Improvement of Stability and Dissolution of Prostaglandin E_1 by Maltosyl-β-cyclodextrin in Lyophilized Formulation

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To improve undesirable pharmaceutical properties of prostaglandin E_1 (PGE_1) in lyophilized formulation, potential use of highly water-soluble maltosyl-β-cyclodextrin (G_2-β-CyD) was examined, comparing it with parent β-cyclodextrin (β-CyD). Inclusion complexation of PGE_1 with G_2-β-CyD in an aqueous solution was estimated by the solubility method, circular dichroism and carbon-13 nuclear magnetic resonance spectroscopies. PGE_1 was freeze-dried with various additives and subjected to stability and dissolution tests. When the amorphous products were stored at 60 °C, decomposition of PGE_1 was significantly decelerated by both G_2-β-CyD and β-CyD, while it was accelerated by mannitol as an inert ingredient. During storage, the rapid dissolving property of PGE_1 was maintained by complexation with G_2-β-CyD, while it tended to decrease by β-CyD, depending on the moisture-adsorbing and wetting properties along with crystallinity change of the additives. The limited data obtained here suggests that G_2-β-CyD may be preferable to β-CyD for the improvement of chemical instability and poor dissolution properties of PGE_1 in dry solids for injections.

Keywords maltosyl-β-cyclodextrin; prostaglandin E_1; inclusion complex; lyophilized formulation; stabilization; solubilization; dissolution

The solubility and chemical instability of naturally occurring prostaglandins, including E-type prostaglandins (PGEs), in aqueous solution have limited dosage form development and result in a substantial challenge to the pharmaceutical scientist.[1] In the currently available PGEs formulations, inclusion complex formations with natural cyclodextrins (CyDs) such as α- and β-CyDs are successfully utilized for the solubilization and stabilization of PGEs.[2,3] However, the promising advantages of these CyDs are limited by their relatively low solubility in water.[4,5] Recently, extensive efforts have been directed toward the development of more hydrophilic and safer CyD derivatives as parenteral drug carriers.[6,7] For example, 6-*O*-α-D-maltosyl-β-CyD (G_2-β-CyD), in which one of the primary hydroxyl groups of β-CyD is substituted by di-saccharide through the α-1,6 glycosidic linkage, has received increased attention in the pharmaceutical field.[8] G_2-β-CyD can be obtained in a high state of purity and has significant advantages over parent β-CyD; higher solubility in water, excellent solubilizing ability for poorly water-soluble drugs and lower hemolytic activity.[9-11] Thus, the present study dealt with the potential use of G_2-β-CyD in the lyophilized formulation in anticipation of improved stability and dissolution of PGE_1 during accelerated storage conditions.

Experimental

Materials G_2-β-CyD and β-CyD were supplied by Ensuiko Sugar Refining Co., Ltd. (Yokohama) and Nihon Shokuhin Kako Co. (Tokyo), respectively. PGE_1 and $PGF_{2\alpha}$ were donated by Ono Pharmaceutical Co., Ltd. (Osaka). Other chemicals and solvents were of analytical reagent grade, and deionized double-distilled water was used throughout the study.

Apparatus The circular dichroism (CD) spectra were obtained by a Jasco-40S recording spectropolarimeter (Tokyo), and expressed in terms of molar ellipticity [θ]. The carbon-13 nuclear magnetic resonance (^{13}C-NMR) spectra were taken on a JNM-FX270 (JEOL, Tokyo). The NMR spectra were recorded for degassed solutions of $PGF_{2\alpha}$ (0.02 M) in the absence and presence of additives (0.02 M for β-CyDs and 0.14 M for mannitol) in 0.1 M sodium borate buffer (pH meter reading of 9.3) in 5 mm spinning tubes at an ambient temperature (about 25 °C) using D_2O solvent. ^{13}C-Chemical shifts were referenced to external tetramethylsilane with accuracy of ±0.014 ppm. All peaks were assigned according to the previous paper.[12] The DSC thermograms were obtained by a Rigaku Denki TAS 100 (Tokyo), using a scanning rate of 10 °C/min. Powder X-ray diffraction patterns were taken on a Geiger Flex 2012 diffractometer, operating under the following conditions: X-ray, Ni-filtered Cu-K_α radiation; voltage, 30 kV; current, 20 mA; time constant, 2 s; scanning speed, 1°/min.

Solubility Studies Solubility measurements were carried out according to Higuchi and Connors.[13] Excess amounts of PGE_1 were added to aqueous solutions containing various concentrations of additives (β-CyD, G_2-β-CyD or mannitol) and were shaken for 10 h at 25 °C. Then, an aliquot was centrifuged and pipetted through a cotton plug. A 0.5 ml of sample was diluted with the mobile phase described below and PGE_1 was assayed by high-performance liquid chromatography (HPLC) under the following conditions: pump and detector, a Hitachi L-6000 liquid chromatograph (Tokyo) with a Hitachi L-4000 UV monitor (Tokyo); column, Tosoh TSK-GEL ODS-120T (5 μm, 4.6 mm diameter × 150 mm, Tokyo); mobile phase, 0.01 M KH_2PO_4 solution–acetonitrile (3:2); flow rate, 1.0 ml/min; detection, 201 nm. PGE_1 was quantitated by measuring peak heights and compared with those of a known amount of internal standard, cortisone 21-acetate. Under this condition, no appreciable degradation of PGE_1 was observed. The apparent 1:1 stability constants of inclusion complexes were calculated from the slope and intercept of the initial straight line portion of the solubility diagrams.

Preparation of Lyophilized Samples The amorphous PGE_1 powders were prepared according to the freeze-drying method.[14] PGE_1 and additives in 1:32 weight ratio, which was reported to be a suitable formulation for parenteral application,[15] were dissolved in water, and the solutions were freeze-dried at about −50 °C (dry ice-acetone) under a 20—30 millitorr chamber pressure over night. Unless otherwise stated, citric acid (6.7%) was added to the solution as a pH lowering agent to prevent the formation of PGB_1.[15]

Stability Studies 7.1 mg (equivalent to 200 μg PGE_1) of solid samples were put in test tubes, sealed tightly with glass-stoppers, and stored in an incubator at 60 °C. Ths storage temperature was chosen for the convenience of kinetic measurements because the degradation rate of PGE_1 was rather slow at room temperature.[16] At appropriate intervals, the samples were withdrawn and intact PGE_1 in the samples was assayed by HPLC according to the method described above.

Measurements of Some Physical Properties of Powder Samples The lyophilized β-CyDs or mannitol (100 mg) was weighed into a test tube which was then sealed tightly with a screw cap, and was placed in an incubator at 60 °C. At appropriate intervals, the moisture contents of the sample powders were determined by the Karl–Fisher method, using a Kyoto-Denshi MKA-3P (Kyoto). Water penetration rates into the test samples, as tentative measure of wettability, were measured according to the method reported previously.[17]

Dissolution Studies The dissolution behaviors of PGE_1 samples in water were examined by employing the dispersed amount method.[18] The powder samples were put into 25 ml water which was kept at 25 °C and stirred at 57 rpm. Samples (0.5 ml) were withdrawn from the flask at appropriate time intervals and filtered through a 0.45 μm membrane filter, diluted with

the mobile phase and assayed by HPLC according to the method described above. The volume in the vessel was replaced with water after each sampling.

Results and Discussion

Evidences of Inclusion Complexation Figure 1 shows the phase solubility diagrams for PGE_1–β-CyDs systems in water. The solubility of PGE_1 increased linearly as a function of β-CyDs concentration, showing a feature of the A_L-type phase solubility diagram.[13] On the other hand, no solubilization of PGE_1 was observed for the mannitol system. These suggest the soluble complex formations of PGE_1 with both β-CyD and G_2-β-CyDs. Then, apparent 1 : 1 stability constants (K') for the complexes were calculated from the straight lines of the solubility diagrams. The K' value for G_2-β-CyD complex ($1060\,M^{-1}$) was smaller than that for β-CyD complex ($1700\,M^{-1}$), probably due to the steric hindrance of the maltosyl group in G_2-β-CyD.[11] However, G_2-β-CyD is a better solubilizer than parent β-CyD, since its intrinsic aqueous solubility (>50%) is much higher than that of β-CyD (about 2%).

Figure 2 shows the CD spectra of PGE_1 in the absence and presence of G_2-β-CyD in phosphate buffer (pH 6.0). PGE_1 exhibits a negative CD band around 292 nm due to $n\rightarrow\pi^*$ transition of C-9 carbonyl chromophore. By the addition of G_2-β-CyD, this negative peak was shifted to a longer wavelength with a slight decrease in optical activity. The β-CyD system also showed similar spectral changes as reported,[19] while no appreciable change was observed for the PGE_1–mannitol system indicating negligible interaction between PGE_1 and mannitol. Although the intrinsic CD of PGE_1 can be compensated by the induced CD of PGE_1 by binding to G_2-β-CyD, the above results suggest that the chromophor of PGE_1 may be located within the hydrophobic cavity of G_2-β-CyD.

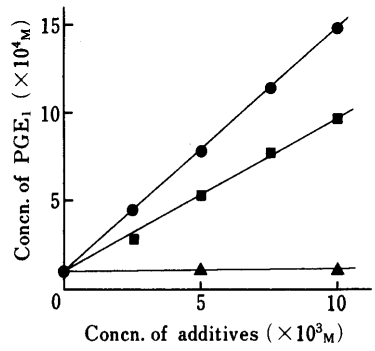


Fig. 1. Phase Solubility Diagrams of PGE_1–β-CyDs or –Mannitol Systems in Water at 25 °C

●, β-CyD; ■, G_2-β-CyD; ▲, mannitol.

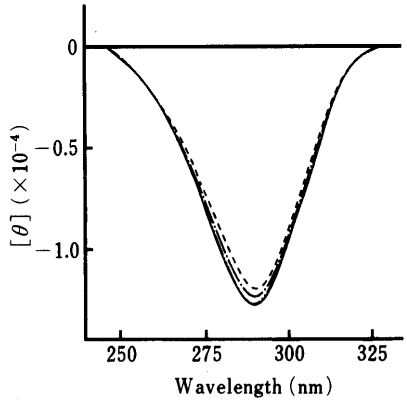
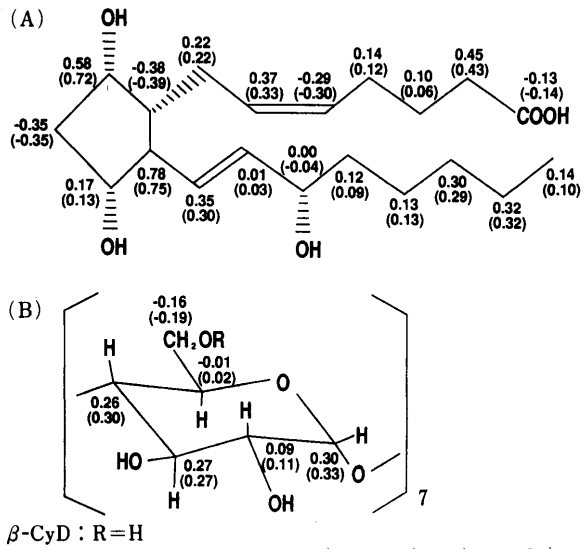


Fig. 2. CD Spectra of PGE_1 in the Presence and Absence of Additives in 0.1 M Phosphate Buffer (pH 6.0)

——, in the absence of β-CyDs; – – –, in the presence of β-CyD (1×10^{-4} M); —·—, in the presence of G_2-β-CyD (1×10^{-4} M); ··—··—, in the presence of mannitol (7×10^{-4} M).

The inclusion mode was further examined by means of the ^{13}C-NMR technique, using a chemically stable $PGF_{2\alpha}$ instead of PGE_1. Figure 3 shows the induced ^{13}C-chemical shift changes of $PGF_{2\alpha}$ and G_2-β-CyD in 0.1 M sodium borate–D_2O solution. It is apparent that the large shift changes were observed around the five-membered ring carbons (C5—C13), whereas the change was smaller in the ω-chain. β-CyD also showed similar shift changes as reported.[12,20] In the case of mannitol, the shift change of $PGF_{2\alpha}$ was negligible ($<\pm0.014$ ppm) even at a higher concentration (0.14 M). Prostaglandin molecules are known to be made up of three moieties, from a dynamic point of view, *i.e.*, a terminal alkyl chain in the ω-chain, an alkyl chain in the α-chain containing carboxyl group, and a five-membered ring and its neighborhood in the order of the reduced conformational freedom.[21,22] Therefore, the above result suggested that β-CyDs preferentially include the most rigid portion of $PGF_{2\alpha}$, *i.e.*, the five-membered ring and its neighborhood. Figure 3(B) shows the ^{13}C-shift changes of G_2-β-CyD induced by the addition of $PGF_{2\alpha}$, where the results on unsubstituted glucose units in the β-CyD ring are listed because ^{13}C-peaks of the maltosyl moiety and maltosyl-substituted glucose unit could not be unequivocally assigned.[23] The C1 and C4 carbons of β-CyDs showed the large shift changes, which may be due to the conformational changes in the macrocycle upon complexation with $PGF_{2\alpha}$.[24] The C3 carbon, which locates inside the cavity, also showed the large shift, supporting that the guest molecule is included within the hydrophobic



β-CyD : R=H

G_2-β-CyD : 1 glucose unit R=-($C_6H_{10}O_4$)-O-($C_6H_{11}O_5$)

6 glucose units R=H

Fig. 3. ^{13}C-NMR Chemical Shift Changes of $PGF_{2\alpha}$ (A), β-CyD (B) and G_2-β-CyD (B) through Inclusion Complexation in Sodium Borate Buffer (pH Meter Reading of 9.3)

A chemical shift change in the downfield direction is expressed as a positive value and an opposite change is expressed as a negative value. The unparenthesized and parenthesized numbers are the induced chemical shift changes for G_2-β-CyD and β-CyD systems, respectively.

cavity of G_2-β-CyD. No appreciable difference of the shift changes between the β-CyD and G_2-β-CyD complexes was observed, suggesting that both complexes have a similar inclusion structure. From the above CD and NMR spectroscopic studies, it can be assumed that the cyclopentanone moiety, *i.e.*, reactive site of PGE_1, is included in the cavity of β-CyDs.

The complex formation of PGE_1 with G_2-β-CyD in the solid state was examined by DSC analysis. Figure 4 shows DSC thermograms of the lyophilized PGE_1–β-CyD complexes. In the case of the physical mixture of PGE_1 and β-CyDs, an endothermic peak due to the melting of PGE_1 was observed around 115 °C. In contrast, the β-CyD and G_2-β-CyD complexes showed no appreciable endothermic peak. This endothermic peak did not disappear in the lyophilized PGE_1–mannitol product, as shown in Fig. 6 where the endothermic peaks due to the meltings of PGE_1 and mannitol (166 °C) were observed. These results indicated that PGE_1 may interact with β-CyDs in the solid state and exist in an amorphous form.[16]

Thermal Stability With increasing temperature, the β-hydroxyketo moiety of PGE_1 is known to be extremely susceptible to dehydration in relatively high acidic and alkaline conditions, giving PGA_1 which is then isomerized consecutively to PGB_1 under alkaline conditions with loss of the pharmacological activity.[25,26] In this study, an attempt was made to evaluate the effect of G_2-β-CyD on the thermal stability of PGE_1 in the solid state. Figure 5 shows the degradation curves of the lyophilized PGE_1–β-CyD complexes in the presence of citric acid at 60 °C. By the addition of citric acid, the dehydration of PGE_1 was decelerated and no decomposition product other than PGA_1 was detected because of the prevention of the isomerization of PGA_1 under the acidic condition. It is obvious that the decomposition of PGE_1 was significantly suppressed by both β- and G_2-β-CyDs, probably due to the inclusion of the β-hydroxyketo moiety of PGE_1, as expected from the ^{13}C-NMR measurements (Fig. 3).

To gain insight into the stabilization mechanism of β-CyDs, the effect of aging on the moisture sorption of additives was examined, and the results are listed in Table I. It can be assumed that the moisture sorption behavior of β-CyDs may also be responsible for the stabilization of PGE_1, *i.e.*, β-CyDs adsorb the headspace moisture of the container and decrease the water vapor responsible for the degradation of PGE_1. Moreover, a suitable acidic en-

TABLE I. Moisture Adsorptions of Lyophilized β-CyDs and Mannitol, Stored at 60 °C

Property	Day	β-CyD	G_2-β-CyD	Mannitol
Moisture adsorbed (%)[a]	0	1.59±0.32	1.80±0.16	1.31±0.33
	1	6.28±0.48[b]	5.64±0.32[b]	2.04±0.15
	3	7.19±0.31[b]	7.10±0.55[b]	2.20±0.17

a) Determined by Karl–Fischer method. *b*) $p<0.05$ *vs.* 0 d.

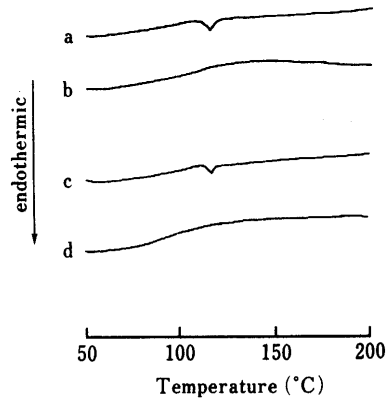


Fig. 4. DSC Thermograms of PGE_1–β-CyD Systems

a, physical mixture of PGE_1 and β-CyD; b, lyophilized PGE_1 with β-CyD; c, physical mixture of PGE_1 and G_2-β-CyD; d, lyophilized PGE_1 with G_2-β-CyD.

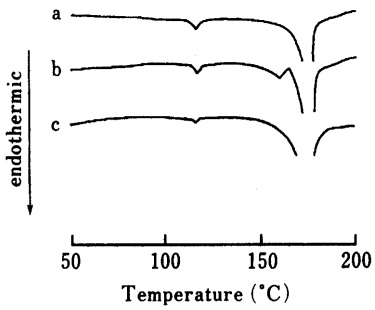


Fig. 6. DSC Thermograms of PGE_1–Mannitol Systems

a, physical mixture of PGE_1 and mannitol; b, lyophilized PGE_1–mannitol product immediately after preparations; c, lyophilized PGE_1–mannitol product after storage of 3 d at 60 °C.

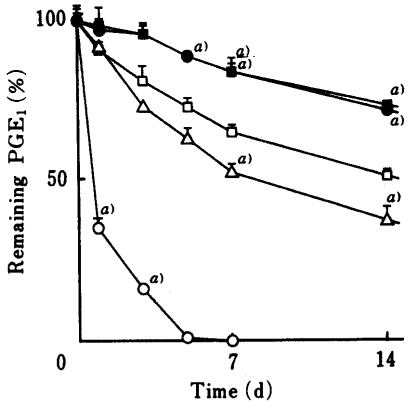


Fig. 5. Effects of β-CyDs and Mannitol on the Stability of PGE_1 Lyophilized Products, Stored at 60 °C

○, PGE_1 alone; □, PGE_1 alone with citric acid; ●, β-CyD complex with citric acid; ■, G_2-β-CyD complex with citric acid; △, mannitol product with citric acid. *a*) $p<0.05$ *vs.* PGE_1 alone with citric acid (□).

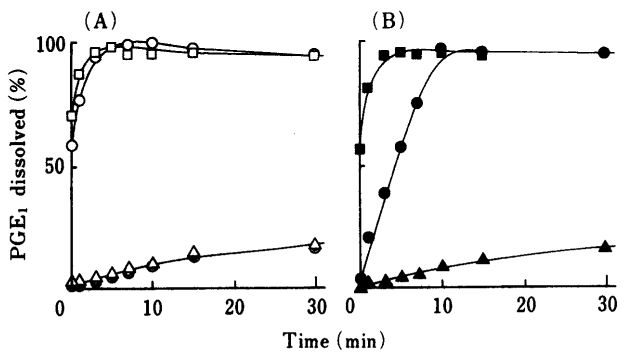


Fig. 7. Dissolution Profiles of PGE_1 from Lyophilized PGE_1 Products in Water at 25 °C, Measured by Dispersed Amount Method (57 rpm)

◎, PGE_1 alone (without lyophilization); ○●, with β-CyD; □■, with G_2-β-CyD; △▲, with mannitol. (A) immediately after lyophilization, (B) after storage of 3 d at 60 °C.

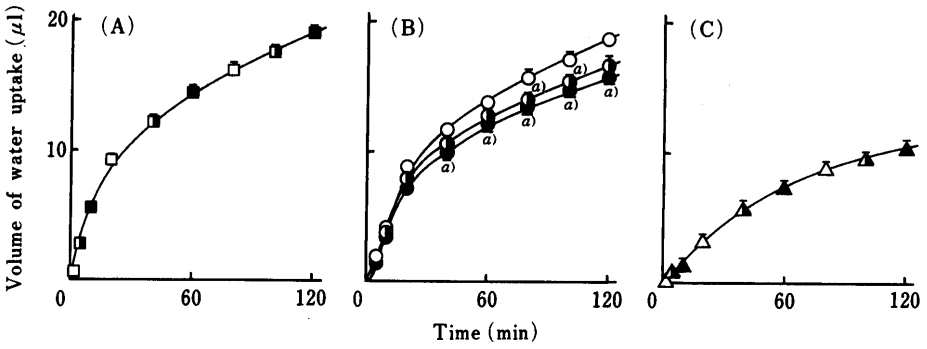


Fig. 8. Water Penetration Behaviors of Lyophilized G_2-β-CyD (A), β-CyD (B) and Mannitol (C) at 25 °C

○□△, immediately after lyophilization; ◑◧▲, after storage of 1 d at 60 °C; ●■▲, after storage of 3 d at 60 °C. *a*) $p<0.05$ *vs.* immediately after lyophilization.

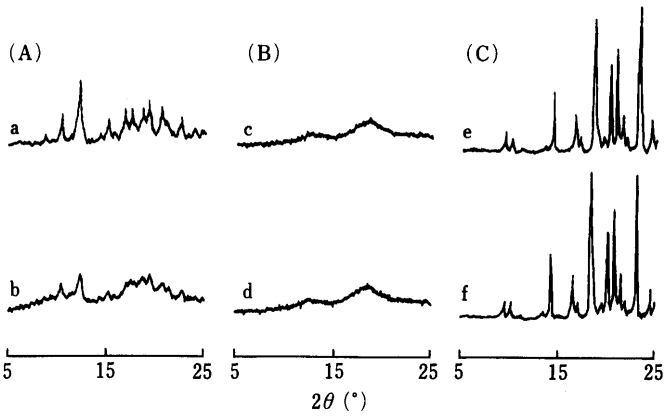


Fig. 9. Powder X-Ray Diffraction Patterns of β-CyD, G_2-β-CyD and Mannitol Alone

(A) β-CyD, (B) G_2-β-CyD, (C) mannitol. a, c, e, after storage of 3 d at 60 °C. b, d, f, immediately after lyophilization.

vironment around PGE_1 can be provided by citric acid, dissolving into the aqueous phase of the surface of solid complexes, since the most stable pH of PGE_1 is reported to be about 3.5.[25] On the other hand, mannitol, which has no inclusion property and relatively low moisture-adsorbing property, was found to rather accelerate the degradation of PGE_1 during storage. As shown in Fig. 6, this degradation behavior of PGE_1 in the mannitol system was also reflected in the DSC thermograms, where the endothermic peak due to the melting of PGE_1 around 115 °C decreased with the passage of time.

Dissolution Behavior The effect of aging on the dissolution behavior of PGE_1 was examined from the viewpoint of quality assurance of dry solid formulations. The dissolution profiles of PGE_1 from the lyophilized powders in water are shown in Fig. 7. Immediately after freeze-drying, both β- and G_2-β-CyD complexes dissolved much more rapidly (about 7-fold) than the PGE_1–mannitol system. The increase in rate may be due to the increase in solubility and the decrease in crystallinity of PGE_1 by inclusion complexation, as expected from Fig. 1 and Fig. 4, respectively. In addition, superior wetting properties of β-CyDs compared with mannitol (see Fig. 8) may also be responsible for the increase in dissolution rate. After being stored for 3 d at 60 °C, the initial dissolution rate of the β-CyD complex tended to decrease, compared to the G_2-β-CyD complex. In this storage condition, no appreciable decomposition of PGE_1 was observed for both β-CyDs systems, while about 20% decomposition of PGE_1 was detected for the mannitol system. The decrease in dissolution rate observed for the β-CyD complex may be ascribed to the increase in crystallinity of β-CyD during the storage, as shown in Fig. 9. Furthermore, as shown in Fig. 8, the wettability of β-CyD decreased significantly with the passage of time, while those of G_2-β-CyD and mannitol were almost constant, which may also be responsible for the decrease in the dissolution of the β-CyD complex.

The above-mentioned results suggest that the fast-dissolving form of the PGE_1–G_2-β-CyD complex with good storage property is superior to that of the parent β-CyD complex, and the present approach may be applicable to other PGEs having undesirable physicochemical properties. Furthermore, in the development of dry solids suitable for injections, G_2-β-CyD has a significant advantage over other hydrophilic β-CyD derivatives with respect to the higher purity and the lack of surface activity.[7,11]

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