Examination of dissolution ratio of β-carotene in water for practical application of β-carotene amorphous solid dispersion

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KI designed the study as the first author, and drafted the manuscript. YN, SM, TI, and YK collected the test data. SO, SM, and YA helped in the interpretation of results. SN directed the research and reviewed the manuscript.

Research highlights

- Decreasing β-carotene ratio in solid dispersion increases its water dissolution ratio
- β-Carotene, when dissolved in water, is absorbed from the gastrointestinal tract
- The dissolution ratio of β-carotene in water correlates with its bioavailability

Abstract

β-Carotene (BC) has an antioxidant effect that removes active oxygen in vivo and can reduce the risk of developing various diseases, but it is almost insoluble in water. Therefore, to develop highly effective BC functional food products, it is essential to increase its water solubility, which in turn can improve its absolute bioavailability. Recently, a BC amorphous solid dispersion (BC-SD) prepared using hot melt extruder technology had increased water solubility and improved absorption from the gastrointestinal tract. However, only a part of the BC in BC-SD could be dissolved in water. In this study, we evaluated whether the dissolution ratio of BC in water could be improved by examining the mixing ratio of BC and base materials in BC-SD. Results showed that by reducing the mixing ratio of BC to the base materials, the dissolution ratio of BC in water increased. It was also found that when BC-SD, which has the highest dissolution ratio, was intragastrically administered to rats, its absolute bioavailability was most increased. These results are useful findings that may help in reducing the costs associated with the BC-SD manufacturing process and will be an important part of our strategy for practical use in the future.

Keywords

β-Carotene, amorphous solid dispersion, hot melt extruder technology, dissolution ratio in water, practical application, absolute bioavailability

Abbreviations

β-Carotene, BC; BC amorphous solid dispersion, BC-SD; PVP, polyvinylpyrrolidone; Suc, sucrose fatty acid ester; PM, physical mixture; PXRD, Powder X-ray diffraction.

Introduction

In recent years, the idea of self-medication has become widespread in developed countries, where increasing medical costs due to aging and technological innovation has become a social problem (Eichhorn et al., 2011). As part of this effort, over-the-counter health foods, nutraceuticals, and medicinal products from plants and other natural sources are increasingly being used. However, among the materials used in functional foods, there are many compounds that are poorly absorbed from the gastrointestinal tract because of their poor water solubility, and hence are not able to fully exert their functions (Aungst, 2017). To solve this problem, it is necessary to improve the water solubility of poorly water-soluble compounds in functional foods, and thereby improve their gastrointestinal absorption (Gupta et al., 2013). In addition, from the viewpoint of the notion that "a healthy diet and medicine are equally important for health," it is necessary to develop health foods with high absorbability and, at the same time, ensure that they are both safe and effective.

β-Carotene (BC), which is classified as a carotenoid, is a naturally occurring red-orange pigment that is abundantly found in common vegetables and fruits (Gul et al., 2015). For example, carrots and spinach contain 5-1030 μg/g and 10 μg/g of BC, respectively (Gul et al., 2015). It has an antioxidant effect that removes active oxygen in vivo and can reduce the risk of developing various diseases, such as liver injury and arteriosclerosis (Kritchevsky, 1999; Kumar et al., 2005). In addition, BC exerts various physiological activities, such as anti-

inflammatory and anti-allergic activities (Bai et al., 2005; Sato et al., 2004). BC is also a precursor of vitamin A and is the main source of vitamin A in vivo (Weber & Grune, 2012). Therefore, ingestion of BC can be expected to exert similar physiological activities as vitamin A, such as maintaining healthy skin and mucous membranes and improving eyesight (Faustino et al., 2016). These findings indicate that BC is a useful compound for incorporation in health foods, pharmaceuticals, and cosmetics. However, BC is extremely hydrophobic and hardly dissolves in hydrophilic solvents owing to its structure, which is composed of carbon and hydrogen (Craft & Soares, 1992). Organochlorine compounds, such as dichloromethane and low-polarity solvents, such as hexane, are commonly used to dissolve BC. Furthermore, ingestion of BC in the form of health foods is associated with low gastrointestinal absorption as it hardly dissolves in the gastrointestinal fluid (Gul et al., 2015). Therefore, it is important to develop a formulation with improved water solubility and absolute bioavailability of BC, and thereby improve the efficacy of BC.

Solid dispersions are one of the most effective techniques for increasing the solubility of poorly water-soluble compounds (Baghel et al., 2016; Tekade & Yadav, 2020). A solid dispersion system is a delivery system in which a compound is dispersed in a biologically inert matrix to enhance its oral bioavailability (Craig, 2002). In our previous study, we prepared a BC amorphous solid dispersion (BC-SD), composed of polyvinylpyrrolidone (PVP) and sucrose fatty acid ester (Suc), using the hot melt extruder technology (Ishimoto et al., 2019).

The developed BC-SD had improved water solubility and showed higher absorption from the gastrointestinal tract compared to crystal BC (Otani et al., 2020). Since our method did not use organic solvents, there are no concerns about residual solvents. In addition, the process can be applied for mass production, and can thus be used in the food industry (Koklesova et al., 2020). However, in BC-SD, only around 25% of the BC in the formulation could be dissolved in water. This may result in wastage of BC, which in turn may be a concern during the practical application of BC-SD. It has been reported that the concentration of the base materials in a solid dispersion reduces the aggregation and nucleation rate of poorly water-soluble compounds (Baghel et al., 2016; Konno & Taylor, 2006; Tekade & Yadav, 2020). In this study, we evaluated if there was any improvement in the dissolution ratio of BC in water by examining the mixing ratio of BC and base materials in BC-SD.

Materials and Methods

Materials

BC was purchased from Nacalai Tesque, Inc. (Kyoto, Japan). PVP (Kollidon 25) was kindly provided by BASF Japan Ltd. (Tokyo, Japan). Suc (S-1670) was provided by Mitsubishi Chemical Foods Co., Ltd. (Tokyo, Japan). Dissolution Test Solution 2 (Nacalai Tesque, Kyoto, Japan) was used as the phosphate buffer. High-performance liquid chromatography (HPLC)-grade acetonitrile, methanol, tetrahydrofuran, analytical-grade n-hexane, dibutylhydroxytoluene, acetic acid, ammonium acetate, sodium acetate, and N-

ethyldiisopropylamine were purchased from FUJIFILM Wako Pure Chemical Corporation (Osaka, Japan). Trans-β-Apo-8-carotenal was used as the internal standard (IS) and was purchased from Sigma-Aldrich Japan Inc. (Tokyo, Japan).

Animals

All animal studies were approved by the Animal Care and Use Committee of the Graduate School of Pharmaceutical Science, Osaka University. All experimental procedures were conducted in accordance with the guidelines of the Guide for the Care and Use of Laboratory Animals (National Research Council, 2011). Every effort was made to minimize animal suffering and to reduce the number of animals used. Male Sprague–Dawley (SD) rats (7 weeks old) were obtained from Charles River Laboratories Japan, Inc. (Yokohama, Japan) and acclimatized under controlled environmental conditions (22 ± 1°C; 50 ± 10% relative humidity; a 12-hour light-dark cycle, lights on at 8:00 AM) for at least 1 week before the start of the experiment. The rats were fed a certified diet MF (Oriental Yeast Co., Ltd.) and water ad libitum. The rats were fasted overnight before the experiment.

Preparation of BC-SD

β-Carotene, PVP, and Suc were mixed in various proportions (Fig. 2A). To prepare BC-SD, the physical mixtures (PM) were heat kneaded at 180 °C using a twin-screw extruder (Technovel Co., Ltd., Osaka, Japan).

Evaluation of physical properties by powder X-ray diffraction (PXRD)

The crystal state of the sample was analyzed using an automated multipurpose X-ray diffractometer SmartLab (Rigaku Co., Ltd., Tokyo, Japan), equipped with a D/teX Ultra high-speed 1D X-ray detector. The X-ray source was CuKα radiation with a CuKβ filter, and the X-ray output was 45 kV and 200 mA. Sample measurements were taken over a diffraction angle range (2θ) of 5–55 ° at 50 °/min. Before measurement, BC-SD was pulverized using a mixer and passed through a 150-μm sieve.

Evaluation of the dissolution ratio of BC in water

BC-SD (10 to 15 mg) was added to phosphate buffer to obtain a solid dispersion with a concentration of 10 mg/mL. A mass of solid dispersion was dispersed by vortex or ultrasonic waves to prepare a uniform suspension, and then diluted with phosphate buffer solution to obtain the desired concentration. After shaking at 37 °C for 1 h (protected from light), the sample was centrifuged and the supernatant was passed through a 0.2-μm filter. The obtained filtrate was used for HPLC analysis. The dissolution ratio of BC in water was defined by the following formula: Dissolution ratio of BC in water (%) = [amount of BC dissolved in water/amount of BC mixed in solid dispersion] × 100.

BC-SD dissolution test

The dissolution test was carried out using the paddle method, as described in the Japanese Pharmacopoeia dissolution. Total 900 mL of phosphate buffer was placed in the container of the dissolution tester, and 190 mg of BC-SD5, 100 mg of BC-SD10, or 55 mg of BC-SD20 was added. Samples were collected 2, 5, 10, 15, 30, 45, 60, and 120 min after the addition of BC-SD, and the solution was passed through a filter (0.2 μm). The obtained filtrate was used for HPLC analysis.

In vivo pharmacokinetic studies

Rats (8-10 weeks old) were intragastrically administered BC-SD solution, prepared in ion-exchanged water to a concentration of 10 mL/kg. The accurate BC dose for rats was calculated by analyzing a part of the sample by HPLC. Based on the analysis, the BC dose was found to be 33 or 75 mg/kg for BC-SD5 and 137 or 252 mg/kg for BC-SD20. Blood was collected from the jugular vein at 1, 2, 3, 4, and 6 h after administration, under isoflurane anesthesia, and from the heart at 8 h after administration. Blood was collected using a heparin-treated syringe, and the collected blood was transferred to a vacuum blood collection tube (plane). Plasma was isolated by centrifuging the blood samples. Extraction of BC from plasma was performed using the same method as previously reported (Otani et al., 2020).

HPLC analysis

The concentration of BC was measured by HPLC. The HPLC conditions for sample analysis of the dissolution test were the same as those previously reported (Ishimoto et al., 2019). In addition, the HPLC conditions used were similar to those reported by Otani et al. for measuring plasma BC concentration after intravenous administration (Otani et al., 2020).

Pharmacokinetic analysis

Pharmacokinetic analysis of BC after intragastric administration was performed using the free software "MOMENT.xls" provided by Kyoto University (TABATA et al., 1999). The absolute bioavailability of BC was calculated using $AUC_{0-\infty}$ (value obtained from the study by Otal et al.) when BC was intravenously administered to SD rats (Otani et al., 2020). Absolute bioavailability of BC was defined by the following formula: $F\ (\%) = [AUC_{po}/D_{po}]/[AUC_{iv}/D_{iv}] \times 100$. The correction absolute bioavailability of BC by dissolution ratio was defined by the following formula: F (%) [corrected by dissolution ratio] = [F (%) for each BC-SD/dissolution ratio of BC in water for each BC-SD] × 100.

Results

Examination of dissolution ratio of BC amorphous solid dispersion

An amorphous solid dispersion of BC was prepared by heat kneading the materials at a BC:PVP:Suc mixing ratio of 10:70:20 (wt. ratio) (BC-SD10). The actual concentration of BC was measured after dissolving different concentrations of BC-SD10 in water. Although the

actual solubility of BC increased depending on the amount of BC-SD10 dissolved in water, not all BC in BC-SD10 dissolved in water (Fig. 1A). When the dissolution ratio (i.e. the ratio of the dissolved BC amount to the amount of BC added to water) was examined, the amount of BC in BC-SD10 showed a dissolution rate of about 25%, regardless of any time point (Fig. 1B). In other words, about 75% of BC in BC-SD10 was precipitated without being dissolved in water.

Decreasing the ratio of BC to the base materials in the solid dispersion increases the dissolution ratio of BC

In case of a solid dispersion in which the mixing ratio of BC to the base materials was changed, it was decided to examine whether it showed a constant dissolution ratio as observed for BC-SD10. BC-SD5 and BC-SD20, with mixing ratios shown in Figure 2A, were prepared. The improvement in the solubility of BC in water is due to the amorphization of BC mixed in the solid dispersion (Ishimoto et al., 2019). PXRD was used to analyze whether the three types of BC solid dispersions with different mixing ratios were amorphized or not (Fig. 2B). In all BC solid dispersions, the peak observed in PM, which was the pre-heat kneading product, disappeared and a halo patter was seen. Therefore, it was confirmed that all BC-SDs were amorphized.

Next, the dissolution ratio of BC when different concentrations of these BC-SDs were added to water was evaluated (Fig. 3A). Even if the amount of BC-SD input was changed, the

dissolution ratio was approximately 56% for BC-SD5 and approximately 12% for BC-SD20, which indicated a constant dissolution ratio, similar to that of BC-SD10. It was confirmed that the lower the mixing ratio of BC in the solid dispersion, the higher was the dissolution ratio of BC in water. Further, to investigate the effect of solid dispersions with different BC mixing ratios on the dissolution characteristics, a dissolution test based on the Japanese Pharmacopoeia was conducted (Fig. 3B). All BC-SDs almost reached the plateau within 2 min after addition to water. Based on these findings, it was concluded that the change in the mixing ratio of BC in the solid dispersion changes the dissolution ratio, regardless of the elution behavior.

Gastrointestinal absorption of BC depends on the amount of BC dissolved in water in BC-SD

BC-SD10, prepared by our method, showed improved gastrointestinal absorption of BC in rats (Otani et al., 2020). Therefore, we evaluated the effects of BC-SD5 and BC-SD20, which had different dissolution ratios from BC-SD10, on the gastrointestinal absorption of BC. First, the plasma BC concentrations at various time points after oral administration of BC-SD5 or BC-SD20 were examined (Fig. 4A, B). Then, $AUC_{0\text{-}8\ h}$ was calculated from Fig. 4A and B, and it was plotted, as shown in Fig. 4C. Since Otani et al. have already reported the $AUC_{0\text{-}8}$ h of BC-SD10, we created a graph using the reported value (Otani et al., 2020). In each BC-SD, it was revealed that $AUC_{0\text{-}8\ h}$ increased in proportion to the BC dose, and a linear correlation

was observed between the dose and the absorption amount in this range. As absorption saturation did not occur in these data, the relationship between BC dose and absorption amount could be evaluated appropriately. Since the doses of BC were different in different formulations, it was difficult to make a simple comparison of BC-SD. Nonetheless, it was observed that BC-SD5, which had a high dissolution ratio in water, tended to have a low dose but a high absorption. On the other hand, BC-SD20, which has a low dissolution ratio in water, tended to have a high dose but low absorption. Figure 4D shows that there was no correlation between the amount of BC in BC-SD orally administered to rats and $AUC_{0\text{-}8\ h}$ ($R^2 = 0.0136$). Next, the amount of BC dissolved in water was calculated by multiplying the amount of BC orally administered to rats by the dissolution ratio of each BC-SD in water, and the correlation between that value and $AUC_{0\text{-}8\ h}$ was examined (Fig. 4E), and a high correlation ($R^2 = 0.8373$) was observed between these two parameters. This suggested that as the amount of BC dissolved in water increased, the amount of BC absorbed also increased. Therefore, although the amount of BC administered to rats was low for BC-SD5, it is considered that the amount of BC-SD5 absorbed was improved because the amount of BC dissolved in water was high.

A positive correlation exists between the dissolution ratio of BC in water and its absolute bioavailability

Next, the effect of changes in the BC mixing ratio in BC-SD on absolute bioavailability was examined (Fig. 5A). It was observed that BC-SD5, which has a high

dissolution ratio in water, had the highest absorption rate. In the correlation diagram showing the dissolution ratio and absorption rate of each BC-SD, it was found that the dissolution ratio of BC in water had a positive correlation with absolute bioavailability (Fig. 5B). These results suggest that the dissolution ratio in water and the absorption rate may improve by lowering the mixing ratio of BC to the base materials.

It was assumed that the absolute bioavailability of BC can be maximized when a BC aqueous solution with 100% dissolution ratio of BC in water is administered. When absolute bioavailability (F) of each BC-SD was corrected by the dissolution ratio of each BC and calculated by averaging it, the absolute bioavailability of BC was approximately 0.11%. This indicated that BC-SD prepared by our method can improve the absolute bioavailability of BC up to this ratio.

Discussion

To ensure that BC is able to exert its beneficial pharmacological effects when incorporated in various health foods, pharmaceuticals, and cosmetics, it is necessary to overcome its low water solubility and bioavailability. In this study, we developed an original formulation technology that heat-kneads the three components BC, PVP, and Suc (Ishimoto et al., 2019). When BC-SD prepared by this method was dissolved in water, the solubility of BC in water has been reported as 117 ± 11 μg/mL, which was the highest value reported in previous studies (Ishimoto et al., 2019). As shown in Fig. 1A, BC-SD showed a solubility of 201 ± 5

μg/mL, which exceeded the value reported in the previous study. Furthermore, it was confirmed that when a high concentration of BC-SD was added to water, the BC solubility was 2.34 ± 0.05 mg/mL (data not shown). Although this formulation technology could remarkably improve the water solubility of BC, it could not address the problem of low dissolution ratio in water. In this study, it was clarified that the dissolution ratio to water was improved to the greatest extent for BC-SD5, in which the BC mixing ratio was reduced (Fig. 3A). Our findings are in line with previously reported studies suggesting that the initial dissolution ratio increases when the mixing ratio to the base materials is reduced, even when other compounds are used (Craig, 2002). In addition to the dissolution of the compound, reducing the aggregation and nucleation rate of the dissolved compound is an important factor for improving the dissolution ratio (Baghel et al., 2016; Konno & Taylor, 2006; Tekade & Yadav, 2020). Polymers, which are mostly used as carriers for solid dispersions, can improve the stabilization of amorphous solid-dispersed compounds by interacting with the compounds (Yen et al., 2010). Therefore, the rate of crystal formation can be suppressed by reducing the molecular motility of the compound (Lindfors et al., 2008; Sethia & Squillante, 2004). It also enhances the physical and chemical stability of the compound in solid dispersions by preventing nucleation and aggregation of the compound, although it is an emulsifier commonly used to improve the dissolution profile of the compound during formulation (Soottitantawat et al., 2004; Tekade & Yadav, 2020). Chen et al. have reported that emulsifiers have the effect of suppressing nucleation and crystal

formation rates by solubilizing compounds and forming micelles (Chen et al., 2015). From these findings, it is considered that BS-SD5, which had a sufficient mixing amount of the polymer and emulsifier with respect to the amount of BC, could maintain a high dissolution ratio as compared with BC-SD10 and BC-SD20. In contrast, it is presumed that the mechanism of action of the compound on dissolution differs between the polymer and the emulsifier. Therefore, it is expected that the dissolution ratio can be further improved by examining the mixing ratio of the polymer and the emulsifier and balancing the respective effects that contribute to the dissolution of the compound.

The major gastrointestinal absorption route of BC is by simple diffusion via micelle formation (Erdman et al., 1993). After phospholipids and bile acids, secreted as bile, promote the dispersion of BC in the gastrointestinal tract, mixed micelles are produced in the small intestinal lumen and finally absorbed only by BC solubilized in small intestinal epithelial cells (Reboul, 2019). Baskaran et al. prepared BC complex mixed micelles and used them to improve absorption of BC in mice and rats (Baskaran et al., 2003; RAJU et al., 2005). However, in our study, the BC-SD was formulated based on the strategy of increasing the amount of BC in the solubilized state in the gastrointestinal tract by increasing the dissolution ratio of BC (Fig. 4). Even if the PM of the poorly water-soluble BC-SD10 is orally administered to rats, the amount of BC absorbed from the gastrointestinal tract could not be detected (Otani et al., 2020). Using a similar strategy, Jain et al. formulated BC-encapsulated solid lipid nanoparticles, which

improved the absorption of BC by increasing the elution of BC (Jain et al., 2019). Thus, overcoming the low solubility of BC in the gastrointestinal tract appears to be a promising strategy to improve the biopharmaceutical properties of BC.

When BC-SD5 was intragastrically administered to rats, the absolute bioavailability of BC showed the greatest improvement (Fig. 5). This value was higher than that reported by Otani et al (Otani et al., 2020). The high absolute bioavailability of BC despite the low proportion of BC in the solid dispersion is an advantageous condition for practical use. When considering the practical application of this BC-SD, it is a considerable cost advantage as it reduces the mixing ratio of BC, which is the most expensive raw material. However, when this BC-SD was administered to rats, the maximum absolute bioavailability was approximately 0.11%. In order to achieve further improvement in the absolute bioavailability of BC, it is necessary to optimize the blending ratio of PVP and Suc, as described above, or to make improvements such as changing the polymer and emulsifier.

Conclusion

BC-SD prepared by our method increases the dissolution ratio of BC in water by reducing the mixing ratio of the base materials to BC. It was also shown that the increase in dissolution ratio leads to an improvement in the absolute bioavailability of BC. These results

are useful findings from the perspective of reduction in costs in the BC-SD associated with the manufacturing process, and will be an important part of the strategy for future practical use.

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Figure Captions

Fig. 1 Solubility and dissolution ratio of BC in water when different concentrations of BC-SD10 were suspended in water

(A) Actual BC concentration relative to theoretical BC concentration when BC-SD10 added to water

(B) Dissolution ratio of BC in water with respect to the theoretical BC concentration when BC-SD10 was added to water.

Fig. 2 Evaluation of physical properties of BC-SD prepared by changing the mixing ratio

(A) Mixing ratio of BC, PVP, and Suc of BC-SD prepared in this study.

(B) Analysis of BC-SD crystal state using powder X-ray diffraction. PM; physical mixture, SD; solid dispersion.

Fig. 3 Water solubility evaluation of BC-SD prepared by changing the BC mixing ratio

(A) Dissolution ratio of BC in water with respect to BC-SD concentration after each BC-SD was added (mean ± S.D., n = 3).

(B) Transition of BC elution after each BC-SD was added (mean ± S.D., n = 3).

Fig. 4 Plasma levels of BC in SD rats after oral administration of BC-SD

(A) Transition of BC plasma concentration after administration of BC-SD5. BC-SD5 was administered at 33 or 75 mg/kg as the BC equivalent (mean ± S.D., n = 3).

(B) Transition of BC plasma concentration after administration of BC-SD20. BC-SD20 was administered at 137 or 252 mg/kg as the BC equivalent (mean ± S.D., n = 3).

(C) $AUC_{0\text{-}8\ h}$ for each BC-SD (mean + S.D. n = 3-5). The value of BC-SD10 is quoted from the report by Otani et al.

(D) Correlation diagram of $AUC_{0\text{-}8\ h}$ with respect to the amount of BC administered to rats.

(E) Correlation diagram of $AUC_{0\text{-}8\ h}$ to the value obtained by multiplying the amount of BC administered to rats by the dissolution ratio of each BC-SD in water.

Fig. 5 BC absolute bioavailability in each BC-SD

(A) Dissolution ratio of BC in water and its absolute bioavailability for each BC-SD

(B) Correlation diagram of BC absolute bioavailability with respect to the dissolution ratio of BC in water for each BC-SD

(C) Absolute bioavailability of BC when the dissolution ratio of BC in water for each BC-SD is assumed to be 100%. These values were calculated by correcting BC absolute bioavailability with the dissolution ratio of each BC-SD in water.

Fig. 1

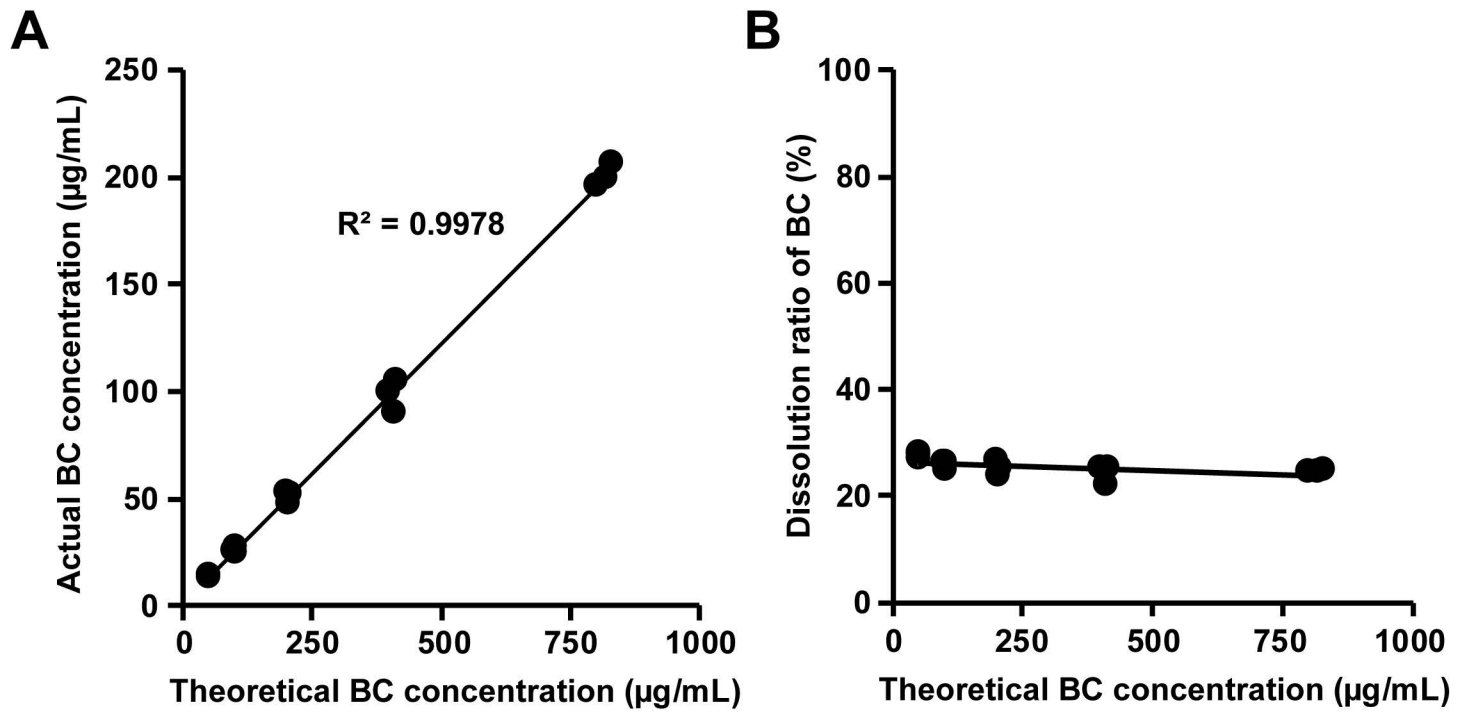

A
250
200
150
100
50
0
Actual BC concentration (μg/mL)
R² = 0.9978
0
250
500
750
1000
Theoretical BC concentration (μg/mL)
B
100
80
60
40
20
0
Dissolution ratio of BC (%)
0
250
500
750
1000
Theoretical BC concentration (μg/mL)

Fig. 2

A

Solid dispersion	Mixing wt. ratio		
	BC	PVP	Suc
BC-SD5	5	70	20
BC-SD10	10	70	20
BC-SD20	20	70	20

B

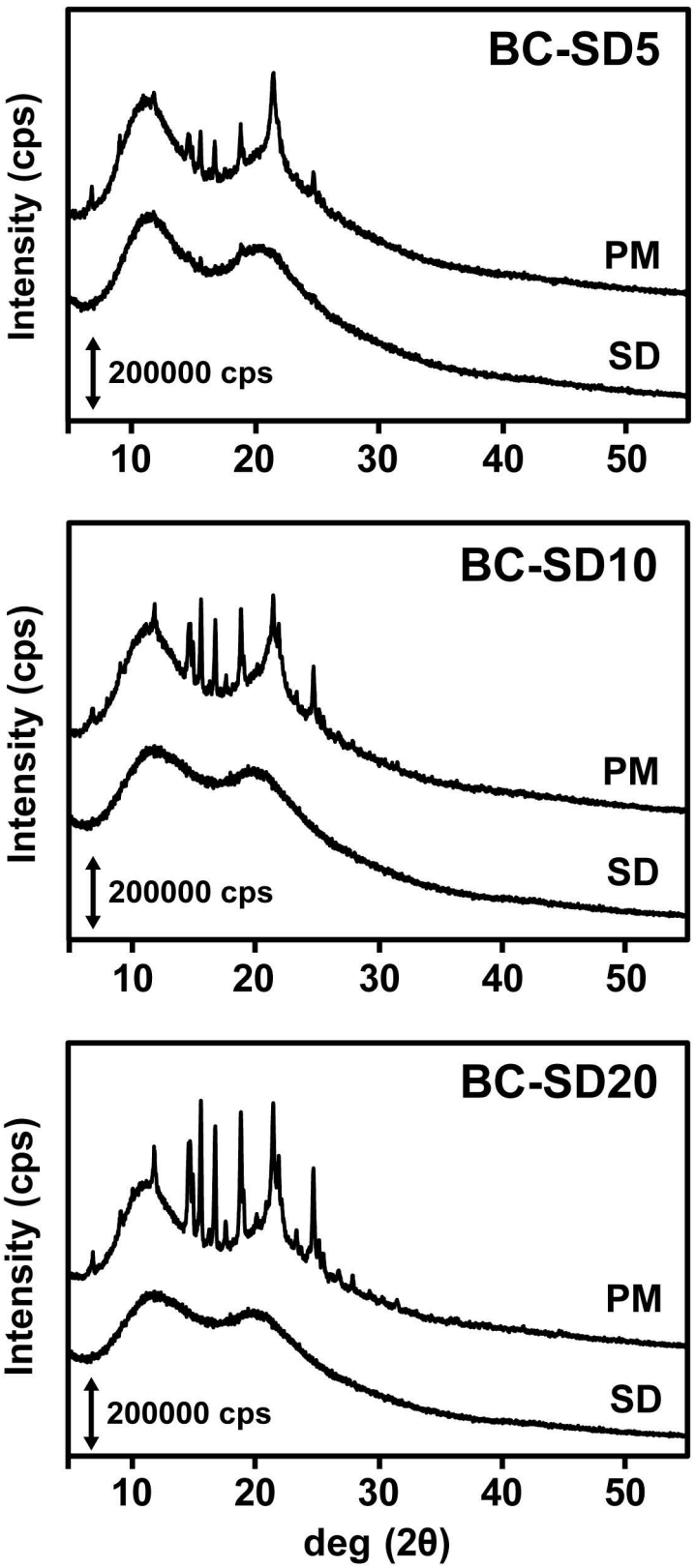


Fig. 3

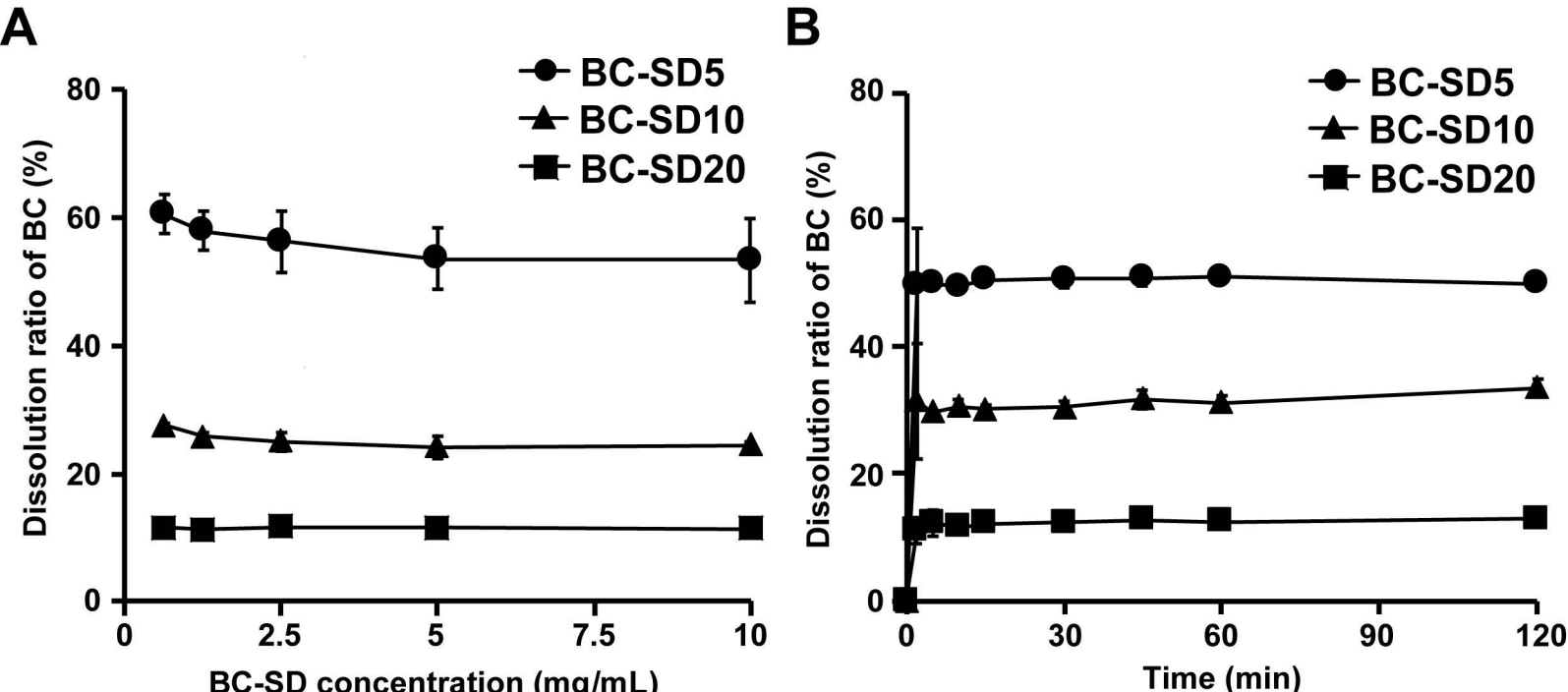

A
BC-SD5
BC-SD10
BC-SD20
Dissolution ratio of BC (%)
0
20
40
60
80
0
2.5
5
7.5
10
BC-SD concentration (mg/mL)
B
BC-SD5
BC-SD10
BC-SD20
Dissolution ratio of BC (%)
0
20
40
60
80
0
30
60
90
120
Time (min)

Fig. 4

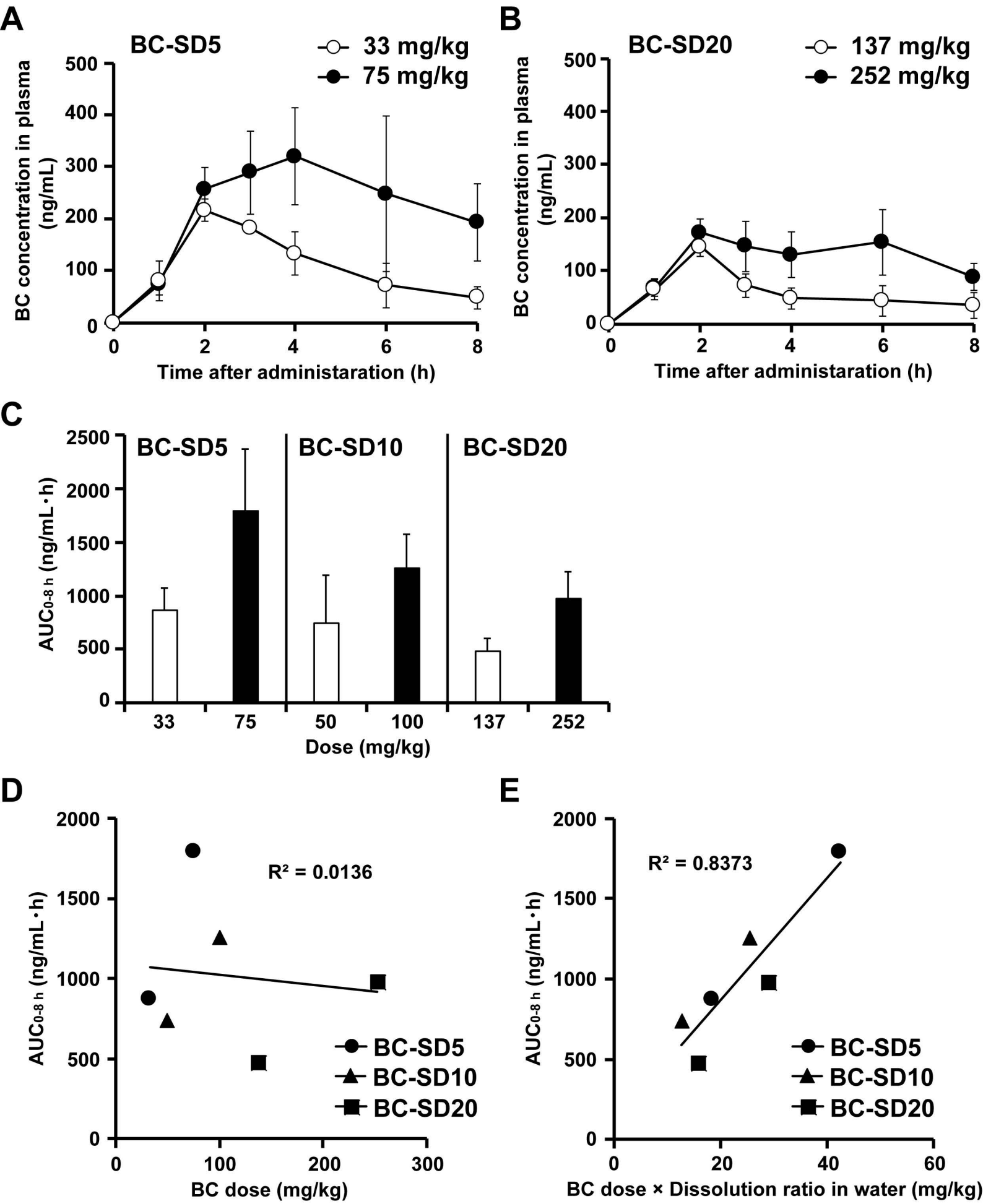
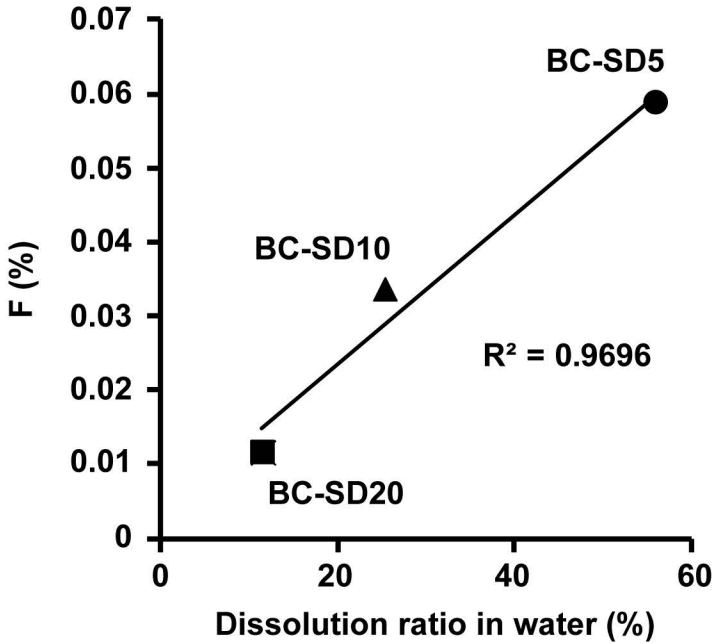

A
BC-SD5
33 mg/kg
75 mg/kg
BC concentration in plasma (ng/mL)
Time after administaration (h)
B
BC-SD20
137 mg/kg
252 mg/kg
BC concentration in plasma (ng/mL)
Time after administaration (h)
C
BC-SD5
BC-SD10
BC-SD20
AUC0-8 h (ng/mL·h)
33
75
50
100
137
252
Dose (mg/kg)
D
R² = 0.0136
AUC0-8 h (ng/mL·h)
BC-SD5
BC-SD10
BC-SD20
BC dose (mg/kg)
E
R² = 0.8373
AUC0-8 h (ng/mL·h)
BC-SD5
BC-SD10
BC-SD20
BC dose × Dissolution ratio in water (mg/kg)

Fig. 5

A

	BC-SD5	BC-SD10	BC-SD20
Dissolution ratio in water (%)	56.3 ± 4.8	25.4 ± 1.5	11.5 ± 0.7
F (%)	0.059 ± 0.026	0.033 ± 0.020	0.012 ± 0.006

B



C

	F (%) [Corrected by dissolution ratio]
BC-SD5	0.104 ± 0.047
BC-SD10	0.132 ± 0.080
BC-SD20	0.101 ± 0.054
Average	0.112 ± 0.060