Enhancement of Diosgenin Distribution in the Skin by Cyclodextrin Complexation Following Oral Administration

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Orally administrated diosgenin, a steroidal saponin found in several plants including *Dioscorea villosa*, recovers skin thickness reduced in ovariectomized mice, and plays an important role in the treatment of hyperlipidemia. Thus, diosgenin is an active element of cosmeceutical and dietary supplements. However, we have already elucidated that the skin distribution and absolute oral bioavailability of diosgenin is very low. The aim of this study is to evaluate the efficacy of diosgenin–cyclodextrin (CD) complexes in improving the skin concentration of diosgenin. The formation of the CD complex was indicated by powder X-ray diffraction (XRD), differential scanning calorimetry (DSC), and scanning electron microscope (SEM) studies. Oral administration of the diosgenin/β-CD complex resulted in a significant enhancement in terms of the skin distribution of diosgenin, maximum plasma level (C_{max}), area under the plasma concentration–time curve (AUC), and absolute oral bioavailability over those of the drug alone. These results suggest that the inclusion complex of diosgenin/β-CD can be used to improve low skin content of diosgenin.

Key words   diosgenin; skin distribution; cyclodextrin; intravenous administration; oral administration

Diosgenin (Fig. 1) is a steroid sapogenin which can be found in several species of the genus *Dioscorea villosa*, *Costus speciosus*, and *Trigonella foenum greaecum*.[1] Previous investigations have shown that diosgenin was effective in treating various diseases such as hyperglycemia and hyperlipidemia.[2,3] It was established as a starting material for the production of steroidal hormones in the pharmaceutical industry.[4] Diosgenin has been applied to hormone replacement therapy in menopausal women.[5,6] Estrogen enhanced the proliferation of estrogen-dependent cancer cells,[7] whereas diosgenin inhibited the proliferation of breast cancer cells.[8] Oral administration of diosgenin improved reduced skin thickness in ovariectomized mice.[9] In B16 melanoma cells, it inhibits melanogenesis by activating the phosphatidylinositol-3-kinase pathway.[10] Thus, diosgenin was noted as an active element of cosmeceutical and dietary supplements. Furthermore, diosgenin was expected for systemic action by oral administration. However, only a few reports have investigated the disposition and skin distribution of diosgenin.

In our previous study, the skin distributions and absolute oral bioavailability of diosgenin was very low.[11] Diosgenin aqueous solubility was found to be 0.95 μg/mL (calculated using Advance Chemistry Development Software V8.14 for Solaris). This low oral bioavailability may be caused by diosgenin's low solubility in water.

Cyclodextrins (CDs) show a remarkable ability to form inclusion complexes with diverse lipophilic molecules that fit inside the cavity. This phenomenon modifies solubility, dissolution rates, and bioavailability of guest molecules. Inclusion complexation of a number of drugs with β-CD has been reported.[12–14]

In the present study, we prepared and characterized complexes of diosgenin with several CDs. The effects of prepared CD inclusion complexes in the oral administration of diosgenin were investigated on skin content and oral bioavailability of diosgenin.

MATERIALS AND METHODS

Materials   Diosgenin was purchased from Sigma-Aldrich (St. Louis, MO, U.S.A.). Polyoxyethylene hydrogenated castor oil 60 (HCO-60) was supplied from Nikko Chemicals (Tokyo, Japan). Sodium pentobarbital was obtained from Kyoritsu Seiyaku Co. (Tokyo, Japan). 6-Methyl diosgenin and other chemicals were obtained from Wako Pure Chemical Industries, Ltd. (Osaka, Japan).

Phase Solubility Study   Solubility studies were performed as described earlier.[15] Diosgenin was dissolved in methanol and dispensed into each test tube. Methanol was removed by evaporation in a 40°C heating block. Different concentrations of α, β, and γ-CD solutions (2 mL) were added to 0.2 mg of diosgenin. Each CD concentrations were ranging from 0 to 10 mM. These sample solutions were shaken in an incubator at 37°C for 24 h. The solution was filtered using 0.2 μm membrane filters. Diosgenin content was determined by a LC/MS system. Separation was achieved by a Michrom Biosources Inc. MXY01-01 (Auburn, CA, U.S.A.) with a Tosoh TSK gel ODS-100V column (2.0×50 mm, 3 μm) (Tokyo, Japan) at room temperature. The mobile phase consisted of methanol (90%) and H_2O (10%) containing 10 mM ammonium acetate. Flow rate was set to 150 μL/min and detection was carried out

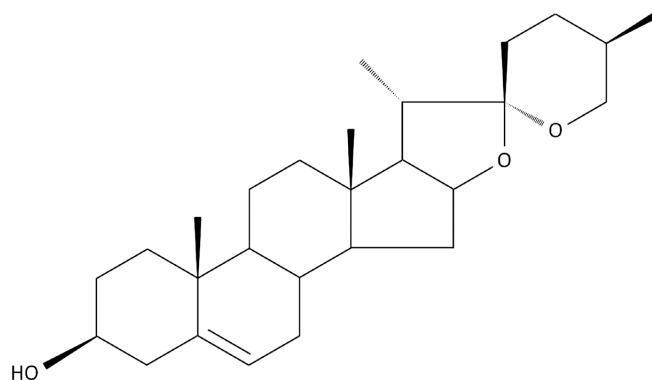Fig. 1.   Molecular Structure of Diosgenin

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using a Thermo Fisher LCQ DECA XP[Plus] mass spectrometer (Waltham, MA, U.S.A.).

Preparation of Mixture and Complex In previous study, it was found that diosgenin and β-CD form 1:2 complexes.[11)] The complexes were prepared by co precipitate method. Briefly, diosgenin (1.6 mmol) and each CD (3.2 mmol) were shaking in 100 mL water at 37°C for 24 h. The solid was collected, dried, and ground into a fine powder using a mortar and pestle. The physical mixture of diosgenin and each CD was prepared by mixing individual components in the mortar and pestle in 1:2 molar ratios of diosgenin and each CD. These samples were subsequently analyzed by powder X-ray diffraction (XRD), differential scanning calorimetry (DSC) and scanning electron microscope (SEM).

Powder X-Ray Diffraction Analysis XRD patterns of diosgenin, CDs, physical mixtures and complexes were recorded on a Rigaku Mini FlexII (Tokyo, Japan) using Ni-filtered, Cu$K\alpha$ radiation, a voltage of 30 kV, and a 15 mA current. The instrument was operated with a scanning rate of 2 degree/min over the 2θ range of 0–30 degree.

Differential Scanning Calorimetry Analysis DSC spectra of diosgenin, CDs, physical mixtures and complexes were obtained using a Rigaku Thermo plus EVO DSC 8230 (Tokyo, Japan). Samples (4 to 5 mg) were weighed in crimped aluminum pans and heated from 50 to 230°C, at a constant scanning rate of 10°C/min with nitrogen purging (50 mL/min).

Scanning Electron Microscope The morphology of diosgenin, CDs, physical mixtures and complexes were determined using a Hitachi High-Technologies S-3000N (Tokyo, Japan), operated at an accelerating voltage of 15 kV. Samples were mounted onto aluminum stub 5×5 mm graphite tape and sputter-coated with gold.

Animals Mail Wistar rats (200 to 250 g) were provided from Japan SLC (Hamamatsu, Shizuoka, Japan). Animals were housed under a 12 h light and dark cycle in a temperature controlled room (25°C). They had free access to food and water. All procedures were approved by the Ethics Committee of Josai University (Sakado, Saitama, Japan) in accordance with the National Institute of Health (Tokyo, Japan).

Pharmacokinetic Studies Intravenous and oral administration studies were performed to compare the pharmacokinetic parameters and bioequivalence of diosgenin. Rats were fasted overnight for at least 12 h prior to dosing. Diosgenin and its CD complex were dissolved or suspended in physiological saline containing 1% HCO-60. In intravenous administration, 60 μg/mL of diosgenin solution was prepared and 120 μg/kg of diosgenin was injected into the tail vein. In oral administration, 50 mg/mL of diosgenin suspension was prepared and 100 mg/kg of diosgenin was administrated. In the physical mixture group, the diosgenin suspension and each CD solution were simultaneously administered. Skin samples were collected at 4, 6, and 8 h after oral administration. At time range from 0 to 120 h, blood was collected in heparinized tubes by tail vein and separated by centrifugation immediately. Each sample was stored at −30°C until analyzed.

Analytical Procedure For the analysis of diosgenin in skin and plasma samples, 6-methyl diosgenin was used as an internal standard. Skin samples (1 cm[2]) were minced and sonicated for 20 min in methanol. Plasma samples were added to three folds of methanol and mixed by a vortex mixer for 1 min. These samples were centrifuged at 15000×*g* for 5 min.
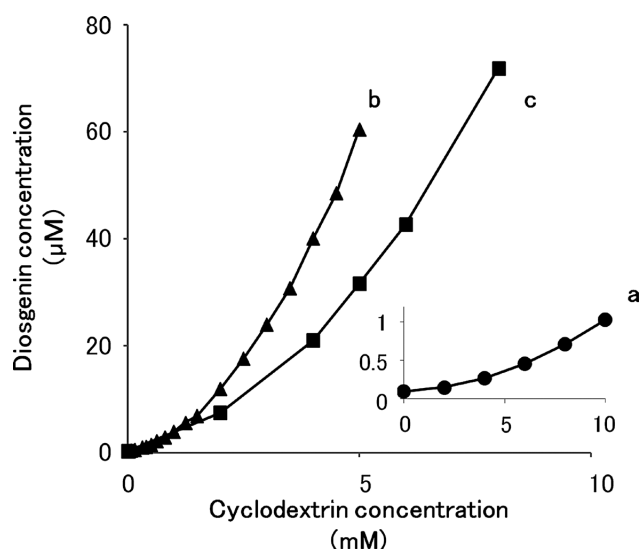

Fig. 2. Phase Solubility Diagrams of Diosgenin/α-CD (a), Diosgenin/β-CD (b), and Diosgenin/γ-CD (c) at 37°C

Analysis was achieved by a LC/MS system.

Pharmacokinetics and Statistical Analysis Pharmacokinetics analysis was performed with least squares methods. The area under the plasma concentration–time curve (*AUC*) was calculated by the liner trapezoidal rule. The absolute bioavailability, was determined as *AUC* oral/*AUC* intravenous, using mean *AUC* values for oral and intravenous doses. To assess the significance of differences between two groups, Dennett's multiple comparison tests was used. A *p* value of less than 0.05 was termed significant.

RESULTS AND DISCUSSION

Phase Solubility Study Figure 2 shows the phase solubility diagrams obtained for diosgenin against each α, β and γ-CD concentration in distilled water, where the solubility curves can be classified as type Ap, suggesting high order complexation. The stability constants (K_{11} and K_{12}) were defined by Eqs. 1 and 2 where [S] and [L] represent molar concentrations of free diosgenin and CD, respectively. The S_0 is the solubility of diosgenin in the absence of CD. The Seq and Leq are total concentration of diosgenin and CD, respectively. The stability constants were determined by analyzing Eqs. 3 and 4 by the nonlinear least square method at 0–10 mM of each CD.[16)]

$$K_{11} = [SL] / S_0[L] \tag{1}$$

$$K_{12} = [SL_2] / [SL][L] \tag{2}$$

$$S_{eq} = S_0 + [SL] + [SL_2] \tag{3}$$

$$L_{eq} = [L] + [SL] + 2[SL_2] \tag{4}$$

These results indicate that the small cavity size of α-CD resulted in low inclusion of diosgenin (K_{11}=100 M^{-1} and K_{12}=860 M^{-1}), whereas β-CD (K_{11}=5680 M^{-1} and K_{12}=1160 M^{-1}) and γ-CD (K_{11}=4550 M^{-1} and K_{12}=510 M^{-1}) have large cavity sizes, which enhance effective interactions with diosgenin.
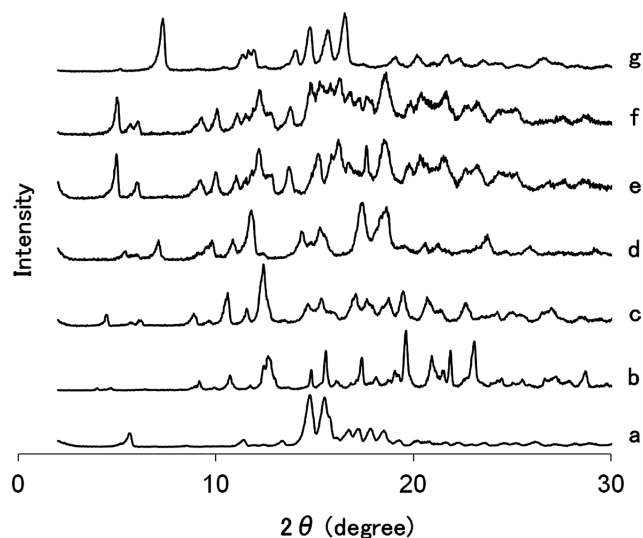
Fig. 3. Powder X-Ray Diffractograms of Diosgenin (a), β-CD (b), Diosgenin/β-CD Physical Mixture (c), Diosgenin/β-CD Complex (d), γ-CD (e), Diosgenin/γ-CD Physical Mixture (f), and Diosgenin/γ-CD Complex (g)
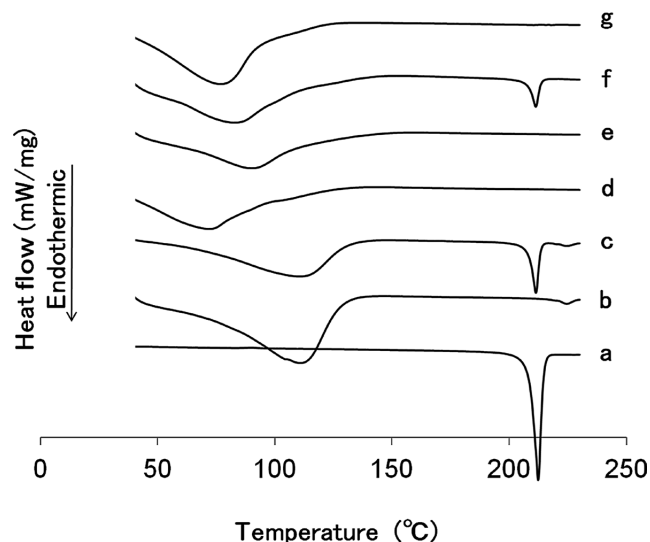

Fig. 4. Differential Scanning Calorimetry Curves of Diosgenin (a), β-CD (b), Diosgenin/β-CD Physical Mixture (c), Diosgenin/β-CD Complex (d), γ-CD (e), Diosgenin/γ-CD Physical Mixture (f), and Diosgenin/γ-CD Complex (g)
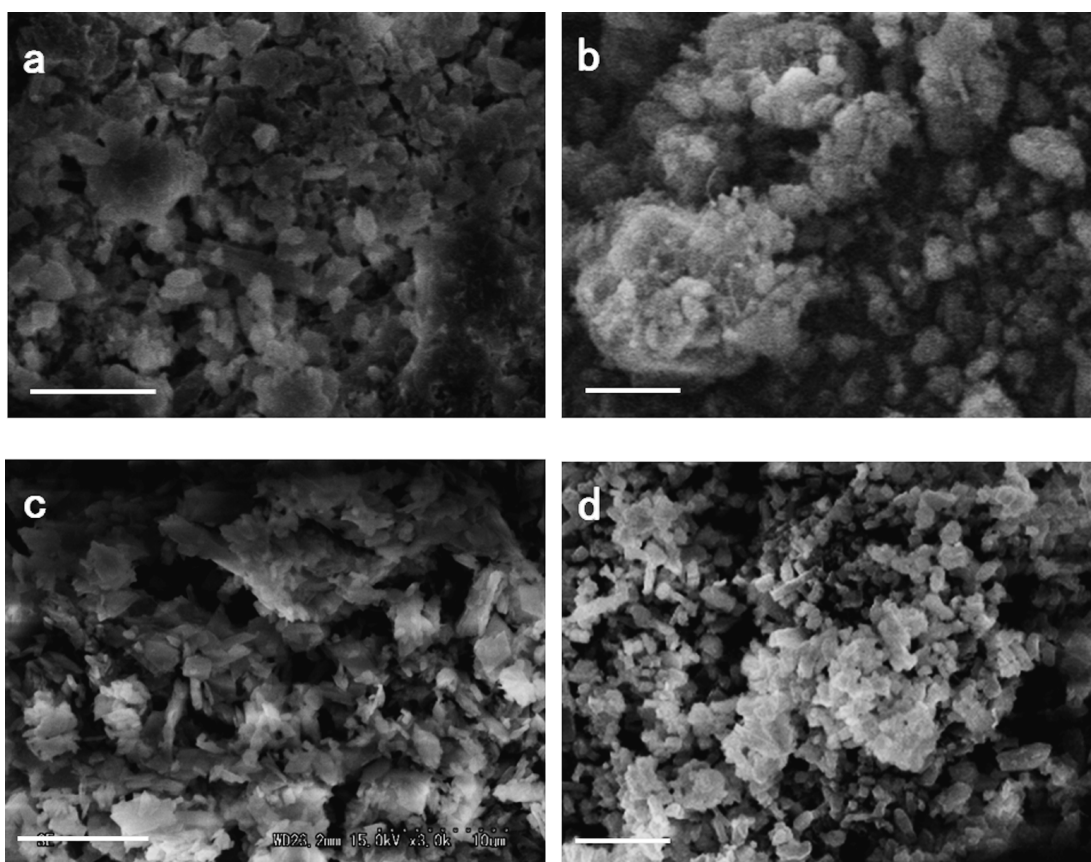

Fig. 5. Scanning Electron Microscopy of Diosgenin (a), Diosgenin/α-CD Complex (b), Diosgenin/β-CD Complex (c), and Diosgenin/γ-CD Complex (d)

Bar was 100 μm.

Binding of diosgenin with γ-CD was less than that with β-CD, suggesting that the cavity size of γ-CD is too large for diosgenin.[17]

Powder X-Ray Diffraction Figure 3 shows XRD patterns of diosgenin-CD systems. XRD patterns of each physical mixture were found to be a combination of diosgenin and each CD. However, XRD patterns of the complex were found to be different from each physical mixture. These results suggest the formation of an inclusion complex between diosgenin and each CD. In the complex of diosgenin/α-CD, the peak was not
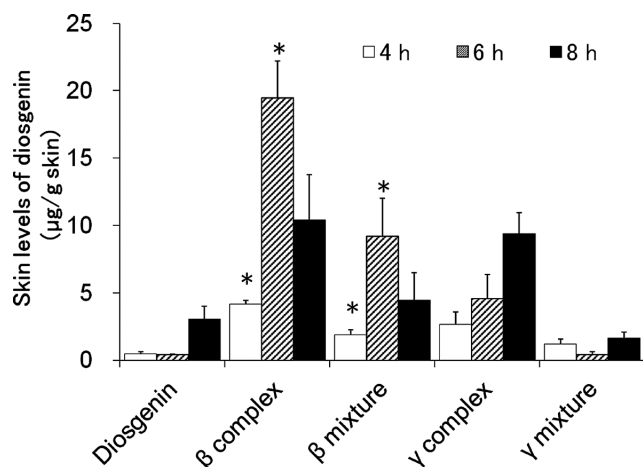
Fig. 6. Skin Diosgenin Levels after Oral Doses of Diosgenin and Its β or γ-CD Complexes to Rats

Mean±S.E., n=3–4, *$p<0.05$ compared to diosgenin.

different from the diosgenin/α-CD physical mixture (data not shown).

Differential Scanning Calorimetry The thermograms of diosgenin–CD systems are shown in Fig. 4. DSC thermograms revealed the endothermic peak of diosgenin at 210°C. With increasing ratios of β or γ-CD in the complex, the diosgenin endothermic peak shifted lower (data not shown). Thermograms of the diosgenin/β-CD (1 : 2) and diosgenin/γ-CD (1 : 2) complexes showed an absence of the characteristic endothermic peak of the drug.[11] This indicates that the inclusion complex has a 1 : 2 (diosgenin/β or γ-CD) formula.

Scanning Electron Microscope In order to investigate whether complexations with each CD could have some influence on particle morphology, SEM was performed for diosgenin and each complex (Fig. 5). It is clear that diosgenin and the diosgenin/α-CD complex have similar crystals. SEM images of each physical mixture have diosgenin and each CD crystal. On the other hand, β and γ-CD complexes presented a parallelogram shape.

Skin Distribution of Diosgenin In Fig. 6, skin distributions of diosgenin were shown at 4, 6, and 8h after oral administration of diosgenin, physical mixture, and complex in rats. After the oral dose, the peak level in skin was observed at 6h. Significant enhancements were found at 4 and 6h in the β-CD complex and β-CD physical mixture groups than the drug alone. After oral administration, however, high diosgenin concentrations were obtained in skin, indicating that orally dosed diosgenin was probably distributed through the systemic circulation to skin.

Concentrations of Diosgenin in Plasma Figure 7a shows the mean diosgenin concentration–time profile after intravenous administration of diosgenin at a dose of 10mg/kg in rats. Furthermore, Fig. 7b shows the results of oral administration of diosgenin alone and formulation at a dose of 100mg/kg. Obtained pharmacokinetic parameters are summarized in Table 1. In diosgenin/β-CD complex groups, significant enhancements were found in C_{max}, AUC, and absolute oral bioavailability compared to drug alone. These data suggest that diosgenin solubility was increased in the intestine because of inclusion complex formation.
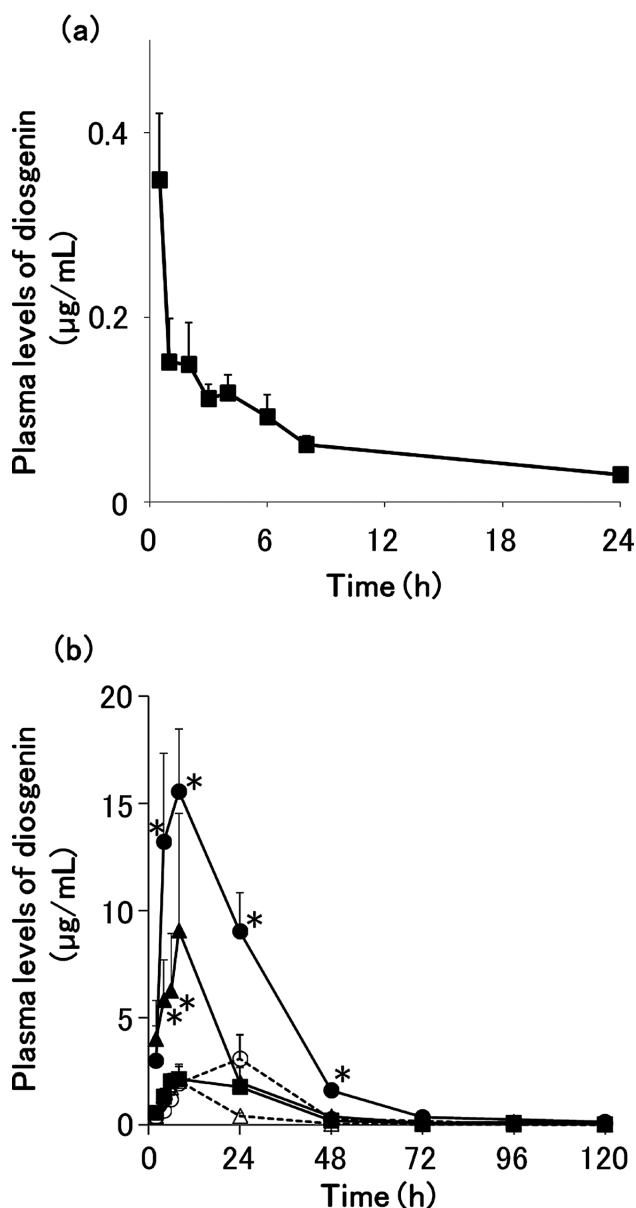

Fig. 7. Plasma Diosgenin Levels after Intravenous (a) and Oral (b) Doses of Diosgenin and Its β or γ-CD Complexes to Rats

Symbols: square, diosgenin; open circle, diosgenin/β-CD physical mixture; circle, diosgenin/β-CD complex; open triangle, diosgenin/γ-CD physical mixture; triangle, diosgenin/γ-CD complex. Mean±S.E., n=4–8, *$p<0.05$ compared to diosgenin.

Table 1. Pharmacokinetic Parameters of Diosgenin and Its β or γ-CD Complexes Following Oral Administration to Rats

	AUC oral ($\mu g \cdot h/mL$)	C_{max} ($\mu g/mL$)	Bioavailability (%)
β Complex	424.6±37.2*	14.7±2.2*	35.5±3.2*
γ Complex	129.7±56.2	2.9±1.3	11.1±4.8
β Mixture	91.9±31.1	3.0±1.0	8.0±2.7
γ Mixture	34.6±9.8	1.5±0.6	3.2±1.0
Diosgenin	53.1±5.8	1.7±0.2	4.3±0.5

Mean±S.E., n=4–8, *$p<0.05$ compared to diosgenin.

CONCLUSION

Diosgenin/β or γ-CD complexes were prepared by coprecipitation, and analyzed using DSC, XRD, and SEM. Results confirmed the inclusion of diosgenin in β and γ-CD cavities. Experimental results of pharmacokinetics of the complex in rats indicated that the β-CD complex significantly enhanced skin distribution and oral bioavailability compared to diosgenin alone. It is expected that orally administrated diosgenin could improve climacteric skin thickness and hyperpigmentation related with skin diseases. In conclusion, our results demonstrated that orally administrated diosgenin/β-CD complex increased skin distribution and absolute bioavailability.

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