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Polymeric nanocapsules with SEDDS oil-core for the controlled and enhanced oral absorption of cyclosporine

Min-Jeong Park¹, Prabagar Balakrishnan¹, Su-Geun Yang*

Department of Drug Development and UNIDDS, School of Medicine, Inha University, 8F A-dong, Jeongseok Bldg., Sinheung-dong 3-ga, Jung-gu, Incheon 400-712, South Korea

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ABSTRACT

Self-microemulsifying drug delivery system (SEDDS) cored-polymeric nanocapsules (NC) were fabricated using emulsion diffusion method for the controlled oral absorption of the poorly water soluble drug, cyclosporine. Poly-DL-lactide (PDLLA) was used as the shell-forming polymer. The NCs in different polymer/oil ratios (from 25/125 to 125/125) were prepared following a solvent-diffusion method. Especially, the SEDDS oil-core compositions, which can form microemulsions on dispersion, were selected based on a pseudo-phase diagram study and further optimized based on the solubility and permeability studies. The prepared NCs were with a mean diameter of 150–220 nm and 9.4–4.5% w/w drug loading. In vivo study in rats showed that the optimized NC(50/125) and NC(100/125) released the drug in controlled way as well as enhanced the bioavailability significantly with AUC_{0–24h} values of 14880.3 ± 1470.6 and 12657.8 ± 754.5 ng h/ml, respectively, compared to that of SEDDS-core solution (9878.9 ± 409.6 ng h/ml). Moreover it was observed that the NCs maintained blood concentration of cyclosporine (>500 ng/ml) for 14–20 h but in the case of control formulation it was only 7.33 h. Our results suggest that the prepared NCs could be a potential carrier for the oral controlled release formulation of cyclosporine.

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1. Introduction

Cyclosporine is an immunosuppressant drug and widely administered in post-allogenic organ transplant to reduce the activity of immune system and therefore the risk of organ rejection. However, cyclosporine shows low oral bioavailability due to its low water solubility (0.02 mg/ml at 25 °C) (Yang et al., 2006a). Clinically, cyclosporine is available in microemulsion formulation, which increases its oral bioavailability (Yang et al., 2006a). However, microemulsions do not provide the possibility of prolonged release, an initial plasma peak of above 1000 ng/ml occurs in human cases. This initial peak has been reported for potential nephrotoxicity (Muller et al., 2000). Therefore, an alternative approach is required to obtain the same reproducible blood profile as the microemulsion and controlled release of cyclosporine to achieve an effective treatment with cyclosporine.

Nanocapsules are nano-sized carrier systems, which are composed of an oily or aqueous core contained by a thin polymer skin (Mayer, 2005). Nanocapsules act as 'reservoirs' in which different active ingredients can be encapsulated, such as, drugs (Ourique

et al., 2008), nucleotides (Bouclier et al., 2008), cells (Krol et al., 2004), imaging agents (Plush et al., 2009; Sharma et al., 2010) and proteins (Morille et al., 2010). Nanocapsules display advantageous as an active substance carrier as they protect the encapsulated drugs from the exterior environment and facilitate their controlled release (Dowding et al., 2005). Nanocapsules designed to have hollow or water-core can entrap hydrophilic drugs with high entrapment efficiency (Yang et al., 2008), whereas oil-cored nanocapsules can be used to encapsulate hydrophobic agents (Hureau et al., 2010). The most remarkable feature of nanocapsules is that they can be utilized "to administer both hydrophilic and hydrophobic drugs at high drug loading efficiency" (Mora-Huertas et al., 2010).

Several methods have been reported to prepare nanocapsules, such as nanoprecipitation, emulsion-diffusion, interfacial-polymerization, and polymer-coating (Mora-Huertas et al., 2010). Among them, interfacial polymerization method using alkyl-cyanoacrylate monomers is one of the fastest methods for the preparation of nanocapsules and is readily scalable (al Khouri et al., 1986). Instantaneous polymerization on the surfaces of oil/water or water/oil (o/w or w/o) emulsions can be used to entrap drugs with high loading efficiency (Couvreur et al., 2002). Poly(isobutylcyanoacrylate) nanocapsules for the oral application of insulin demonstrated a high entrapment efficiency (>80%) with good resistance against acidic media (Aboubakar et al., 1999; Mesiha et al., 2005). Oil-cored poly(alkylcyanoacrylate) nanocapsules are viewed as a potential carrier for hydrophobic drugs, such

* Corresponding author at: Department of Drug Development and NCEED, School of Medicine, Inha University, South Korea. Tel.: +82 32 890 1183; fax: +82 32 859 1199.

E-mail addresses: Sugeun.Yang@Inha.ac.kr, orosyang@gmail.com (S.-G. Yang).

¹ Authors contributed equally.

as, lipiodol (Damge et al., 2000), indomethacin (Miyazaki et al., 2003), azidothymidine (Hillaireau et al., 2006). Interfacial polymerization allows the formation of well-defined capsular structures and high drug loading efficiencies for a range of drugs. However, unwanted reactions between monomers and drug molecules hampered the development of poly(alkylcyanoacrylate) nanocapsules (Couvreur et al., 2002).

A research group led by Briancon has developed an emulsion–diffusion method based on pre-formed, biodegradable polymers that overcomes this problem (Guinebreteire et al., 2002). In this method, metastable emulsion known as “quasi-emulsion” is preformed with water and partially water-miscible solvents, such as ethyl acetate. Lipophilic drugs, oil and polymer were dissolved together into ethyl acetate and formed the internal oil phase and this metastable emulsion is broken down by adding water, which induces the instantaneous diffusion-out of ethyl acetate from the internal phase and the disposition of polymer at the interface of emulsion to form a capsular wall (Fig. 3A) (Mora-Huertas et al., 2010). Medium chain triglycerides (MCT) can be used as the core-forming oil (Guterres et al., 2000), and poly-DL-lactic acid, Eudragit, and poly(ϵ -caprolactone) as the shell-forming polymer (Mora-Huertas et al., 2010). Poloxamer and polyvinyl alcohol (PVA) have both been employed as quasi-emulsion stabilizer, though they must be removed subsequently by a centrifuge process (Nehilla et al., 2008).

In this study, SEDDS cored-polymeric nanocapsules were prepared for the oral delivery of cyclosporine. Basically, poly-DL-lactide (PDLLA) was selected as a capsule-forming polymer. SEDDS oil-core was composited based on the design method of self-micro emulsifying drug delivery system (SEDDS); tertiary phase diagram (Hirunpanich and Sato, 2009), solubilization capacity and membrane permeability (Liu et al., 2003). The prepared nanocapsules with the SEDDS oil-core were characterized in terms of its particle size, entrapment efficiency and density. The in vivo absorption characteristics of the prepared NCs were examined in rats.

2. Materials and methods

2.1. Materials

Poly-DL-lactide (PDLLA, Lactel®, inherent viscosity; 0.26–0.54 dl/g) was purchased from Durect Co. (Pelham, AL). Cyclosporine was kindly supplied by CKD Pharm. Co. (Seoul, Korea). Polyoxyl 40 hydrogenated castor oil 40 (CreRH40, Cremophor RH 40®) was purchased from BASF (Ludwigshafen, Germany). Medium chain mono-diglyceride (Capmul® MCM) was purchased from Arbutec Co. (Columbus, OH). Medium chain triglycerides (MCT), Tween 20 (Tw20) and triacetin (TA) were purchased from Sigma–Aldrich Co. (St. Louis, MO). All other reagents and solvents used were of pharmaceutical grade.

2.2. Determination of SEDDS oil-core composition

2.2.1. Pseudo-ternary phase diagram

The SEDDS-core composition was screened via a pseudo-ternary phase diagram study which allows getting the most appropriate compositions for the enhanced bioavailability of water-insoluble drugs (Hirunpanich and Sato, 2009). In our earlier study we found that MCT oil alone, which is generally used as core forming oil, is not suitable for solubilization and oral absorption enhancement of cyclosporine (Yang, 2006). Therefore, the composition of SEDDS core was screened via phase-diagram study. A mixture of MCT and MCM was selected as the oil (O_m), and CreRH40, Tw20, and TA was employed as the surfactant (S_m). The effect of surfactant mixture on the pseudo-ternary phase diagram was systematically observed

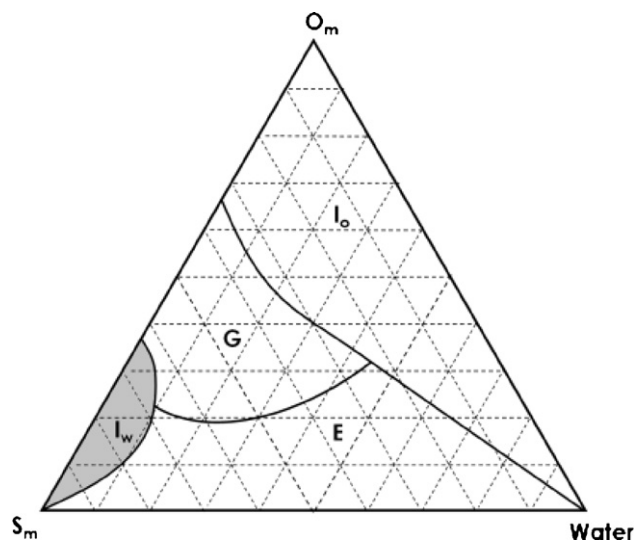


Fig. 1. Ternary-phase diagram of oil (O_m , 2.5:1 mixture of MCT and MCM), surfactant (S_m , 2:1:1 mixture of CreRH40, Tw20 and TA) and water. Each phase was classified as isotropic oil phase (I_o), isotropic aqueous phase (I_w), gel phase (G) and o/w emulsion phase (E). SEDDS-core compositions (I–VI) were determined from the isotropic aqueous region (I_w), and named as followings; composition I (●), composition II (○), composition III (▼), composition IV (△) and composition V (■).

at room temperature. The oil phase (O_m) and surfactant mixture (S_m) were mixed at varying weight ratios (i.e. 9:1, 8:2, 7:3, 6:4, 5:5, 4:6, 3:7, 2:8, or 1:9 (w/w)) using a vortex mixer and then titrated with water, mixed again, and allowed to stand overnight at room temperature. Phases in each mixture were then examined. Compositional ratios of O_m , S_m and water were observed on the area of isotropic oil region (Figs. 1 and 2A) and selected as the SEDDS core composition to further optimize for the preparation of NCs (Table 1).

2.2.2. Solubilization capacity of the SEDDS oil-core

The solubility study was performed in the mixture of oil mixture and surfactant mixture at different ratio as described in Table 1. The solubility study in the SEDDS core composition (oil and surfactant mixture, SEDDS) of NCs was determined according to earlier reported study (Yang, 2006). Briefly, 300–400 mg of cyclosporine was added into 1 g of each oil mixture in a round-bottom polyethylene tube, probe-sonicated (Sonic dismembrator, Fisher Scientific, PA, USA) for 1 min, stored for 2 weeks at room temperature and centrifuged at $15,000 \times g$ for 15 min. Supernatants were filtered through 0.45 μm filters and analyzed by high performance liquid chromatography (HPLC) after appropriate dilution with a mixture of acetone and methanol at 1:1 ratio (v/v) (Yang et al., 2006b). The HPLC analysis was performed using Waters HPLC system (Alliance 2610, Waters, MA, USA) equipped with a reverse phase C_{18} column (Capcellpak® UG120, Shiseido, Japan) at 70 °C temperature. The wavelength of the UV detector was set at 210 nm. Mixture of acetonitrile, methanol and (pH 2.5) phosphate buffer solution (65:15:20, v/v) was used as the mobile phase at a flow rate of 1.0 ml/min.

Table 1
SEDDS oil-core compositions.

Formula no.	I	II	III	IV	V
Oil ^a :Surfactant ^b	1.2:4	1.4:4	1.75:4	2.3:4	3:4

^a Mixture of MCT and MCM.

^b Mixture of CreRH40, Tw20 and TA.

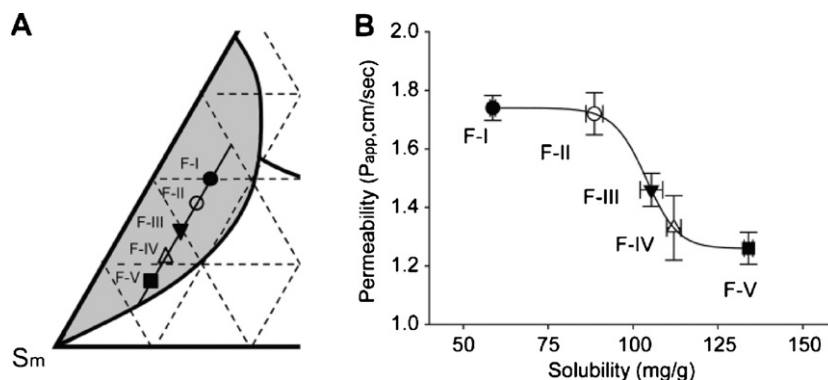


Fig. 2. Elicitation of SEDDS-core composition. (A) Compositions of SEDDS-core were extracted based on the mid points of I_w region. (B) Solubility and permeability (cm/s) profiles of cyclosporine in different core compositions. F-III with solubility over 100 mg/ml and permeability over 1.4 cm/s was selected for the SEDDS-core composition.

2.2.3. In vitro permeability study

In vitro permeability of the SEDDS core compositions was performed by Parallel artificial membrane permeability assay (PAMPA). The passive permeability of cyclosporine from the different SEDDS-core composition was estimated using an artificial membrane (Liu et al., 2003). Hydrophobic Immobilon-P filters (pore size 0.45 μ m) in a 96-well plate (Millipore, Bedford, MA, USA) were impregnated with 5 μ l of an artificial membrane solution (5% egg lecithin in *n*-dodecane) to form a lipid bilayer on the filters. The filter plate was then placed on a polystyrene 96-well reservoir plate (Whatman, Clifton, NJ, USA) that had been pre-filled with Hanks Balanced Salt Solution buffered with 25 mM HEPES to pH 7.4 (HBSS buffer). 10 mg of the SEDDS-core formulas (Table 1), containing 1 mg of cyclosporine and 2 μ Ci of 3 H-labeled cyclosporine, was dispersed in HBSS buffer. 5 mM of 14 C-mannitol (5 μ Ci equivalents) was then added and the mixtures were placed on the donor side and incubated at room temperature. After 120 min of formulation placement, aliquots (100 μ l) of the sample (HBSS buffer) from the receiver compartment were taken. Radioactivity of the taken samples (3 H and 14 C) was determined using a liquid scintillation counter (LSC, System 1400, Wallac, Finland). The apparent permeability (P_{app}) of cyclosporine was determined by dividing the rates of cyclosporine passage through the filters by the initial concentration of cyclosporine added in the donor compartment.

2.3. Preparation of cyclosporine NCs

NCs were prepared by emulsion–diffusion method reported earlier with slight modifications (Guinebreteire et al., 2002). To optimize the polymer ratio, NCs were prepared using different amount of polymer. Briefly, 25–100 mg of PDLLA and 125 mg of SEDDS core-forming (oil and surfactant mixture, SEDDS) mixture (Table 2) were dissolved in 5 ml of water-saturated with ethyl acetate in a glass vial. This solution was emulsified with 10 ml of aqueous phase saturated with ethyl acetate and probe-sonicated for 10 s to form a quasi-emulsion (Sonic dismembrator, Fisher Scientific, PA, USA). 50 ml of water was then added to the emulsion to diffusion-out ethyl acetate from oil droplets and polymer disposition at interface between water and oil phase. The ethyl acetate was removed under reduced pressure. The free cyclosporine was removed by ultrafiltration (Vivaspin® 100 kDa, Sartorius S.B. GmbH, Germany) at 2500 \times g for 30 min. The prepared NCs were named as shown in Table 2.

2.4. Characterization of cyclosporine NCs

2.4.1. Transmission electron microscopy

The morphology of the NCs was observed under an Energy-Filtering Transmission electron microscopy (TEM 1010, JEOL,

Tyoko) operating at 200 kV accelerating voltage. NCs in suspension were dropped onto a carbon-film-coated 200-mesh copper grids (Electron Microscopy Sciences, Hatfield, PA, USA), which were rendered hydrophilic by glow-discharge for 20 s and negatively stained with a drop of 2% phosphotungstic acid solution. This negative staining reveals NCs as white particles in a dark surrounding on TEM images.

2.4.2. Particles size and its stability (DLS) analysis

Particle size and polydispersity of the prepared NCs were determined by dynamic light scattering method using a DLS-7000 (Otsuka Electronics, Japan) fitted with a 488 nm argon laser at a fixed angle of 90°. NCs were diluted with distilled water and measurements were performed at 25 °C.

Particle size stability was analyzed after diluting the concentrated NCs suspension 500-fold in water, phosphate buffer and 0.1 N HCl over a period of 8 h. The samples were kept at 100 rpm stirring.

2.4.3. Determination of NC densities

The density of NCs was determined by isopycnic centrifugation in a density gradient of colloidal silica (Percoll®, Pharmacia LKB, Sweden). NCs fabricated at different ratios of polymer to oil were added to 7 ml of Percoll® 45% (v/v) containing 0.15 M NaCl and centrifuged to generate density gradients using a 25° angle head rotor (Optima L-100 XP, Beckman-Coulter, CA) at 20,800 \times g for 90 min. Density marker beads (Pharmacia LKB, Sweden) were used as external standards and treated in the same manner as experimental samples. Heights of the bands were measured from the meniscus to band centers using a Vernier Caliper. The density of NCs was calculated using a standard curve obtained using the standard marker beads. The density of SEDDS-core solution and polymer-shell were separately estimated and used as controls.

2.4.4. Drug loading and entrapment efficiency

The NCs pellets, obtained after centrifugation at 13,000 \times g for 10 min and dried under nitrogen, was weighed and dissolved in dichloromethane, vortexed for 1 min, filtered through a 0.22 μ m nylon membrane filter and subjected to HPLC analysis to assess the drug concentration. The entrapment efficiency (EE) was calculated as the mass ratio of the entrapped drug in NC compared to the amount used in their preparation using the following equation:

$$EE(\%) = \frac{\text{weight of cyclosporine in nancapsules}}{\text{weight of cyclosporine fed initially}} \times 100$$

Table 2
Characteristics of PDLLA nanocapsules obtained from different ratio of polymer shell and SEDDS oil-core.

Nanocapsules	Shell/Core ratio	Mean diameter (nm)	PI ($\times 10^{-1}$) ^a	DLC (%)	EE (%)
NC(25/125) ^b	0.2	159	2.24	9.4 \pm 0.4	91.7 \pm 3.6
NC(50/125)	0.47	202	2.01	6.6 \pm 0.8	80.7 \pm 7.7
NC(75/125)	0.6	216	1.83	5.5 \pm 0.4	77.2 \pm 4.0
NC(100/125)	0.8	220	1.85	4.5 \pm 0.5	72.4 \pm 4.9

^a PI, polydispersity index.

^b 25/125 represents 25 mg of PDLLA and 125 mg of oil-core.

Drug loading content (DLC) was calculated using the following equation:

$$\text{DLC}(\%) = \frac{\text{weight of cyclosporine in nanocapsules}}{\text{weight of nanocapsules}} \times 100$$

2.5. In vivo oral absorption of cyclosporine

Sprague-Dawley rats weighing 200–250 g were purchased from Daehan-Biolink Co. (Chungbuk, South Korea). Rats were housed under scheduled lighting and provided with rodent chow (Purina, South Korea) and water. Animal experiments were performed in accordance with the guidelines and protocols approved by the Institute of Laboratory Animal Resource, School of Medicine, Inha University. NCs and SEDDS oil-core were individually administered orally using oral gavage at a dose of cyclosporine 5 mg/kg under 12 h fasting conditions. SEDDS-core formula (F-III, Table 1) was dispersed in appropriated amount of water, which forms o/w microemulsions, before administration. Approximately 0.2 ml of blood was drawn from a jugular artery cannula in EDTA tubes at predetermined time intervals (0, 0.5, 1, 1.5, 2, 4, 6, 8, 10, and 24 h) and the same volume of saline was supplied via a jugular vein cannula. Cyclosporine concentration in the whole blood was measured using CYCLO-Trac SP[®] RIA kits (DiaSorin Inc., MN, USA); a double-antibody competitive-binding assay.

3. Results and discussion

3.1. SEDDS oil-core compositions of NCs

Pseudo-ternary phase diagram was constructed in the absence of cyclosporine to optimize the SEDDS oil-core composition for the preparation of NCs. The phase diagram of the system containing the mixture of MCT and MCM as oil (O_m) and the mixture of CreRH40, Tw20, and TA as surfactant (S_m) is shown in Fig. 1. Four distinctive phases, an isotropic oil phase (I_o), an isotropic aqueous phase (I_w), a gel phase (G), and an o/w emulsion phase (E) were observed in the phase diagram. SEDDS-core compositions (SEDDS) were determined from the isotropic aqueous region, which implies the formation of thermodynamically stable emulsions (Patel and Sawant, 2009). Several lines at different O_m to S_m ratios were drawn from left vertex (S_m) of diagram to the opposite side and SEDDS oil-core compositions were derived based on intersections between these lines and a line crossing in the middle of the isotropic aqueous region, as shown in Fig. 2A. The derived compositions are summarized in Table 1. All these compositions formed microemulsions in water and 0.1 N HCl with a particle size of less than 100 nm.

The solubility profile of cyclosporine on the selected SEDDS core composition and the permeability profile of the selected SEDDS core compositions are reported in Fig. 2A. The solubility study was performed keeping the surfactant ratio constant with increased oil ratio in the tested SEDDS core composition formulas (Table 1). It was observed that increasing the oil ratio resulted in significantly higher drug solubility of the SEDDS core compositions. Though, high oil ratio showed higher solubility for the drug and that would

be a favorable SEDDS core for the preparation of the NCs, we may need to consider their drug permeability profile, since high oil ratio in the core may affect the permeation of drug in vivo from the NCs. Hence, in vitro permeability study using PAMPA assay was performed to optimize the SEDDS core composition, which provides good solubility and permeability profile to prepare nanocapsules. The P_{app} of cyclosporine for the SEDDS core compositions ranged from 1.19×10^{-6} to 1.74×10^{-6} cm/s, and that of ¹⁴C-mannitol was $1.52 \times 10^{-7} \pm 6.99 \times 10^{-9}$ cm/s indicating that the artificial membrane was tight enough to estimate the permeability of cyclosporine. It was observed that with increased oil content in the SEDDS core composition permeability was significantly decreased. Formula III (F-III) was selected as the SEDDS-core for further studies, as it showed P_{app} value of 1.46×10^{-6} cm/s, which showed good drug solubilization capacity (over 100 mg/g) and comparable permeability.

3.2. Formation of SEDDS oil-core/polymer-shell structured NCs

The NCs were prepared by emulsion diffusion method. The representative TEM photomicrographs of the prepared NC (50/125) showed a well defined SEDDS core-polymer shell capsular structure (Fig. 3B and Fig. S1). Fig. S1 shows a well-defined core-shell particle structure. An oil droplet was observed inner side of the polymer wall, which could be due to the free oil from the break-up of a certain number of NCs during the sample preparation or by the TEM work-up. However, its presence strongly suggests that the capsule core consists of oil.

The results of the density gradient study shown in Fig. 4 suggest the formation of core-shell NCs by emulsion diffusion method. The density of the set of NCs prepared with different ratio of SEDDS-core/polymer mixture had a density intermediate between those of SEDDS-core and polymer-shell (Fig. 5).

Similar results were reported for Mygliol/PLA NCs prepared by emulsion diffusion method (Quintanar-Guerrero et al., 1998). A single buoyant band observed in the prepared NCs suggested the formation of nanocapsules in the SEDDS-core/polymer ratios used (Table 2). Moreover, it was observed that the density of the NCs increased with the increased polymer ratio.

Furthermore, a fainter and wider band of particles was observed at low polymer to oil ratio, which suggests a heterogenic size distribution of NCs at low polymer levels. The formation of NCs could be considered as a two-step process (Fig. 3A); first construction of a quasi-emulsion and the second is diffusion of solvent from the emulsion, which leads to the disposition of polymer on the surface of emulsion particles. Quasi-emulsion was prepared by emulsifying a partially water-miscible solvent (ethyl acetate) in water. In the present study, probe-sonication rather than homogenization was used to produce quasi-emulsions and this method proved suitable in terms of obtaining homogeneous, small NCs (Table 2). The subsequent addition of water to quasi-emulsions broke down this equilibrium and triggered diffusion-out of ethyl acetate from the emulsion droplets to water. The diffusion-out velocities of each component are differentiated by their affinities to the solvents (ethyl acetate and water) and cause the formation of the SEDDS

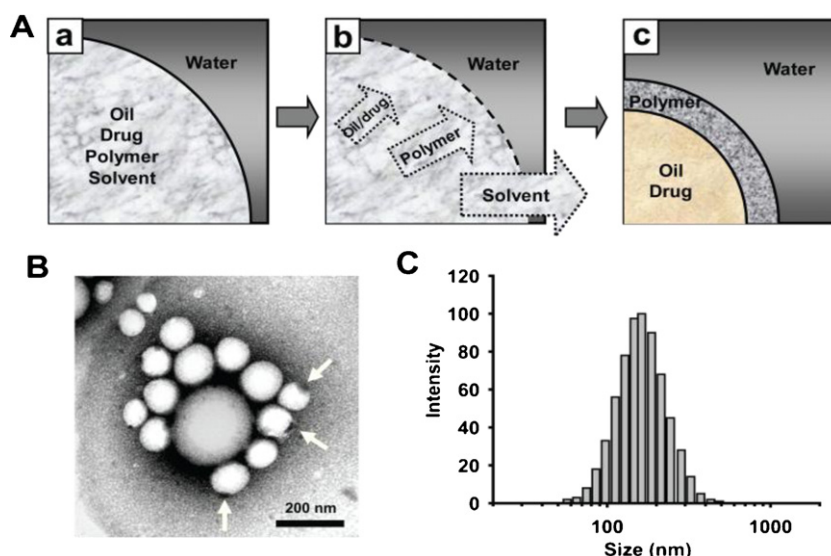


Fig. 3. (A) Schematic diagram for the formation mechanism of SEDDS core-polymer shell nanocapsules made by quasi-emulsion diffusion method, (a) metastable quasi-emulsion composed of internal oil phase (ethyl acetate) which is dissolving oil, drug and polymer, and external water phase, (b) instantaneous diffusion-out of ethyl acetate after applying additional water to the quasi-emulsion, and (c) formation of polymer wall on the interface. (B) Representative TEM image of core-shell nanocapsules, image was taken after negative staining with 2% phosphotungstic acid. Arrow indicates the leaked oil droplets. (C) Particle size distribution of NC (25/125).

core-polymer shell structure. In this process, there could be loss of the components of SEDDS core to water particularly surfactants as the drug encapsulation and efficiency is lower than 100%.

3.3. Characterization of nanocapsules

The effect of the different shell/core ratio used for the preparation of NCs was evaluated in terms of particles size distribution, homogeneity of suspension, drug loading and drug content. The particle sizes of NCs prepared at different polymer to oil ratios are shown in Table 2, and a representative size distribution of

NC(25/125) is shown in Fig. 3C. The mean particle sizes of NCs were around 200 nm, which concurs with our TEM observations (Fig. 3B).

Particle size increased with polymer ratio, but polydispersity (PI) decreased (Fig. 5A and Table 2). These results were highly correspondent with the previous density study. A lower PI (<0.1) value indicates a unimodal distribution, whereas a PI value of >0.3 indicates heterogeneity of particle size. NCs produced in this study were homogeneous with PI < 0.3 (Kristl et al., 2008). In general, the thickness of the membrane of NCs depends on the polymer concentration. It should increase with the polymer concentration in the organic phase, i.e. with an increasing polymer/oil ratio. This thickness could be estimated considering the measured mean size of particles. The results showed slight difference on the membrane thickness when the proportion of polymer in function of oil was increased by a factor of 2 (shell/core ratio from 0.2 to 0.47), however, further increase did not show any tremendous increase in the particle size (Table 2). This result could mean that there is a limit for the polymer concentration to affect the particle size or thickness of the shell, i.e. up to some shell/core ratio it can affect the particle size/shell thickness beyond that it may not affect the NC size. However, these results should only be considered as estimations. The real thickness of the NC membrane should be confirmed by further investigations such as microscopy analyses.

The particle size stability over a period of 8 h in different media showed some insight on the stability of the NCs. As the inner core lost a large amount of surfactant while preparing the NCs by diffusion method, it was important to know that whether the encapsulated SEDDS-core of the nanocapsules retain its ability to form the same particle size emulsion. The particle size stability study of NCs (Table 3) showed that in acidic condition (0.1 N HCl)

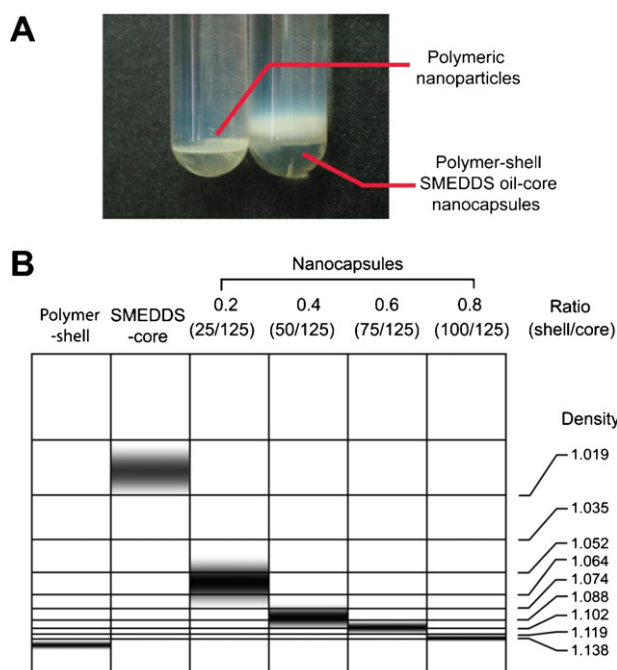


Fig. 4. Density gradient of NCs, SEDDS-core solution and polymer-shell. (A) Representative photo image of polymeric nanoparticles and NCs after density gradient centrifuge at 20,800 × g for 90 min. (B) Density diagram of NCs with different core shell ratios, SEDDS-core solution and polymer-shell.

Table 3
Pharmacokinetic parameters (mean ± SD) of cyclosporine following oral administration of NCs and SEDDS oil-core formulations to rats (n = 3–4).

Parameters	Nanocapsules (NCs)		SEDDS core
	NC(50/125)	NC(100/125)	
AUC _(0–24h) (ng hr/ml)	14880 ± 1470	12657 ± 754	9878 ± 409
C _{max} (ng/ml)	1061 ± 94	956 ± 301	1425 ± 84
T _{max} (h)	3.3 ± 0.3	5.7 ± 2.2	1.7 ± 0.2
CL (ng/ml)	236 ± 40	154 ± 89	310 ± 29

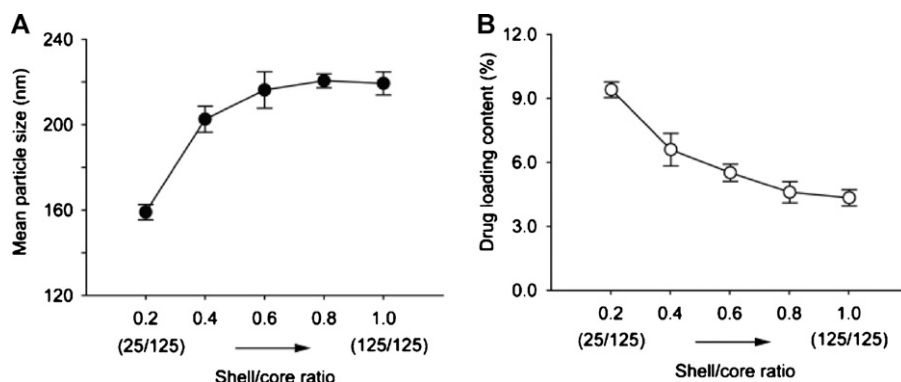


Fig. 5. Influence of polymer shell to SEDDS core ratio on the particle size and cyclosporine loading content. As the amount of polymer shell increased, particle size increased (A) and drug loading content decreased (B).

the particles size increased significantly compared to initial size both in the stirred and unstirred samples. Moreover, the particle size distribution of the samples except 0 h showed another small peak of around 4.5 μm (Fig. S2), indicating the formation of crude emulsion by SEDDS core released from nanocapsules. This study partly showed that the encapsulated SEDDS did not retain the nano sized emulsion formation capability but formed partly crude emulsion after its release.

DLC and DEE values of NCs are shown in Table 2. NCs showed high DLC and DEE values. Our preliminary study showed that MCT-cored NCs had a DLC of only 2.8% (w/w) (data not shown). However, a DLC of 9.4% w/w was obtained using F-III (Table 1). This suggests that the drug solubilizing capacity of the SEDDS-core markedly affects the DLC of NCs. Interestingly, DLC and DEE were decreased as the polymer ratio increased (Fig. 5B). X-ray diffraction study was performed to analyze the state of drug in the optimized NCs (NC(50/125)). Fig. S3 shows the X-ray pattern of cyclosporine powder, blank NC and cyclosporine NC. Cyclosporine specific peaks, which appeared in powder form (A) disappeared in NC, suggesting that cyclosporine in the NCs does not exist in crystal form, but in solubilized form (Table 4).

3.4. Oral absorption of cyclosporine from the NC formulation

The blood cyclosporine concentration vs. time profiles observed for SEDDS-core solution, NC(50/125) and NC(100/125) are shown in Fig. 6. NCs showed significantly different blood profile of cyclosporine compared with the SEDDS-core formulation. In this study SEDDS-core was used as control as we found in our earlier reported study that its oral bioavailability and pharmacokinetic

profile was comparable and equivalent to the commercial SEDDS formulation Sandimmune Neoral® (Yang et al., 2006a). So the SEDDS core was used as control in this study. Non-compartmental analysis of the blood concentrations showed a significant difference in the pharmacokinetic parameters of tested NCs compared to that of control formulation (Table 3). Whole blood concentration–time profile for CsA was analyzed by conventional non-compartmental pharmacokinetic techniques using WinNonlin program (Mountain View, CA, USA). The peak plasma concentrations (C_{max}), the time of the maximum plasma concentration reached (T_{max}) and the terminal time concentration ($C_{24\text{h}}$) were directly determined from the data. The area under the whole blood CsA concentration–time curve from time zero to the last point ($\text{AUC}_{0-24\text{h}}$) was calculated using the trapezoidal rule. NCs displayed sustained oral absorption with significantly higher bioavailability with AUC of 14880 ± 1470 for NC(50/125) and 12657 ± 754 for NC(100/125) than SEDDS core 9878 ± 409 , whereas the SEDDS-core solution showed faster cyclosporine absorption (T_{max} (1.7 ± 0.2 h) and elimination (310 ± 29 ng/ml) than NCs (T_{max} : 3.3 ± 0.3 h, CL: 236 ± 40 ng/ml for NC(50/125) and T_{max} : 5.7 ± 2.2 h, CL: 154 ± 89 ng/ml for NC(100/125)). Moreover it was observed that the NCs maintained blood concentration of cyclosporine (>500 ng/ml) for 14–20 h but in the case of control formulation it was only 7.33 h (Table 3).

PDLLA have been used in controlled and sustained release formulations (Schmidmaier et al., 2001). The drug release mechanism from PDLLA matrices have been described by several authors (Yen et al., 2001). It has been reported that drug release from PLA systems is governed by both diffusion of drugs in the matrix as well as the matrix erosion resulting from degradation and dissolution of smaller molecular weight polymer at the surface (Tamada and

Table 4
(A) Particle size (nm) with stirring at 100 rpm in different media. (B) Particle size (nm) without stirring in different media.

Time (h)	Water	0.1 N HCl	Phosphate buffer (pH 7)
(a)			
0	285 \pm 3	282 \pm 1	288 \pm 2
1	281 \pm 2	292 \pm 4	304 \pm 1
2	288 \pm 2	283 \pm 5	308 \pm 2
3	284 \pm 4	306 \pm 2	312 \pm 3
4	299 \pm 3	320 \pm 3	318 \pm 1
8	313 \pm 1	354 \pm 2	334 \pm 2
(b)			
0	285 \pm 3	282 \pm 1	288 \pm 3
1	278 \pm 4	880 \pm 55	290 \pm 5
2	279 \pm 2	1591 \pm 30	289 \pm 5
3	293 \pm 3	1766 \pm 110	281 \pm 2
4	288 \pm 5	2422 \pm 45	290 \pm 4
8	290 \pm 2	2690 \pm 69	297 \pm 3

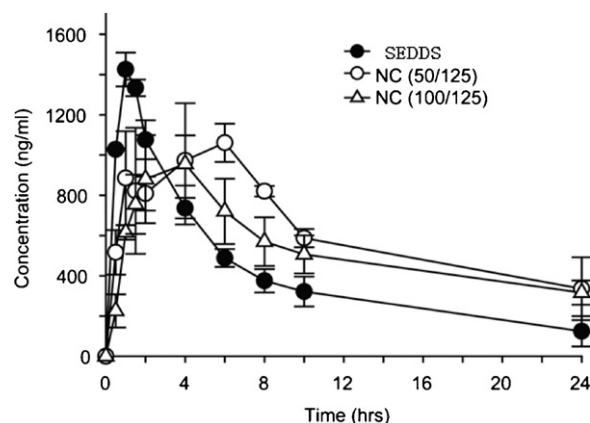


Fig. 6. Whole blood concentration–time profiles of cyclosporine after oral administration of NC(50/125), NC(100/125) and SEDDS-core solution in rats ($n = 3-4$).

Langer, 1993). Moreover, it has been reported that the chemical interaction between the entrapped compounds and poly(lactic acid) based polymers may have a strong effect on the degradation and thus on drug release, however, it has been reported that the degradation of PDLLA polymers takes several days even in gastric and intestinal fluid (Tommy, 2003). Therefore, in this study the possible mechanism for the drug release could be the diffusion out of the SEDDS core and partial matrix erosion or swelling of the nanocapsules in vivo as the polymer shell thickness was ~ 10 nm (Yen et al., 2001). This explains the sustained drug release with low or no initial burst effect observed for the prepared NCs formulations compared to control formulation. The particle size stability study also support this assumption, that in unstirred acidic media (0.1 N HCl) NCs particle size was significantly increased in 1 h indicates either the particle swelled or destabilized in the acidic condition in 1 h (Table 3B), which lead to the release of SEDDS inner core either by diffusion out or by breaking NCs and formation of crude emulsion. This particle size variation was not observed in stirred acidic media this is probably due to the fact that the stirring force might have facilitated the diffusion out of SEDDS inner core or breaking the swelled NCs and formation of much smaller emulsion particles compared to unstirred condition. The NCs particle size was not tremendously varied in unstirred media compared to stirred media, this support the above assumptions (Table 3). Moreover, due to the loss of large amount of surfactants during the preparation of NCs, the SEDDS core forms partly crude emulsion with big particle size and this could also be the reason for no burst effect and sustained oral absorption of drug from NCs. However, The SEDDS core of the NCs composed of MCT/MCM, which are known to enhance the oral absorption of poorly absorbed drugs (Cho et al., 2004). It has been reported that the MCTs were primarily absorbed directly into the portal blood (Araya et al., 2005; von Corswant et al., 1998). Moreover, the digested lipids of MCTs form lipophilic particles with bile salts and overcome the barrier of aqueous diffusion layer in the gastrointestinal (GI) tract thereby enhancing the absorption of the drug (Araya et al., 2005; von Corswant et al., 1998). There were other attempts to increase the oral bioavailability of cyclosporine, such as nanoparticles (Dai et al., 2004), microemulsion (Hirunpanich and Sato, 2009) and solid dispersion (Onoue et al., 2010), these formulations showed similar pharmacokinetic profile to Sandimmune Neoral® or more than $2 \mu\text{g/ml}$ of C_{max} , such a high peak cyclosporine blood concentration produce adverse reactions (Kahan, 2004). In this study the aim was not only increasing the absorption of the drug but also sustaining oral absorption of drug. Our results showed that the formulated NCs not only increased the oral absorption but also sustained the oral absorption by providing controlled release of the drug. However, further studies are required with respect to understand the clear mechanism behind the sustained release of this SEDDS core NCs.

The in vivo absorption of drug from the designed formulations is of fundamental importance to obtain a therapeutic concentration in blood or tissue and to avoid toxic concentrations. Maintaining drug concentration in a therapeutic window is a challenging problem for water-insoluble drugs, such as cyclosporine (therapeutic range: 120 ng/ml to $1.92 \mu\text{g/ml}$) and it is critical that the therapy is effective without toxic events (Buchler and Johnston, 2005; Kahan, 2004). Acute side effects, such as, fever; anorexia, fatigue, renal toxicity, hyperthermia, and tremor have been associated with high peak cyclosporine values. On the other hand, low blood concentrations may cause acute organ rejections (Kahan, 2004). Hence, a controlled release formulation of cyclosporine that helps maintaining therapeutic blood levels of cyclosporine and preventing side effects and organ rejection episodes is of paramount important. In this respect, we believe that the SEDDS core-polymer shell NCs offer an excellent formulation model for the controlled oral delivery of cyclosporine (Fig. 6). Our results suggest that the prepared

NCs could be an excellent carrier for controlled oral delivery of cyclosporine.

4. Conclusion

In conclusion, the results obtained from this study revealed that NCs with SEDDS-core, which was selected based on SEDDS technology, could be designed to give a sustained pharmacokinetic profile. It was observed that the increased oil content in the SEDDS core composition affected the drug permeation in vitro and the increased polymer content in NCs affected the drug absorption in vivo. The optimized formulations NC(25/125) and NC(50/125) showed controlled drug release profile and comparable bioavailability in comparison with those of the simple emulsion which was made from SEDDS-core.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.ijpharm.2012.10.018>.

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