Preparation of Four Types of Coenzyme Q10/γ-Cyclodextrin Supramolecular Complexes and Comparison of Their Pharmaceutical Properties

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In this study, we prepared four kinds of complexes of Coenzyme Q10 (CoQ10) with γ-cyclodextrin (γ-CyD) by the kneading methods and the solubility methods with or without heating, and compared their pharmaceutical properties. Differential scanning calorimetrical curves and powder X-ray diffraction patterns showed that the complexes formed pseudorotaxane-like supramolecular structure, although included free γ-CyD and CoQ10, when prepared by the kneading method and solubility method without heating. At the preparation process, a heating improved the complexation of CoQ10 with γ-CyD in the both methods. The dispersion rate of CoQ10 in water increased in the order of CoQ10 alone≈physical mixture with γ-CyD≪solubility/heating product<solubility product<kneading/heating product<kneading product, possibly due to submicron-ordered particle formulation. Of the various ointments containing CoQ10 alone, the release of CoQ10 from hydrophilic ointment was fastest. In addition, the release rate of CoQ10 from hydrophilic ointment in the solubility/heating product was markedly increased, compared to that in the CoQ10/γ-CyD physical mixture and the other complexes. The fast release of CoQ10 from hydrophilic ointment could be involved in propylene glycol in the ointment. These results suggest that supramolecular complexes of CoQ10 with γ-CyD can be prepared by various methods, and among various complexes the pseudorotaxane-like CoQ10/γ-CyD complexes prepared by the solubility method with heating have the potential for preparation of ointments.

Key words Coenzyme Q10; cyclodextrin; supramolecule; pseudorotaxane; hydrophilic ointment; heating

Cyclodextrins (CyDs) are cyclic oligosaccharides consisting of usually six, seven, or eight glucose units (α-, β- and γ-CyDs, respectively) bound by 1,4-glycosidic linkages. CyDs can generally form inclusion complexes with a number of lipophilic substances as guests and thus have been utilized for improving their water solubility, stability and bioavailability.[1—4] The drug/CyD complexes are prepared by various methods and a lot of studies have been reported so far, *e.g.*, kneading, coevaporation, freeze drying, solubility and spray drying methods, *etc.*[5—7] In addition, it has been demonstrated that the preparation methods or the preparation conditions can significantly influence the characteristics of the resulting complexes.[8—10] Al-Marzouqi *et al.* reported that benzocaine/β-CyD complexes prepared by the supercritical fluid technology, kneading, co-evaporation, co-grinding or sealed-heating methods had different physicochemical properties.[11] Toropainen *et al.* reported that the crystal structures or dissolution properties of budesonide/γ-CyD complexes depended on the preparation conditions, *e.g.* temperature during the supercritical fluids process.[12] Thus, the methods and their conditions for the preparation of CyD complexes are diverse and complicated.

Coenzymes Q (ubiquinones) are a group of benzoquinone derivatives with one to twelve mono-unsaturated *trans*-isoprenoid units in the side chain, among which the 10 unit homologue (Coenzyme Q10, CoQ10, Fig. 1) in the most common in animals. CoQ10 is a fat-soluble, vitamin-like, benzoquinone compound that functions primarily as an antioxidant, a membrane stabilizer and a cofactor in the oxidative phosphorylation production of adenosine triphosphate (ATP).[13] It has also been shown to help preserve myocardial sodium-potassium ATPase activity and to stabilize myocardial calcium-dependent ion channels.[14,15] Due to the lipophilic property of CoQ10, however, it is not well absorbed when taken orally or applied percutaneously by humans because of its water insoluble property.[16] Therefore, formulations that could improve aqueous solubility of CoQ10 and enhance its oral or percutaneous bioavailability should be prepared.

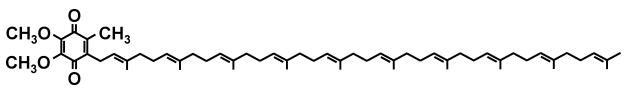

Fig. 1. Chemical Structure of CoQ10

Lutka *et al.* reported that γ-CyD and methyl-β-CyD increased CoQ10 solubility in aqueous solution and stabilized CoQ10 in solid state through an inclusion complexation.[17—20] Likewise, we previously reported that heptakis(2,6-di-*O*-methyl)-β-CyD significantly enhanced the low solubility and oral bioavailability of CoQ10 in rats through inclusion complexation by the kneading method.[21] In addition, among α-, β- and γ-CyDs, γ-CyD significantly increased the solubility of CoQ10 at lower CyD concentrations, and the dispersion rate of CoQ10 was significantly increased by the complexation with γ-CyD, when prepared by the kneading method, probably due to soluble complex formation and/or submicron-sized particle formation, which was reflected in the enhanced oral absorption in dogs.[22] Recently, we reported that CoQ10 forms the pseudorotaxane-like complex with γ-CyD, when it was prepared by the solubility method.[23] Despite many studies on the formation of CoQ10/γ-CyD complexes prepared by various methods were

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reported so far, little is known about comparison of those pharmaceutical properties. Additionally, we previously reported that the CoQ10/γ-CyD complexes slightly had a free CoQ10 and γ-CyD, when prepared by the kneading method.[22] Therefore, the improvement of the kneading method for the formation of the complete CoQ10/γ-CyD complex has been required.

In this study, we prepared complexes of CoQ10 with γ-CyD, *i.e.*, kneading methods and solubility methods with and without heating, and compared those pharmaceutical properties such as the dispersion rate and the release rate of CoQ10 from various ointment bases.

Experimental

Materials γ-CyD was obtained from Nihon Shokuhin Kako (Tokyo, Japan). CoQ10 was donated by Nisshin Pharma (Tokyo, Japan). Polypropylene glycol (PPG) (molecular weight=1000) was obtained from Wako Pure Chemicals Ind. (Osaka, Japan). All other chemicals and solvents were of analytical reagent grade and double distilled water was used throughout the study. Various ointment bases were used as a JP grade.

Preparation of Solid Complexes of CoQ10 with γ-CyD The solid complexes were prepared by four kinds of methods. The kneading method; the amounts of CoQ10 and γ-CyD (molar ratio=1 : 5) were weighed and triturated with ethanol/water (1 : 1, v/v) solution in a mortar and the slurry was thoroughly kneaded for 2—3 h while adding adequate volume of mixed solvent. After evaporation of the solvent, the solid complexes were dried at room temperature for 3 d under reduced pressure. The kneading method with a heating; the same slurries of CoQ10 and γ-CyD were kneaded at 50 °C, above the melting point of CoQ10 (48 °C) for 2—3 h under the same conditions as that is the kneading method. The solubility method[23—25]; CoQ10 (44.2 mg, 51.2 μmol) was added to 2.0 ml of aqueous γ-CyD (232 mg/ml) solution (molar ratio of CoQ10 : CyDs=1 : 7), and then the suspensions were sonicated by a US-4 sonicator (AS ONE, Osaka, Japan) for 30 min followed by the incubation at 25 °C for 5 d under nitrogen gas atmosphere and in the dark. After centrifugation (4492×***g***, 10 min), the supernatant was removed and then the resulting precipitates were washed by 2.0 ml of diethyl ether twice in order to remove the free CoQ10, followed by drying under the reduced pressure overnight. The solubility method with heating; the same suspension of CoQ10 and γ-CyD was incubated at 50 °C for 5 d under the same conditions as that in the solubility method. Physical mixture was prepared by simply mixing powder of each component of CoQ10 and γ-CyD using an agate mortar and a pestle in a molar ratio of 1 : 5 (CoQ10 : γ-CyD).

Powder X-Ray Diffraction Studies Powder X-ray diffraction patterns were measured by a Rigaku RINT 2500 VL X-ray diffractometer (Tokyo, Japan) with a Ni filtered Cu$K\alpha$ radiation, a voltage of 40 kV, a current of 40 mA, a scanning speed of 1°/min, a time constant of 2 s, and a scan range of 2θ=5—35°, a divergent slit of 1.74 mm (1°), a scattering slit of 0.94 mm (1°) and a receiving slit of 0.15 mm.

Differential Scanning Calorimeter (DSC) Studies The DSC analysis was carried out using a SII EXSTAR DSC 6200 (Seiko Instruments, Tokyo, Japan), with a sample weight of 2 mg (equivalent to CoQ10) and a heating rate of 10 °C/min under nitrogen atmosphere.

High Performance Liquid Chromatography (HPLC) Studies HPLC was measured under the following conditions: a JASCO PU-1580 pump and a JASCO UV-970 UV detector (Tokyo, Japan) at 275 nm; a Hitachi D-2500 ChromatoIntegrator (Tokyo, Japan); a Tosoh TSK gel ODS-80TS column (5 μm, 4.6×75 mm, Tokyo, Japan); a mobile phase of ethanol/methanol (3 : 2, v/v); and a flow rate of 1.0 ml/min.

Dispersion Rate Studies The *in vitro* dispersion rate was measured by the dispersed-amount method.[26] The powder sample (equivalent to 30 mg CoQ10, <100 mesh) was added to 100 ml of degassed water at 37 °C, and stirred at 100 rpm. At appropriate intervals, an aliquot (1.0 ml) of the dispersion medium was withdrawn with a syringe and the suspensions were filtered using membrane filters (pore size 0.8 μm, DISMIC-25 CS, Toyo Roshi, Tokyo, Japan). Five hundred microliters of ethanol solution containing 100 mg/ml of tocopherol acetate as an internal standard were added to 0.5 ml of the filtrate. The CoQ10 concentration was measured by HPLC under the condition described above.

Size Distribution Studies The aqueous suspensions containing supramolecular complexes of CoQ10 with γ-CyD (equivalent to 30 mg CoQ10, <100 mesh) were vigorously agitated for 30 s. The particle sizes of the resulting suspensions were determined by quasi-elastic (dynamic) light scattering using a Zetasizer Nano (Malvern Instruments, Worcestershire, U.K.) at 25 °C.

Preparation of Ointments Containing CoQ10 or Its Supramolecular Complexes with γ-CyD Hydrophilic ointment without preservatives and macrogol ointment were prepared according to the JP XV. Plastibase 50 W of a JP grade was used. Carbopol gel was prepared by adding aqueous diisopropanolamine solution (5% w/v) to aqueous carbopol gel 934 P solution (1% w/v). The resulting ointment bases were mixed with CoQ10, its physical mixture with γ-CyD or the CoQ10/γ-CyD complexes (equivalent to 1% w/w CoQ10), and then the kneaded for 10 min at 50 °C.

Release Studies from Ointments We used nitrocellulose membrane (0.8 μm, Millipore, MA, U.S.A.) as a separating membrane and the Frantz type diffusion cell (Hanson Research Co., CA, U.S.A.). Ointments containing CoQ10, its physical mixture with γ-CyD or the CoQ10/γ-CyD complexes (0.5 g) were applied to a donor phase and then water was added to an acceptor phase. At appropriate intervals, an aliquot of the 1.0 ml dissolution medium from acceptor phase was withdrawn and analyzed for CoQ10 by HPLC.

Solubility Studies in Propylene Glycol CoQ10, its physical mixture with γ-CyD or the CoQ10/γ-CyD complex (equivalent to 2 mg CoQ10) was added to 1 ml of propylene glycol, and then the suspension was sonicated for 10 min followed by the incubation at 25 °C for 5 d. After centrifugation (20000×***g***, 5 min), the supernatant was withdrawn, and determined the concentration of CoQ10 in the supernatant by HPLC.

Results and Discussion

We previously reported that when γ-CyD solution was added to CoQ10, yellow precipitates were provided, that is pseudorotaxane-like supramolecular complexes[24,27] of CoQ10 and γ-CyD were found to be formed.[23] We also reported that when the CoQ10/γ-CyD complex (molar ratio=1 : 5) was prepared by the kneading method at room temperature, the resulting product slightly included free CoQ10 and free γ-CyD due to incomplete complexation.[22] Hence, to improve the complexation efficiency, we attempted to prepare the CoQ10/γ-CyD complex with heating at above the melting point of CoQ10 (50 °C), because this incomplete complexation was presumed to result from less dispersibility of CoQ10 in solvent due to its hydrophobic property.

To confirm the complexation of CoQ10 with γ-CyD, we measured the DSC thermographs (Fig. 2). The CoQ10 showed an endothermic peak at 47 °C in the DSC curve due to the melting of CoQ10 (Fig. 2a). The peaks of the kneading product and the solubility product without heating did not

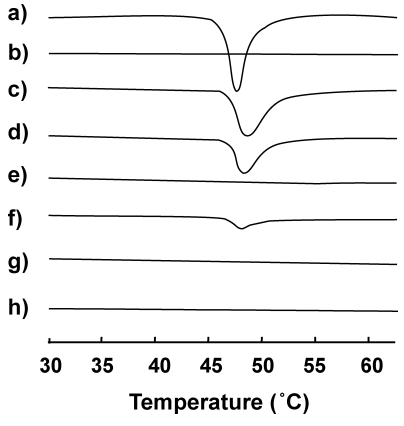

Fig. 2. DSC Thermograms of CoQ10/γ-CyD Systems

(a) CoQ10 alone, (b) γ-CyD alone, (c) physical mixture of CoQ10 and γ-CyD, (d) kneading product, (e) kneading/heating product, (f) solubility product, (g) solubility/heating product, (h) polypropylene glycol (PPG) polypseudorotaxane with γ-CyD.

completely disappear, suggesting the resulting product had the low amount of the residual free CoQ10, which may be due to crystallization of CoQ10 (Figs. 2d, f). However, the endothermic peak of CoQ10 in the kneading/heating product and the solubility/heating product completely disappeared by the complexation with γ-CyD (Figs. 2e, g), compared with that of the corresponding physical mixture (Fig. 2c). These DSC patterns were consistent with that with the polypseudorotaxane of PPG with γ-CyD as a positive control (Fig. 2h). These results suggest the complexes of CoQ10 with γ-CyD can be prepared by the kneading method and the solubility method, and the heating improves the complexation efficiency of CoQ10 with γ-CyD in both methods.

To investigate the crystal structures of CoQ10 complexes with γ-CyD, we measured the powder X-ray diffractograms (Fig. 3). Generally, three types of crystal packing of CyD complexes were well known, *i.e.*, a channel type, a cage type and a layer structure.[28,29] The powder X-ray diffractograms are useful for a confirmation of the complexation with CyDs, because they can provide information to distinguish between the herringbone packing of free CyDs and the different packing of inclusion complexes.[30] Figure 3 shows powder X-ray diffraction patterns of γ-CyD complexes with CoQ10, in comparison with those of PPG which was used for a positive control as a polypseudorotaxane with γ-CyD (Fig. 3h).[31,32] The X-ray diffraction pattern of the physical mixture of CoQ10 and γ-CyD was simply a superposition of the diffraction patterns of each component (Fig. 3c). The diffraction patterns of the kneading/heating product, the solubility product and the solubility/heating product (Figs. 3e—g) were different from those of physical mixture (Fig. 3c), but they were almost the same as that of PPG/γ-CyD polypseudorotaxane (Fig. 3h). These diffraction patterns of the kneading/heating product, the solubility product and the solubility/heating product are known to be the tetragonal columnar channels of the linearly aligned γ-CyD cavities in the crystalline phase.[33,34] Therefore, these diffraction patterns were indexed on the basis of the two-dimensional tetragonal unit cells with dimensions $a=b=24.13$, 24.07 and 24.13 Å, respectively, as shown in Table 1. The *d*-spacings of the *hkl* (200) reflection were used to calculate the unit cell dimensions as shown in Fig. 3e. All values of the calculated *d*-spacings (d_{cal}) were in excellent agreement with those observed values (d_{obs}), confirming that pseudorotaxane-like supramolecular complexes of CoQ10 with γ-CyD formed the tetragonal structure. Therefore, these data suggest that the isoprenoid chain of CoQ10 is included in the tetragonal columnar channels formed by the stacking of γ-CyD molecules in the crystalline part of the solid complex as shown in Fig. 4. Meanwhile, the X-ray diffraction pattern of the kneading product was superposition of the diffraction patterns of CoQ10, γ-CyD and

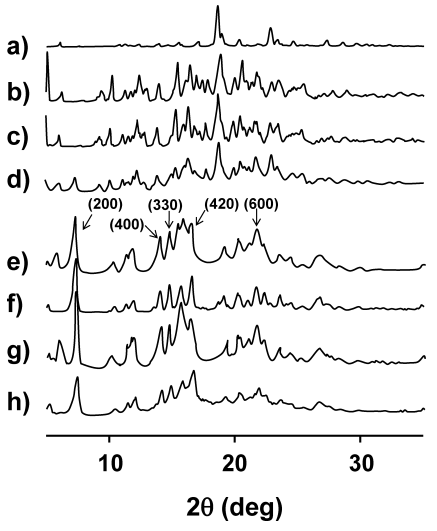

Fig. 3. Powder X-Ray Diffraction Patterns of CoQ10/γ-CyD Systems

(a) CoQ10 alone, (b) γ-CyD alone, (c) physical mixture of CoQ10 and γ-CyD, (d) kneading product, (e) kneading/heating product, (f) solubility product, (g) solubility/heating product, (h) polypropylene glycol (PPG) polypseudorotaxane with γ-CyD.

Table 1. Crystallographic Characteristics of Kneading/Heating Product, Solubility Product, Solubility/Heating Product of CoQ10 with γ-CyD

Kneading/heating product

2θ	(*hkl*)	d_{obs} (Å)	d_{cal} [a] (Å)
7.32	(200)	12.07	12.07
14.04	(400)	6.30	6.03
14.78	(330)	5.99	5.69
16.52	(420)	5.36	5.40
21.70	(600)	4.09	4.02

Solubility product

2θ	(*hkl*)	d_{obs} (Å)	d_{cal} [b] (Å)
7.34	(200)	12.03	12.03
14.08	(400)	6.28	6.02
14.80	(330)	5.98	5.67
16.54	(420)	5.35	5.38
21.68	(600)	4.10	4.01

Solubility/heating product

2θ	(*hkl*)	d_{obs} (Å)	d_{cal} [c] (Å)
7.32	(200)	12.07	12.07
14.06	(400)	6.29	6.03
14.78	(330)	5.99	5.69
16.56	(420)	5.35	5.40
21.68	(600)	4.10	4.02

a) Calculated assuming a tetragonal unit cell with $a=b=24.13$ Å, packing diameter 12.07 Å. *b*) Calculated assuming a tetragonal unit cell with $a=b=24.07$ Å, packing diameter 12.03 Å. *c*) Calculated assuming a tetragonal unit cell with $a=b=24.13$ Å, packing diameter 12.07 Å.

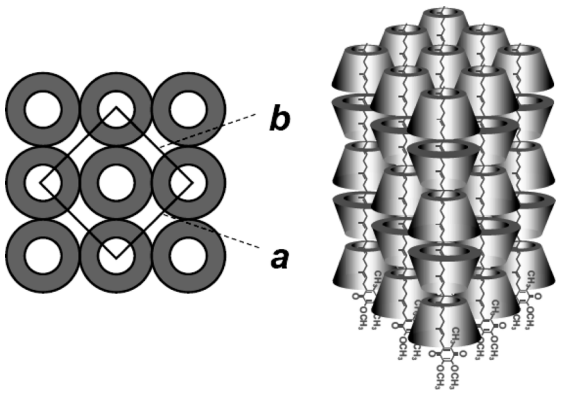

Fig. 4. Schematic Representation of Crystal Packing Structures of CoQ10/γ-CyD Supramolecular Complex

pseudorotaxane-like supramolecular complex, due to incomplete complexation of CoQ10 and γ-CyD. Hence, these results support the hypothesis that heating improves the formation of the supramolecular complexes of CoQ10 with γ-CyD in the solubility method and kneading method.

To calculate the stoichiometry of complexes prepared by the solubility method, we measured ^{1}H-NMR spectra of the solubility product and the solubility/heating product after dissolving the solid complexes in DMSO-d_6. Figure 5 shows ^{1}H-NMR spectrum of CoQ10/γ-CyD supramolecular complex in DMSO-d_6 at 25 °C as the representative example. The anomeric proton peak of γ-CyD and ethylene protons peak of CoQ10 were used for calculation of stoichiometry of complexes (Fig. 5). The number of γ-CyD molecule encapsulating a CoQ10 molecule in the solubility product and the solubility/heating product was estimated to be 5.0 and 4.6, respectively. Additionally, these facts suggest that the coverage of the long isoprenoid side chain of CoQ10 by γ-CyD was approximately 100%, when assumed that the two isoprenoid repeat units are included in one γ-CyD cavity.

Next, we evaluated some pharmaceutical properties of CoQ10 complexes. At first, we determined the dispersion rate using the dispersed amount method[23,26] with a slight modification. Figure 6 shows the dispersion rate profiles of the CoQ10 complexes with γ-CyD in water at 37 °C. Here we could not detect the CoQ10 dissolved completely in water after filtration of samples through a filter membrane (pore size 0.2 μm) under the present experimental conditions, because of extremely low aqueous solubility of CoQ10. Thereby, we determined the concentration of CoQ10 in the filtrate through a membrane filter (pore size 0.8 μm). Actually, the dispersion rate of CoQ10 was markedly low (Fig. 6). Likewise, the physical mixture of CoQ10 with γ-CyD did not exhibit any increase in dispersion. Meanwhile, the dispersion rate of CoQ10 complex with γ-CyD increased in the order of solubility/heating product<solubility product< kneading/heating product≪kneading product (Fig. 6). It is acknowledged that the dispersion rate of the particles is associated with the particle sizes. Therefore, we next measured the particle size of the CoQ10/γ-CyD complexes by a quasi-elastic (dynamic) light scattering method. As shown in Table 2, the mean particle sizes of the kneading products, the kneading/heating product, the solubility product and the solubility/heating product after agitation were 457 nm, 604 nm, 626 nm and 1105 nm, respectively, reflecting the results of the dispersion rate. These results suggest the dispersion rate of the CoQ10 complexes with γ-CyD higher than that of CoQ10 alone or its physical mixture may be due to submicron-ordered particle formulation as well as the different dispersion properties of the complexes prepared by the kneading method and solubility method with and without heating. Interestingly, the dispersion rate of the kneading product was the highest among four types of the complexes, in spite of its incomplete complexation. This may be ascribed to the incomplete complexation of CoQ10 with γ-CyD. In general, the channel inclusion complexes are less soluble in water due to a stacking of CyD molecules and a lack of hydration of the complexes with H_2O molecules. The powder X-ray diffractograms (Fig. 2) and ^{1}H-NMR spectrum (Fig. 5) demonstrated that the kneading/heating products, the solubility products and the solubility/heating products formed the pseudorotaxane-like channel type structure with approximately 100% coverage of CoQ10 by five γ-CyDs, although the complexation of the kneading product was incomplete. Therefore, these results may be explained by the presumption that incomplete complexation with a few γ-CyDs per one CoQ10 molecule increased the dispersion rate, and in sharp contrast the complete complexation with five γ-CyDs per one CoQ10 molecule decreased the dispersion rate due to its channel type structure. In fact, our previous studies demonstrated that the phase solubility diagrams showed a mixed pattern of the A_p-type diagram at the region of lower γ-CyD concentration and the B_s-type diagram at the region of higher γ-CyD concentration, respectively,[22] defined by Higuchi and Connors.[25] These findings strongly suggest that incomplete complexation of CoQ10 with γ-CyD may be associated with its rapid dispersion property.

CoQ10 is applicable as an ointment, however, not well absorbed when applied to skin.[35] Then, we evaluated the re-

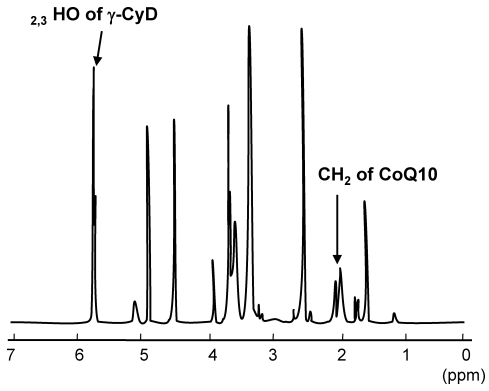

Fig. 5. ^{1}H-NMR Spectrum of CoQ10/γ-CyD Supramolecular Complex in DMSO-d_6 at 25 °C

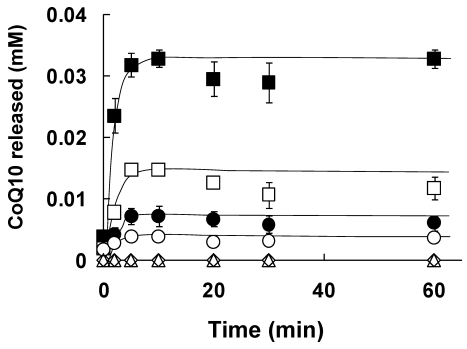

Fig. 6. Dispersion Profiles of CoQ10, Its Physical Mixture with γ-CyD and Its Complexes with γ-CyD in Water at 37 °C

◇, CoQ10 alone; △, physical mixture with γ-CyD; ■, kneading product; □, kneading/heating product; ●, solubility product; ○, solubility/heating product. Each point represents the mean±S.E. of 3—7 experiments.

Table 2. Particle Sizes of CoQ10 Complexes with γ-CyD in Water at 25 °C

Complex	Particle size (nm)
Kneading product	457±6
Kneading/heating product	604±8
Solubility product	626±8
Solubility/heating product	1105±19

Each value represents the mean±S.E. of 4—5 experiments.

lease of CoQ10 from ointment bases. First, to find the optimal ointment bases regarding the release of CoQ10 from ointment bases, we compared the release profiles of CoQ10 from various ointment bases containing 1% w/w CoQ10 in water at 32 °C (Fig. 7). The release rate of CoQ10 from ointment bases increased in the order of plastibase 50 W≈carbopol gel<macrogol ointment<hydrophilic ointment. These results indicate that the release rate of CoQ10 strikingly depends on the type of ointment bases, and the release of CoQ10 from macrogol ointment and hydrophilic ointment are fairly favorable. Next, we evaluated the release profiles of CoQ10 from macrogol or hydrophilic ointment containing CoQ10 alone, the CoQ10/γ-CyD physical mixture and the supramolecular complexes (equivalent to 1% w/w CoQ10) in water at 32 °C. As shown in Fig. 8a, the release rate of CoQ10 from macrogol ointment increased in the order of CoQ10 alone<solubility product<physical mixture≈solubility/heating product<kneading product≈kneading/heating product. Thus, the complexes prepared by the kneading methods (kneading product and kneading/heating product) showed the high release rate, compared to the complexes prepared by the solubility methods (solubility product and solubility/heating product), probably due to the formation of soluble complexes as shown in Fig. 6. Meanwhile, the release rate of CoQ10 from hydrophilic ointment increased in the order of CoQ10 alone<physical mixture<kneading product<kneading/heating product<solubility product<solubility/heating product (Fig. 8b), totally inconsistent with the results shown in macrogol ointment. Interestingly, the complexes prepared by the solubility methods with and without heating showed the high release rate in spite of the low dispersion ability. These results suggest that supramolecular complexes of CoQ10 with γ-CyD, in particular the solubility/heating product improved the release rate of CoQ10 from hydrophilic ointment.

To gain insight into the mechanism by which the release of CoQ10 from hydrophilic ointment containing the solubility/heating product, we examined the effects of additives on the release profile of CoQ10 from hydrophilic ointment containing supramolecular complexes by removing a constituent such as monoglyceride, white petrolatum and propylene glycol from hydrophilic ointment (Fig. 9). The lack of monoglyceride or white petrolatum was slightly decreased the release rate of CoQ10. Meanwhile, the lack of propylene glycol in hydrophilic ointment was remarkably decreased the release rate of CoQ10. These results indicate that propylene glycol plays a pivotal role for the enhancement of the release rate of CoQ10 from hydrophilic ointment containing the supramolecular complexes. In addition, solubility of CoQ10 in propylene glycol increased in the order of CoQ10 alone≈physical mixture<kneading product<solubility product≈kneading/heating product<solubility/heating product (Table

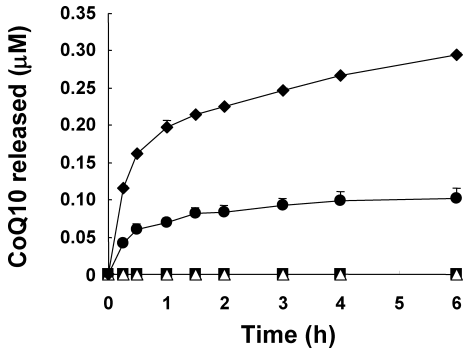

Fig. 7. Release Profiles of CoQ10 from Different Ointment Bases Containing 1% w/w CoQ10 in Water at 32 °C

◆, hydrophilic ointment; ●, macrogol ointment; ■, plastibase 50 W; △, carbopol gel. Each point represents the mean±S.E. of 3 experiments.

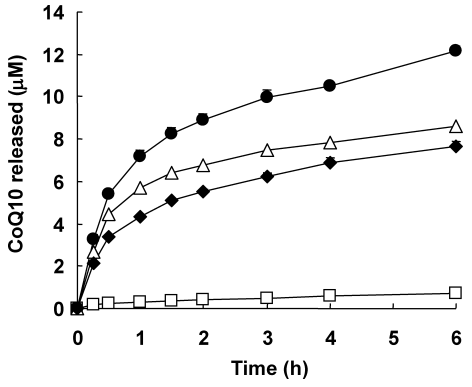

Fig. 9. Release Profiles of CoQ10 from Hydrophilic Ointment Containing Solubility/Heating Product (Equivalent to 1% w/w CoQ10) in Water at 32 °C

●, hydrophilic ointment; △, without monoglyceride; ◆, without white petrolatum; □, without propylene glycol. Each point represents the mean±S.E. of 3 experiments.

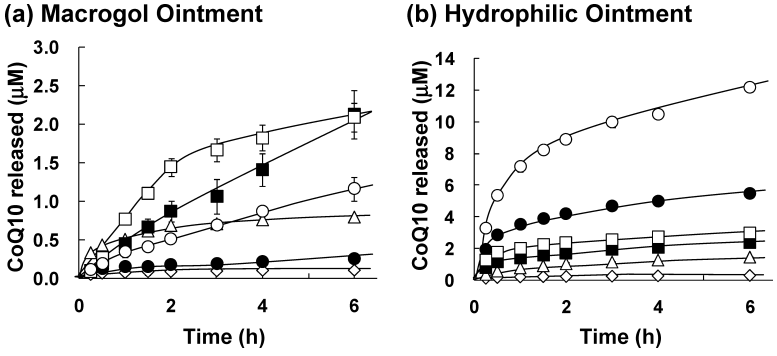

Fig. 8. Release Profiles of CoQ10 from Macrogol (a) and Hydrophilic (b) Ointment Bases Containing CoQ10, Its Physical Mixture with γ-CyD and Its Complexes with γ-CyD (Equivalent to 1% w/w CoQ10) in Water at 32 °C

◇, CoQ10 alone; △, physical mixture with γ-CyD; ■, kneading product; □, kneading/heating product; ●, solubility product; ○, solubility/heating product. Each point represents the mean±S.E. of 3 experiments.

Table 3. Solubility of CoQ10, Its Physical Mixture with γ-CyD and Its Complexes with γ-CyD in Propylene Glycol at 25 °C

System	Solubility (mg/ml)
CoQ10 alone	1.35±0.20
Physical mixture	1.65±0.14
Kneading product	8.59±0.62
Kneading/heating product	18.59±0.45
Solubility product	14.79±0.58
Solubility/heating product	24.87±1.24

Each value represents the mean±S.E. of 3 experiments.

3), which coincided with the order of the release rate of CoQ10 from hydrophilic ointment, suggesting the involvement of solubility of CoQ10 in propylene glycol in the release rate of CoQ10 from hydrophilic ointment. Collectively, these findings suggest that propylene glycol affects the pseudorotaxane-like supramolecular structure of CoQ10 and γ-CyD, resulting in the high solubility in hydrophilic ointment followed by the high release rate of CoQ10 from hydrophilic ointment. Hereafter, further elaborate study should be required to clarify the detailed mechanism for the different release rate of CoQ10 between the kneading product and solubility product with and without heating and to reveal *in-vivo* transdermal absorption of CoQ10 after application of hydrophilic ointment containing CoQ10/γ-CyD complexes to skins.

In conclusion, the present study showed that the CoQ10 forms the pseudorotaxane-like complex with γ-CyD by kneading method and solubility method with or without heating. In the solubility method and kneading method used in the present study heating improved the complexation of CoQ10 with γ-CyD. Moreover, some pharmaceutical properties were different by the distinct preparation method, especially the solubility/heating product of CoQ10 with γ-CyD markedly improved the release rate of CoQ10 from hydrophilic ointment. These results suggest that supramolecular complexes of CoQ10 with γ-CyD can be prepared by various methods, and among various complexes the pseudorotaxane-like CoQ10/γ-CyD complexes prepared by the solubility method with heating have the potential for preparation of ointments.

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