluted with distilled water to form bortezomib microemulsion at ten different ratios from 9 : 1 to 1 : 9. The viscosity of the bortezomib microemulsion was evaluated using Brookfield DVII viscometer (Model DVII+, Middleboro, U.S.A.). The measurements were conducted with 1.5, 3 and 30 rpm of spindle rotating speed at 25°C and were carried out in triplicate. The droplet sizes of bortezomib microemulsion were measured using Zetasizer 3000 HS.

Ion conductivity of bortezomib microemulsion was measured using ion conductivity meter (HI 8633, Hanna Instruments, Woonsocket, U.S.A.). Both distilled water and isotonic 0.9 wt% NaCl solution were used for dilution to measure ion conductivity.

The morphology of bortezomib microemulsion was observed using transmission electron microscope (JEM-2100F, JEOL, Tokyo, Japan). After dilution with water (weight ratio of SMEDDS to water=1 : 50), a sample drop was placed on a copper grid. Samples were subsequently negative stained with 1% phosphotungistic acid solution for 30 s and dried at room temperature.

Stability Studies Bortezomib SMEDDS filled in hard gelatin capsules was placed at room temperature for 24 h. The

stability of the formulation was assessed by analyzing concentration of bortezomib with the HPLC method.

Ex vivo stability of the formulation was also evaluated. Sprague-Dawley rats were fasted for 12 h prior to sacrifice. The tissues of stomach and intestine were excised from rats and homogenized with 10 mM phosphate buffer (pH 7.4) at 3000 rpm for 3 min. Then tissue juices were centrifuged at 10000 rpm for 10 min and the supernatants were used. *Ex vivo* tissue juice (1 mL) was spiked into bortezomib microemulsion which consisted of 4 mg of drug, 200 mg of L'CL-blank-SMEDDS and 3800 mg of water. The concentration of drug was analyzed by HPLC at 10, 20, 30 and 60 min.

Pharmacokinetic Studies Animal studies were performed in compliance with the regulations of the Institutional Laboratory Animal Resources of Sungkyunkwan University. Male Sprague-Dawley rats (8–10 week, body weight 240–290 g) (Hyochang Science, Daegu, Korea) were kept in plastic cages with free access to water and standard rat diet. Rats were housed at 23±2°C/50% relative humidity (RH) with a 12-h light/dark and acclimatized for at least 1 week prior to the experiment.

Rats were anesthetized by intraperitoneal injection (50 mg/kg) of Zoletil 50 (Virbac Laboratories, Carros, France) and cannulated in the jugular vein with polyethylene (PE) tubing (0.58 mm i.d. and 0.96 mm o.d., Natume Co., Tokyo). Recovery period more than 48 h was given to the rats. Rats were divided into three groups for three different dosage forms; group 1 for bortezomib in distilled water, group 2 for bortemzomib microemulsion and group 3 for bortezomib in Labrasol solution.

Bortezomib in distilled water was prepared by adding 8 mg of the drug into 100 mL of distilled water. For the bortemzomib microemulsion, blank SMEDDS containing Cremophor EL, Labrasol and Labrafil was prepared in the weight ratio of 3 : 1 : 1. After addition of 20 mg of drug to 8 g of blank SMEDDS, bortemzomib SMEDDS was diluted with distilled water to correspond to 0.1 mg/mL of drug concentration. Bortezomib in Labrasol solution was prepared by adding 5 mg of the drug into 0.025% Labrasol aqueous solution. Each dosage form was orally administered to the rats using appropriate feeding needles and dose of the drug was 0.2 mg/kg. Because of the difference in formulations, administered volume of dosage forms varied in the range of 2 to 2.86 mL/kg. Blood samples (0.3 mL) were collected from the jugular vein at predetermined time points (0, 5, 15, 30 min, 1, 2, 4, 8, 12 and 24 h after administration) and equal volumes of heparinized saline (50 IU/mL) were replaced. Plasma samples were harvested by centrifugation at 15000 rpm for 10 min and were acidified with 50% formic acid for stabilization. Samples were stored at −20°C until analysis and were analyzed within a week.

Drug concentrations in the plasma samples were determined using an LC-MS/MS system. Agilent 6430 coupled with Agilent 1200 HPLC system (Agilent Technologies, Santa Clara, CA, U.S.A.) and Luna C8 column (150×2.00 mm, Phenomenex, Torrence, CA, U.S.A.) were utilized in the present study. A mixture of aqueous formic acid and acetonitrile (in the volume ratio of 55 : 45) was used as the mobile phase. Analytical conditions are as follows; injection volume—10 μL, flow rate of the mobile phase—0.3 mL/min and column oven temperature—30°C. The multiple reaction monitoring was on the basis of transition of m/z, 367.2→226.1 for the drug and 189.1→76.8 for antipyrine (internal standard). The analytical method was validated in the range of 0.5–500 ng/mL (with r^2=0.9997 for the linearity) and the lower limit of quantification (LOQ) was 0.5 ng/mL. Pharmacokinetic parameters were calculated using non-compartmental analysis in WinNonlin software (Pharsight, Cary, NC, U.S.A.).

Statistical Analysis All statistical analyses were conducted using one-way ANOVA with Minitab™ software (release 13.32, Minitab Inc., State College, PA, U.S.A.). p Values smaller than 0.05 were considered statistically significant.

Results and Discussion

Solubility Studies The solubility of bortezomib in various pharmaceutical vehicles are shown in Table 1. Among the eight types of oil tested, Capryol 90 showed the highest solubility (43.70±0.44 mg/mL). Labrafill M2125CS (37.09±2.72 mg/mL) and Labrafil M1944CS (34.67±0.72 mg/mL) exhibited similar solubility. These three excipients were considered as candidates for the oil phase of SMEDDS for further studies. Among the surfactants used in solubility studies, Labrasol (46.46±0.88 mg/mL) and Transcutol HP (46.44±1.86 mg/mL) showed highest solubility followed by Cremophor EL (16.37±0.36 mg/mL). Therefore, these three surfactants were selected for further studies.

Table 1. Solubility of Bortezomib in Various Pharmaceutical Vehicles at 37.5°C (n=3, Mean±S.D.)

Catergory	Name (Brand name)	Solubility (mg/mL)
Oil	Propylene Glycol Monocaprylate (Capryol 90)	43.70±0.44
Oil	Linoleoyl Polyoxyl-6 Glycerides (Labrafil M2125CS)	37.09±2.72
Oil	Oleoyl Polyoxyl-6 Glycerides (Labrafil M1944CS)	34.67±0.72
Oil	Propylene Glycol Dicaprylate (Labrafac PG)	6.28±0.07
Oil	Caprylic/Capric Triglyceride (Miglyol 812)	1.99±0.02
Oil	Medium-chain Triglycerides (Labrafac WL1349)	1.45±0.05
Oil	Cotton Seed Oil	0.22±0.04
Oil	Castor Oil	0.01±0.06
Surfactant	Caprylocaproyl Polyoxyl-8 Glycerides (Labrasol)	46.46±0.88
Surfactant	Diethylene Glycol Monoethyl Ether (Transcutol HP)	46.44±1.86
Surfactant	Polyoxyl 35 Castor Oil (Cremophor EL)	16.37±0.36
Surfactant	Polyethylene Glycol 400 (Lipoxol 400 MED)	3.42±1.06
Surfactant	POE(5) Sorbitan Monooleate (Tween 20)	0.75±0.08
Surfactant	POE(20) Sorbitan Monooleate (Tween 80)	0.74±0.14
Surfactant	Polyoxyl 40 Hydrogenated Castor Oil (Cremophor RH40)	0.01±0.08

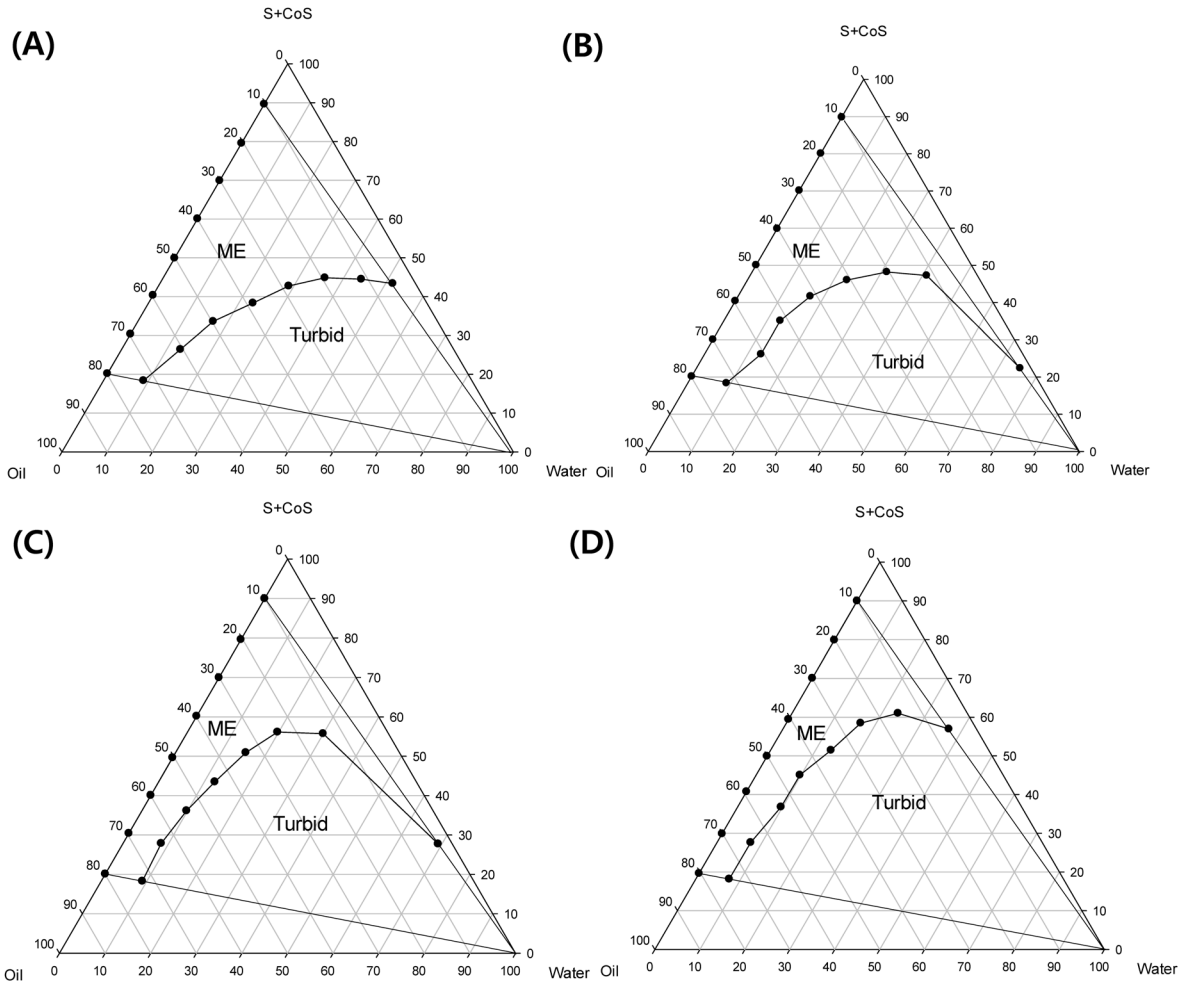

Fig. 1. Pseudo-ternary Phase Diagrams of Microemulsions Composed of (A) Capryol 90, Labrasol, Transcutol HP and Water at S/CoS Ratio of 1 : 3; (B) at S/CoS Ratio of 1 : 1; (C) at S/CoS Ratio of 3 : 1; and (D) at S/CoS Ratio of 5 : 1

ME: microemulsion area, the marker (●) on the border line indicates the compositions which were experimentally prepared.

Identifying solubility of the drug in various pharmaceutical vehicles should be carried out to optimize formulation. In the case of the drug with low aqueous solubility such as bortezomib, the importance of its solubility is even more emphasized. However, there were only a few studies which dealt with solubility of bortezomib. Six vehicles were chosen on the basis of results of solubility studies. It was expected that high solubility of the drug in these vehicles would contribute not only to solubilization but also to physical stability of formulations.[14] Selected excipients have been commonly used for preparation of microemulsion and there has been many studies which employed Capryol 90, Labrafil M2125CS or Labrafil M1944CS as oil phase.[15–17] In addition, the efficiency of self-microemulsification is much more related to the hydrophilic–lipophilic balance (HLB) value of the surfactants. Surfactants with HLB value greater than 10 are greatly superior at providing fine, uniform microemulsion droplets.[18] Labrasol (HLB: 14) and Cremophor EL (HLB: 12) had potential of self-microemulsification due to their high HLB values.

Construction of Pseudo-ternary Phase Diagrams Among various combinations of six selected vehicles, Capryol 90-Labrasol-Transcutol HP mixture (CLT-Blank-SMEDDS) and Labrafil M1944CS-Cremophor EL-Labrasol mixture (L'CL-Blank-SMEDDS) showed large microemulsion area. Pseudo-ternary phase diagrams of CLT and L'CL were shown in Figs. 1 and 2. Area of microemulsion region was summarized in Table 2.

In the case of CLT-Blank-SMEDDS, Capryol 90, Labrasol and Transcutol HP were used as oil, surfactant, and cosurfactant, respectively. The investigated weight ratio of surfactant and cosurfactant in S/CoS mixture were 1 : 3, 1 : 1, 3 : 1 and 5 : 1. Phase behavior showed that the area of microemulsion increased as the ratio of cosurfactant increased in S/CoS mixture (Table 2) which corresponds to a reported literature.[19] Considering that larger area of microemulsion region in the diagram indicates formation of stable microemulsion, the surfactant to cosurfactant weight ratio of 1 : 3 was selected for further studies. The percentage area of microemulsion region of CLT was 31.95% when the ratio was 1 : 3.

For L'CL-Blank-SMEDDS, Labrafil M1944CS, Cremophor EL and Labrasol were used as oil, surfactant and cosurfactant, respectively. When the weight ratio of surfactant to cosurfactant increased from 1 : 3 to 3 : 1, it produced a result opposite to what was observed in CLT. The area of microemlulsion region increased with decreasing surfactant proportion in S/CoS mixture (Table 2). In both cases of CLT and L'CL, larger amount of Labrasol in S/CoS mixture led to larger area of microemulsion region regardless of its role in SMEDDS (sur-

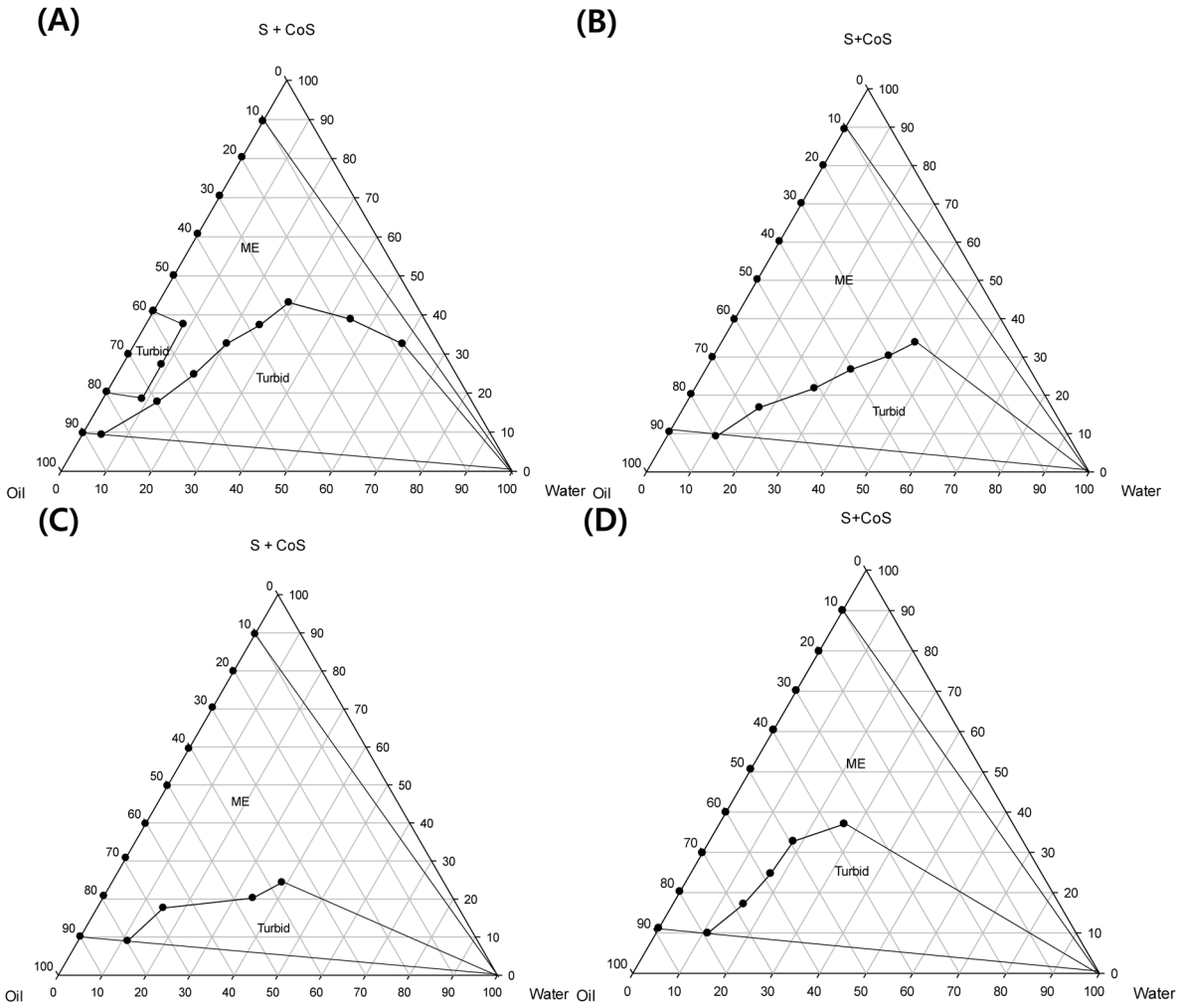

Fig. 2. Pseudo-ternary Phase Diagrams of Microemulsions Composed of (A) Labrafil M1944CS, Cremophor EL, Labrasol and Water at S/CoS Ratio of 1 : 3; (B) at S/CoS Ratio of 1 : 1; (C) at S/CoS Ratio of 3 : 1; and (D) at S/CoS Ratio of 5 : 1

ME: microemulsion area, the marker (●) on the border line indicates the compositions which were experimentally prepared.

Table 2. Percentage Area of Microemulsion Region in Phase Diagram

	Composition	
Surfactant : Cosurfactant	CLT (Capryol 90/Labrasol/Transcutol HP)	L'CL (Labrafil M1944CS/Cremophor EL/Labrasol)
1 : 3	31.95%	36.28%
1 : 1	30.50%	54.46%
3 : 1	25.20%	62.90%
5 : 1	23.79%	52.98%

factant or cosurfactant). The percentage area of microemulsion region was the largest when the weight ratio of surfactant to cosurfactant was 3 : 1 (62.90% of total area of phase diagram). L'CL-Blank-SMEDDS with the weight ratio of surfactant to cosurfactant=3 : 1 was selected for further studies on the basis of the area of microemulsion region. The weight ratio of Labrafil M1944CS : Cremophor EL : Labrasol in chosen L'CL-Blank-SMEDDS was 2 : 6 : 2.

One of the advantages of the microemulsion over coarse emulsion is its thermodynamic stability.[20] It is clear that the microemulsion formation depends on the amount of surfactant and cosurfactant in the system which led to low interfacial tension between oil phase and water phase. Nevertheless, microemulsion could be formed with relatively low amount of surfactant and cosurfactant when optimized combination was established.

Dispersibility Studies Dispersibility of L'CL-Blank-SMEDDS was investigated by dilution with various media. The investigated weight ratios of blank SMEDDS to dilution media were 1/10, 1/20, 1/50 and 1/100. As shown in Fig. 3, the median droplet size was maintained between 20 nm to 30 nm regardless of dilution media type, which indicated that blank SMEDDS spontaneously formed microemulsion with various media. When the drug was incorporated into the L'CL-Blank-SMEDDS, microemulsion showed similar median droplet size to blank SMEDDS as displayed in Fig. 3(B). The transmission

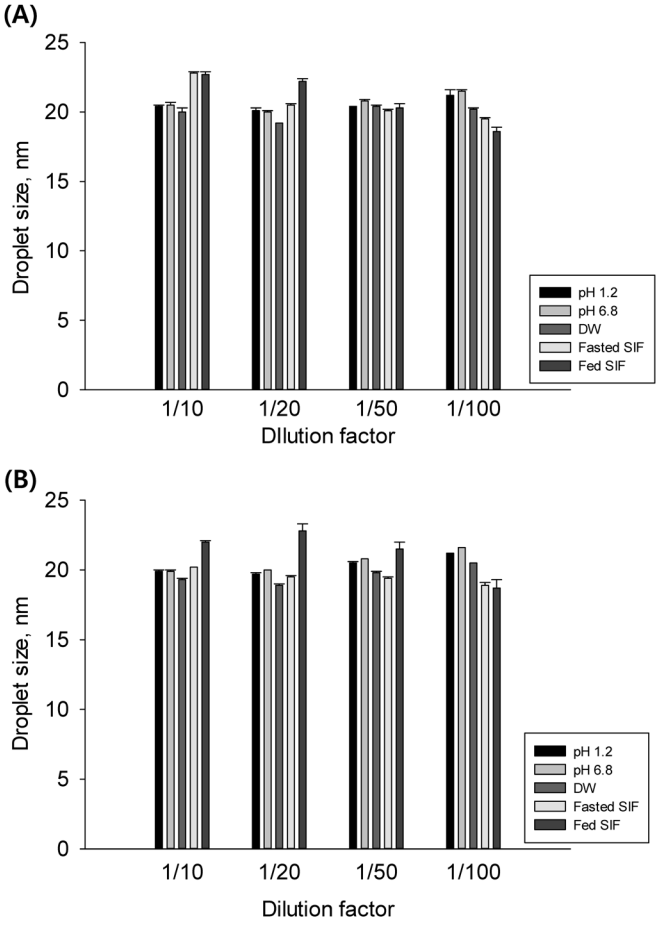

Fig. 3. Effect of Dilution on Droplet Size of L'CL Microemulsion (A) without Bortezomib; (B) with Bortezomib (n=3, Mean±S.D.)

The weight ratio of Labrafil M1944CS : Cremophor EL : Labrasol in L'CL Microemulsion=2 : 6 : 2.

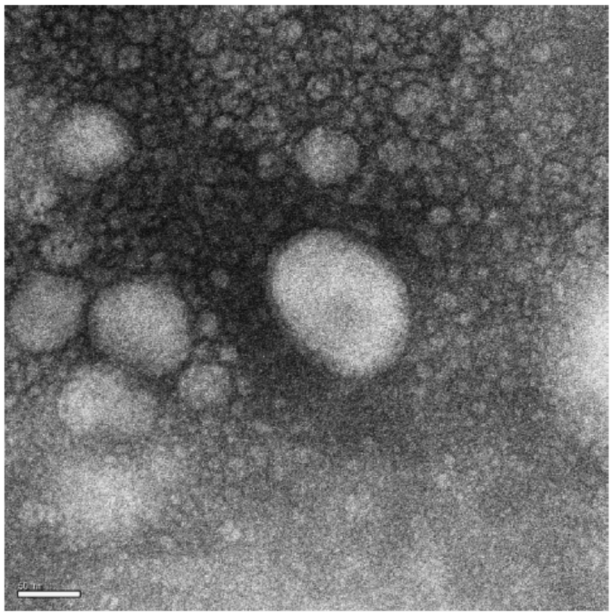

Fig. 4. Representative TEM Image of Bortezomib Microemulsion

Weight ratio of SMEDDS to water=1 : 50, Scale bar: 50 nm.

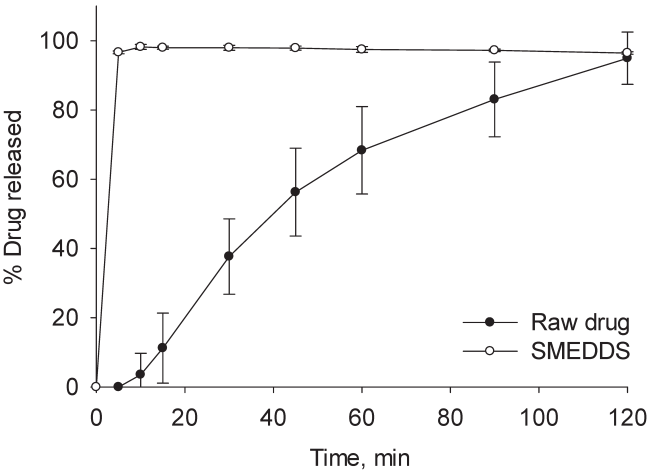

Fig. 5. *In vitro* Release of Bortezomib SMEDDS in pH 1.2 Medium (n=3, Mean±S.D.)

electron microscope (TEM) image of bortezomib microemulsion with 1 : 50 weight ratio of SMEDDS to water was shown in Fig. 4.

The formation of microemulsion with nano-sized droplets was due to the optimized combination of the vehicles selected on the basis of phase diagrams. The droplet size is an important parameter of the colloid system and microemulsion with bigger droplet size causes agglomeration of globules that may result in unstable system.[21,22] In addition, the droplet size of microemulsion was a crucial factor in self-emulsification performance because it is concerned with the rate and extent of drug release, as well as absorption.[23] It was confirmed that L'CL-Blank-SMEDDS and drug loaded L'CL-SMEDDS (bortezomib SMEDDS) could form stable and nano-sized microemulsion which was not affected by pH and compositions of the media.

Drug Release Behavior As shown in Fig. 5, approximately 100% of bortezomib was released from SMEDDS within 5 min. The drug release maintained a plateau level for 120 min without any sign of precipitation. Results indicated that release of bortezomib from SMEDDS was significantly faster than that of raw drug powder in pH 1.2 medium. In addition, the solubility of bortezomib SMEDDS in distilled water was 12.46 mg/mL at 2 h and 15.80 mg/mL at 24 h (data not shown), which were significantly higher than the reported aqueous solubility of the drug (0.5–1 mg/mL).[3] In the case of the raw drug powder, it took 120 min to reach 100% release of the drug and large variations among the samples at each time point were detected. Not only the aqueous solubility but also the release rate may influence the absorption and bioavailability of the drug.[24] When in contact with the dissolution media, bortezomib SMEDDS resulted in spontaneous formation of a microemulsion with nano-sized droplets which led to faster release rate. It was supposed that solubilization effect of surfactants of SMEDDS also contributed to the results.[25]

Viscosity, Size, Ion Conductivity and Morphology of Bortezomib Microemulsion Bortezomib SMEDDS was diluted with distilled water to investigate its physicochemical properties and behaviors in gastrointestinal environment as microemulsion.

The viscosity of bortezomib microemulsion was measured at 1.5, 3.0 and 30 rpm and the results were displayed in Fig. 6. Viscosity of bortezomib SMEDDS decreased with increasing rotation speed with all water proportion. Newtonian fluid had

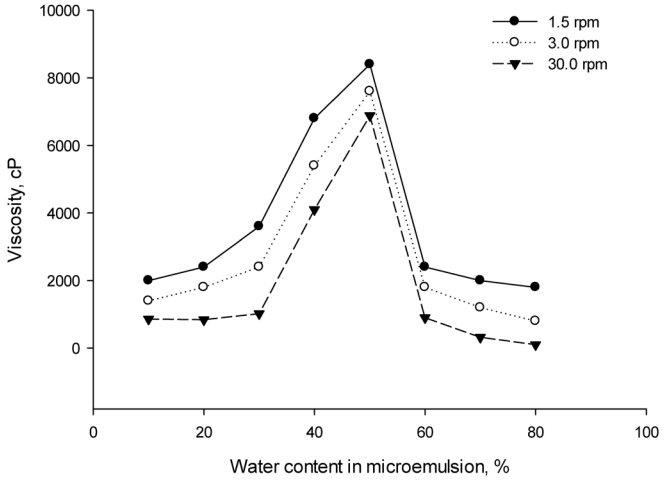

Fig. 6. Effect of Water Content on Viscosity of Bortezomib Microemulsion (n=2)

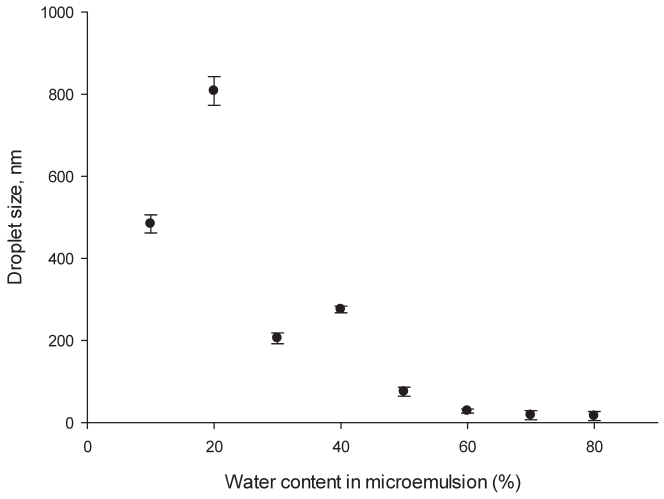

Fig. 7. Effect of Water Content on Median Droplet Size of Bortezomib Microemulsion (n=3, Mean±S.D.)

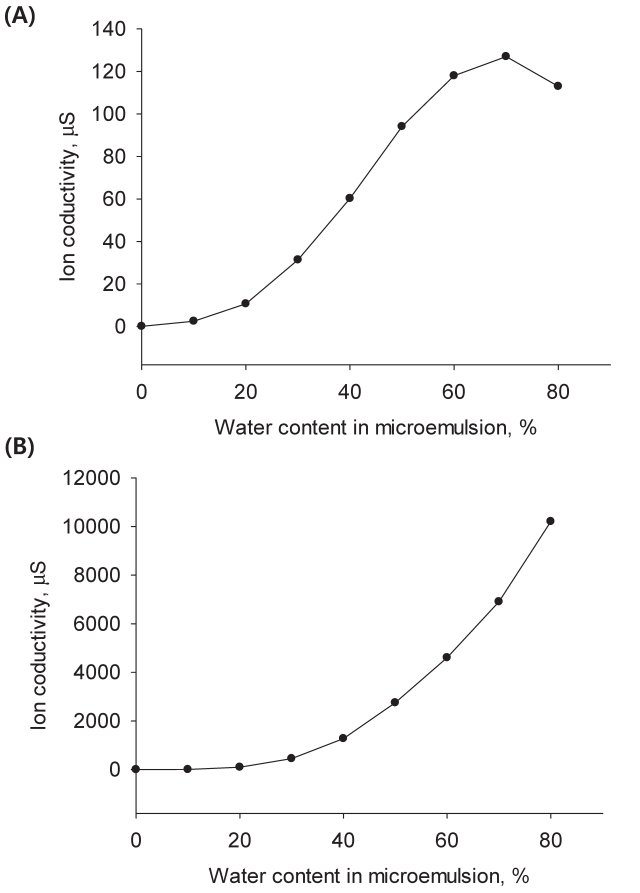

Fig. 8. Effect of Water Content on Ion Conductivity of Bortezomib Microemulsion

(A) without 0.9 wt% NaCl; (B) with 0.9 wt% NaCl (n=2).

the constant viscosity regardless of shear rate (*e.g.*, water) and it is a decisive feature of microemulsion due to its equilibrium structure.[26,27] However, change in viscosity of bortezomib SMEDDS was observed with different rotation speed, which indicated non-newtonian flow. It was relatively high viscosity of the bortezomib SMEDDS that resulted in this rheological behavior.

A steep increase in viscosity was observed with water proportion of 30–50%. When the weight ratio of water is lower than 30% or higher than 60%, the viscosity was lower and not susceptible to change in the ratio. The isolated droplets of water in oil phase (or the reverse) spontaneously led to a low viscosity.[28] The increase in viscosity is presumably associated with compositional and structural effects derived from the interfacial packing.[29] Therefore, it was supposed that diluted bortezomib SMEDDS was in a bicontinuous state which is the state of phase inversion from W/O emulsion to O/W emulsion.[30]

Viscosity values decreased back to original levels at point where the water proportion exceeded 50%. When water content in microemulsion was 50%, the maximum viscosity value was reached due to percolation transition, which represents the transition from bicontinuous state to O/W microemulsions.[30]

As shown in Fig. 7, the droplet size of microemulsion showed decreasing trend with the increasing water proportion, which corresponds to reported results.[31] When small amount of water was added, W/O emulsion with the larger droplet size was formed. It was thought that coalescence of water droplets occurred with increase in water content from 10 to 20%, which led to increase in droplet size. Droplet size decreased to 200–300 nm as the ratio of water in the microemulsion increased, which suggested formation of bicontinuous state. When water content was higher than 60%, droplet size was maintained at 20 nm and it was thought that stable O/W microemulsion was formed.[32]

The ion conductivity of bortezomib microemulsion was shown in Fig. 8. Because of the electrolytes, diluting with isotonic 0.9% NaCl solution resulted in higher ion conductivity than with distilled water. Regardless of the composition and type of diluents, the ion conductivity increased steeply when the water content in microemulsion was higher than 30%.

The increase of conductivity can be explained by the existence of network of conductive channels. This can explain why W/O microemulsion shows limited conductivity compared to O/W emulsion.[33] As water amount increases, the formation of water channels is triggered in an oil phase due to the attractive interactions between microdroplets of water phase in the W/O microemulsion.[33]

When the weight ratio of water to SMEDDS was higher

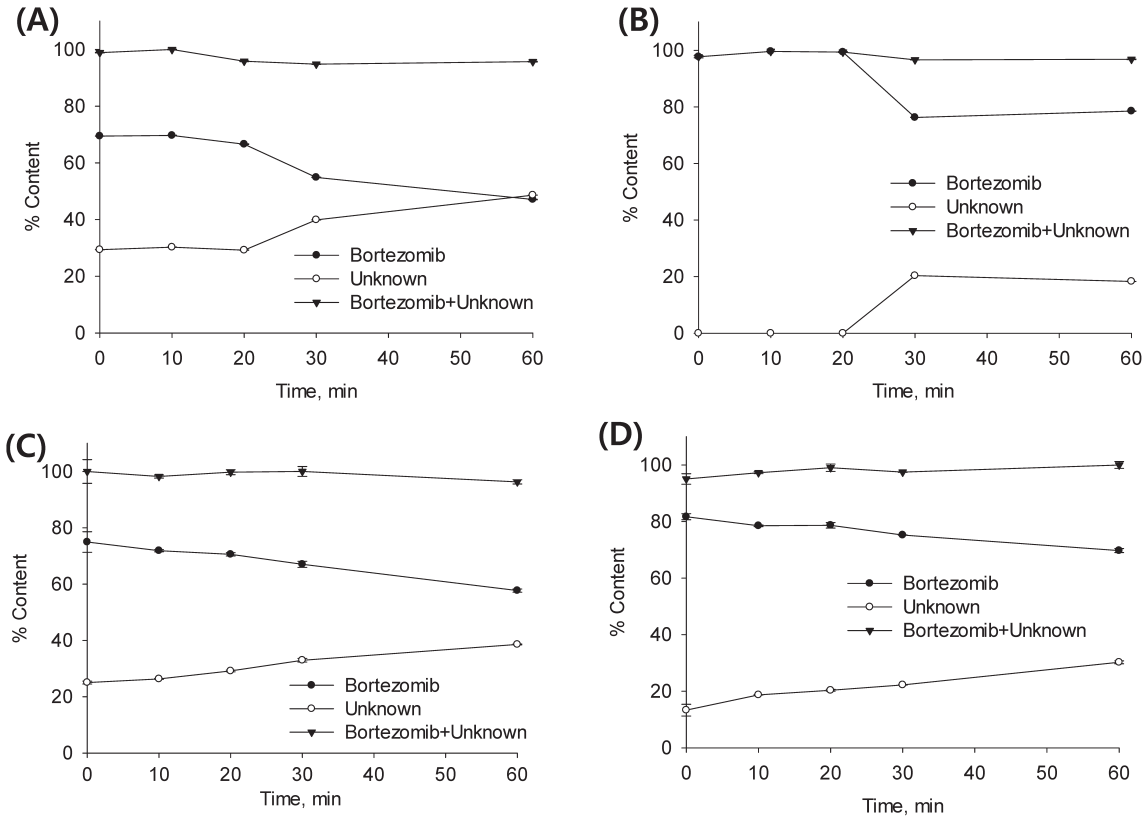

Fig. 9. Stability of (A) Bortezomib Microemulsion Exposed to Gastric Tissue Juice; (B) Bortezomib Raw Material Exposed to Gastric Tissue Juice; (C) Bortezomib Microemulsion Exposed to Intestinal Tissue Juice; and (D) Bortezomib Raw Material (Bortezomib in Water) Exposed to Intestinal Tissue Juice (n=3, Mean±S.D.)

'Unknown' indicates the unknown peak detected in HPLC chromatograms.

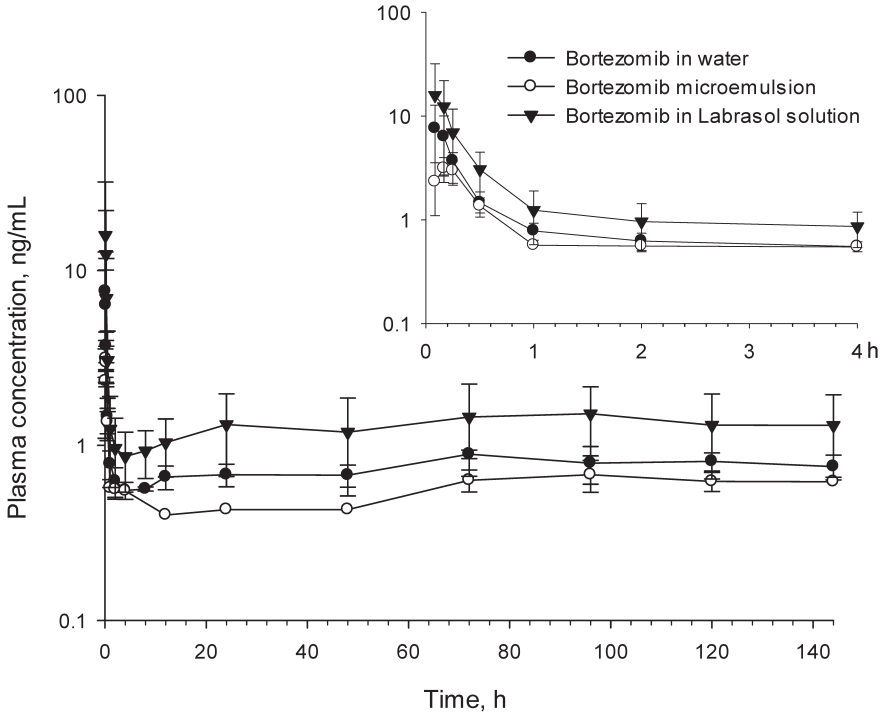

Fig. 10. Bortezomib Plasma Concentration *versus* Time Curves after Oral Administration (n=5, Mean±S.D.)

than 0.3, the ion conductivity increased steeply, which indicated phase inversion of microemulsion. It was inferred that the conductivity and viscosity data have confirmed the continuous structural transitions during the increase of water phase fraction. The results of viscosity, droplet size and ion conductivity of SMEDDS indicate that a bi-continuous state was formed

Table 3. Pharmacokinetic Parameters of Bortezomib after Oral Administration

Parameter	Bortezomib in distilled water	Bortezomib microemulsion	Bortezomib in Labrasol® solution
$t_{1/2}$ (h)	91.72±22.09	277.74±249.65	168.97±52.39
t_{max} (h)	0.12±0.08	0.18±0.07[c)]	0.10±0.04[c)]
C_{max} (ng/mL)	7.92±4.8	3.44±0.81[c)]	15.59±13.67[c)]
AUC_{0-24h} (ng·h/mL)	15.97±2.19[a,b)]	8.62±4.41[a,c)]	27.58±6.68[b,c)]
V_z/F (L/kg)	134.6±11.92[a)]	246.44±100.99[a,c)]	107.34±33.96[c)]
Cl/F (mL/min/kg)	17.79±5.19	16.85±10	8.05±3.26

a) Bortezomib in distilled water *vs.* Bortezomib microemulsion ($p<0.05$). *b*) Bortezomib in distilled water *vs.* Bortezomib in Labrasol® solution ($p<0.05$). *c*) Bortezomib microemulsion *vs.* Bortezomib in Labrasol® solution ($p<0.05$).

when the weight ratio of water to SMEDDS ranged from 0.3 to 0.6.

Stability Studies The drug in SMEDDS was stable after storage at 25°C for 24 h (99.22±5.64%). It was reported that bortezomib was stable for up to 15 d when stored at 4°C in the original packaging.[34)] However, the content of the drug was gradually decreased with time when the raw drug was in contact with gastrointestinal tissue juice as shown in Fig. 9. Considering mass balance of the sample, the unknown peak detected in HPLC chromatograms was inferred to main degradant of the drug.

The degradation was more pronounced in intestinal tissue juice than in gastric tissue juice. Previous study reported that bortezomib showed significant degradation under stress conditions of heat, acid and oxidation but not under basic environment.[35)] Because no sign of degradation in dissolution medium (pH 1.2 medium) and in vehicles used for solubility studies was detected, it was thought the main reason of degradation was related to compositions of tissue juice including enzymes, not compositions of SMEDDS.

In addition, raw drug was relatively stable compared to bortezomib microemulsion in gastric tissue juice. In intestinal tissue juice, the stability of raw drug was similar to that of bortezomib SMEDDS. Considering that bortezomib SMEDDS was stable for 24 h as previously mentioned, it was suspected that generation of interfacial between oil and water phase of microemulsion affected drug stability.

Pharmacokinetic Studies Bortezomib plasma concentration *versus* time curves after oral administration of bortezomib in various dosage forms were displayed in Fig. 10 and the pharmacokinetic parameters acquired by non-compartmental analysis were summarized in Table 3.

Considering time to reach C_{max} (T_{max}) (0.10–0.18 h) values, the drug appears to be absorbed quickly with all dosage forms after oral administration. Plasma concentration–time curves have two phases, distribution phase and elimination phase and the drug was eliminated bi-exponentially. It took less than 30 min for drug distribution and then plasma concentration maintained the plateau until 144 h, the last sampling point. The elimination half-lives were markedly long ranging from 91.75 to 277.74 h. As there was no significant difference among elimination half-lives of treatments, a broad range of half-lives was a result of modeling with extremely long half-lives. There have been researches which reported similar plasma concentration *versus* time curve patterns; short distribution phase and extremely long elimination phase.[36,37)] Therefore, it is logical to presume that the results from the present study are reliable.

Among the three different dosage forms, it was bortezomib in Labrasol solution which showed the highest area under the curve from 0 to 24 h (AUC_{0-24h}) and maximum plasma concentration (C_{max}) values. The C_{max} of bortezomib in Labrasol solution was 15.59±13.67 ng/mL, which was 1.9- and 4.5-fold higher than bortezomib in water and bortezomib microemulsion, respectively. The AUC_{0-24h} of bortezomib in Labrasol was 27.58±6.68 ng·h/mL, which was 1.7- or 3.3-fold higher than that of bortezomib in distilled water and bortezomib microemulsion, respectively. Therefore, Labrasol has some effects on improvement in bioavailability of the drug, but microemulsion containing Labrasol caused the reduction in bioavaiability of the drug due to the drug stability problem.

Bortezomib was stable in SMEDDS but when bortezomib microemulsion was formed and exposed to gastric environments, degradation of the drug occurred. Therefore, it is conceivable that actual dose of bortezomib microemulsion exposed to the rats was less than expected. As mentioned in stability section, generation of the interface between oil and water was thought to be the reason of drug degradation. Therefore, drug in Labrasol solution might have better stability than SMEDDS. In addition, drug in Labrasol solution led to improvement in bioavailability, which implies it could be promising oral delivery system for bortezomib.

Conclusion

The present study investigated the preparation of SMEDDS containing bortezomib. It was confirmed that physically stable SMEDDS with nano-sized droplets could be formulated on the basis of optimized combination of oils, surfactants and cosurfactants. It was thought that the fast release of the drug from the SMEDDS was attributed to fast dispersibility and nano-sized droplets of SMEDDS. In the pharmacokinetic study, bortezomib microemulsion failed to improve bioavailability of the drug due to drug stability in the formulation. However, bortezomib in Labrasol solution showed significantly increased AUC_{0-24h} and C_{max} values compared to those of drug suspension. The result implies that colloidal delivery system containing Labrasol could be an effective strategy for oral delivery of bortezomib.

Acknowledgments This study was supported by Grants from the Korean Health Technology R&D Project, Ministry for Health, Welfare & Family Affairs, Republic of Korea (Grant number A092018) and from Handok Inc. (Seoul, Korea).

Conflict of Interest The authors declare no conflict of interest.

References

1) Chen D., Frezza M., Schmitt S., Kanwar J., Dou Q. P., *Curr. Cancer Drug Targets*, **11**, 239–253 (2011).
2) Leveque D., Carvalho M. C. M., Maloisel F., *In Vivo*, **21**, 273–278 (2007).
3) Stella V. J., Nti-Addae K. W., *Adv. Drug Deliv. Rev.*, **59**, 677–694 (2007).
4) Lü S., Chen Z., Yang J., Chen L., Zhou H., Xu X., Li J., Han F., Wang J., *Int. J. Lab. Hematol.*, **32**, e123–e131 (2010).
5) Spernath A., Aserin A., *Adv. Colloid Interface Sci.*, **128–130**, 47–64 (2006).
6) Cho H.-J., Ku W.-S., Termsarasab U., Yoon I., Chung C.-W., Moon H. T., Kim D.-D., *Int. J. Pharm.*, **423**, 153–160 (2012).
7) Yin Y.-M., Cui F.-D., Mu C.-F., Choi M.-K., Kim J. S., Chung S.-J., Shim C.-K., Kim D.-D., *J. Control. Release*, **140**, 86–94 (2009).
8) Elshafeey A. H., Bendas E. R., Mohamed O. H., *AAPS PharmSciTech*, **10**, 361–367 (2009).
9) Bachhav Y. G., Patravale V. B., *Int. J. Pharm.*, **365**, 175–179 (2009).
10) Bachhav Y. G., Patravale V. B., *AAPS PharmSciTech*, **10**, 476–481 (2009).
11) Rowe R. C., Sheskey P. J., Quinn M. E., "Handbook of Pharmaceutical Excipients," 6th ed., Pharmaceutical Press, London, 2009, pp. 557–561.
12) Prasad Y. V. R., Puthli S. P., Eaimtrakarn S., Ishida M., Yoshikawa Y., Shibata N., Takada K., *Int. J. Pharm.*, **250**, 181–190 (2003).
13) Lin Y., Shen Q., Katsumi H., Okada N., Fujita T., Jiang X., Yamamoto A., *Biol. Pharm. Bull.*, **30**, 1301–1307 (2007).
14) Borhade V., Pathak S., Sharma S., Patravale V., *Int. J. Pharm.*, **431**, 138–148 (2012).
15) Kang B. K., Lee J. S., Chon S. K., Jeong S. Y., Yuk S. H., Khang G., Lee H. B., Cho S. H., *Int. J. Pharm.*, **274**, 65–73 (2004).
16) Oh D. H., Kang J. H., Kim D. W., Lee B.-J., Kim J. O., Yong C. S., Choi H.-G., *Int. J. Pharm.*, **420**, 412–418 (2011).
17) Hu L., Wu H., Niu F., Yan C., Yang X., Jia Y., *Int. J. Pharm.*, **420**, 251–255 (2011).
18) Singh A. K., Chaurasiya A., Awasthi A., Mishra G., Asati D., Khar R. K., Mukherjee R., *AAPS PharmSciTech*, **10**, 906–916 (2009).
19) Bali V., Ali M., Ali J., *Colloids Surf. B Biointerfaces*, **76**, 410–420 (2010).
20) He C.-X., He Z.-G., Gao J.-Q., *Expert Opin. Drug Deliv.*, **7**, 445–460 (2010).
21) Dixit A. R., Rajput S. J., Patel S. G., *AAPS PharmSciTech*, **11**, 314–321 (2010).
22) Shen H., Zhong M., *J. Pharm. Pharmacol.*, **58**, 1183–1191 (2006).
23) Constantinides P. P., Scalart J.-P., Lancaster C., Marcello J., Marks G., Ellens H., Smith P. L., *Pharm. Res.*, **11**, 1385–1390 (1994).
24) Cui S.-X., Nie S.-F., Li L., Wang C.-G., Pan W.-S., Sun J.-P., *Drug Dev. Ind. Pharm.*, **35**, 603–611 (2009).
25) Patel A. R., Vavia P. R., *AAPS J.*, **9**, E344–E352 (2007).
26) Fanun M., "Microemulsions: Properties and Applications," Chap. 6, CRC Press, Boca Raton, 2008.
27) Moulik S., Paul B., *Adv. Colloid Interface Sci.*, **78**, 99–195 (1998).
28) De Gennes P., Taupin C., *J. Phys. Chem.*, **86**, 2294–2304 (1982).
29) Fanun M., *J. Mol. Liq.*, **135**, 5–13 (2007).
30) Georges J., Chen J., *Colloid Polym. Sci.*, **264**, 896–902 (1986).
31) Trotta M., Gallarate M., Carlotti M. E., Morel S., *Int. J. Pharm.*, **254**, 235–242 (2003).
32) Morales D., Gutiérrez J. M., Garcia-Celma M., Solans Y., *Langmuir*, **19**, 7196–7200 (2003).
33) Djordjevic L., Primorac M., Stupar M., Krajisnik D., *Int. J. Pharm.*, **271**, 11–19 (2004).
34) Vanderloo J. P., Pomplun M. L., Vermeulen L. C., Kolesar J. M., *J. Oncol. Pharm. Pract.*, **17**, 400–402 (2011).
35) Venkataramanna M., *J. Chromatogr. Sep. Tech.*, **3**, 1–6 (2012).
36) Hemeryck A., Geerts R., Monbaliu J., Hassler S., Verhaeghe T., Diels L., Verluyten W., Van Beijsterveldt L., Mamidi R. N., Janssen C., De Coster R., *Cancer Chemother. Pharmacol.*, **60**, 777–787 (2007).
37) Kupperman E., Lee E. C., Cao Y., Bannerman B., Fitzgerald M., Berger A., Yu J., Yang Y., Hales P., Bruzzese F., Liu J., Blank J., Garcia K., Tsu C., Dick L., Fleming P., Yu L., Manfredi M., Rolfe M., Bolen J., *Cancer Res.*, **70**, 1970–1980 (2010).