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Comparison of a solid SMEDDS and solid dispersion for enhanced stability and bioavailability of clopidogrel napadisilate

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ABSTRACT

The intention of this study was to compare the physicochemical properties, stability and bioavailability of a clopidogrel napadisilate (CN)-loaded solid dispersion (SD) and solid self-microemulsifying drug delivery system (solid SMEDDS). SD was prepared by a surface attached method using different ratios of Cremophor RH60 (surfactant) and HPMC (polymer), optimized based on their drug solubility. Liquid SMEDDS was composed of oil (peceol), a surfactant (Cremophor RH60) and a co-surfactant (Transcutol HP). A pseudo-ternary phase diagram was constructed to identify the emulsifying domain, and the optimized liquid SMEDDS was spray dried with an inert solid carrier (silicon dioxide), producing the solid SMEDDS. The physicochemical properties, solubility, dissolution, stability and pharmacokinetics were assessed and compared to clopidogrel napadisilate (CN) and bisulfate (CB) powders. In solid SMEDDS, liquid SMEDDS was absorbed or coated inside the pores of silicon dioxide. In SD, hydrophilic polymer and surfactants were adhered onto drug surface. The drug was in crystalline and molecularly dispersed form in SD and solid SMEDDS, respectively. Solid SMEDDS and SD greatly increased the solubility of CN but gave lower drug solubility compared to CB powder. These preparations significantly improved the dissolution of CN, but the latter more increased than the former. Stability under accelerated condition showed that they were more stable compared to CB powder, and SD was more stable than solid SMEDDS. They significantly increased the oral bioavailability of CN powder. Furthermore, SD showed significantly improved oral bioavailability compared to solid SMEDDS and CB powder. Thus, SD with excellent stability and bioavailability is recommended as an alternative for the clopidogrel-based oral formulation.
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1. Introduction

Clopidogrel is a thienopyridene class anti-platelet oral drug for the treatment of patients with a high risk of myocardial infarction and stroke (Fox & Chelliah, 2007). It has been approved for several indications including patients with stroke events, peripheral vascular disease, atherosclerotic events, cardiovascular disease and myocardial infarction (Fox & Chelliah, 2007). Clopidogrel, a prodrug, needs to be converted into its active metabolite by cytochrome P450 enzymes to exert its pharmacological effect. The active metabolite binds to the P2Y12 adenosine diphosphate receptor of platelets and results in reduced ADP-mediated activation of the glycoprotein IIb/IIIa complex (Shim et al., 2010). Since clopidogrel is extensively metabolized by hepatic enzymes and hydrolysis following oral administration, it is difficult to determine its plasma concentration.

Clopidogrel bisulfate (CB) is widely used in the pharmaceutical industry due to its crystalline form and good water solubility; its base form is in the oil phase and is difficult to purify and

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formulate (El Ahmady, Ibrahim, Hussein, & Bustami, 2009). However, this bisulfate salt form has been reported to have poor stability and significantly degrades under accelerated moisture and heat conditions (Gomez, Adams, & Hoogmartens, 2004). Hence, the more stable clopidogrel napadisilate (CN) was developed to improve stability; however, CN shows very poor aqueous solubility compared to the CB salt (Kim et al., 2011). Therefore, to increase the solubility and bioavailability of CN, new approaches are needed.

In the last ten years, lipid-microemulsion formulations, particularly self-microemulsifying (SMEDDS) or self-emulsifying drug delivery systems have received much attention to improve the oral bioavailability of poorly water soluble drugs (Seo et al., 2013; Woo, Song, Hong, Lim, & Kim, 2008). SMEDDS is an isotropic mixture of oil and surfactants, which forms an emulsion with mild agitation in the GI fluid. SMEDDS is a desired technology to increase the water solubility of poorly water-soluble drugs as they have large solubilization capacity and small droplet size, which allows enhanced permeation and bioavailability of the formulated drug across the GI membrane. However, there are a few drawbacks with SMEDDS technology, as these preparations are sensitive to humidity and temperature, have a high production cost and are incompatible with encapsulation in soft gelatin. Therefore, solid SMEDDS, which have the advantages of SMEDDS and overcome these disadvantages, have been investigated in the last few years and reported (Balakrishnan et al., 2009a; Breitkreitz, Sabin, Polla, & Poppi, 2013). Several methods have been employed to prepare solid SMEDDS, including the extrusion/spheronization method or wet granulation in a high shear mixer (Franceschinis et al., 2005; Nazzal & Khan, 2006). Spray drying could also be a good method for the industrial application of solid SMEDDS.

Solid dispersion (SD) is one of the most promising techniques to increase the solubility and dissolution of poorly water soluble drugs (Joe et al., 2010; Yan et al., 2012). Incorporation of a poorly water-soluble drug into hydrophilic polymers has been reported to increase the solubility and dissolution of the drug. The SD technique provides a way to reduce the particle size of a poor water soluble drug to nearly the molecular level. As the hydrophilic polymer dissolves, the poor water soluble drug is presented as very fine particles for quick absorption (Lim et al., 2010). The SD is prepared by either solvent evaporation, where the drug and polymer are dissolved in a common solvent followed by removal of the solvent, or by the melting method, where the drug and polymer mixtures are prepared by melting together, followed by cooling (Shahzad, Sohail, Arshad, Hussain, & Shah, 2013). However, in the solvent evaporation method, the conventional way of preparing SD requires organic solvents, which result in solvent residue toxicity. Moreover, using conventional methods, the drug/polymer ratio will be higher and that will increase the bulk of the required dose of the drug (Lee et al., 2013). The melting method also has disadvantages, such as thermal degradation, sublimation and phase separation (Shahzad et al., 2013). The novel surface attached SD technique seems to be promising approach to prepare SD without the complications found in the conventional SD techniques such as a low drug/polymer ratio; moreover, water is used as the solvent (Li et al., 2010; Park et al., 2009).

The objectives of the present study were to compare the physicochemical properties, stability and bioavailability in rats of a clopidogrel napadisilate (CN)-loaded solid dispersion (SD) and solid self-microemulsifying drug delivery system (solid SMEDDS). The SD and solid SMEDDS were prepared by the spray drying technique and characterized by scanning electron microscopy (SEM), differential scanning calorimetry (DSC) and powder X-ray diffraction (PXRD). Furthermore, their dissolution, stability and pharmacokinetics in rats were compared with those of CN and CB powders.

2. Materials and methods

2.1. Materials

Clopidogrel napadisilate (monohydrate form; CN) and clopidogrel bisulfate (CB) were kindly provided by Hanmi Pharm. Co. (Hwasung, South Korea). Peceol, Labrafil M 2125 CS, Capryol PGMC, Capryol 90, Plurol Oleique CC 497, Transcutol P, Transcutol HP, Lauroglycol 90, Labrasol were obtained from Gattefosse (Saint-Priest Cedex, France). Peanut oil, corn oil, soybean oil, sesame oil, castor oil, cotton seed oil, sorbitan monolaurate 20 (Span 20), sorbitan monooleate 80 (Span 80), polysorbate 20 (Tween 20), polysorbate 80 (Tween 80) and PEG 400 were purchased from Daejung Chem. Co. (Siheung, South Korea). Cremophor ELP, Cremophor EL, Cremophor RH40, Cremophor RH60, Lutrol L 44 and polyvinylpyrrolidone (PVP, K30) were obtained from BASF (Ludwigshafen, Germany). Hydroxypropylmethylcellulose (HPMC, 2208), sodium carboxymethylcellulose (Na-CMC), polyethylene glycol 6000 (PEG 6000), polyvinylalcohol (PVA) and HPC-L (hydroxypropylcellulose-low viscosity) were purchased from Shin-Etsu Co. (Tokyo, Japan). Hydroxypropyl-β-cyclodextrin (HP-β-CD) and β-cyclodextrin (β-CD) were purchased from Roquette (Lestrem, France). Dextran was supplied by Sigma-Aldrich Co. (St. Louis, MO, USA). Silicon dioxide (Aerosil® 200) was obtained from Degussa (Frankfurt, Germany). All other chemicals and solvents were of reagent grade and were used without further purification.

2.2. Animals

Male Sprague-Dawley rats (6–8 weeks old, 280 ± 20 g), which were purchased from Nara Biotech (Seoul, South Korea), had free access to usual standard laboratory diet and tap water. All through the experiment, the animals were encaged under controlled conditions of 23–24 °C/50–60% RH. The protocols for the animal studies were implemented consistent with the NIH Policy and Animal Welfare Act under the approval by the Institutional Animal Care and Use Committee (IACUC) at Hanyang University.

2.3. Solubility studies

Solubility studies were executed by introducing an excess amount of CN (approximately 50 mg) in a 2 ml micro tube (Axygen MCT-200) carrying 1 ml of surfactant or an aqueous solution of the 1% hydrophilic polymer. The mixture was vortexed and kept for 7 days at 25 °C in a shaking water bath to promote solubilization. Then, the samples were centrifuged at 10,000 × g for 10 min (Hanil Science Industrial Co.; Incheon, South Korea) to separate the undissolved CN. The supernatant was diluted with acetonitrile for quantification of CN by an HPLC system (Agilent 1260 Infinity; Agilent Technologies, Santa Clara, CA, USA) consisting of Chem Station software, a G1311C 1260 Quat Pump and a G1314B 1260 VWD VL detector. The column was a Capcell Pak C18 MG column (Shiseido, Japan, 4.6 mm I.D. × 250 mm, 5 μm). The mobile phase consisted of acetonitrile and triple distilled water (85:15, volume ratio). The eluent was monitored at 220 nm with a flow rate of 1.0 ml/min.

In addition, a solubility study was carried out with CN powder, CB powder and the selected formulations such as solid SMEDDS and SD by the solubility method as described above.

2.4. Construction of pseudo-ternary phase diagrams

The microemulsion forming fields produced under dilution and mild agitation of SMEDDS were determined from ternary phase diagrams. On the basis of solubility study, peceol, Cremophor RH60 and Transcutol HP were chosen as the oil, surfactant and co-surfactant,

Table 1
Composition and drug solubility of various solid dispersions.

Formulations	I	II	III	IV	V	VI	VII	VIII	IX	X
Drug (g)	5	5	5	5	5	5	5	5	5	5
HPMC (g)	5	4.5	4	3.5	3	2.5	2	1.25	0.675	0.25
Cremophor RH60 (g)	0	0.5	1	1.5	2	2.5	2	1.25	0.675	0.25
Water (ml)	500	500	500	500	500	500	500	500	500	500
Drug solubility (mg/ml)	1.98 ± 0.18	2.25 ± 0.05	2.16 ± 0.08	2.34 ± 0.13	4.77 ± 0.66	5.63 ± 0.74	6.53 ± 0.60	2.95 ± 0.96	1.64 ± 0.09	1.38 ± 0.02

Each value represents the mean ± S.D. ($n = 3$).

respectively. The formulation (0.2 ml) was poured into 300 ml of water in a glass beaker at 37 °C and gently stirred (300 rpm) with a magnetic bar. The tendency to emulsify spontaneously and also the propagation of the emulsion droplets were observed. The tendency to develop a microemulsion was considered "good" when droplets spread smoothly in water and formed a fine microemulsion, and it was considered "bad" when there was poor or no emulsion development with the prompt coalescence of oil droplets, especially when stirring was terminated (Balakrishnan et al., 2009b; Kim, Kang, Oh, Yong, & Choi, 2012). The phase diagram was constructed identifying the good self-microemulsifying region. All studies were performed thrice with similar observations being made between repeats. The self-emulsifying property was visually assessed after infinite dilution using purified water.

2.5. Preparation of CN-loaded solid SMEDDS formulations

The liquid SEDDS formulation was prepared by dissolving CN in a mixture of the surfactant, oil and co-surfactant at room temperature. The final mixture was vortexed until a clear solution was achieved. This formulation was visually observed for turbidity and phase separation. Particle size and zeta potential analysis were performed.

Moreover, silicon dioxide (0.75 g) was well-suspended in 100 ml of ethanol by magnetic stirring and then 1 g of liquid SMEDDS (equivalent to 100 mg of CN) was added to it. The mixture was stirred with a magnetic stir bar for 15 min to obtain a stabilized suspension of silicon dioxide. The suspension was kept stirring while spray dried using a Büchi mini spray dryer (B-290; Flawil, Switzerland) under the following conditions: inlet temperature, 70 °C; outlet temperature, 40 °C; aspiration, 100%; drying air flow, 500 Nl/h; feeding rate of the suspension, 6 ml/min.

2.6. Preparation of CN-loaded SD

A Büchi mini spray dryer was employed to prepare the CN-loaded surface attached SDs. Various ratios of Cremophor RH60 and HPMC in water were prepared, and 5 g of CN was pre-sieved through 50 mesh screen and then added to each solution (Table 1). The resultant suspension was kept stirring while spray dried under the following conditions: inlet temperature, 150 °C; outlet temperature, 75–85 °C; aspiration, 100%; feeding rate of the suspension, 5 ml/min.

2.7. Physicochemical characterization

2.7.1. Loading efficacy

The SD and solid SMEDDS equivalent to 5 mg CN was completely dissolved in 100 ml acetonitrile and filtered through 0.45 μm membrane filter and assayed for the content of drug by the HPLC method as mentioned above. The loading efficacy (%) was calculated as followings: loading efficacy (%) = $C_a/C_t \times 100$, where C_a and C_t were the actual and theoretical drug content in these formulations, respectively.

2.7.2. Droplet size of the emulsion

The droplet size of the emulsion was measured by a Zetasizer Nano ZS (Malvern Instruments; Malvern, UK) dynamic light scattering particle size analyzer at a wavelength of 635 nm and a scattering angle of 90° at 25 °C. All tests were repeated three times and the readings of the *z*-average diameters were used. The *z*-average diameter, also referred to as the harmonic intensity-weighted average hydrodynamic diameter, of the emulsions was derived from the cumulated analysis by Automeasure software (Malvern Instruments; Malvern, UK).

2.7.3. Morphological analysis

The shape and surface of the solid SMEDDS and SD formulations were inspected using an S-4800 scanning electron microscope (Hitachi; Tokyo, Japan). The samples were secured on a brass stub using double-sided adhesive tape and made electrically conductive by coating with platinum (6 nm/min) in a vacuum (0.8 Pa) using an EmiTeck Sputter Coater (K575K) for 4 min at 15 mA.

2.7.4. Solid state characterization

The physical state of CN in solid SMEDDS and SD formulations was characterized by differential scanning calorimetry (DSC Q200; TA Instruments, New Castle, DE, USA). The samples (about 3.00 mg) were placed in standard aluminum pans, and dry nitrogen was used as the effluent gas. All samples were scanned at a temperature ramp speed of 10 °C/min and heat flow from 40 to 250 °C. Furthermore, X-ray powder scattering measurements were carried out with an X-ray diffractometer (MPD for bulk(powder); PAN analytical, Netherlands) at room temperature using monochromatic Cu $K\alpha$ radiation ($\lambda = 1.5406$ Å) at 30 mA and 40 kV over a range of 2θ angles from 3° to 40° with an angular increment of 0.15°/s.

2.8. Drug release

Drug release studies were accomplished using a dissolution apparatus II with 900 ml of phosphate buffer (pH 6.8) as the dissolution medium at 37 ± 0.5 °C. The speed of the paddle was set at 100 rpm. The CN-loaded solid SMEDDS, SD formulation, CN and CB powders (equivalent to 50 mg of clopidogrel base) were inserted in the dissolution tester (Vision G2 Classic 6; Hanson Technology, Chatsworth, CA, USA). At specific time intervals, an aliquot (1 ml) of sample was collected, filtered through a 0.45 μm nylon syringe filter and assayed for the content of clopidogrel by high performance liquid chromatography (HPLC) as mentioned above. An equivalent volume (1 ml) of fresh dissolution medium was added to compensate for the loss due to sampling.

2.9. Stability

To investigate the comparative stability of solid SMEDDS, SD, CN and CB, they were kept under accelerated conditions of 50 °C/75% RH for 4 weeks. The water content, drug content and degradants were analyzed at pre-determined time intervals. Water contents were analyzed using a thermogravimetric analyzer (TGA Q50, TA Instruments; New Castle, DE, USA). To determine the drug contents and the impurities of clopidogrel, the compounds were dissolved in the mobile phase and filtered through a membrane filter (0.45 μm). The resulting solution (10 μl) was analyzed by HPLC equipped with an Ultron ES-OVM column (4.6 mm I.D. × 150 mm, 5 μm; Shinwa Chemical Industrial Co., Kyoto, Japan). The mobile phase consisted of phosphate buffered solution (pH 4.8) and acetonitrile (80:20, volume ratio). The eluent was monitored at 220 nm with a flow rate of 1.0 ml/min. (Durga Rao, Kalyanaraman, Sait, & Venkata Rao, 2010; Gomez et al., 2004; Kim et al., 2011)

2.10. In vivo test

The pharmacokinetic study of two formulations, CN and CB powder at a dose of 30 mg/kg clopidogrel base, was performed in rats. Male Sprague-Dawley rats weighing 280 ± 20 g were fasted for 10–12 h prior to the experiments but allowed free access to water. Twenty-four rats were randomly divided into four groups. The 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 ketamine in the supine position. The rats in each group were administered with SD, solid SMEDDS, CN and the CB powder enclosed in small hard gelatin capsules (#9, Suheung capsule Co.; Seoul, Korea). Then, 0.4 ml of blood was sampled from the femoral artery at particular time intervals and 0.2 ml of plasma was obtained by centrifuging the blood samples at 3000 × *g* for 10 min. Plasma samples were kept at −20 °C until further procedure. Plasma (180 μl) samples were mixed with 20 μl atorvastatin (128 μg/ml in methanol) as an internal standard before the addition of 1.5 ml of methanol to precipitate the plasma proteins. After vortex mixing, the mixture was centrifuged at 3000 × *g* for 10 min, and the supernatant was shifted to a clean test tube and evaporated using a vacuum centrifugal evaporator. The residue was reconstituted in 80 μl of the mobile phase. Then, the resulting solution was analyzed by HPLC as described above. All pharmacokinetic parameters including maximum plasma concentration (C_{max}), time to reach the maximum plasma concentration (T_{max}), area under the whole blood concentration-time curve (AUC), elimination rate constant (K_{el}) and half-life ($t_{1/2}$) were estimated by the WinNonlin™ (Pharsight Corp.; Mountain view, CA, USA) program (Li et al., 2008). Moreover, Student's *t*-tests were applied to evaluate the differences between the formulations. Values were denoted as mean ± SD and the data were considered statistically significant at $P < 0.05$.

3. Results and discussion

3.1. Selection of carriers

Self-microemulsifying drug delivery systems (SMEDDS), a mixture of oil, surfactant/co-surfactant and drug in monophasic liquid state, are widely used to improve poorly water-soluble drugs (Kang, Oh, Oh, Yong, & Choi, 2012). This system should form a fine emulsion when introduced to the aqueous phase with mild agitation. Therefore, to select the SMEDDS composition for CN, its solubility in various vehicles was studied; the results are presented in Fig. 1. Among the oils tested, peceol (glyceryl monooleate) showed the highest solubility for CN (Fig. 1A). Moreover, peceol has been widely used in pharmaceutical applications due to its solubilizing capability, rheological behavior and low toxicity (Ganem-Quintanar, Quintanar-Guerrero, & Buri, 2000). Thus, peceol was selected as the oil phase for further studies. Cremophor RH60 (PEG-60 hydrogenated castor oil, HLB 15-17) and Transcutol HP (diethylene glycol monoethyl ether, HLB 4.2) were also chosen as the surfactant and co-surfactant, respectively. Cremophor RH60 is widely used as an emulsifying and solubilizing agent (Balakrishnan et al., 2009a); moreover, it showed the highest drug solubility among the tested surfactants (Fig. 1B). Generally, non-ionic surfactants are considered less toxic than ionic surfactants. They are usually accepted for oral ingestion (Pouton & Porter, 2008). Transcutol HP showed no enhancement in drug solubility; however, when it was mixed with Cremophor RH60, it had a suitable HLB value and viscosity to form a fine microemulsion. Furthermore, Transcutol HP is a powerful solubilizing agent used in several dosage forms on account of its ability to solubilize many drugs (Ganem-Quintanar et al., 2000).

On the other hand, the solid dispersion (SD) was prepared using a hydrophilic polymer and a surfactant by the spray drying technique. To screen the polymer and surfactants for the preparation of SD, the drug solubility in various surfactants (Fig. 1B) and polymers (Fig. 1C) was determined (Oh, Park, Kang, Yong, & Choi, 2011b). Cremophor RH60 was selected as the surfactant, since it showed higher solubility than the others (Fig. 1B). Among the tested hydrophilic polymers, HPMC most significantly improved drug solubility (to about 4,500 μg/ml; approximately three-fold) (Fig. 1C). Therefore, it was selected as the carrier to prepare the SD.

3.2. Preparation of solid SMEDDS

A series of SMEDDS were prepared, and their self-emulsification properties were visually observed. The pseudo-ternary phase diagram was constructed in the absence of CN to identify the optimized concentrations of oil, surfactant and co-surfactant in the liquid SMEDDS formulation (Oh et al., 2012). The phase diagram of the system containing Cremophor RH60 as the surfactant, peceol as the oil and Transcutol HP as the co-surfactant is shown in Fig. 2A. From the pseudo-ternary phase diagram, these components gave a reasonably large self-emulsification region. Spontaneous emulsion formation was not efficient when the volume of surfactant was less than that of the oil in liquid SMEDDS. The efficiency of emulsification was good when the concentration of the surfactant/co-surfactant was more than 65% v/v of the liquid SMEDDS formulation. The formulations surrounding the self-micro emulsification domain exhibited poor emulsion forming ability. It has been reported that the drug incorporated in the liquid SMEDDS may have some effect on self-emulsifying performance (Pouton, 2000). However, in our study, no significant differences were found in self-microemulsifying performance when compared with the corresponding formulations with CN.

The assessment of self-emulsification can be performed visually by observing the emulsification and droplet formation of the liquid SMEDDS formulations (Balakrishnan et al., 2009b). The droplet size of the formed emulsion of liquid SMEDDS plays a crucial factor as it determines the bioavailability of the drug. In this study, no significant difference in the droplet size was observed with increasing the surfactant concentration from 30% to 90% v/v in the liquid SMEDDS formulation (Fig. 2B). It was observed that increasing the co-surfactant (Transcutol HP) concentration from 20% to 50% v/v in the SMEDDS formulation decreased the *z*-average diameter of the emulsion but with more than 50% Transcutol HP, the *z*-average diameter did not change (Fig. 2C). It was observed that the liquid SMEDDS formulation composed of 20% peceol, 30% Cremophor RH60 and 50% Transcutol HP gave the smallest *z*-average diameter of the liquid SMEDDS formulations tested. Furthermore, 10% (w/w) drug was entirely dissolved in this formulation since we could use

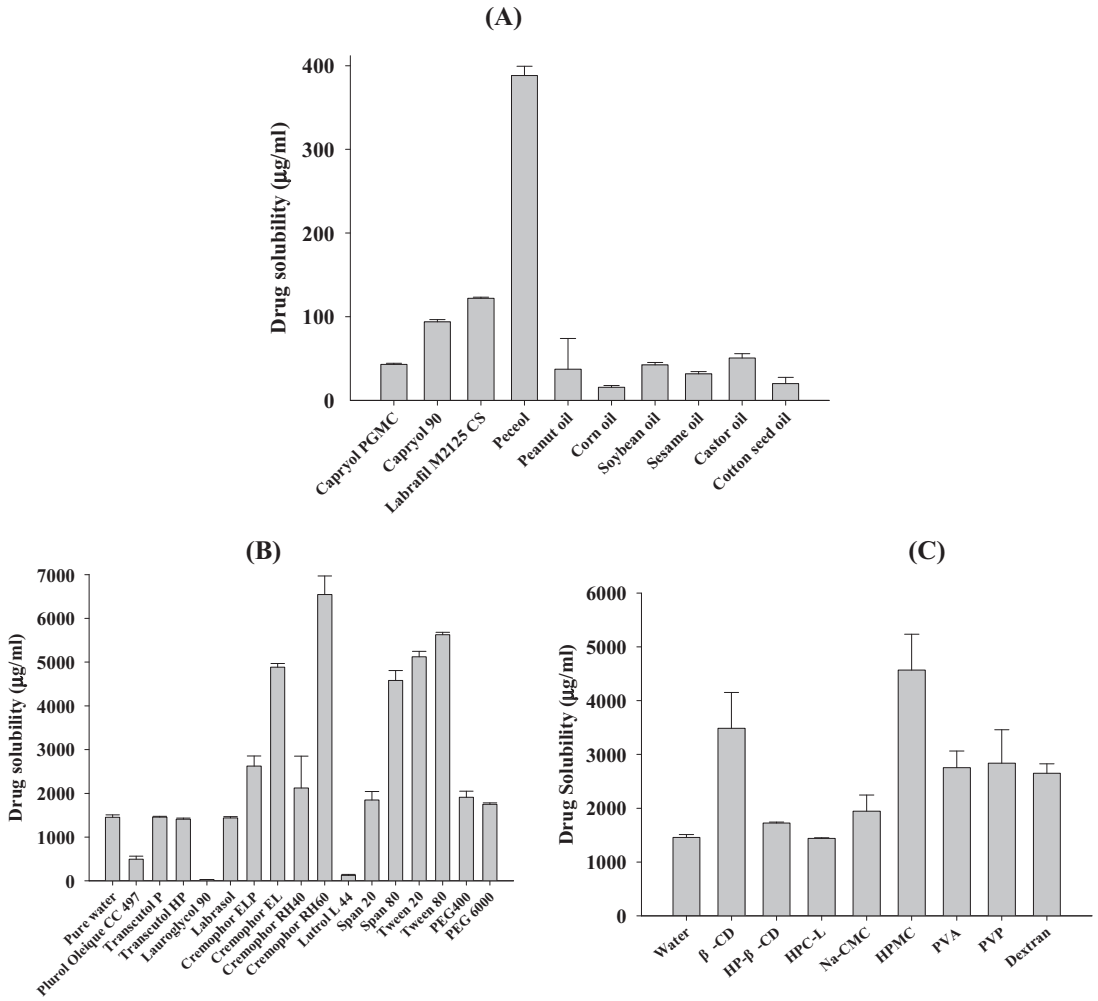

Fig. 1. Aqueous solubility of clopidogrel napadisilate: (A) oil; (B) 1% surfactant solution; (C) 1% polymer solution. Each value represents the mean ± S.D. ($n = 3$).

a sufficient volume of oil (peceol) to dissolve the drug. Therefore, this composition was used as an optimal liquid SMEDDS in this study. Moreover, the ethanolic solution containing 0.75 g silicon dioxide and 1 g liquid SMEDDS was spray-dried to produce the solid SMEDDS. Similarly, this optimal solid SMEDDS formulation composed of 75 mg silicon dioxide, 20 mg peceol, 30 mg Cremophor RH60 and 50 mg Transcutol HP was chosen for further studies.

The *z*-average particle size of the liquid and solid SMEDDS were assessed. Both *z*-average particle size of liquid and solid SMEDDS were around 150 nm. Liquid SMEDDS loaded with the drug (161.9 ± 13.3; PDI, 0.356 ± 0.016) gave a larger particle size than liquid SMEDDS without the drug (127.9 ± 12.3; PDI, 0.307 ± 0.005), but these were not significantly different. Moreover, the *z*-average particle sizes of liquid and solid SMEDDS (158.1 ± 14.7; PDI, 0.299 ± 0.005) were similar. Their polydispersity index (PDI) further confirmed the self-emulsification property of liquid and solid SMEDDS (Oh et al., 2012).

3.3. Preparation of SD

In conventional solid dispersion systems such as solvent evaporation and solvent wetting, a high drug/polymer ratio is normally used. However, in this surface attached solid dispersion technique, large amounts of the surfactant/hydrophilic polymer mixture are not used for presenting the solubilization state on the drug surface (Lee et al., 2013; Marasini et al., 2013). To determine the ratio between the polymer and surfactant in SD, formulations were prepared at various ratios of the surfactant to the polymer while keeping the amount of drug constant (Table 1). Furthermore, to determine the proper ratio of the drug to the surfactant/polymer, the SD formulations were prepared with different ratios, and the CN solubility was checked (Table 1). Comparing formulations I-VI, the formulation showed increased solubility of the drug with an increased surfactant ratio (Tran et al., 2013). SD prepared at a 1:1 surfactant/polymer ratio (formulation VI) showed the maximum solubility of CN.

In addition, among formulations VI–X, which were composed of different amounts of the carrier (surfactant/polymer ratio, 1:1), SD with a carrier/drug ratio of 0.8 (formulation VII) gave the significantly highest drug solubility. Our results suggest that an optimized amount of surfactant/polymer mixture is required to formulate SD without compromising drug solubility. Thus, formulation VII was selected for further studies, as it showed almost 4.5-fold higher solubility of CN in water (6.52 ± 0.60 vs. 1.46 ± 0.55 mg/ml).

3.4. Comparison of solid state characteristics

Scanning electron micrographs of the CN powder, solid carriers, solid SMEDDS and SD are shown in Fig. 3. The CN-loaded solid SMEDDS and SD prepared in this study gave almost 100% loading efficiency. The CN powder (Fig. 3A) was composed of even-surfaced rectangular crystals. On the other hand, Fig. 3B shows typical features of silicon dioxide with a highly rough and porous surface, which probably allows the ingress of the aqueous phase into the

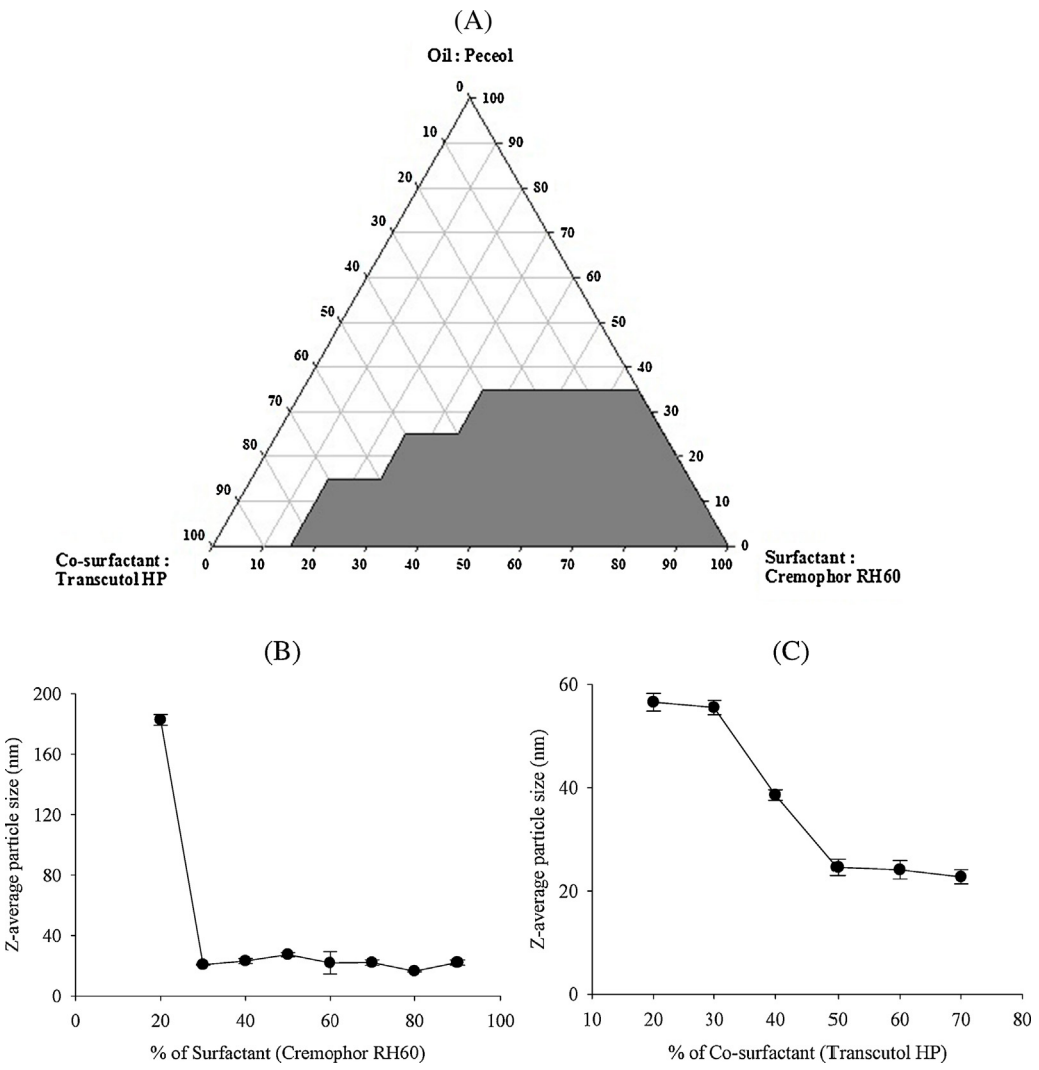

Fig. 2. Formulation of liquid SMEDDS: (A) Pseudo-ternary phase diagram using peceol as an oil, Cremophor RH60 as a surfactant and Transcutol HP as a co-surfactant; (B) effect of the percentage volume ratio of surfactant to oil on the droplet size of the emulsion formed from the oil/surfactant mixture; (C) effect of the co-surfactant percentage volume ratio on the mean emulsion droplet diameter of formulations containing 30% of a constant surfactant volume. Each value represents the mean ± S.D. ($n = 3$).

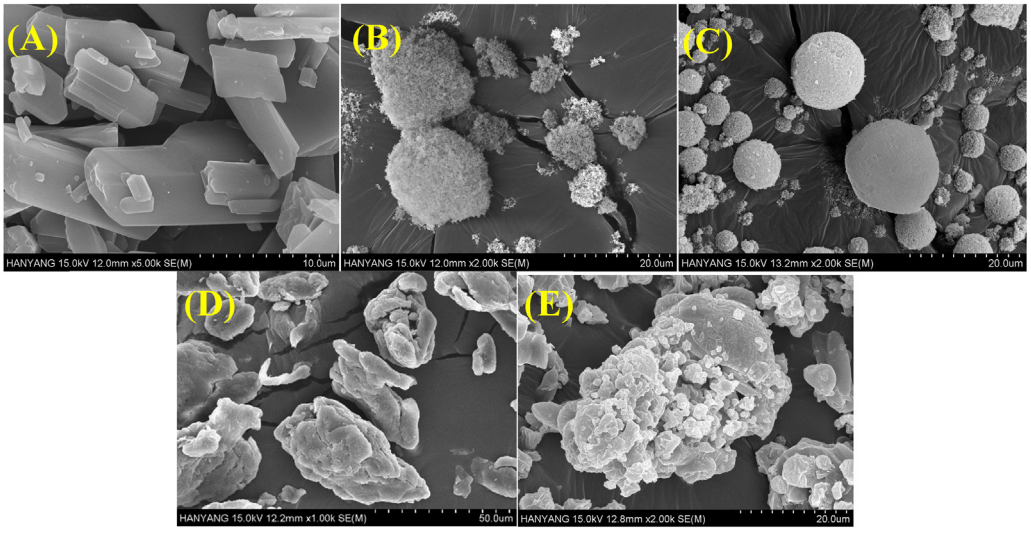

Fig. 3. Scanning electron micrographs: (A) clopidogrel napadisilate monohydrate; (B) silicon dioxide; (C) solid SMEDDS; (E) HPMC; (E) SD.

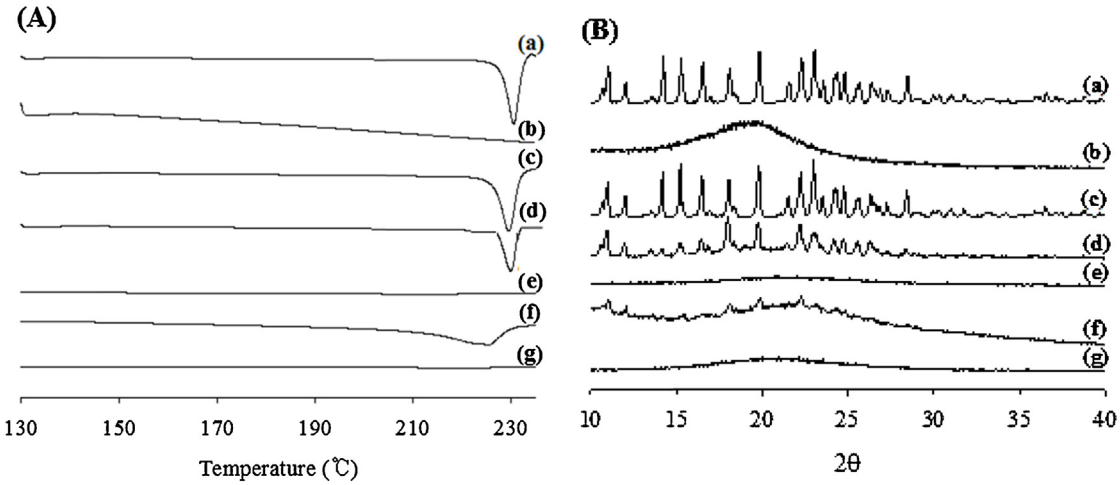

Fig. 4. Differential scanning calorimetric thermograms (A) and X-ray powder diffraction (B): (a), clopidogrel napadisilate (CN); (b), HPMC; (c), physical mixture of CN and HPMC; (d) SD; (e) silicon dioxide; (f) physical mixture of CN and silicon dioxide; (g) solid SMEDDS.

matrix. Furthermore, solid SMEDDS (Fig. 3C) was composed of relatively smooth-surfaced silicon dioxide particles, indicating that the liquid SMEDDS was absorbed or coated inside the pores of silicon dioxide. The HPMC was irregular in shape with a rough surface (Fig. 3D), and SD showed a relatively coarse and fissured surface (Fig. 3E), suggesting that the carriers were adhered onto the drug surface (Oh et al., 2012; Woo et al., 2008).

DSC is a widely utilized thermoanalytical technique used to monitor endothermic processes (i.e. melting, phase transition, chemical degradation) as well as exothermic processes of prepared solid formulations to reveal drug/carrier interactions. The thermal behavior of the CN powder, HPMC, silicon dioxide, the physical mixture and solid SMEDDS are presented in Fig. 4A. The DSC curves show that CN had a sharp endothermic peak at around 230 °C, indicating its exact melting point and indicating its crystalline nature (a). HPMC (b) showed no peaks in the tested region. A small endothermic peak corresponding to the drug was also detected in the physical mixture of CN and silicon dioxide (c). SD (d) showed an endothermic peak for the drug even though it was smaller than that of the drug or the physical mixture, indicating that the drug was present in crystalline form (Cho & Choi, 2013). Silicon dioxide (e) showed no peaks in the tested region but the physical mixture of the drug and silicon dioxide showed small crystalline peaks (f), suggesting no interaction between silicon dioxide and CN. On the contrary, solid SMEDDS (g) showed no intrinsic peak of the drug, indicating that the drug was present in a molecularly dissolved state (Balakrishnan et al., 2009a; Seo et al., 2013).

From the powder X-ray diffraction profiles shown in Fig. 4B, CN (a) showed a typical crystalline pattern, but carriers such as HPMC (b) and silicon dioxide (e) gave no peaks. In SD (d), the features of all the major representative peaks detected in CN (a) and the physical mixture (c) appeared. Our results indicate that the drug was present in unchanged crystalline form in the SD. Similarly, the physical mixture of the drug and silicon dioxide (f) showed a weak crystalline pattern similar to CN. However, the intrinsic peak of the drug disappeared in solid SMEDDS (g). Thus, in agreement with the DSC results, the drug existed as an amorphous form in solid SMEDDS (Oh et al., 2011a; Seo et al., 2013).

3.5. Comparison of solubility and dissolution

The solubility test was performed with CN powder, CB powder, solid SMEDDS and SD (Fig. 5A). The napadisilate salt form with an aqueous solubility of about 1 mg/ml was poorly water-soluble, but the bisulfate salt form was not (88.7 ± 7.8 mg/ml). The solid SMEDDS and SD improved the solubility of CN. SD enhanced the solubility more than the solid SMEDDS (6.52 ± 0.60 vs. 2.52 ± 0.35 mg/ml); however this formulation had low drug solubility compared to the CB powder.

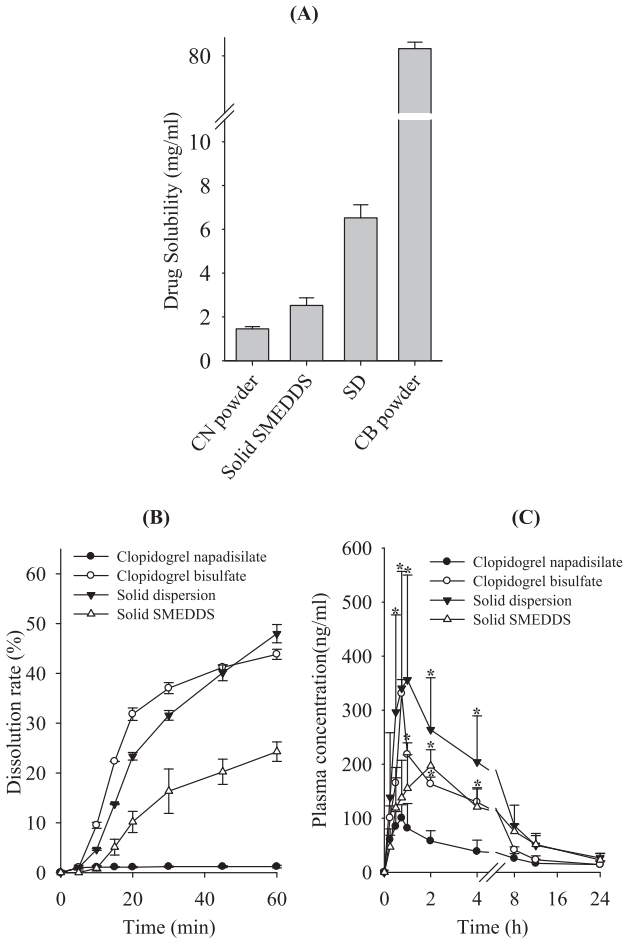

Fig. 5. Comparison of SD, solid SMEDDS, CN powder and CB powder: (A) aqueous solubility of drug; (B) dissolution; (C) plasma concentration–time profiles after oral administration in rats. * $P < 0.05$ when compared with CN powder. Each value represents the mean ± S.D. ($n = 3$ or 6).

Table 2
Pharmacokinetic parameters.

Parameter	CN powder	CB powder	Solid SMEDDS	SD
AUC (h ng/ml)	527.26 ± 147.43	1295.09 ± 310.88	1521.30 ± 191.50*	2297.57 ± 631.78*,#
C_{max} (ng/ml)	99.97 ± 38.52	310.21 ± 67.05*	198.28 ± 30.85*	350.48 ± 102.69*
T_{max} (h)	0.99 ± 0.35	1.00 ± 0.44	1.93 ± 1.11	1.33 ± 0.50
$t_{1/2}$ (h)	4.37 ± 1.25	2.46 ± 0.72	3.66 ± 2.06	3.52 ± 1.01
K_{el} (h^{-1})	0.17 ± 0.05	0.29 ± 0.09	0.28 ± 0.15	0.21 ± 0.06

Each value represents the mean ± S.D. ($n = 6$).
* $P < 0.05$ compared with CN powder.
# $P < 0.05$ compared with CB powder.

To evaluate whether the two preparations affected the dissolution profile of CN, dissolution studies were performed on CB, CN, SD and solid SMEDDS in phosphate buffer (pH 6.8) (Fig. 5B). CB powder showed a fast dissolution profile as it is freely soluble. However, the CN powder showed a very slow dissolution profile compared to that of CB. The solid SMEDDS improved the drug release rate compared to the CN powder. Generally, solid SMEDDS induce the fast release of poorly water-soluble drugs, since the free energy required to a form microemulsion is very low in the case of self-emulsifying systems, hence, the spontaneous formation of an interface between water and oil droplets is possible (Balakrishnan et al., 2009a). However, solid SMEDDS increased the drug release rate to a lesser extent than SD due to its limited solubility. SD showed a similar dissolution profile to the CB powder. However, SD had a higher dissolution rate than the CB powder at 60 min. In conventional SDs, the drug will exist in amorphous form; however in the surface attached method, the hydrophilic polymer and surfactants cover the surface of drug crystals. Therefore, the surface of the drug crystal is converted from a hydrophobic surface to a hydrophilic surface, and upon contacting water, these drug crystals interact with the aqueous microenvironment, thereby dispersing the drug into a supersaturated solution (Balakrishnan et al., 2009b). Recrystallization due to a rising degree of supersaturation because of the interfacial tension between the small drug particles was prevented by the surfactant Cremophor RH60 while solubilizing the drug. Thus, the highest degree of dissolution was achieved with the prepared SD (Kang et al., 2012). These results suggest that the prepared solid SMEDDS and SD significantly improved the dissolution of CN, but the latter increased the drug dissolution rate compared to the former.

3.6. Comparison of bioavailability

Fig. 5C shows the change in the mean plasma concentration of SR 26334, the major metabolite of clopidogrel after the oral administration of CN powder, CB powder, SD and solid SMEDDS at a dose of 30 mg/kg clopidogrel base in rats. Clopidogrel has no activity in itself as a prodrug; its metabolites including 2-oxo-clopidogrel and the thiol derivative produce the in vivo effects (Mitakos & Panderi, 2002, 2004). However, these metabolites are undetectable in plasma as they are highly labile. In addition, this drug is metabolized to its major inactive metabolite, SR 26334 [(*S*)-(2-chlorophenyl)-6,7-dihydrothieno[3,2-c]pyridine-5(4*H*)-acetic acid; clopidogrel carboxylic acid derivative] in animals (Antić, Filipić, & Agbaba, 2007; Savi et al., 2006; Silvestro et al., 2010). Hence, the quantification of this inactive metabolite is an indirect approach to determine the pharmacokinetics of clopidogrel (Silvestro et al., 2010; Singh, Sharma, Barot, Mohan, & Lohray, 2005). The CN powder gave a lower total plasma concentration of SR26334 compared to the CB powder. Particularly, from 1 h to 4 h, the plasma concentrations of CN powder were significantly lower compared with CB powder ($P < 0.05$), due to its lower drug solubility and dissolution. The SD and solid SMEDDS formulations showed higher plasma concentrations than CN powder. Similarly, at 0.5–4 h and 2–4 h, the plasma concentrations of SD and solid SMEDDS formulations were significantly higher compared with CN powder, respectively. Furthermore, SD had a relatively higher plasma concentration than solid SMEDDS and the CB powder, even if they were not significantly different. Our results indicate that the relatively higher plasma concentrations of the SR26334 with SD were due to the increase in solubility and dissolution of clopidogrel from SD (Balakrishnan et al., 2009a; Seo et al., 2013).

The pharmacokinetic parameters are shown in Table 2. The SD and solid SMEDDS formulations showed significantly higher AUC and C_{max} compared to the CN powder. In particular, the AUC values of SD and solid SMEDDS were about 4.3- and 2.9-fold higher than that of the CN powder, respectively. The C_{max} values were about 3- and 2-fold higher than that of CN powder, respectively. SD gave 1.7-fold higher AUC value compared to the CB powder, but solid SMEDDS showed a similar AUC value to CB powder. However, the T_{max}, K_{el} and $t_{1/2}$ values of SD and solid SMEDDS were not significantly different from those of CN and CB powder. Thus, the two formulations significantly increased the oral bioavailability of CN powder. Furthermore, the SD improved the oral bioavailability of the CN powder to a greater degree than solid SMEDDS. Our results suggest that the enhanced oral bioavailability of clopidogrel in the SD was due the marked increase in the absorption of the drug through its improved solubility and dissolution (Woo et al., 2008).

3.7. Comparison of stability

The stability of the drug in the formulations was evaluated by the drug content, water drug content, hydrolyzed degradant and racemized degradant in the SD and solid SMEDDS formulations compared to that of the CN powder and CB powder under accelerated stress conditions of 50 °C/75%RH for 4 weeks (Agrawal, Kaul, Paradkar, & Mahadik, 2003; Kim et al., 2011). The water and drug contents were calculated as the following: water (or drug) content % = $[W_t/W_0] \times 100$ where W_0 and W_t are the water (or drug) content in each sample at 0 and t weeks, respectively.

Fig. 6A shows the drug content of the formulations after 4 weeks. The CB powder showed a tremendous decrease in the drug content (~50%) over a period of 4 weeks. CN powder and solid SMEDD showed about a 7% decrease in the drug content at the end of 4 week. However, SD showed no significant drug loss, suggesting that it was the most stable among the formulations tested in this study; it improved the stability of CN. Fig. 6B shows the water content of the formulations over a period of 4 weeks; it was observed that CB had an 8-fold higher water content (802 ± 38%) indicating its hygroscopic property. The CN powder had a lower water content than the CB powder, suggesting that CN powder was not hygroscopic. The water content was slightly increased in the order CB powder < SD < solid SMEDDS, even though there were no significant differences. This might be due to the fact that the carriers in these formulations affected the water content only a little. The CB powder showed a hydrolyzed degradant content of around 30% at 4 weeks (Fig. 6C). However, the CN power, SD and solid SMEDDS contained less than 0.5% hydrolyzed degradants. On the other hand, it was observed that the CB powder and solid SMEDDS showed about 2.8%

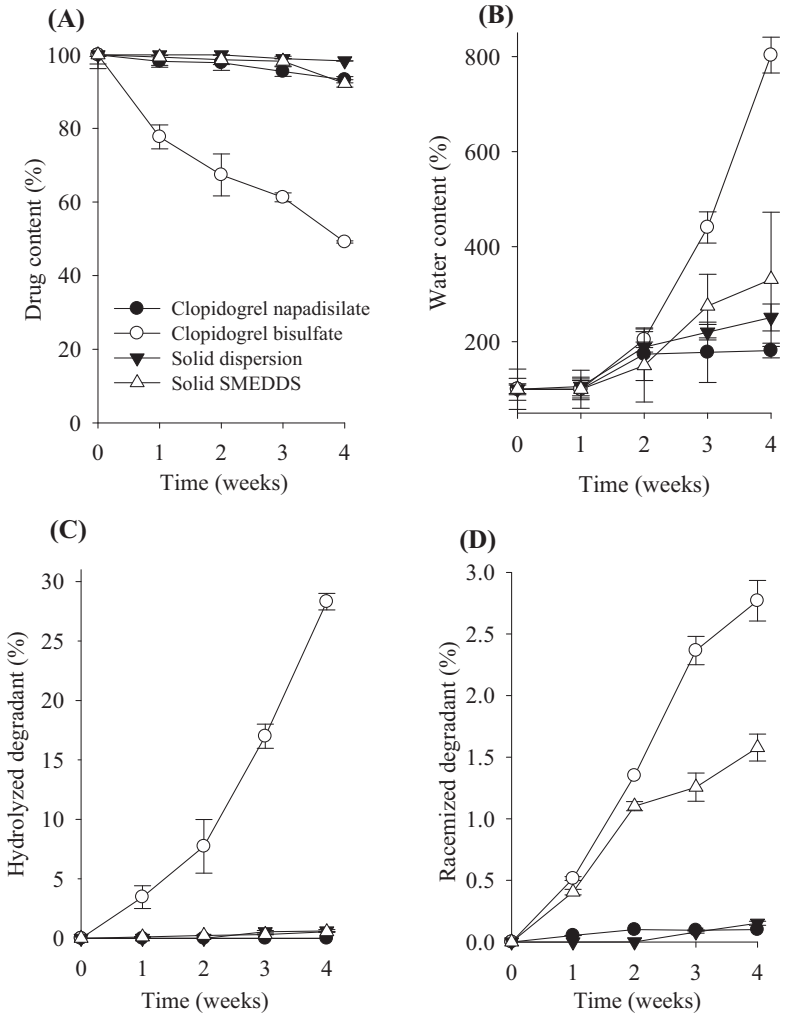

Fig. 6. Stability under accelerated conditions of 50 °C/75% RH for 4 weeks: (A) drug content; (B) water content; (C) hydrolyzed degradant; (D) racemized degradant. Each value represents the mean ± S.D. ($n = 5$).

and 1.6% racemized degradants, respectively (Fig. 6D). CN and SD showed less than 0.1% degradants. Thus, the CB powder produced a lot of hydrolyzed and racemized degradants under these conditions (50 °C/75% RH) due to humidity, suggesting that this bisulfate form was unstable. However, the CN powder was little affected by humidity, indicating that the CN powder is more stable than the CB powder. Furthermore, the SD formulation improved the stability of the CN powder, while solid SMEDDS could not (Agrawal et al., 2003; Kim et al., 2011). Thus, the SD formulation could be a good alternative to the CB and CN salt commercial formulations.

In this study, two formulations, i.e. a clopidogrel napadisilate (CN)-loaded solid dispersion (SD) and a solid self-microemulsifying drug delivery system (solid SMEDDS) were developed as novel alternative products to improve the stability and bioavailability of clopidogrel. In the solid SMEDDS, the drug existed as an amorphous form by absorbing or coating CN-loaded liquid SMEDDS inside the pores of silicon dioxide (carrier) (Balakrishnan et al., 2009a,b; Seo et al., 2013). The SD, in which the crystalline drug was unchanged, was produced by adhering a hydrophilic polymer and surfactants onto the surface of CN (Joe et al., 2010; Li et al., 2010; Yan et al., 2012). Thus, the two formulations employed different mechanisms to enhance the solubility and bioavailability of a poorly water-soluble drug but improved both parameters. On contact with gastric fluid in the GI tract, solid SMEDDS spontaneously form an amorphous drug-loaded nanoemulsion, leading to enhanced solubility and permeation, which allows enhanced bioavailability of CN (Kang et al., 2012; Kim et al., 2012). Moreover, the SD was changed from the hydrophobic crystalline drug to hydrophilic form by the attachment of a hydrophilic polymer and surfactants on the drug surface, leading to improved solubility and bioavailability of CN without a change in crystallinity (Joe et al., 2010; Oh et al., 2011a,b; Yan et al., 2012). In this study, SD increased the solubility, dissolution and bioavailability of CN to a greater extent compared to the solid SMEDDS formulation, even though both significantly improved these parameters. In particular, the former formulation underwent no different changes in dissolution and bioavailability compared to the CB powder even though it had lower drug solubility. Furthermore, the SD and SMEDDS formulations were stable under accelerated conditions (50 °C/75% RH) for 4 weeks, while the CB powder was not (Kim et al., 2011). Thus, a CN-loaded SD could solve both the low oral bioavailability of CN powder and the instability of CB powder.

4. Conclusions

From the comparison of CN-loaded SD and solid SMEDDS, SD enhanced the solubility, dissolution and bioavailability of CN to a greater extent than the solid SMEDDS formulation due to

different solubility-improving mechanisms. SD was very stable and had similar oral bioavailability to the CB powder. Thus, the SD system is more suitable for poorly water-soluble clopidogrel napadisilate than the SMEDDS system. Furthermore, SD showed excellent stability and bioavailability and can be recommended as an alternative for clopidogrel-based oral formulations.

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