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Comparative studies on physicochemical stability of cyclosporine A-loaded amorphous solid dispersions

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ABSTRACT

The present study aimed to evaluate the physical stability on amorphous solid dispersion (SD) of cyclosporine A (CsA) employing hydroxypropyl cellulose (HPC). SD formulations (5–30% CsA) of CsA such wet-milled SD (WM/SD) and freeze-dried SD (FD/SD) were prepared, and both SD formulations were stored at 40 °C/75% relative humidity for 8 weeks. Transitions in morphology, dissolution behavior, crystallinity and thermal behavior of CsA were evaluated. There was at least 84-fold improvement in initial dissolution rate of SD formulations compared with that of amorphous CsA powder, although their dissolution rate was gradually decreased under accelerated conditions. In particular, aged FD/SD with a drug load of 30% exhibited highly limited dissolution as evidenced by 40% reduction of solubility after 8 weeks of storage. In contrast, aged WM/SD exhibited less reduction in dissolution rate compared with FD/SD. No significant changes were seen in crystallinity and thermal behavior after aging of SD formulations for 8 weeks; however, electron microscopic observations revealed aggregation of drug molecules/particles in the aged FD/SD, possibly leading to the reduced dissolution. From these findings, stability on CsA-loaded SD might be variable depending on the preparation methodology, and the wet-milling approach could be a viable option for preparing efficacious SD formulations with improved stability.
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Cyclosporine A (CsA) is a lipophilic cyclic undecapeptide of fungal origin, which has immunomodulatory effects on various T-lymphocyte functions, such as regulating transcription of a number of genes including those for key pro-inflammatory cytokines and the interleukin-2 receptor (Underwood et al., 2001). CsA has been applied to the treatment of patients with allograft rejection in various organ transplantations, including kidney, liver, heart, lung and pancreas, and autoimmune diseases such as rheumatoid arthritis (Calderon et al., 1992). According to the biopharmaceutics classification system (Amidon et al., 1995), CsA is classified as class II drug owing to its low solubility and high membrane permeability (Wu and Benet, 2005). Absorption of CsA is limited by its poorly water-soluble character, attributable to its high molecular weight and very high lipophilicity (log P = 2.92) (el Tayar et al., 1993), so it shows poor bioavailability and high variability. Our group previously prepared an amorphous solid dispersion (SD) formulation of CsA using hydroxypropyl cellulose (HPC) by a wet-milling process for inhalation therapy (Onoue et al., 2009). The amorphous SD formulation exhibited rapid dissolution in water and onset of pharmacological effect after intratracheal administration in an experimental rat asthma model.

SD technique is one of the preferable strategies for improving the solubility of poorly water-soluble drugs. It can be defined as a distribution of active ingredients in molecular, amorphous particles (clusters) and/or microcrystalline forms surrounded by inert carriers (Chiou and Riegelman, 1971). On the basis of their molecular arrangement, there are mainly two different types of solid dispersion, which are two-phase system and one phase system. Two-phase system is composed of drug and carrier amorphous/crystalline particles as observed in wet-milled SD (WM/SD) formulations; however, in one-phase system defined as solid solution, the drug is dispersed into polymer matrix at the molecular level. Although there was great interest in SD systems, their

Abbreviations: ANOVA, one-way analysis of variance; CsA, cyclosporine A; DSC, differential scanning calorimetry; ESI, electrospray ionization; FD/SD, freeze-dried solid dispersion; HPC, hydroxypropyl cellulose; RH, relative humidity; SIR, selected ion recording; SQD, single quadrupole detector; TEM, transmission electron microscopy; WM/SD, wet-milled solid dispersion; PXRD, powder X-ray diffraction.

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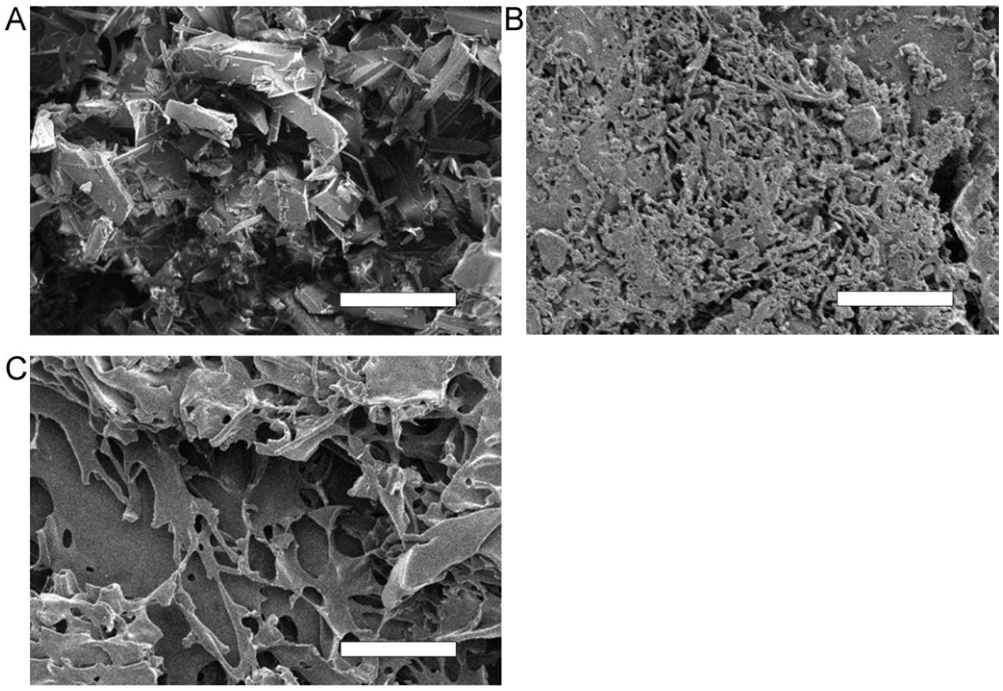

Fig. 1. Scanning electron microscopic images from cyclosporine samples, including (A) amorphous CsA, (B) WM30/SD and (C) FD30/SD. Bar represents 50 μm.

commercial use has been very limited because of manufacturing difficulties and stability problems. During pharmaceutical processing and storage, crystalline materials gradually appeared (Van den Mooter et al., 2006) or polymorphic transitions for amorphous SD formulations were observed (Morris et al., 2001). Therefore, various attempts have been made to avoid drug re-crystallization and/or stabilize the active ingredient in the amorphous state (Ambike et al., 2004; Dhumal et al., 2007). Generally, some polymers could improve the physical stability to keep API in an amorphous state, mechanisms of which are thought to involve increase in glass transition temperature (Ambike et al., 2004), decrease in mobility of drug molecules (Shimpi et al., 2005) and formation of hydrogen bonding between drugs and polymers (Gupta et al., 2004). However, the stability of amorphous CsA within SD formulation has not been fully clarified.

Thus, the main objective of this study was to investigate the physical stability of HPC-based amorphous SD formulations of CsA, which include (i) WM/SD as two-phase SD systems and (ii) freeze-dried SD (FD/SD) as one-phase systems. Physicochemical properties of each formulation were characterized focusing on morphology by scanning electron microscopy (SEM) and transmission electron microscopy (TEM), dissolution behavior and crystallinity by powder X-ray diffraction (PXRD), and thermal behavior by differential scanning calorimetry (DSC). Each SD formulation was aged at 40 °C/75% relative humidity (RH) for 8 weeks to assess possible transition in the physicochemical properties such as morphology, crystallinity and dissolution.

The WM/SD was produced according to a previously reported procedure (Onoue et al., 2009). Briefly, CsA suspended in hydroxypropyl cellulose (HPC-SSL) solution (3 mg/mL) was micronized using a rotation/revolution mixer (ARV-250, Thinky Co., Ltd., Tokyo, Japan) containing zirconia (zirconium oxide) balls with a diameter of 0.5 mm (Nikkato Co., Ltd., Osaka, Japan). CsA was weighed into the vessel of the rotation/revolution mixer to ensure concentrations of CsA at 5% (WM5/SD) and 30% (w/w) (WM30/SD) against the total weight of mixture. CsA suspension was processed by 4-step wet-milling with the indicated pulverizing condition as follows: the first step, 1000 rpm for 2 min with 0.3 mL of HPC-SSL solution; the second step, 2000 rpm for 2 min with 1.3 mL of HPC-SSL solution; the third step, 2000 rpm for 2 min with 10 mL of HPC-SSL solution; and the last step, 400 rpm for 1 min with 10 mL of HPC-SSL solution. After micronization by wet-milling, the CsA suspension in a 20 mL vial was frozen with liquid nitrogen, and freeze-dried using a FD-81 freeze dryer (Tokyo Rikakikai, Tokyo, Japan). The FD/SD was prepared by a solvent evaporation method using dioxane as a volatile organic solvent. In this experiment, FD/SD loaded with 5% (FD5/SD), 15% (FD15/SD) or 30% (FD30/SD) CsA was produced. Briefly, CsA and carrier powder were weighed and mixed at CsA levels of 5, 15 and 30% (w/w) of the total weight of mixture. After mixing, the mixture was completely dissolved in a common solvent, dioxane, to maintain the concentration of mixture at 20 mg/mL. The solvent was frozen at −80 °C for 24 h, and then frozen samples were freeze-dried for 24 h using an FD-1000 freeze dryer (Tokyo Rikakikai, Tokyo, Japan).

The shape and morphology of the SD formulations were evaluated by SEM (Fig. 1). In amorphous CsA (Fig. 1A), rod-shaped particles, with a diameter of approximately 50 μm, were observed. Particle shapes of both SD formulations revealed clear changes compared with CsA raw powder material (Fig. 1B and C). The WM/SD consisted of fine particles micronized through the process of wet-milling (Fig. 1B), and the particles exhibited the appearance of fine flaky freeze-dried material as reported previously (Onoue et al., 2009). Theoretically, the WM/SD formulations could be in a two-phase SD system, in which wet-milled drug particles were dispersed into carrier particles, separating two components. Then, the drug dispersed in SD formulation could exist as amorphous particles (clusters) or crystalline particles (Chiou and Riegelman, 1971). According to the SEM image from FD/SD (Fig. 1C), individual particles of CsA and carrier were negligible, and the uniform surface of FD/SD was observed. During the preparation process with solvent evaporation method, CsA and carrier polymer were dissolved in organic solvent very well, so that the solid solution with one-phase system could be formed in theory.

Fig. 2 demonstrates the dissolution patterns of amorphous CsA, WM/SD with drug loads of 5% (WM5/SD) and 30% (WM30/SD), and FD/SD with drug loads of 5% (FD5/SD), 15% (FD15/SD) and

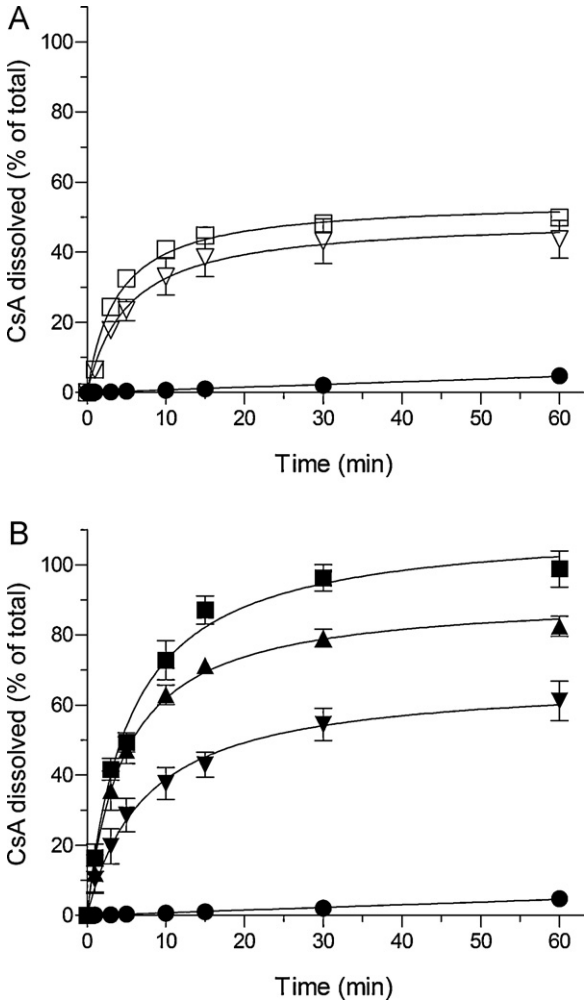

Fig. 2. Dissolution profiles of CsA-loaded SD formulations and amorphous CsA in distilled water. (A) Wet-milled SD formulations. □, WM5/SD; ▽, WM30/SD; and ●, amorphous CsA. (B) Freeze-dried SD formulations. ■, FD5/SD; ▲, FD15/SD; ▼, FD30/SD; and ●, amorphous CsA. Data represent mean ± SE of 3 experiments.

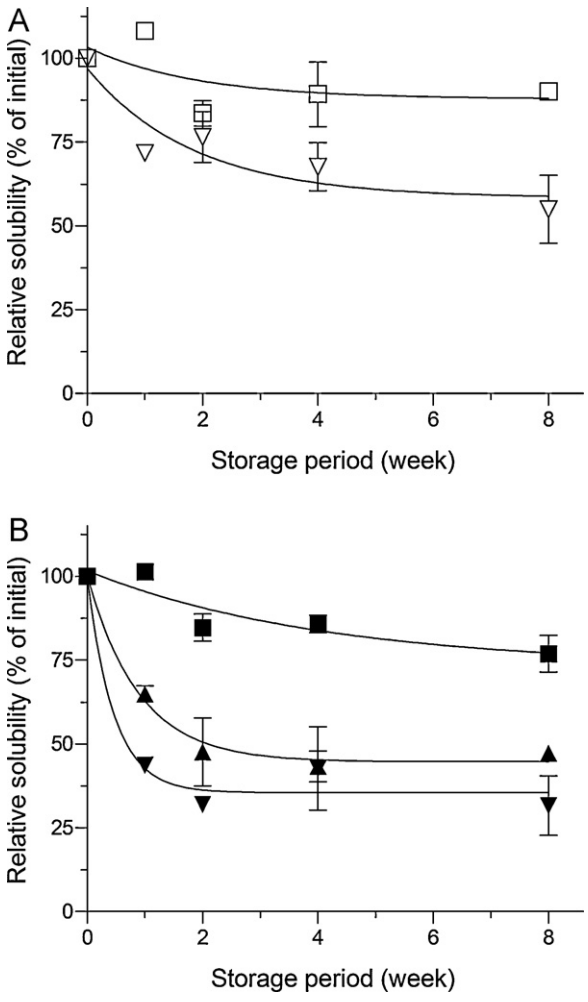

Fig. 3. Transition in dissolution behavior of SD formulations under accelerated conditions. Each SD formulation was stored at 40 °C/75% RH for the indicated period and subjected to dissolution test in distilled water. The released CsA (% of initial) from each aged SD formulation at 60 min was plotted over the storage periods. (A) Wet-milled SD formulations. □, WM5/SD; and ▽, WM30/SD. (B) Freeze-dried SD formulations. ■, FD5/SD; ▲, FD15/SD; and ▼, FD30/SD. Data represent mean ± SE of 3 experiments.

30% (FD30/SD), examined up to 60 min. WM5/SD and WM30/SD exhibited 125- and 84-fold higher initial dissolution rates than amorphous CsA, although they reached similar maximal levels at 49.9% and 43.7%, respectively (Fig. 2A). Interestingly, there appeared to be marked improvement in dissolution behavior of CsA by the FD/SD approach; in particular, the FD5/SD achieved almost complete release (98.8%) of CsA at 60 min (Fig. 2B). The initial dissolution rates of FD/SD such as FD5/SD, FD15/SD and FD30/SD were found to be 214-, 201- and 101-fold higher than that of amorphous CsA, respectively. Thus, dissolution behavior of FD/SD could be variable depending on the drug loading amount, although FD/SD exceeded WM/SD in terms of dissolution characteristics. In the SD formulations, the improved dissolution rate could be attributable to at least two possible factors. First, the higher specific surface area than amorphous CsA powder was obtained by pharmaceutical process, as evidenced by the SEM observations on amorphous CsA and the WM/SD. Second, hydrophilic polymer might improve the wettability and dispersibility of CsA itself, so that the rapid emission of drug particles/molecules could be achieved. In a one-phase SD system like FD/SD, the drug can be dispersed molecularly into polymer matrix; therefore, the drug molecules are emitted with ease when hydrophilic polymers are dissolved in water. This might explain in part the faster and greater drug release from the FD/SD than from the WM/SD.

A stability problem of SD formulations during storage is the main reason for their few marketed products, despite the numerous research papers (Ford, 1986). In general, an amorphous state often has higher solubility and dissolution rate than crystalline material due to a highly energetic state. However, amorphous materials sometimes exhibit transition of crystal forms during long-term storage and/or manufacturing processes, eventually leading to impaired dissolution profiles. These previous observations prompted us to carry out stability testing on the SD formulations of CsA, with focus on possible transition in the dissolution of inner CsA. Each SD formulation was stored at 40 °C/75% RH for up to 8 weeks and subjected to dissolution testing. The released CsA from each aged SD formulation at 60 min was plotted over the storage periods (Fig. 3). There was slight decrease in dissolution rate of WM5/SD under accelerated conditions, whereas ca. 45% reduction of dissolution rate was observed in the WM30/SD after 8 weeks of storage (Fig. 3A). Storage of FD/SD under accelerated conditions also resulted in marked impairment of dissolution behavior (Fig. 3B). Notably, there appeared to be drastic decrease in the dissolution rates of FD15/SD and FD30/SD by 53% and 69%, respectively, after storage at 40 °C/75% RH for 8 weeks, and they

seemed to reach a plateau at 2 weeks. Although the aged WM5/SD exhibited less decrease in the dissolution rate, drug release from the FD5/SD was significantly impaired during the aging process as evidenced by ca. 23% reduction in dissolution rate at 8 weeks. Thus, release of CsA from SD formulations decreased gradually without any morphological changes by SEM experiments (data not shown). In the SD formulations, the polymer matrix could enable the drug particles/molecules to avoid aggregation, and interaction between drug and polymer molecules is believed to stabilize the amorphous state. Therefore, a high level of carrier polymers might be efficacious for stabilization of inner drug in an amorphous state, and this might also have contributed to much higher stability on SD formulations with a drug load of 5% than at 15% or higher. In addition, physicochemical stability on the SD formulations was likely to be dependent on formulation type, and the two-phase SD system (WM/SD) might exceed the one-phase SD system (FD/SD) in terms of a stable dissolution profile.

Since drastic transition in the drug-release profiles was observed in aged SD formulations, solid-state characteristics of WM30/SD and FD30/SD were further evaluated before and after storage at 40 °C/75% RH for 8 weeks. To clarify possible changes in crystallinity of inner CsA during storage, PXRD and DSC analyses were conducted (Fig. 4). According to the X-ray diffraction (XRD) patterns of crystalline/amorphous CsA and initial/aged SD formulations with a drug load of 30% (Fig. 4A), several intense and characteristic peaks were observed in crystalline CsA, the patterns of which were indicative of tetragonal crystal form (Bertacche et al., 2006). In contrast, both initial and aged SD formulations exhibited a halo pattern, suggesting an amorphous state of CsA in both WM/SD and FD/SD. Thus, in spite of the impaired dissolution behavior, an amorphous state of inner CsA seemed to be maintained even under accelerated conditions. In addition to the PXRD, thermal behavior of SD formulations was also assessed to ascertain the changes in physicochemical status of inner CsA during storage (Fig. 4B). Crystalline CsA exhibited a melting endothermic peak at 110 °C; however, in amorphous CsA, there was no endothermic peak at 110 °C. DSC thermogram of amorphous CsA exhibited a new endothermic peak at 127 °C, and this shift might be attributable to liquid-to-solid phase transition of CsA caused by thermal stress in the thermal analysis. The DSC thermogram of both WM/SD and FD/SD did not show any thermal events even after 8 weeks of storage. Within SD formulations, inner CsA particles/molecules could interact with carrier polymers, possibly leading to prevention of phase transition and molecular mobility. From these findings, decrease in dissolution rate of aged SD formulation might not be attributable to transition in crystallinity of CsA since inner CsA was still amorphized completely even after long-term storage.

In addition to the crystallinity, aggregation of drug particles/molecules in the SD formulations also affects the dissolution properties. Recent study demonstrated that glucagon formed aggregates in solid glucagon/γ-cyclodextrin powder, resulting in impaired release profiles (Matilainen et al., 2009). In the present study, aggregation property of CsA in the SD formulations with a drug load of 30% was evaluated by TEM observation on water-suspended SD formulations (Fig. 5). According to the TEM image from the WM30/SD (Fig. 5A-1), there were very fine rod-shaped particles of amorphous CsA contained in hydrophilic polymer. In contrast, the FD30/SD exhibited no visible particles, suggesting uniform distribution of CsA molecules in the FD30/SD (Fig. 5B-1). After storage at 40 °C/75% RH for 8 weeks, slight growth of fine CsA particles was seen in the aged WM30/SD (Fig. 5A-2), and fine particles also appeared even in the aged FD30/SD (Fig. 5B-2). These observations suggest the aggregation of amorphous CsA under accelerated conditions, and the morphological changes might lead to poor dissolution profiles owing to the reduction of surface

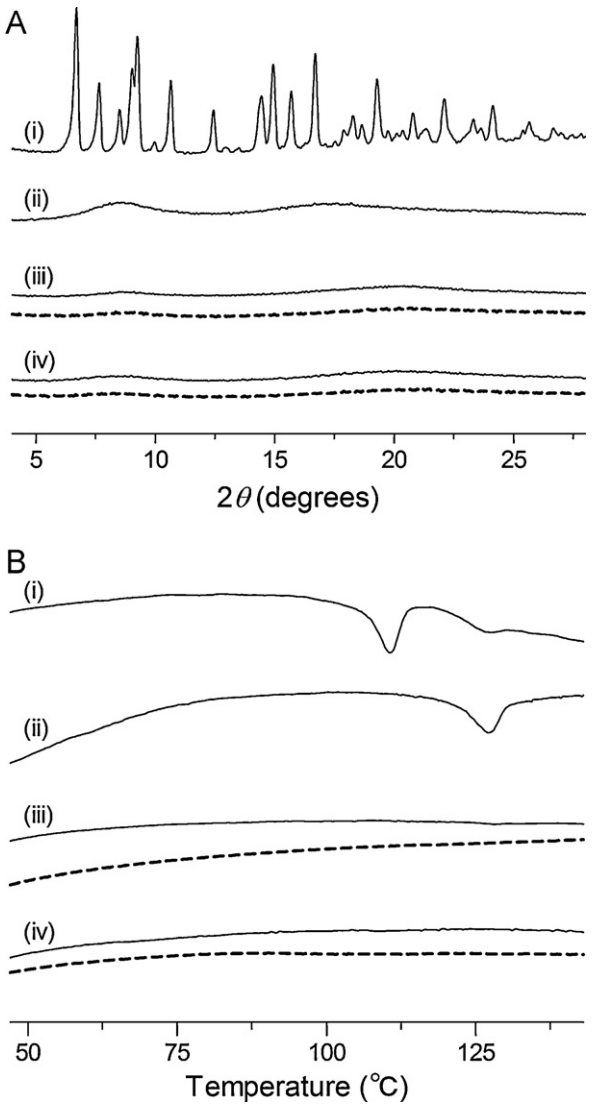

Fig. 4. Crystallinity evaluation on SD formulations using (A) powder X-ray diffraction patterns and (B) DSC thermograms. (i) Crystalline CsA, (ii) amorphous CsA, (iii) WM30/SD, and (iv) FD30/SD (solid line, initial samples; dotted line, samples aged for 8 weeks).

area and dispersibility of drug particles/molecules. In particular, the FD/SD prepared as a one-phase SD system might partly change into a two-phase SD system during 8 weeks of storage, and the formation of visible aggregates would result in a significant impact on its dissolution behavior. This might be part of the reason for better dissolution profile of the aged WM/SD than the aged FD/SD, while gradual growth of CsA particles in the WM/SD also led to moderate decrease of dissolution rate. From these observations, prevention of the aggregation within SD formulations of CsA might be important for stable dissolution.

In conclusion, we demonstrated the differences in dissolution behavior and its stability between two SD formulations of CsA, WM/SD as a two-phase SD system and FD/SD as a one-phase SD system. The WM/SD approach with relatively low drug load might be a stable solubilizing technique for poorly water-soluble chemicals, and further improvement in the preparation method might lead to successful development of more stable SD formulations.

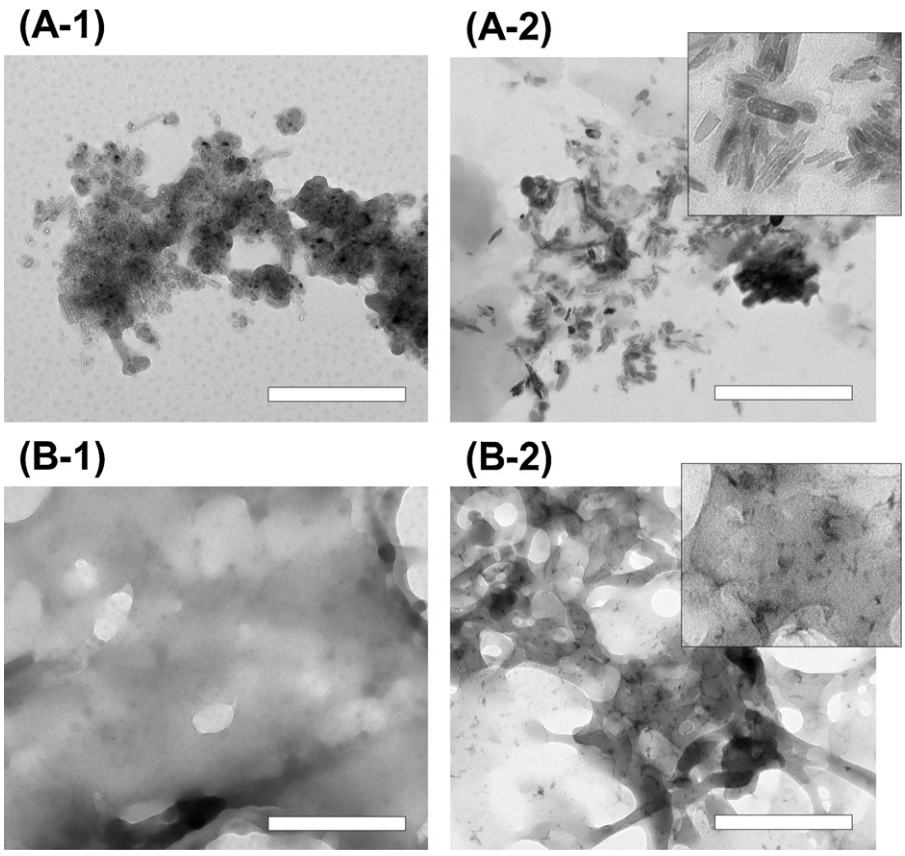

Fig. 5. Transmission electron microscope images of SD formulations dispersed into water, including (A-1) WM30/SD, (A-2) WM30/SD aged for 8 weeks, (B-1) FD30/SD and (B-2) FD30/SD aged for 8 weeks. Bar represents 500 μm. The inset highlights the visible aggregates.

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