Constitution of Colloidal Particles Formed from a Solid Dispersion System

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Colloidal particles, produced upon the dispersion into water of a solid dispersion system composed of (*R*)-1-[2,3-dihydro-1-(2′-methylphenacyl)-2-oxo-5-phenyl-1*H*-1,4-benzodiazepin-3-yl]-3-(3-methylphenyl)urea (YM022), hydroxypropylmethylcellulose 2910 (TC-5E) and polyoxyethylene hydrogenated castor oil 60 (HCO-60) at a weight ratio of 1:3.5:0.5, were characterized with respect to their physicochemical properties. From the IR absorption spectroscopic analysis, it was found that the colloidal particles, composed of YM022, TC-5E and HCO-60, have a combined weight ratio of 1:0.2:0.3. Although colloidal particles were prepared from solid dispersion systems with various weight ratios of YM022/TC-5E/HCO-60, they showed a similarity in combined ratio, suggesting the presence of specific interactions among the components. Particle diameter seemed to be affected by the composition of colloidal particles. Smaller particles contained less TC-5E which was related to the large portion of YM022 in colloidal particles. The amount of HCO-60 incorporated into colloidal particles was not affected by the particle diameter and correlated with the amount of YM022 at a fixed ratio (1.0:0.3). This suggested that TC-5E might function as a 3-dimensional framework for solid dispersion, while HCO-60 might interact with YM022 resulting in a fixed weight ratio between YM022/HCO-60 in colloidal particles.

Key words solid dispersion; colloidal particle; poorly water-soluble drug; water-soluble polymer; molecular interaction; water-insoluble particle

YM022, (*R*)-1-[2,3-dihydro-1-(2′-methylphenacyl)-2-oxo-5-phenyl-1*H*-1,4-benzodiazepin-3-yl]-3-(3-methylphenyl)urea (Chart 1-1), possesses an activity to suppress gastric-acid secretion through selective antagonistic action on the gastrin receptor.[1,2] YM022 has extremely low solubility in water, that is, both α- and β-form crystals have solubility in water less than 1 μg/ml at 20 °C. To improve the bioavailability after oral administration of YM022, we formulated a solid dispersion system using the solvent method, which significantly improved it.[3]

A solid dispersion is generally prepared from a mixture of drug substances and water-soluble polymers.[4–11] We developed a new spray dried solid dispersion(SD5) composed of YM022, hydroxypropylmethylcellulose 2910 (TC-5E) (Chart 1-3) and polyoxyethylene hydrogenated castor oil 60 (HCO-60) (Chart 1-2), which produced colloidal particles after reconstitution in purified water. The colloidal particles had an average diameter of approximately 160 nm, YM022 was present in them in amorphous form at a concentration determined to be 67—77% of total weight (YM022 : total weight = 1 : 1.3—1.5). The colloidal particles reconstituted from SD5 were composed of multiple components but not of a drug substance itself. These particles were shown to be much more stable than those prepared from conventional solid dispersion systems. This result was related to the physicochemical properties and the bioavailability of drugs reported.[12]

These observations suggested that the present solid dispersion system offered advantages over conventional systems in providing a formulation to improve the absorption profile of poorly water-soluble drugs. In the present study, we further characterized colloidal particles formed from the SD5 system with respect to their physicochemical properties.[13]

Experimental

Reagents YM022 was obtained from the Chemical Technology Laboratories of Yamanouchi Pharmaceutical Co., Ltd. (Takahagi, Ibaragi). TC-5E and HCO-60 were purchased from Shin-Etsu Chemical Co., Ltd. (Tokyo) and Nikko Chemicals Co., Ltd. (Tokyo), respectively. All other reagents were of Japanese Pharmacopoeia XIII grade.

Preparation of Solid Dispersion Systems A series of solid dispersion systems, SD1.5, SD5, SD5H, SD5S and SD10T, were prepared according

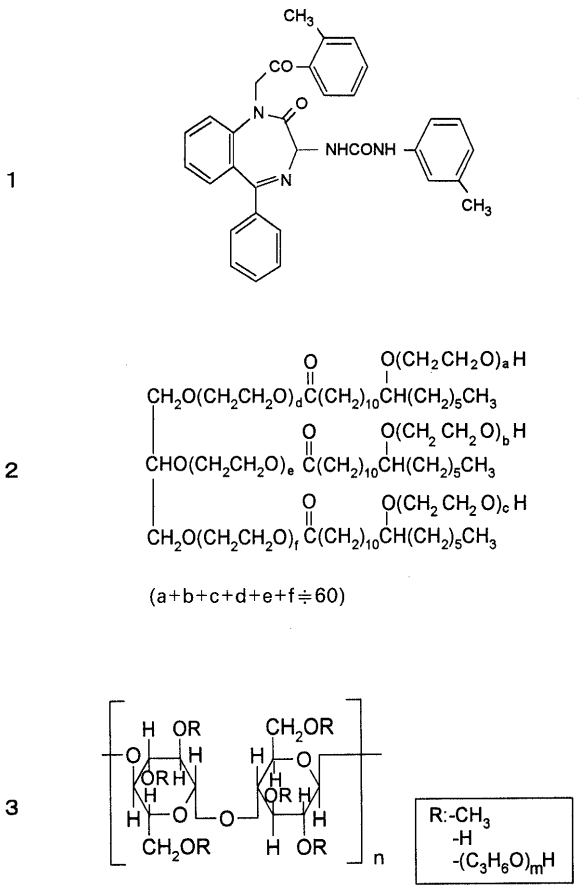

Chart 1. Molecular Structures
(1) YM022, (2) HCO-60, (3) TC-5E.

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Table 1. Formulation of YM022 Solid Dispersion (g)

	SD1.5	SD5	SD5H	SD5S	SD10T
YM022	10	10	10	10	10
TC-5E	2.0	35	30	40	85
HCO-60	3.0	5.0	10	—	5.0

to the formulas illustrated in Table 1. All components were dissolved in a mixed solvent (450 g) of methylene chloride and methanol at the weight ratio of 4:1. The powder sample was obtained by spray drying of the solution using a spray dryer (DL-41, Yamato Kagaku Co., Ltd., Tokyo) at an inlet temperature of 120 °C and subsequent sieving through one hundred-mesh screen.

Preparation of Colloidal Particles Three different solid dispersion systems (500 mg), SD5, SD5H or SD10T, were added to purified water (40 ml). The resultant mixtures were incubated (150 strokes/min) at 20 °C for 30 min (M-100D, Taiyo Kagaku Kogyo Co., Ltd., Tokyo) and then centrifuged at 50000 rpm (250000 *g*) for 30 min (L-70, Beckman, CA, U.S.A.) to allow fine particles to settle. The obtained sediment was subsequently dried under reduced pressure at 40 °C for 15 h.

Infrared (IR) Absorption Spectroscopic Analysis IR absorption spectra of samples were determined by the chloroform-solution method using Fourier transform (FT) IR spectroscopic apparatus (FT-IR System, Perkin Elmer Japan, Kanagawa).

Quantitative Analysis of TC-5E in Colloidal Particles The amount of TC-5E incorporated in the colloidal particles was determined from the IR spectrum. The IR absorption spectroscopic analysis was performed under the constant YM022 concentration of 2.9×10^{-2} M. Solid mixtures composed of YM022, TC-5E and HCO-60 at varying weight ratios (1:0.5:0.0, 1:0.4:0.1, 1:0.3:0.2, 1:0.2:0.3, 1:0.1:0.4) were used to determine the calibration curve, and IR absorption intensities at 1077 and 1097 cm^{-1} were used to determine the concentration of TC-5E in colloidal particles.

Gas Chromatographic Analysis of Methoxyl Group of TC-5E The methoxyl group of TC-5E was determined by gas chromatographic analysis (GC-14A, Shimadzu Co., Kyoto) according to the method described in the section of Hydroxypropylmethylcellulose 2208 in Japanese Pharmacopoeia XII.[14]

Analysis of Particle Diameters (1) The solid dispersion containing YM022 (10 mg) was dispersed in purified water (200 ml) and incubated at 20 °C for 30 min with stirring. The diameter of colloidal particles was then determined by a laser-diffraction scattering particle analyzer (LA-910, Horiba Co., Kyoto).

(2) The solution (4 ml) of YM022 in methanol (2.5 mg/ml) was mixed with 200 ml of various solvents at 20 °C. Solvents used were purified water, 0.01% TC-5E aqueous solution and 0.01% HCO-60 aqueous solution. The particle diameter for the obtained dispersion was determined at 1 min-intervals by the instrument described above.

Fractionation of Colloidal Particles The size of colloidal particles was fractionated by the following method: SD5 (500 mg), containing 100 mg YM022, was dispersed in 40 ml of purified water. After shaking at 20 °C for 1 h, the mixture was used for successive centrifugation processes to obtain different sediments. The conditions for centrifugation were 5000 rpm (2500 *g*) for 30 min, 10000 rpm (10000 *g*) for 30 min and, finally, 50000 rpm (250000 *g*) for 30 min. Sediments containing colloidal particles with distributions of various sizes were obtained following each centrifugation process and were subsequently dried under reduced pressure at 40 °C for 15 h.

HPLC Analysis of YM022 YM022 was determined using HPLC[3] (LC-6A, Shimadzu Co., Kyoto) under the following conditions: column YMC A-302 ODS (4.6 mm i.d. × 150 mm), detection wavelength 240 nm, mobile phase pH 5.5 phosphate buffer/acetonitrile (10:15, v/v), flow rate 1.0 ml/min, column temperature: 40 °C. Pyrene was used as an internal standard.

Results and Discussion

Characterization of Colloidal Particles from a Solid Dispersion System Colloidal particles, formed upon dispersion of SD5 into purified water, were water-insoluble particles with an average diameter of approximately 160 nm.[12] While SD5 was prepared from a mixture of YM022, TC-5E and HCO-60 at an initial weight ratio of 1.0:3.5:0.5, the weight ratio of YM022 relative to obtained colloidal particles was calculated to be 1.0:1.5 from the HPLC analysis. It can be assumed that 33% of colloidal particles by weight might result from the incorporation of either TC-5E or HCO-60, or both, into particles.

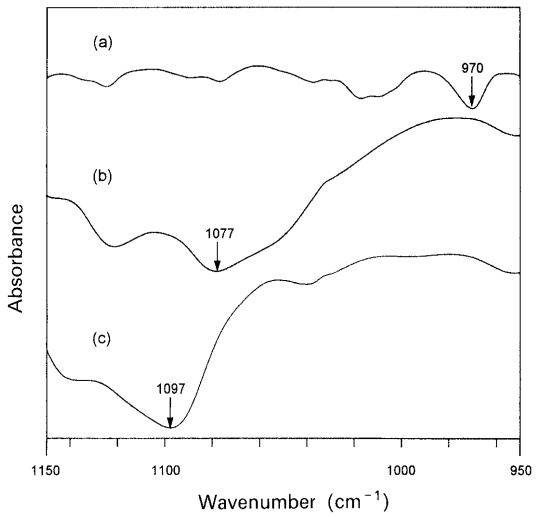

Fig. 1. FT-IR Spectra of YM022, TC-5E and HCO-60 Obtained by $CHCl_3$ Solution Method

(a) YM022, (b) TC-5E, (c) HCO-60.

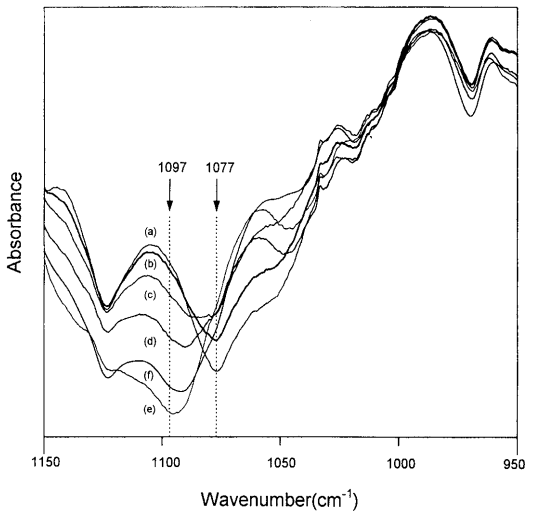

Fig. 2. FT-IR Spectra of Various Mixtures of YM022, TC-5E and HCO-60 Obtained by $CHCl_3$ Solution Method

(a) YM022:TC-5E:HCO-60=1.0:0.5:0.0. (b) YM022:TC-5E:HCO-60=1.0:0.4:0.1. (c) YM022:TC-5E:HCO-60=1.0:0.3:0.2. (d) YM022:TC-5E:HCO-60=1.0:0.2:0.3. (e) YM022:TC-5E:HCO-60=1.0:0.1:0.4. (f) Colloidal particles formed from SD5.

To determine the composition of the colloidal particles produced, the IR absorption spectra were measured. Figure 1 shows IR absorption spectra for YM022, TC-5E and HCO-60, showing a characteristic absorption peak at 970, 1077 and 1097 cm^{-1}, respectively. Figure 2 shows IR absorption spectra of YM022 chloroform solutions containing TC-5E and HCO-60 in a varied weight ratio. The total amount of TC-5E and HCO-60 was fixed as 1/3 of the total weight of solid components,

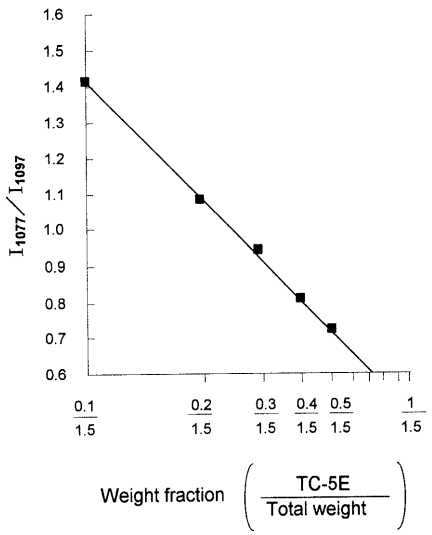

Fig. 3. Relationship between Absorption Intensity Ratio (I_{1077}/I_{1097}) and Weight Fraction of TC-5E (TC-5E/Total Weight) in Various Mixtures of YM022, TC-5E and HCO-60

and the weight ratio of TC-5E relative to the total weight was varied from 1/15 to 5/15. By calculating absorbance ratios (I_{1077}/I_{1097}) from the characteristic peaks of TC-5E and HCO-60 at 1077 and 1097 cm^{-1}, and plotting them against the logarithm of the weight ratios of TC-5E relative to total weight (TC-5E/total weight), the standard curve ($y=-0.416\ln x+0.458$, $R^2=0.997$) was obtained as shown in Fig. 3, where $y=I_{1077}/I_{1097}$ and $x=\log$ (weight fraction of TC-5E). Using this relationship, it was possible to estimate the weight ratio of TC-5E and HCO-60 in an unknown complex system. Based on the absorbance ratio ($I_{1077}/I_{1097}=1.1$) for colloidal particles prepared from SD5, the weight ratio of TC-5E and HCO-60 was determined to be approximately 0.2:0.3. Thus, the weight ratio of YM022/TC-5E/HCO-60 in colloidal particles was estimated as 1.0:0.2:0.3.

The concentration of the methoxyl group derived from TC-5E in colloidal particles was determined by gas chromatographic analysis, and the obtained value was 3.85% of the total weight. The average concentration of the methoxyl group in 50 different lots of TC-5E was determined as 28.9% ($\pm$0.24%S.D.).[15] From these values, the concentration of TC-5E in colloidal particles was calculated to be 13.3% of the total weight. The weight ratio of YM022 and TC-5E, then, was estimated to be 1.0:0.199, and the remaining 0.301 part by weight was therefore perceived as HCO-60, showing good agreement with the data obtained from IR absorption spectroscopic analysis. With the formation of colloidal particles, some parts of TC-5E and HCO-60 were removed from the SD5 system. The amount of TC-5E and HCO-60 dissolved in water from SD5 were estimated as 94% and 40% respectively. The remainder of these compounds was incorporated in particles together with YM022, resulting in the formation of water-insoluble nanoparticles.[16]

Effect of Composition of Solid Dispersion System on Colloidal Particle Formation Three different solid dispersion systems, SD5S, SD10T and SD5H, were used to investigate the effect of composition on the formation of colloidal particles after dispersion into purified water at 20 °C. The SD5S system was composed only of YM022 and TC-5E, while the SD10T and SD5H systems were composed of YM022, TC-5E and HCO-60 with increasing contents of TC-5E and HCO-60, respectively. The addition of SD5, SD5H and SD10T into purified water resulted in a colloid-like cloudy solution. Subsequent stirring of these solutions for 30 min resulted in the formation of colloidal particles which had varied particle size distribution patterns as shown in Fig. 4a—c. The larger particles with diameters of several microns, as shown in Fig. 4b and 4c, had a tendency to transform into colloidal particles with diameters less than 1 μm by further dispersion with ultrasonic treatment, while in the SD5S system prepared without HCO-60, no colloidal particles were formed upon the dispersion of the sample into purified water (Fig. 4d). Subsequent treatment of this solution with ultrasonic sonication did not change the distribution of particle size.

Table 2 shows the compositions of uncentrifugated colloidal particles obtained from different solid dispersion systems, SD5, SD5H and SD10T, determined from HPLC analysis and IR absorbance ratios (I_{1077}/I_{1097}). The composition of colloidal particles was not dramatically altered even when prepared from SDs of YM022/TC-5E/HCO-60 at varying initial component ratios. These results indicate that the incorporation of a definite amount of TC-5E and HCO-60 into the particles was essential for the colloidal particle formation.

Relationship between Colloidal Particles and Particle Size The composition of colloidal particles was investigated in the SD5 system. Colloidal particles were obtained by dispersing SD5 into purified water and subsequently fractionated by the centrifugation method with different conditions (5000, 10000 and 50000 rpm) (Table 3). For colloidal particles fractionated by the centrifugation at 5000 rpm (2500 ***g***), the ratio of total weight of the particles relative to the amount of YM022 (total weight/YM022) was 1.9, and the ratio of YM022, TC-5E and HCO-60 was estimated as 1.0:0.5:0.3 from IR absorption spectroscopic analysis. Also shown in Table 3 is that the total weight/YM022 ratio decreased from 1.9 to 1.6 with decreasing size of colloidal particles. This was primarily due to reduction of the amount of TC-5E incorporated into the particles. The weight ratio of YM022 and HCO-60, on the other hand, was constant (1.0:0.3) in the particles in spite of different particle size distributions, indicating specific interactions between these two components.

Roles of HCO-60 and TC-5E on the Colloidal Particle Formation In the solid dispersion formed from the SD5 system, YM022 and HCO-60 were expected to be present within a structural unit[17,18] formed by TC-5E. The roles of TC-5E and HCO-60 in the formation of colloidal particles after dispersing SD5 into purified water were investigated by inspecting the changing of colloidal particle size with the passage of stirring. Four ml of methanol solution of YM022 (2.5 mg/ml) was added to 200 ml purified water at 20 °C, and the particle diameter was measured at 1-min intervals (Fig. 5). When the YM022 methanol solution was added dropwise to purified water, fine particles with diameters of less than 100 μm were

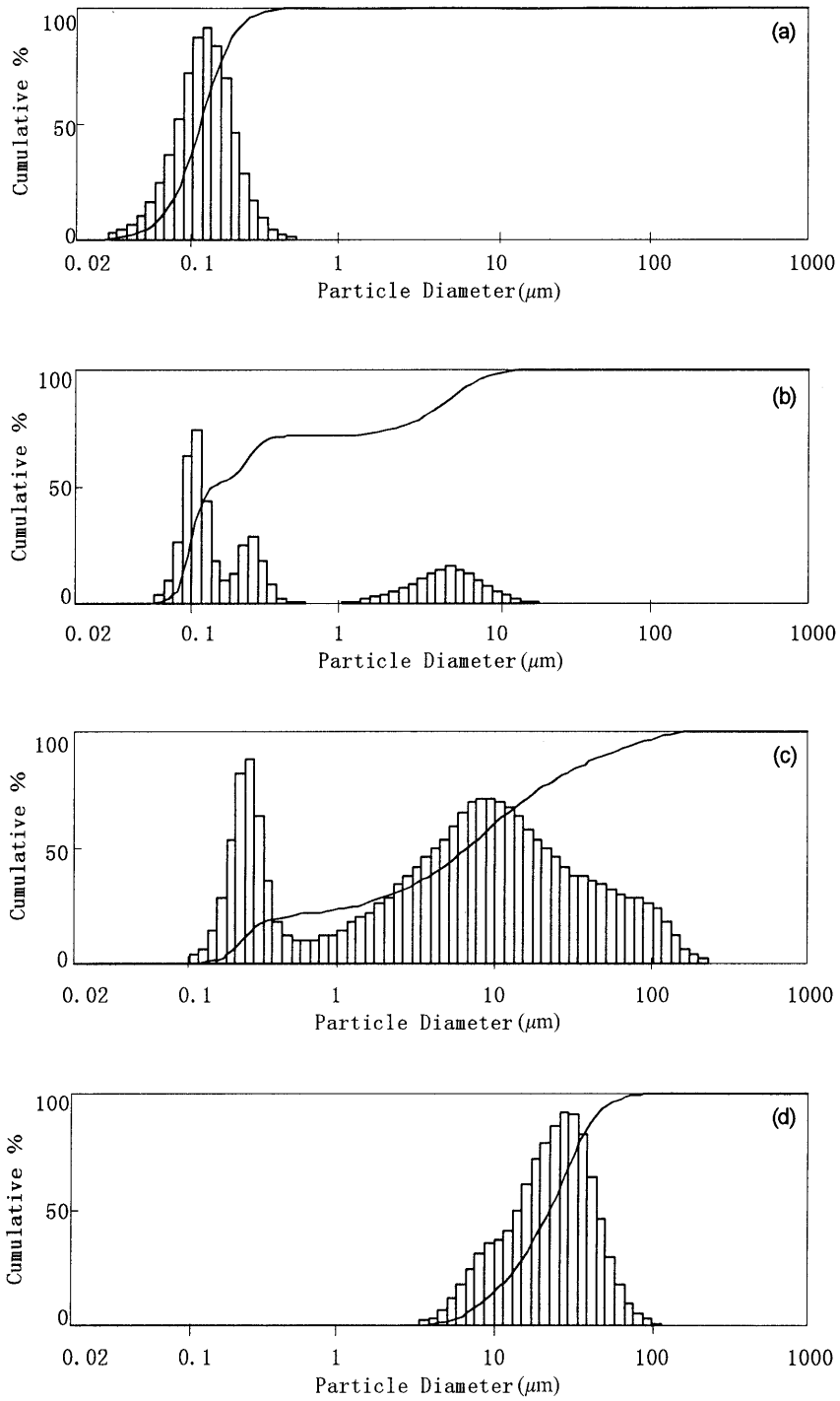

Fig. 4. Particle Size Distribution Curves of Various Solid Dispersions in Water after Stirring for 30 min
(a) SD5, (b) SD5H, (c) SD10T, (d) SD5S.

Table 2. Composition of Colloidal Particles Formed from Various Solid Dispersions

Colloidal particles formed from	Total weight / Weight of YM022	Composition ratio[a] YM022 : TC-5E : HCO-60
SD5	1.5	1.0 : 0.2 : 0.3
SD10T	1.4	1.0 : 0.1 : 0.3
SD5H	1.6	1.0 : 0.2 : 0.4

a) Determined from IR spectra (I_{1077}/I_{1097}).

Table 3. Composition of Fractionated Colloidal Particles Formed from SD5

Centrifugal condition (rpm)	Total weight / Weight of YM022	Composition ratio[a] YM022 : TC-5E : HCO-60
5000	1.9	1.0 : 0.5 : 0.3
10000	1.5	1.0 : 0.2 : 0.3
50000	1.6	1.0 : 0.1 : 0.3

a) Determined from IR spectra (I_{1077}/I_{1097}).

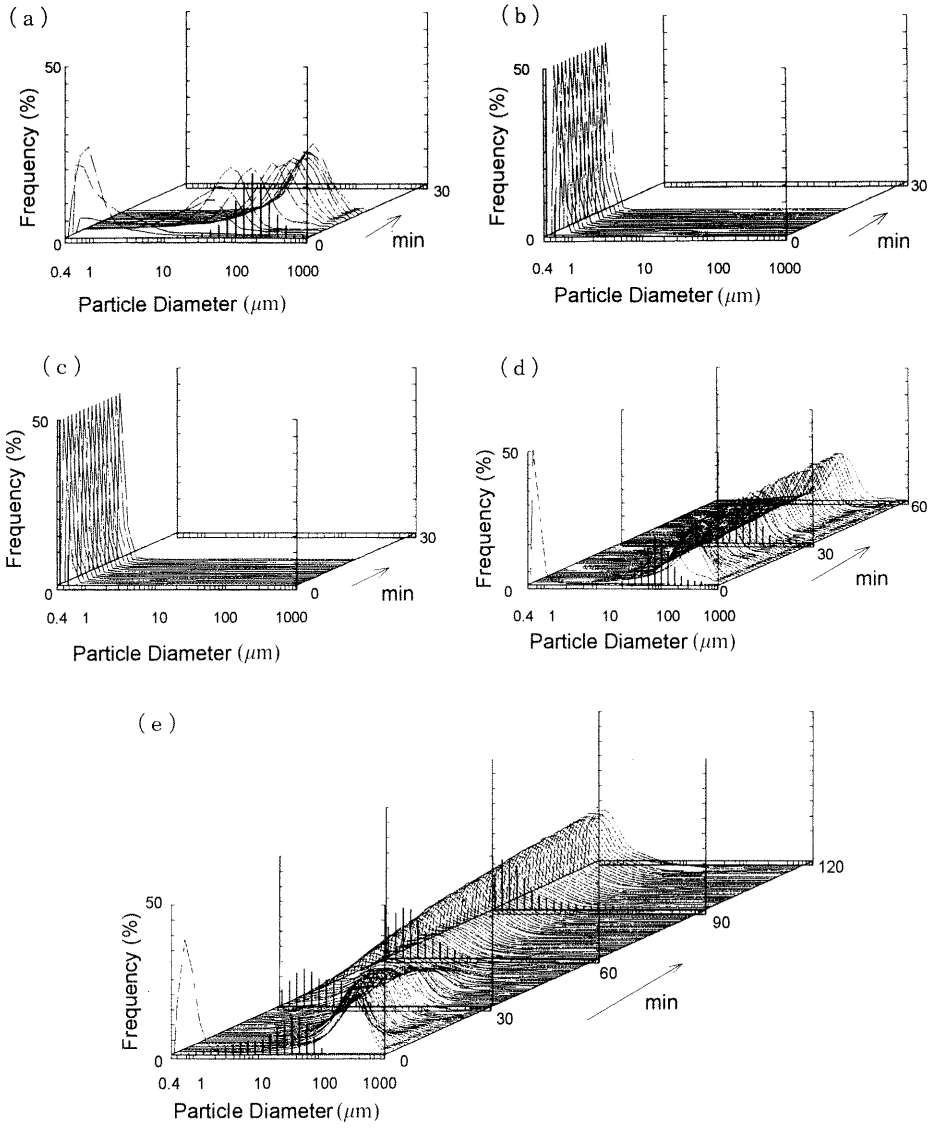

Fig. 5. Time Dependence of Particle Size Distribution Curves of Colloidal YM022 Particles in Various Solutions

(a) Water, (b) 0.01% TC-5E solution, (c) 0.01% HCO-60 solution, (d) water (100 mg TC-5E was added at $t = 5$ min), (e) water (100 mg HCO-60 was added at $t = 5$ min).

precipitated within a few minutes due to low solubility of the drug substance in water (Fig. 5a). When YM022 methanol solution was added to the TC-5E or HCO-60 aqueous solutions, however, the average diameter of crystallized particles was approximately 0.6 μm and the size did not change with the stirring time. Thus, the presence of TC-5E and HCO-60 effectively suppressed the growth of crystallized particles (Fig. 5b, 5c).

In study of the difference in function of TC-5E and HCO-60, one of these substances was subsequently added to the YM022 suspension to learn the effect of these components on the particle size of coprecipitate. The addition of HCO-60 (100 mg) resulted in reversal of the particle diameter from 200—300 μm to approximately 0.6 μm (Fig. 5e), whereas the addition of TC-5E had no impact on the YM022 particle size reduction (Fig. 5d). These observations suggested the difference in roles of TC-5E and HCO-60 in solid dispersions of the SD5 system. Colloidal particles could be made up of a 3-dimensional framework of TC-5E molecules, and HCO-60 could act as a molecular carrier of YM022 in the solid dispersion system and in the colloidal particles through physicochemical interactions.[19]

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