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Development of self-microemulsifying bilayer tablets for pH-independent fast release of candesartan cilexetil

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The aim of this study was to design self-microemulsifying tablets for pH-independent fast release of poorly soluble candesartan cilexetil (CDC). To improve the solubility of CDC, a self-microemulsifying drug delivery system (SMEDDS) was prepared composed of Capryol 90, Tween 80 and tetraglycol at a ratio of 5:35:60. Drug containing SMEDDS was adsorbed onto Fujicalin and Neusilin UFL2, respectively, used as solidification carriers and subsequently compressed into tablets (self-microemulsifying tablet, SMET). SMET using Fujicalin® exhibited immediate CDC release in pH 1.2 medium while Neusilin® UFL2-based SMET showed fast release, especially at pH 6.5. Thus, optimized SMET could be produced with one layer of Fujicalin and the other layer with Neusilin UFL2, demonstrating CDC release of 75% of the initial dose within 15 min in all pH conditions (1.2, 4.5, and 6.5). The average diameter of emulsion droplets formed from SMET was less than 200 nm. It was thus expected that Fujicalin® and Neusilin® UFL2-based bi-layer SMET would overcome low oral bioavailability of CDC due to its limited solubility at physiological pH conditions in the gastrointestinal tract.

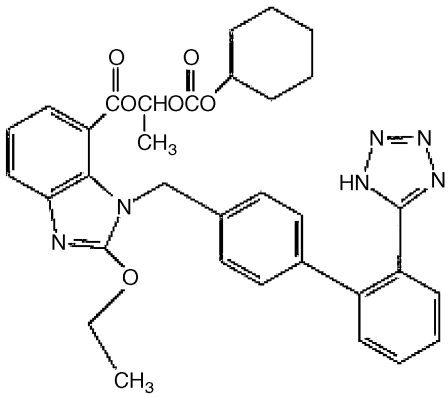
1. Introduction

Candesartan cilexetil (CDC), which belongs to the family of angiotensin II receptor antagonists, is selective for angiotensin II type 1 (AT_1) receptor with an empirical formula of $C_{33}H_{34}N_6O_6$ (Fig. 1). It is a prodrug developed for oral use and is rapidly converted to the active drug, candesartan (CD) during absorption from the gastrointestinal tract (Ogihara et al. 1994). Because CDC is practically insoluble in water with a solubility of less than 50 μg/mL (Product Monograph of Atacand®, AstraZeneca Canada), it shows limited dissolution under physiological conditions and conventional CDC tablets have a significantly low bioavailability of approximately 14% after oral administration. Improvement in dissolution rate is therefore expected to enhance the absorption and bioavailability of CDC, thereby increasing the efficacy and therapeutic outcome with CDC (Nekkanti et al. 2009).

To resolve the solubility issue, a self-microemulsifying drug delivery system (SMEDDS) was introduced. Self-emulsifying systems have gained considerable interest after the successful launching of lipid-based formulations of cyclosporine A (Neoral®, Sandimmun®), and two HIV protease inhibitors, ritonavir (Norvir®) and saquinavir (Fortovase®) (Thi et al. 2009). SMEDDS can be described as an isotropic mixture of oil, surfactant, cosurfactant (or solubilizer) and drug that can form fine oil-in-water microemulsions under mild agitation following dilution with aqueous media present in the gastrointestinal tract when orally administered (Craig et al. 1997). The dispersed phase of the microemulsion formed provides solubilized drug and a large interfacial surface for enhanced drug absorption (Khoo et al. 1998; Tosco et al. 2008).

Previous research published by Nekkanti et al. (2010) has reported SMEDDS formulations of CDC, composed of Miglyol 812, Tween 80 and Labrasol, filled into hard gelatin capsules. The amount of the drug solubilized in this SMEDDS was 230–256 mg/g. However, in our laboratory setting, much lower amount of the drug (< 38 mg/g) could be solubilized with the same SMEDDS. Thus, we attempted to improve SMEDDS formulations for CDC. Moreover, to overcome the disadvantages (e.g. difficulty of process control, leakage of the encapsulated components and high production cost) of liquid SMEDDS-filled hard gelatin capsule dosage forms proposed by Nekkanti et al. (2010), a tablet form of SMEDDS might be desirable.

To produce tablets from SMEDDS formulations, solidification of SMEDDS liquid is critical and generally porous carriers are employed having a large surface area for drug loading. (Cannon 2005; Ito et al. 2005; Sharma et al. 2005; Ito et al. 2006; Patil and Paradkar 2006; Jannin et al. 2008). Additionally, to replace the liquid SMEDD formulation with a tabulated dosage form, the incorporated drug should have immediate release characteristics seen with SMEDDS formulations. Therefore, in the present study, fast release tablet dosage forms of candesartan cilexetil efficiently solubilized in self-microemulsifying drug delivery systems (self-microemulsifying tablets, SMET) were developed to control the dissolution rate of the drug.



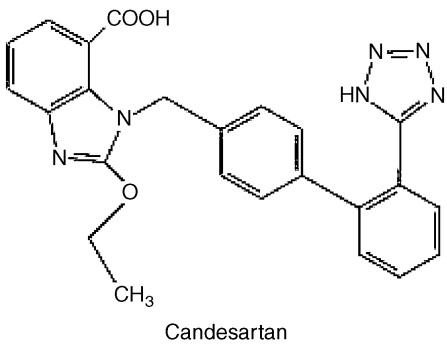


Fig. 1: Structures of the prodrug candesartan cilexetil and the angiotensin II receptor antagonist candesartan

2. Investigations, results and discussion

2.1. Solubility of CDC

Solubility studies were performed to identify suitable oil, surfactant and cosolvents that possessed good solubilizing capacity for CDC. Screening suitable vehicles having maximal solubilizing potential for the drug under investigation is very important in order to achieve optimum drug loading, avoid precipitation of the drug on dilution in the gut lumen and also minimize the final volume of SMEDDS (Tauboll et al. 1990; Kale and Patravale 2008). The results of solubility studies with CDC in various vehicles are shown in Table 1. It is evident that CDC had the highest solubility in Capryol 90, Tween 80, and tetraglycol (7.86 ± 0.86, 43.11 ± 2.24, and 115.07 ± 6.06 mg/mL), respectively and thus, these were selected as oil, surfactant and cosolvent, respectively for further studies. Capryol 90 has an HLB value of 6 and has frequently been used in many other SMEDDS as the oil phase due to its great solubilizing ability (Bachhav and Patraval 2009; Balakrishnan et al. 2009). Also, Tween 80, a non-ionic surfactant with a relatively high HLB value of 15, had the highest solubilizing potential for CDC. As surfactants with HLB values of 12–15 tend to generate self-microemulsification more easily, Tween 80 was thus chosen to obtain good dispersing and self-emulsifying performances (Constantinides 1995), along with its low toxicity and non-reactivity under various pH conditions.

2.2. Construction of pseudo-ternary phase diagram

Phase diagrams were constructed in the presence of CDC to obtain the optimum concentrations of oil, surfactant, and cosurfactant. SMEDDS forms fine oil-in-water emulsions with only gentle agitation upon its introduction into aqueous media. Since the free energy required to form an emulsion is very low, the formation is thermodynamically spontaneous (Craig et al. 1997).

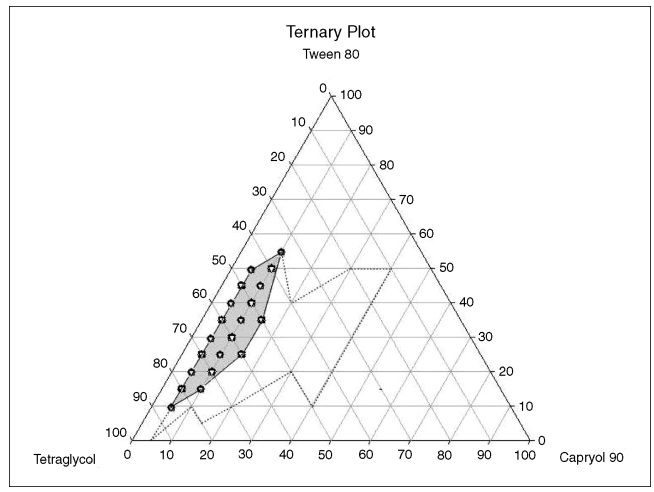


Fig. 2: Pseudoternary phase diagrams of the Capryol 90/Tween 80/tetraglycol system at 37 °C

Surfactants form a layer around emulsion droplets and reduce surface tension between the oil phase and aqueous phase as well as providing a mechanical barrier to coalescence (Kang et al. 2004). Furthermore, the lipid mixtures with different surfactant and cosurfactant ratios lead to the formation of SMEDDS with different properties. To identify self-microemulsifying regions and to select suitable concentrations of oil, surfactant and secondary surfactant for the formulation of SMEDDS, a visual test was designed to measure the apparent spontaneity of microemulsion formation. Figure 2 shows the pseudo-ternary phase diagrams consisting of Capryol 90, Tween 80 and tetraglycol. The gray area indicates clear O/W microemulsion in the system. As seen in this area, Tween 80 produced a transparent microemulsion region in the range of less than 55% of total formulation. In all cases, it was observed that the greater amount of oil (i.e. Capryol 90) in SMEDDS caused a difficulty in the formation of microemulsions upon dilution. Hence, it was decided to keep Capryol 90 concentrations as low as possible while obtaining maximum solubilizing capabilities of SMEDDS. From this point of view, three isotropically stable SMEDDS formulations composed of Capryol 90:Tween 80:tetraglycol = 5:35:60, 10:40:50, and 10:30:60 were selected from the pseudo-ternary phase diagram for the next study to estimate physicochemical properties (Table 2).

2.3. Evaluation of SMEDDS characteristics

2.3.1. Dispersibility

In the present study, purified water was used as a dispersion medium to test dispersibility. The reason for this is that there is no considerable difference in the dispersion behavior of SMEDDS in water, simulated gastric or intestinal fluid (Jaydeep 2011). All the formulations in Table 2 passed the dispersibility test in grade 1 and did not show any phase separation and drug precipitation after 24 hour. They were emulsified within 1 min and especially, formulation 1 demonstrated the fastest emulsification time (12.5 ± 3.5 s).

2.3.2. Solubility of CDC in SMEDDS formulations

Solubility of CDC in SMEDDS formulations 1, 2 and 3 were 70.97 ± 3.07, 55.93 ± 0.52 and 65.26 ± 1.34 mg/mL, respectively which are much improved compared to solubility in water (less than 0.05 mg/mL). The different ratios of each component in three formulations could have attributed to the differences in solubility because the relative solubility of the drug in vari-

Table 1: Solubility (mg/mL) of candesartan cilexetil in various vehicles at 25 °C saturated for 48 h

Oil	Solubility	Surfactant	Solubility
Miglyol 810	1.16 ± 0.72	Lauroglycol 90	4.91 ± 0.18
Miglyol 812	2.50 ± 1.95	Cremophor EL	26.79 ± 1.13
Labrafac CC	0.68 ± 0.03	Tween 80	43.11 ± 2.24
Labrafil 1944 CS	1.87 ± 0.20	Tween 20	30.95 ± 4.06
Labrafil 2125 CS	2.04 ± 0.11	Cosolvent	Solubility
Myvacet 9–45	1.51 ± 0.20	PEG 400	28.38 ± 0.68
Maisine 35–1	2.35 ± 0.13	PEG 300	26.57 ± 1.84
Capryol 90	7.86 ± 0.86	Labrasol	31.30 ± 1.23
Labrafac PG	1.05 ± 0.05	Propylene carbonate	9.65 ± 0.24
Triacetin	4.39 ± 0.01	Ethyl alcohol	14.26 ± 0.49
Labrafac lipophile WL1349	1.34 ± 0.28	Transcutol P	79.53 ± 4.80
Peceol	1.88 ± 0.20	Tetraglycol	115.07 ± 6.06

Data are expressed as mean ± SD (n = 3)

ous components would contribute to drug entrapment in a given microemulsion. The solubility of CDC was higher in tetraglycol and Tween 80 as compared to Capryol 90 and thus it may be the reason that formulation 1 had the highest solubilization capacity towards CDC among the three formulations tested.

2.3.3. Particle size of microemulsion and PDI determination

Droplet size distribution following self-microemulsification is a critical factor in evaluating efficiency of a self-microemulsion system. The smaller the droplet size, the larger the interfacial surface area for drug absorption will be (Gershanik and Benita 2000). The mean particle size and polydispersity index (PDI) for all the SMEDDS tested are summarized in Table 2. The results show that particle sizes in water of SMEDDS 1, 2 and 3 were less than 100 nm, which fulfill the criteria of microemulsion and low PDI. Of these, formulation 1 had the smallest particle size of 72.04 nm. The PDI values of SMEDDS 1, 2 and 3 were evaluated to be 0.187, 0.141, and 0.157, respectively, which indicates uniformity of droplet size within the formulations (Babbota et al. 2007). In addition, there were no considerable differences for particle properties evaluated in the three different media, which demonstrates that physical properties of the formulations were not greatly affected by pH.

2.3.4. Zeta potential measurement

All SMEDDS formulations displayed negative zeta potential values to be −10.8 ± 0.9, −11.6 ± 1.1, and −12.9 ± 1.1 mV for SMEDDS formulations 1, 2, and 3, respectively and the actual values were quite similar among the formulations (Table 2). Tween 80 and tetraglycol used in this study are nonionic and thus it was expected that they did not contribute to any charge to the microemulsion particle (Lu et al. 2008; Cui et al. 2009).

2.4. Selection of optimal SMEDDS

Formulation 1 was considered as the optimal self-microemulsifying system for CDC, based on good dispersibility, the highest solubilizing capacity, the smallest particle size and low PDI value.

2.5. Formulation and evaluation of SMET of CDC

2.5.1. SEM

Appearance of intact Fujicalin showed spherical and rather rough textures with a particle size of approximately 110 μm. On the other hand, Neusilin UFL2 and Aeroperl 300 showed much smaller particle sizes of 5 and 30 μm, respectively (Fig. 3 a, c and e). After the adsorption of liquid SMEDDS onto solid carrier materials, obvious changes in the surface of solid carriers could be identified as shown in Fig. 3b, d and f. The surface of three carriers seen as white were partly covered with liquid SMEDDS and similar texture was previously reported elsewhere (Ahmad et al. 2005). From the SEM observations, it was confirmed that liquid SMEDDS was effectively retained in the micropores as well as on the surface of the carriers.

Table 2: Composition of SMEDDS formulations (% v/v) and their characteristic values

Formulation number		1	2	3
Composition	Capryol 90 (%)	5	10	10
	Tween 80 (%)	35	40	30
	Tetraglycol (%)	60	50	60
	CDC (mg/mL)	50	50	50
Saturated solubility of CDC* (mg/mL)		70.97 ± 3.07	55.93 ± 0.52	65.26 ± 1.34
Visual observation grade		I	I	I
Emulsification time* (s)		12.5 ± 3.5	30.0 ± 6.1	25 ± 2.7
Droplet size* (d.nm)	pH 1.2	90.63 ± 2.64	118.33 ± 18.69	120.70 ± 1.41
	pH 6.5	75.40 ± 3.79	105.06 ± 22.26	127.75 ± 17.04
	Water	72.04 ± 7.59	89.73 ± 19.76	99.51 ± 1.57
Polydispersity index	pH 1.2	0.142	0.132	0.194
	pH 6.5	0.145	0.218	0.130
	Water	0.187	0.141	0.157
Zeta potential* (mV)		−10.8 ± 0.9	−11.6 ± 1.1	−12.9 ± 1.1

* Data indicate mean ± SD (n = 3)

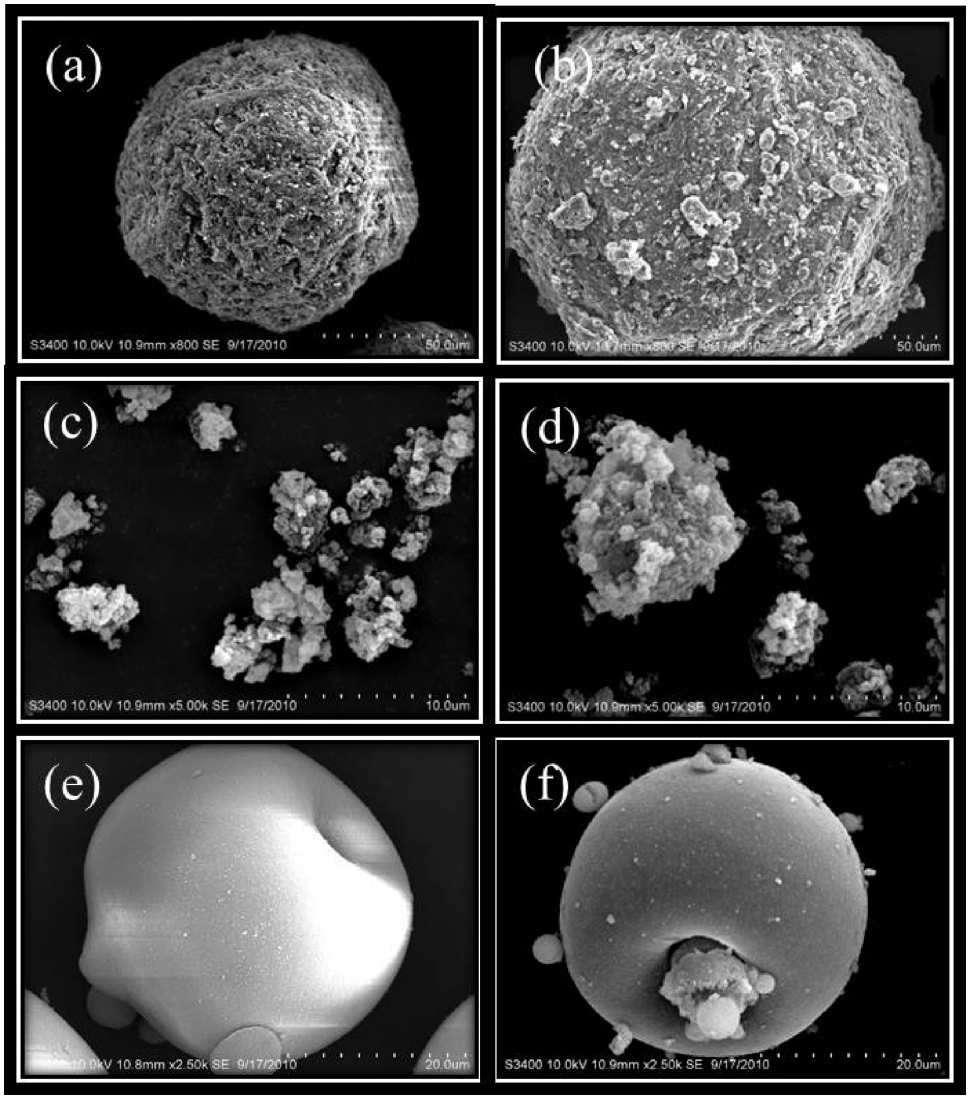


Fig. 3: Scanning electron micrographs of excipients after adsorption of SMEDDS: (a) Fujicalin before adsorption (x500); (b) Solid SMEDDS using Fujicalin (x500); (c) Neusilin UFL2 before adsorption (x5000); (d) Solid SMEDDS using Neusilin UFL2® (x5000); (e) Aeroperl 300 before adsorption (x2500); (f) Solid SMEDDS using Aeroperl 300® (x2500)

2.5.2. Drug release studies

The compositions of SMET for CDC described in Table 3 were prepared on the basis of our preliminary formulation screening. The selection of disintegrant (Polyplasdone) and lubricant (magnesium stearate) was made based on our perception and experience. As for a diluent, we evaluated three possible excipients such as Pearitol (direct compressible mannitol), Flowlac (direct compressible lactose) and USP grade mannitol. Of these, Pearitol was found to be the best diluent for every solid SMEDDS in terms of tablet hardness and disintegration time.

Although the solubility of CDC was greatly enhanced, there was still a need for the addition of a solubilizer to maintain a good sink condition (i.e., below 10% of saturation solubility) since solubility of CDC in a form of bulk powder remained unchanged (Washington 1990). Tween 20 was selected as a solubilizer due to its ability to increase the aqueous solubility of CDC (Table 1). The different amount of Tween 20 added to the release medium was due to the difference in solubilities between CDC in pH 1.2 and pH 6.5 media. The disintegration of all SMETs regardless of adsorbing carriers was completed within 5 min and it was therefore considered that the impact of tablet disintegration on the release of CDS was minimal.

The CDC releases from each SMET formulation in pH 1.2 simulated gastric juice and pH 6.5 phosphate buffer are presented in Fig. 4 and 5, respectively. As shown in Fig. 4, using Fujicalin as an adsorbent, CDC release was immediate and completed within 10 min in pH 1.2. The cumulative amount of CDC released from liquid SMEDDS in 3 min was 96.8%, while that of CDC from Fujicalin-based SMETs in 3 min was 96.1%, comparable to liq-

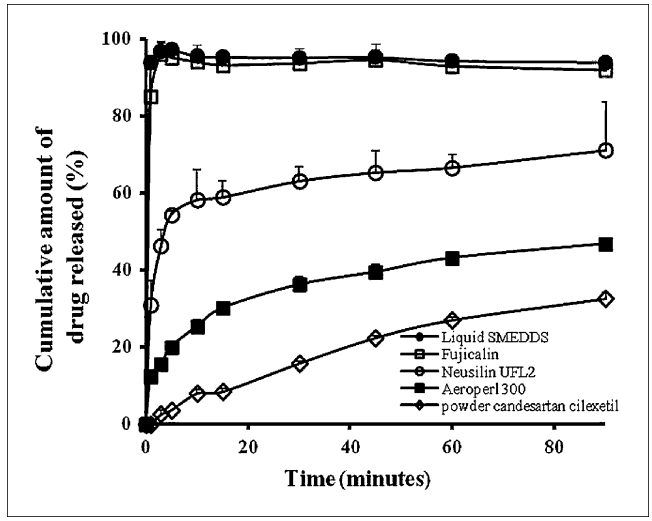


Fig. 4: Dissolution profiles of candesartan cilexetil in self-microemulsifying tablets in simulated gastric juice (pH 1.2) with 0.5% Tween 20. Data are expressed as mean ± SD (n = 3)

Table 3: Tableting formulations for development of self-microemulsifying tablets (SMET)

SMET Formulation		SMEDDS (μL)	Fujicalin (mg)	Neusilin UFL2 (mg)	Aeroperl 300 (mg)	Pearitol (mg)	Polyplasdone (mg)	Mg. Stearate (mg)	Hardness* (kp)
Fujicalin-based		123	250			100.75	25	1.25	3.13 ± 0.18
Neusilin-based		123		117.9		232.85	25	1.25	5.55 ± 0.89
Aeroperl-based		123			142.3	208.45	25	1.25	5.30 ± 1.38
Bi-layer	First layer	61.5	125			50.375	12.5	0.625	4.87 ± 0.75
	Second layer	61.5		58.95		116.425	12.5	0.625	

* Hardness data are expressed as mean ± SD (n = 3)
All formulations contain 8 mg of candesartan cilexetil

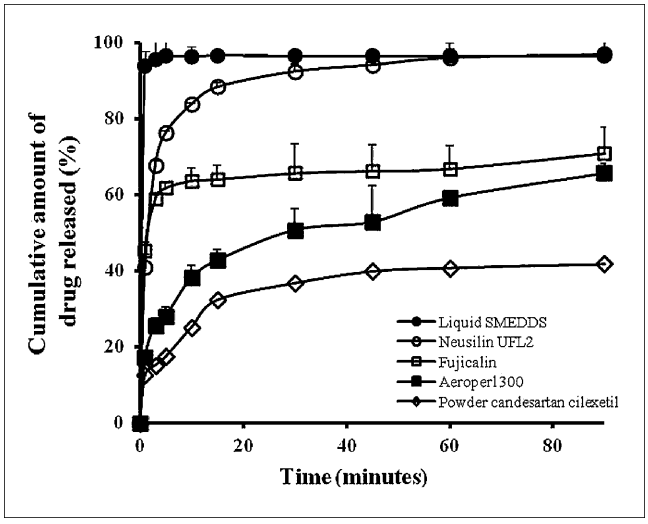


Fig. 5: Dissolution profiles of candesartan cilexetil in self-microemulsifying tablets in 0.05 M phosphate buffer (pH 6.5) with 0.35% Tween 20. Data are expressed as mean ± SD (n = 3)

uid SMEDDS. Moreover, this value was evaluated to be 38 times greater than that measured with bulk powders of CDC. On the other hand, the release of CDC from Neusilin UFL2-based SMETs in pH 6.5 was the fastest and greatest and the amount of CDC released was estimated to be 92.5% at 30 min. However, under the same conditions, the amount of CDC release from Fujicalin-based SMETs was 66% at 30 min.

One of the tested adsorbents, Fujicalin, is a dibasic calcium phosphate anhydrous designed as a direct compression excipient. Fujicalin has porous spheres (mean pore size: 73.5 Å, mean particle size: 115 μm) with a specific surface area of 40 m^2/g and an oil adsorbing capacity of 0.7 mL/g, and is ideally suited for direct compression formulations, especially for difficult-to-compress materials like oils (Takami et al. 1996; Schlack et al. 2001). Furthermore, Fujicalin is soluble in acidic conditions such as simulated gastric juice, leading to rapid disintegration of Fujicalin-based SMETs with an average of 2 min (Kang et al. 2011). High water adsorption capacity (1.2 mL/g) and fast erosion of Fujicalin in pH 1.2 media allows for a fast release rate of CDC equal to that obtained from liquid SMEDDS. However, Fujicalin is insoluble at pH 6.5. Because the surface area available following the collapse of Fujicalin-based tablets in the release medium is smaller than those formed from Neusilin UFL2-based SMETs, CDC release from Fujicalin-based SMET was slower in pH 6.5 media compared to Neusilin-based SMETs.

Neusilin UFL2 is a synthetic, amorphous form of magnesium aluminometasilicate and has a very small bulk density (0.08 g/mL) due to its great porosity. Since the mean particle size of Neusilin UFL2 is approximately 2–8 μm, it has a very large specific surface area of about 300 m^2/g and great oil adsorbing capacity (3.2 mL/g, Fuji Chemical Product Information). According to the earlier findings, adsorbent size may be the primary factor that influences drug release from solid SMEDDS. In Agarwal's report (Agarwal et al. 2009), the release of griseofulvin decreased with increasing adsorbent size because of the entrapment of drug in deeper voids of larger particles and a narrow surface area interacting with the aqueous medium. For this reason, the release of CDC adsorbed onto Neusilin UFL2 was comparably rapid in pH 1.2 and quite fast in pH 6.5 medium. The drug release from Neusilin-based SMETs was slower in pH 1.2 than in pH 6.5. The reason for this may be attributed to the fact that CDC exists mainly as zwitterionic and neutral forms (H_2A^+, H2A) at pH 1–2.8 and as a dianionic form (A^{-}) at pH around 6.5 and higher including physiological pH after partial hydrolysis under acidic and basic conditions (Rao et al. 2007; Tosco et al. 2008). Thus, the compound was anticipated to exist as being more soluble in pH 6.5 compared to pH 1.2. Moreover, since Neusilin has the smallest particle size among the carriers tested the solubility effect caused by ionization might be greater. In the case of Aeroperl 300, it has larger particle size of about 30 μm and similar specific surface area (300m^2/g) to Neusilin UFL2 (Product Information of Aeroperl 300, Degussa GmbH, Frankfurt, Germany). This implies the existence of a number of intraparticular pores, which contribute to the creation of a surface area equivalent to Neusilin UFL2. In spite of this, comparatively low levels of cumulative CDC release (36% in pH 1.2 and 51% in pH 6.5 media) in 30 min compared to Neusilin UFL2 could be observed and this may be attributed to the entrapment of the formulation components in the tortuous pores through which the drug must migrate. A similar observation was reported for the release of loratadine from self-emulsifying formulations carried by porous polystyrene beads (Sharma et al. 2005; Patil et al. 2006).

Based on the findings mentioned above, a bilayer tablet design composed with the first layer containing Fujicalin and the second layer containing Neusilin UFL2 (Table 3) was prepared. This bilayer system could provide practically pH-independent dissolution of CDC, thereby facilitating the opportunity of drug dissolution regardless of the physiological pH. Theoretically, pH-independent release is considered to be desirable for more consistent *in vivo* delivery because pH values in the GI tract vary with location and food intake (Qui et al. 2009). As shown in Fig. 6, at all pH values, the cumulative amount of CDC dissolved from the bilayer SMET containing Fujicalin and Neusilin UFL2 approached 75 % within 15 min. The release study clearly revealed pH-independent CDC release profiles from Fujicalin and Neusilin UFL2-based bilayer SMET. This result may stem from the fact that the Fujicalin- and Neusilin UFL2-based SMET in bilayered SMETs caused the immediate release of CDC at pH 1.2 and pH 6.5, respectively. Thus, to obtain rapid release of CDC throughout pH variation from pH 1.2 to 6.5, combination of Fujicalin- and Neusilin UFL2-based SMETs was necessary since low CDC release in pH 1.2 and pH 6.5 could be enhanced by Neusilin UFL2- and Fujicalin-based SMET portion, respectively.

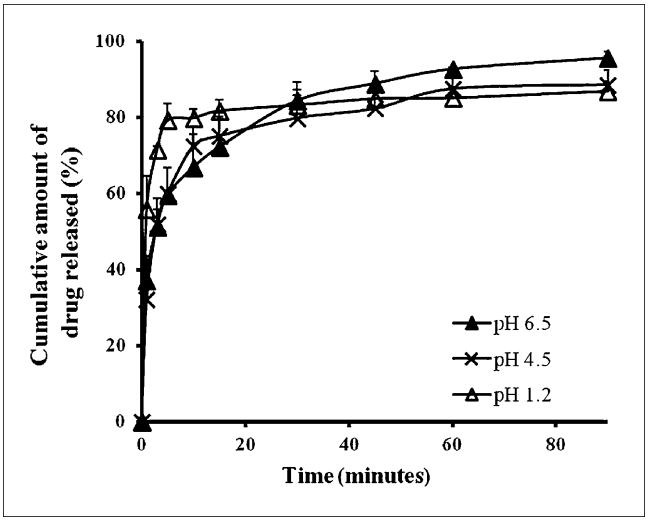


Fig. 6: Dissolution profiles of candesartan cilexetil in self-microemulsifying bi-layer tablets at different conditions (pH 1.2, pH 4.5, and pH 6.5) containing 0.35% Tween 20. Data are expressed as mean ± SD (n = 3)

2.5.3. Size of droplets formed from SMETs

The average diameter of the microemulsion droplets formed from liquid SMEDDS and SMETs are presented in Fig. 7. The droplet sizes of the microemulsions formed from liquid SMEDDS and bi-layer SMETs were 125.4 nm and 158.1 nm in pH 1.2, and 70.8 nm and 181.5 nm in pH 6.5, respectively. The particle sizes produced from bi-layered SMETs were less than 200 nm. This indicates that a proper microemulsion system could be formed from the SMETs. For liquid SMEDDS, the mean droplet sizes of microemulsion obtained from release experiments increased in pH 1.2 or slightly decreased in pH 6.5 compared to those presented in Table 2 (Formulation 1). The reason for this may be due to the difference in measuring conditions. The reason for greater droplet sizes observed with SMET formulations may be due largely to reduced dispersibility by physical attraction between oil, surfactant and carrier materials. The size of microemulsion droplets produced by Fujicalin-based SMET at pH 6.5 was greater than 500 nm. The reason for this may be due to insoluble property of Fujicalin.

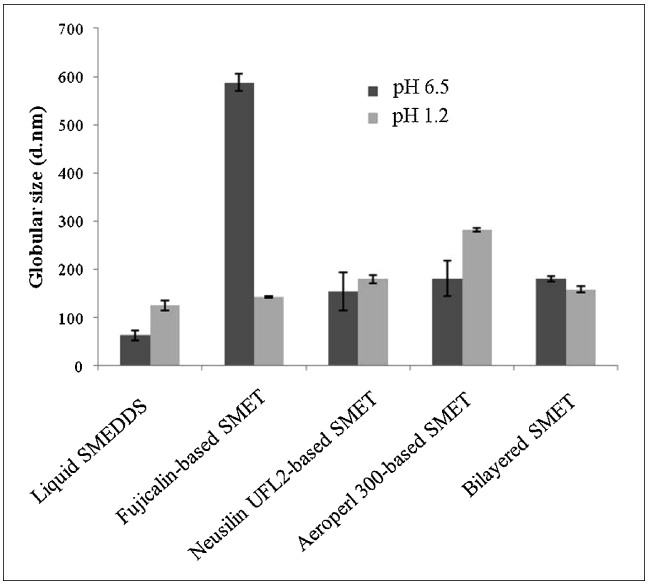


Fig. 7: Droplet sizes of microemulsions formed from self-microemulsifying tablets. Mean ± SD (n = 3)

2.6. Conclusions

In this study, self-microemulsifying bi-layer tablets for pH-independent fast release of CDC were successfully prepared by adsorbing liquid SMEDDS onto solid carriers and using selected excipients. The optimized bilayer tablet consisted of one layer of Fujicalin based- and the other layer of Neusilin UFL2-based SMET; we demonstrated pH-independent fast formation of microemulsions leading to increased release of CDC. This effect was derived from the different properties of the solid carriers. Fujicalin layers provided the immediate release of drug which readily dissolves in an acidic medium and Neusilin UFL2 played a critical role in increasing CDC release at pH 6.5 due to the large surface area. To conclude, CDC release was greatly improved by SMEDDS and drug-incorporated SMEDDS could be formulated into bi-layer tablets for pH-independent immediate release which would ultimately result in overcoming low oral bioavailability of CDC due to its limited solubility in physiological pHs of the gastrointestinal tract.

3. Experimental

3.1. Materials

CDC was a kind gift from Whanin Pharmaceutical Company (Seoul, Korea). Miglyol 810, Miglyol 812, Labrafac CC, Labrafil 1944 CS, Labrafil 2125 CS, Capryol 90, Labrafac PG, Labrafac lipophilic WL1349, Peceol, Lauroglycol 90, Labrasol, Maisine 35–1 and Transcutol P were gifts from Masung Co., LTD (Seoul, Korea) and Cremophor EL, Myvacet 9–45, and Polyplasdone XL were supplied by BASF (Seoul, Korea). Tween 80 and tetraglycol were purchased from Sigma-Aldrich Company (St. Louis, MO). Propylene carbonate, Tween 20, sodium chloride, sodium acetate anhydrous, acetic acid glacial, and ethyl alcohol were purchased from Dae Jung Chemicals (Seoul, Korea). Triacetin, PEG300, PEG400, potassium phosphate monobasic, sodium phosphate monobasic, sodium phosphate dibasic, and magnesium stearate were from Duksan Chemicals (Seoul, Korea). Fujicalin, Neusilin UFL2, mannitol (USP grade), Pearitol, and Flowlac were kindly supplied by Samjin Pharmaceutical Company (Seoul, Korea) and Aeroperl 300 was purchased from Evonik Degussa Korea LTD. (Seoul, Korea). HPLC grade acetonitrile and methanol were purchased by J. T. Baker (Phillipsburg, NJ). Distilled and deionized water was used for the preparation of all solutions.

3.2. Solubility studies for CDC

To select suitable oil and surfactant as compositions of SMEDDS, the solubilities of CDC in various oils, surfactants and cosolvents were measured. An excess amount of CDC (approximately 250 mg) was added to 1 mL of each vehicle placed in a glass vial. After capping the vial, the resulting mixture was vortexed for 5 min and then the mixture was stirred with a magnetic stir bar under the constant rate for 48 h at 25 °C, followed by centrifugation for 15 min at 15,000 rpm to remove the undissolved drug. The supernatant was taken and diluted with the mobile phase appropriately and the drug concentration was determined by (HPLC). Oil, surfactant and cosolvent that showed higher solubility for CDC were selected as the compositions of SMEDDS.

3.3. Pseudoternary phase diagram and formulation of CDC-loaded SMEDDS

On the basis of the solubility study presented in Table 1, Capryol 90 was selected as oil, while Tween 80 and tetraglycol were used as surfactant and cosolvent, respectively. To determine appropriate ratios of these three components, existence of self-emulsifying fields that can be microemulsified under dilution and gentle agitation was identified from a ternary phase diagram. One milliliter of the mixtures of oil, surfactant and cosolvent containing 50 mg of CDC at certain weight ratios was introduced into a fixed volume of water (100 mL) in a glass beaker at 37 °C and the contents were mixed by magnetic stirring. The tendency to form a microemulsion was evaluated visually for phase appearance (Nakajima 1997).

3.4. Preparation of CDC SMEDDS

In accordance with the microemulsion domain in the phase diagram, a series of self-microemulsifying systems were prepared with varying concentrations of oil, surfactant, and cosolvent. In all formulations, the concentration of CDC was constant (i.e. 50 mg/mL). The mixture was sealed in a capped

Table 4: Classification of the SMEDDS formulation in accordance to comparative grades (Singh et al. 2008)

Grade	Dispersibility and appearance	Time of self-ME*
I	Rapid forming microemulsion, which is clear or slightly bluish in appearance	< 1 min
II	Rapid forming, slight less clear emulsion, which has a bluish white appearance	< 2 min
III	Bright white emulsion (similar to milk in appearance)	< 2 min
IV	Dull, grayish white emulsion with a slight oily appearance that is slow to emulsify	> 3 min
V	Exhibit poor or minimal emulsification with large oils droplets present on the surface	> 3 min

* ME: microemulsification

glass vial and stored at room temperature until subjected to further characterization. Detailed compositions of SMEDDS formulations are summarized in Table 2.

3.5. Characterization of SMEDDS formulations

3.5.1. Dispersibility test

To evaluate the efficiency of self-emulsification and appearance of the microemulsion dispersibility, a study was performed using a USP dissolution tester with dissolution apparatus 2. The dispersibility was observed in terms of the five grading systems and self-emulsification time shown in Table 4 (Singh et al. 2008). One milliliter of SMEDDS formulation selected from the phase diagram was added to 500 mL of water at $37 \pm 0.5\,^{\circ}C$ and a standard dissolution paddle set to rotate at the constant speed of 50 rpm provided gentle agitation.

3.5.2. Emulsion droplet size and zeta potential

A 0.2 g of SMEDDS formulation containing drug was dispersed in 100 mL purified water, 0.1 mol/L HCl (pH 1.2) and pH 6.5 phosphate buffer, at $37 \pm 0.5\,^{\circ}C$. The resulting microemulsions were prepared by gentle agitation for 20 min using a magnetic stirrer. One milliliter of the medium was taken and centrifuged at 12,000 rpm for 10 min to remove undissolved components. The droplet size and zeta potential of the microemulsion formed from SMEDDS were determined by using a Zetasizer Nano ZS (Malvern Instruments, UK) at a wavelength of 635 nm and a scattering angle of 90° at 25 °C. Zetasizer Nano ZS measures the diffusion coefficient of particles moving under Brownian motion, and converts the measured diffusion coefficient into a hydrodynamic diameter which is the size of a sphere that has the same diffusion behavior.

3.5.3. Saturated solubility of CDC in SMEDDS formulations

To measure saturated solubility of the drug in SMEDDS formulations, excess amount of CDC was added to 1 mL of each SMEDDS formulation and levels of the drug in SMEDDS were evaluated by the procedure described in the solubility study.

3.6. Preparation of self-microemulsifying tablet (SMET)

The SMETs were prepared in three steps by adsorbing liquid SMEDDS to porous solid carriers, blending powdered SMEDDS with pharmaceutical excipients and compressing them into tablets.

3.6.1. Powdering of SMEDDS using adsorbents

Free-flowing powder forms of SMEDDS (solid SMEDDS) were obtained by mixing liquid SMEDDS with solid carriers. In this experiment, three inorganic carriers, Fujicalin®, Neusilin® UFL2 and Aeroperl® 300, were selected as adsorbents to load SMEDDS with CDC. The SMEDDS formulation containing 8 mg of CDC (i.e. 0.123 mL of SMEDDS) was blended with the appropriate amounts of adsorbents using mortar and pestle for 10 min. The tablet weight of SMET formulations was 500 mg. The weight of 0.123 mL of SMEDDS loaded with 8 mg of CDC measured to be 123 mg. Thus, the SMEDDS concentration in SMETs was calculated to be 24.6%. To confirm whether the liquid SMEDDS was efficiently held by these carriers, the microscopic structure of the SMEDDS-retaining solid carriers was observed by scanning electron microscope (SEM, Hitachi, Japan).

3.6.2. Mixing of solid SMEDDS with excipients

The solidified SMEDDS were blended with a diluent for 5 min. Pearitol was selected as a diluent of the SMETs since it resulted in good compressibility compared to Flowlac or conventional mannitol tested in this study (data not shown). A disintegrant (Polyplasdone) and lubricant (magnesium stearate) were also added to the powder mixtures before direct compression. Four compositions of the SMETs tested are described in Table 3.

3.6.3. Compression of the mixtures composing SMETs

SMETs were prepared by compressing the tablet mixture between flat-faced 13 mm plates equipped in Riken Hydraulic Press (Model P-16B-027, Riken Seiki Co., Ltd., Tokyo, Japan) under a 200 kgf/cm^2 compaction force. Only one tablet portion of the tablet mixture was introduced manually into the die to obtain uniformity of tablet weight and drug content. The mechanical strength of the tablets was measured by using a manual hardness tester (Yita Seisakusho Ltd., Japan). Disintegration of SMETs in water was evaluated using a USP disintegration test apparatus (KDIT-200, Kuk Je Engineering Co., Seoul, Korea).

3.7. In vitro CDC release study

In vitro drug release studies were carried out on bulk powder of CDC, liquid SMEDDS with CDC, and SMETs using a USP Type 2 dissolution test apparatus (DST-600A, Fine Scientific Instruments, Korea). In this study, two types of the dissolution medium were used including simulated gastric juice with 0.5% Tween 20 (pH 1.2) and 0.05 M phosphate buffer containing 0.35% Tween 20 (pH 6.5). The volume of dissolution medium was 900 mL maintained at 37 °C with a paddle speed set at 50 rpm. A 0.5% of Tween 20 was added to 900 mL of pH 1.2 medium. This means that 900 mL of pH 1.2 medium contains 4.5 g of Tween 20. Based on CDC solubility in 1 mL Tween 20 (i.e. ~ 31 mg/mL as shown in Table 1), roughly 4.5 g of Tween 20 can solubilize around 140 mg of CDC. Likewise, the pH 6.5 medium containing 0.35% Tween 20 should solubilize around 98 mg of CDC. Since the dose of CDC was 8 mg in all formulations for dissolution experiments, the saturation solubility would be 8 mg/900 mL. Due to the solubilizing capacities increased by the addition of Tween 20, a good sink condition should be maintained. All SMET formulations tested in this study (Table 4), along with CDC bulk powder and liquid SMEDDS loaded with CDC contained 8 mg of CDC. Aliquots were withdrawn periodically and replaced with fresh and pre-warmed dissolution medium. The collected samples were centrifuged at 12,000 rpm for 10 min and the supernatants were used to assay CDC by HPLC. The cumulative amount of CDC released from each preparation was obtained by the following equation (Turgeon 1998):

$$\text{Cumulative amount released (\%)} = \sum_{t=0}^{t} \frac{M_t}{M_{total}} \times 100$$

where M_t is the amount of CDC released at time t, and M_{total} is the total amount of CDC initially loaded.
After the *in vitro* dissolution test, sizes of microemulsion particles formed from SMEDDS and SMETs were determined by Zetasizer.

3.8. HPLC assay

Levels of CDC were measured with a Younglin YL9100 HPLC system (Younglin Instrument Co., LTD, Anyang, Korea) and mobile phase composed of 10 mM potassium dihydrogen phosphate: methanol: acetonitrile (2:80:18 v/v/v) at a flow rate of 1.0 mL/min. The drug was separated on Capcell Pak column (150 mm x 4.6 mm, 5 μm, Shiseido, Tokyo, Japan) maintained at 25 °C. The detection wavelength was set at 260 nm.

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