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European Journal of Pharmaceutics and Biopharmaceutics 55 (2003) 191–198
European Journal of Pharmaceutics and Biopharmaceutics
www.elsevier.com/locate/ejpb

Research paper

Complex formation of sericoside with hydrophilic cyclodextrins: improvement of solubility and skin penetration in topical emulsion based formulations

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Received 4 March 2002; accepted in revised form 18 December 2002

Abstract

The main objective of this study was to devise novel methods for improving the solubility of the anti-inflammatory triterpenoid sericoside, the main component of *Terminalia sericea* extract, thus enabling its incorporation into topical formulations. Sericoside was stabilized by complex formation with hydrophilic derivatives of β- and γ-cyclodextrins in a molar ratio of 1.0:1.1. The complex of extract and cyclodextrin was equilibrated in water at 25 °C for approximately 24 h. The dehydrated complexes of *T. sericea* extract and cyclodextrin were characterized by differential scanning calorimetry, thermogravimetry analysis and X-ray diffraction. Complex formation with β-cyclodextrin as well as γ-cyclodextrin derivatives was detectable using these three analytical tools; however, only complexes with γ-cyclodextrin derivatives showed stability upon storage after incorporation into topical o/w or w/o formulations. Furthermore, a *T. sericea* extract/γ-cyclodextrin complex incorporated in an o/w formulation resulted in a 2.6-fold higher percutaneous penetration of sericoside in in vitro excised pig skin as compared to pure *T. sericea* extract. For the first time, the virtually insoluble anti-inflammatory active sericoside was incorporated into a topical emulsion based formulation in a stable manner, resulting in efficient skin penetration.

Keywords: *Terminalia sericea* extract; Sericoside; Inclusion complex formation; γ-Cyclodextrin; Hydroxypropylated-γ-cyclodextrin; Methylated-β-cyclodextrin; Hydroxypropylated-β-cyclodextrin; Water solubility; Percutaneous in vitro penetration

1. Introduction

Sericoside, the D-glycosyl-ester of 2α,3β,19α,24-tetrahydroxy-olean-12-ene-28-oic acid [1], is a member of a novel class of anti-inflammatory triterpenoids found in the roots and bark of the plant species *Terminalia* (*Combretacea*) [2–4], which is distributed throughout regions of Africa, Asia, Australia and tropical America [5–7]. *Terminalia sericea* extract (TSE) contains approximately 86% sericoside (Fig. 1), 10% arjunglucoside, the C_4 isomer of sericoside, and 4% other components like sericic acid, the aglycon of sericoside [1,8]. It is a light yellow powdered extract which has been washed with different solvents to achieve its high sericoside content.

Sericoside from TSE is practically insoluble in water and oil components frequently used in emulsion based formulations; however, it is easily solubilized in ethanol [8]. An emulsion based o/w formulation with 6.0% ethanol permitted incorporation of 0.1% TSE. Attempts to incorporate higher amounts of TSE resulted in crystallization of sericoside. To improve its solubility in formulations, complex formation with cyclodextrins (CDs) in an equimolar ratio of 1.0:1.1 TSE/CD was achieved.

The main objective of this study was to improve the solubility of sericoside through complex formation with CD derivatives [9–14]. Accordingly, TSE was equilibrated with γ-, HP-γ-, HP-β- and M-β-CD in an aqueous solution for 48 h at 25 °C. After evaporation, the TSE/CD complex was characterized by differential scanning calorimetry (DSC), X-ray diffraction analysis (XRD) and thermogravimetry analysis (TG) [15–18]. The extent of penetration of sericoside in the TSE/CD complex in comparison with

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doi:10.1016/S0939-6411(02)00194-7

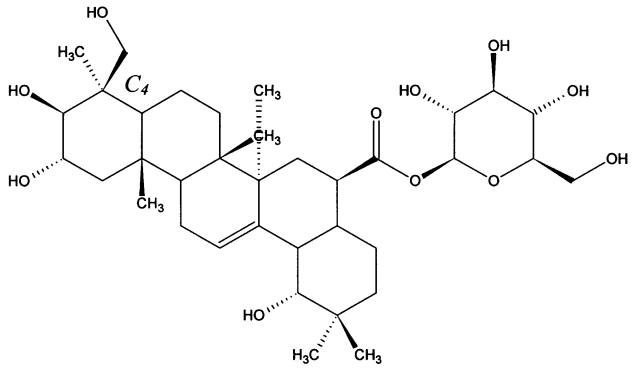

Fig. 1. Structure of sericoside, the main component of TSE. The C_4 isomer is arjunglucoside.

that of pure TSE was investigated by in vitro percutaneous penetration of excised pig skin.

2. Materials and methods

2.1. Materials

A plant extract of *T. sericea* (*Combretacea*) containing 86% sericoside was obtained from Indena (Milano, Italy). This extract also contains approximately 10% arjunglucoside, the C_4 isomer of sericoside. All CDs utilized were obtained from Wacker (Burghausen, Germany). 2-Hydroxypropyl-γ-cyclodextrin (HP-γ-CD) and 2-hydroxypropyl-β-cyclodextrin (HP-β-CD) substitution degrees were specified at 0.5–0.7 (HP-γ-CD) and 0.6–0.9 (HP-β-CD). Methylated-β-cyclodextrin (M-β-CD) had a substitution degree of 1.6–1.9. These values were determined by the supplier using ^{1}H-nuclear magnetic resonance analysis (NMR) and were confirmed by matrix assisted laser desorption-ionization time of flight mass spectrometry (MALDI-TOF). The topical formulations employed as vehicles were derived from standardized o/w and w/o formulations. Fresh deionized water was used for the preparation of complexes and topical formulations. Raw materials were used in the form in which they were obtained.

2.2. Preparation of a solid TSE/CD complex

To prepare solid TSE/CD inclusion complexes 7.50 mmol TSE was equilibrated in 100 ml of deionized water with all the above-mentioned β-CD and γ-CD derivatives in a 1.0:1.1 molar ratio. The resulting mixture was stirred at room temperature (+25 °C) for 2 days. The clear solution was subjected to evaporation at room temperature and low pressure (Vortex-Genie 2, Scientific Industries, Bohemia, NY).

The white powder obtained was stored at +40 °C for 1 day, after which the samples were stored in sealed glass containers at +25 °C for further investigations.

2.3. XRD analysis

The XRD samples, in powder form, were measured on a PSD supply unit (Stoe, Darmstadt) with a germanium monochromator. Three experiments were done in parallel. The adjusted parameters were: CU Kα1 radiation, 40 kV voltage and a 30 mA current. The samples were placed between two polyethylenterephthalate (PETP) films and measured with a vertical rotating sample holder.

2.4. DSC analysis

DSC curves were obtained using a Mettler Toledo DSC 30 (Mettler, Giessen) analyzer and a Mettler Toledo AT 261 detector. Three parallel DSC analyses were carried out. Aliquots of approximately 8 mg of each sample were placed in an aluminium vessel of 40 μl capacity and sealed with a non-perforated aluminium cover. An empty sealed pan was used as a reference and nitrogen (quality 5.0) with a flow of 35 ml/min was used. DSC curves were measured by heating the samples from −50 to +300 °C at a rate of 10 °C/min.

2.5. TG analysis

TG curves were obtained using a Mettler Toledo TGAA SD TA 851^e (Mettler, Giessen) analyzer with an autosampler. Three parallel experiments were carried out. DSC curves were measured by heating the samples from +25 to +150 °C at a rate of 10 °C/min under a nitrogen (quality 5.0) flow of 40 ml/min. Aliquots of about 8 mg of each sample were placed in an aluminium vessel (40 μl capacity) and sealed with a non-perforated aluminium cover. The covers were punctured with a needle by the autosampler directly before measuring.

2.6. Solubility studies

Solubility measurements of sericoside, the main component of the TSE extract, were carried out at 25 °C using CDs in aqueous solutions from 0 to 5% (w/w). Arjunglucoside constitutes up to 10% of TSE, therefore it is probable that this isomer will also undergo complex formation with CD, yielding other complexes besides those containing sericoside. CD studies were carried out using 0.1 M acetic acid/sodium acetate buffer (pH 5). Under these conditions it was assumed that sericoside is in a stable, undissociated state. An excess of TSE (1.2 g) was added to 15 ml CD solutions in stoppered flasks. The mixtures were vortexed for approximately 1 min and agitated for 24 h at +25 °C on a shaker (GFL, Burgwedel). After equilibration, a 10 ml aliquot of each sample suspension was centrifuged at 5000 rev./min for about 5 min. The aqueous phase was stored at +6 °C until analysis by high pressure liquid chromatography/mass spectrometry (LC/MS). The apparent stability constant (K_C) of sericoside/CD ($K_{S/CD}$) and sericoside-arjunglucoside/CD ($K_{SA/CD}$) was estimated from the slope

of the straight line on the phase solubility diagram according to the following equation: K_C = slope/S_0(1 − slope) [19,20]. The solubility values (S_0) were determined by LC/MS.

2.7. Stability studies in topical formulations

To ensure the stability of sericoside in topical formulations, TSE/CD complexes were incorporated in various o/w and w/o pool formulations. A w/o cream containing polyglyceryl-2 dipolyhydroxystearate was used as well as a polyglyceryl-3 diisostearate emulsifier system (formula no. 1, see Table 3). Additionally, an o/w cream containing glyceryl stearate citrate and a dipentaerythrityl hexacaprylate/hexacaprate emulsifier system (formula no. 2, see Table 3) and an o/w lotion containing glyceryl lanolate and triacetearet-4 phosphate (formula no. 3, see Table 3) were used. All pool formulations were free of raw materials which are well known to form stable complexes with the above-mentioned CD derivatives. Indeed, interactions between pool formulation and CD derivatives could not be completely excluded. At first formulations were manufactured by the usual design. After cooling to +30 °C the equilibrated aqueous TSE/CD solution was incorporated into the formulations with gentle stirring and subsequent homogenization. The test samples were stored in sealed glass vessels for 6 months at +6, +25 and +40 °C. They were screened for crystal formation by microscopy directly after manufacturing and after 1, 3 and 6 months.

2.8. Percutaneous penetration of excised pig skin in vitro

The procedure was carried out in accordance with the guidelines for percutaneous absorption/penetration [21]. Gently dry shaven, 3–4 mm thick back skin discs from 100 kg female pigs were sliced with a dermatome. The experiments were carried out in a suitable penetration cell (Franz-type, Fig. 2) at 32 °C. The receptor phase was equipped with 0.9% NaCl, 0.1% gentamycin buffer (pH 7.4) and 1.0% bovine serum albumin. This was poured into the lower penetration cell and stirred at 150 rev./min with a Multipoint HP magnetic stirrer (Variomag, Daytona Beach, FL). Skin discs were positioned on the lower penetration cell. Approximately 20 mg/skin disc of o/w formulation no. 2 (see Table 3) with a complex of 0.25% TSE and CD in a 1.0:1.25 molar ratio was applied. A ratio of 1.0:1.25 was used to avoid crystallization of sericoside due to interactions of CD with other components like the skin layers during the equilibration time of 20 h. Also, the possibility that water evaporated out of the formulation during the diffusion time could not be excluded since the upper penetration cell was not really airtight. In contrast solubility studies (see Section 2.6) on the formulation with a 1.0:1.1 molar ratio of TSE/CD were carried out using various parameters (only formulation, no skin disk, airtight storage). Two treated slices were measured respectively against an untreated slice (without application of any formulation). Slices were treated with the formulation containing the TSE/CD complex as well as with the formulation containing ethanol and CD. The upper penetration cell was positioned on the lower cell and the skin disc was equilibrated for 20 h. The long equilibration time was chosen because of the single formulation application so that there would be a guaranteed TSE penetration into the skin. After equilibration the upper penetration cell was placed in 15 ml 2-propanol to prevent adhesion of residues to the glass. An aliquot of 1 ml was removed and investigated by means of LC/MS.

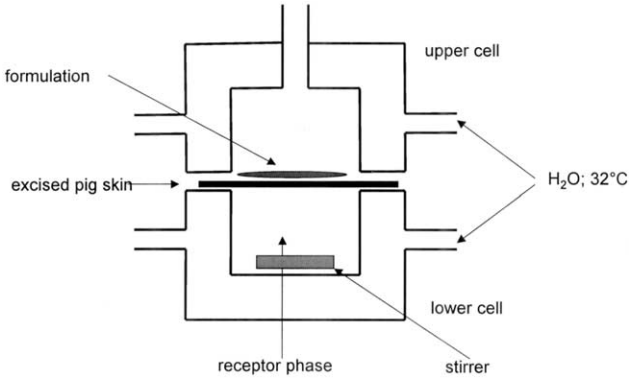

Fig. 2. Schematic diagram of the penetration cell (Franz cell).

- The receptor phase was diluted 1:20 with distilled water and an aliquot of 1 ml was removed for LC/MS measurements. The non-absorbed o/w formulation was removed from the skin disc by scraping with a spatula and dissolved in 2-propanol. A 1 ml aliquot was removed for LC/MS analysis.
- Stratum corneum samples were obtained by 18-fold adhesive tape stripping with Tesafilm Type 4129 (Beiersdorf, Hamburg). The film was applied to the stratum corneum for 10 s, after which the 18 strips were divided into six fractions of three strips each. These fractions were then dissolved in 2-propanol for subsequent investigation by LC/MS analysis.
- The epidermis was isolated by placing the epidermal side of the tissue sample on a hot Ceran plate at 80 °C for 45 s. After this, the epidermis was removed and extracted in 2-propanol. A 1 ml aliquot was removed and analyzed.
- The dermis was cut into pieces to increase the surface area for dissolution in 2-propanol. The extraction took place over approximately 48 h at +40 °C. After cooling, a 1 ml aliquot was removed for investigation by LC/MS.

3. Results and discussion

The aim of these investigations was to improve the solubility of sericoside from TSE. The solubility of a poorly soluble substance such as sericoside normally improves after complex formation with CDs. By means of XRD analysis and DSC analysis the samples were screened for the

presence of a TSE/CD complex and the amounts of unbound sericoside were determined. The water content of TSE and TSE/CD complexes was determined by TG analysis.

3.1. XRD analysis

The samples were investigated by XRD analysis to determine whether a TSE/CD complex had formed. Fig. 3 shows the XRD patterns of TSE and the CD derivatives in the following order: γ-CD, HP-γ-CD, M-β-CD and HP-β-CD. The TSE powder diffraction pattern (Fig. 3A) produced sharp peaks at 2 Theta (2θ) diffraction angles of 11°, 13°, 14°, 15° and 16°. This suggests that the main component sericoside from TSE was present in a crystalline state. The XRD pattern of crystalline γ-CD (Fig. 3B) gave sharp peaks in the range of 8° to 29° 2 Theta (2θ). In contrast, the XRD spectra of the alkylated and hydroxyalkylated CD (Fig. 3C–E) derivatives were characterized by waves without sharp peaks. The XRD patterns in Fig. 3 were controls used to subtract peaks corresponding to raw materials remaining in the reaction mixture (Fig. 4). Fig. 4 shows the XRD patterns of TSE and corresponding complexes with CDs. In contrast to the TSE pattern (Figs. 3A and 4A), the XRD spectra of TSE/CD complexes (Fig. 4B–E) are characterized by amorphous halos. Sharp peaks indicating the

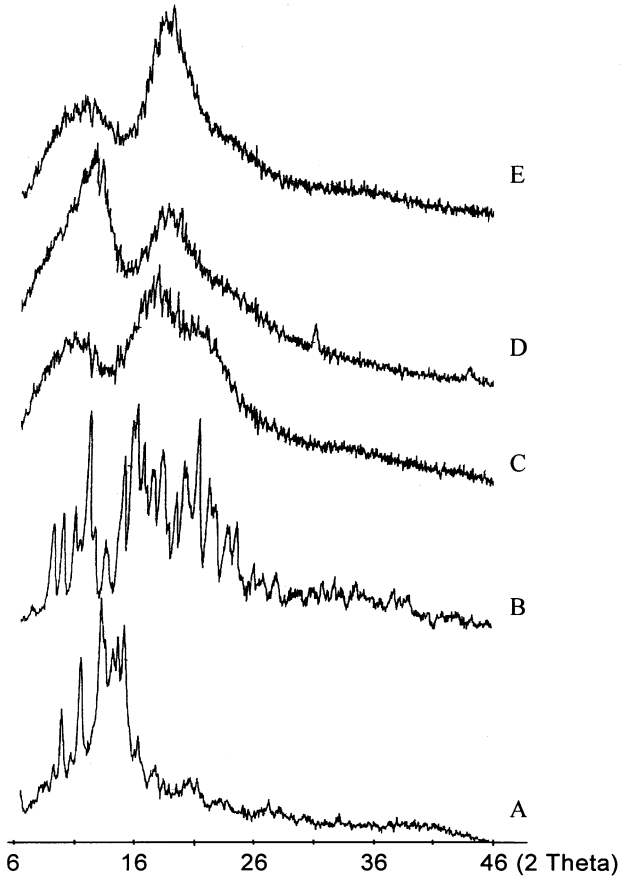

Fig. 3. XRD patterns of (A) TSE, (B) γ-CD, (C) HP-γ-CD, (D) M-β-CD, and (E) HP-β-CD.

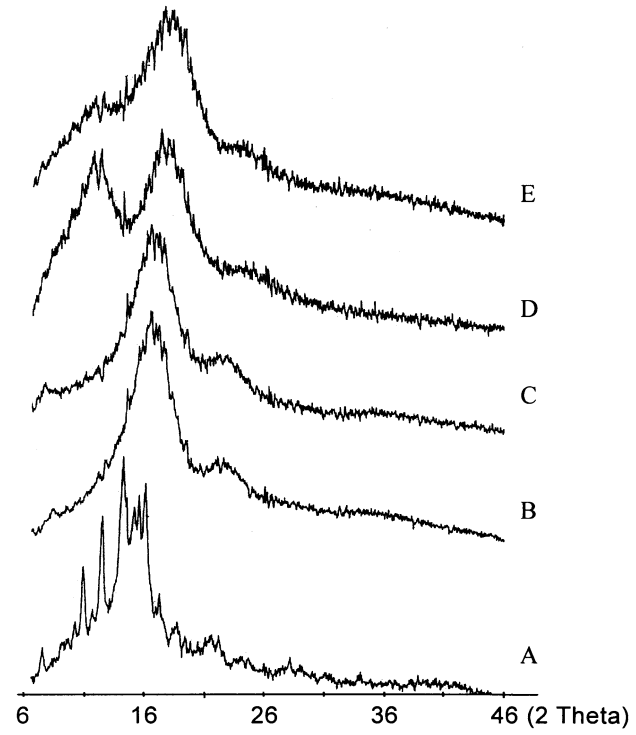

Fig. 4. XRD patterns of (A) TSE, (B) TSE/γ-CD complex, (C) TSE/HP-γ-CD complex, (D) TSE/M-β-CD complex, and (E) TSE/HP-β-CD complex.

crystalline state of TSE are not visible. These results show that TSE, especially sericoside as the main component of TSE, was no longer present as a crystalline material and that a CD complex had formed. The amorphous halos of the TSE/γ-CD spectra are unusual, since the crystalline state of pure γ-CD normally gives sharper peaks in XRD patterns and a crystalline spectra after complex formation between crystalline TSE and crystalline CD was expected. The γ-CD pattern in Fig. 3 supports the assumption that an amorphous impurity of γ-CD was responsible for this occurrence. Perhaps changes in the orientation of γ-CD or different γ-CD crystal sizes were responsible for a decrease in the intensity of the peaks which were still sharp; however, this phenomenon is more likely due to the presence of an amorphous impurity, since it was only detected in γ-CD samples.

3.2. DSC analysis

DSC analysis was carried out to confirm data obtained from XRD studies. In addition, this method allowed verification of complex formation between TSE and CD complexes and also detection of any remaining unbound TSE-derived sericoside. The DSC curves of TSE were obtained over a range of −50 to +300 °C to show the water content of TSE and the melting point of sericoside. All curves show an endothermic peak which indicates the presence of water in pure TSE as well as corresponding TSE/CD complexes. An endothermic peak at +206 °C in

the curve of pure TSE (Fig. 5A) indicates the melting point of sericoside. This peak is not present on the DSC curves of corresponding TSE/CD derivatives (Fig. 5B–E). These results substantiate the XRD data suggesting that complex formation of TSE-derived sericoside with CDs generates amorphous material. This is confirmed by a total integration of TSE-derived sericoside in the CD cavity. TSE/CD curves show low endothermic peaks at +267 °C (Fig. 5B), +228 °C (Fig. 5C), +196 °C (Fig. 5D) and +230 °C (Fig. 5E), suggesting incomplete complex formation.

3.3. TG analysis

Water content was determined by TG analysis. TG curves for determination of the water content of TSE and corresponding TSE/CD systems showed that pure TSE powder contained 3.7% water; the water content of TSE/CD complexes was as follows: γ-CD 6.0%, HP-γ-CD 4.2%, M-β-CD 3.2% and HP-β-CD 4.2%. The percentage of water in the TSE/CD complex was independent of the amount of pure TSE added; thus, conversion of individual weights of TSE and CD derivatives upon their incorporation into the TSE/CD complex in formulations was unnecessary. Incorporation of the TSE/CD complex in formulations for percutaneous penetration studies was required and, for this, mass was calculated from the single weights of TSE and CDs, respectively.

3.4. Solubility studies

The molar ratio of the sericoside/CD complex ($K_{S/CD}$) and the apparent stability constant (K_C) were estimated from phase solubility diagrams (see Section 2.6). Sericoside derived from TSE is nearly insoluble in water; the intrinsic solubility (S_0) of sericoside at pH 5 and +25 °C is 3.4 mmol/l. The concentration of sericoside at +25 °C was notably affected by the presence of CD: 5% (w/w) γ-CD and HP-γ-CD solutions increased the sericoside content to 22.7 and 19.2 mmol/l, respectively, whereas 5% (w/w) M-β-CD and HP-β-CD increased the solubility of sericoside to 18.4 and 19.1 mmol/l. There were no significant differences in the solubilizing effects of the hydrophilic CDs. In all cases, a phase solubility curve obeying Higuchi and Connors of A_L-Type [19,20] was observed. This profile is characterized by formation of a 1:1 complex. In contrast, ratios of 1:2 (γ-CD and HP-γ-CD), 1:2.6 (M-β-CD) and 1:2.2 (HP-β-CD) were calculated. The explanation for the higher calculated complex formation is that TSE was equilibrated with CDs, but only sericoside content, the main component of TSE, was determined. The C_4 isomer arjunglucoside as well as sericoside formed complexes with CD derivatives; accordingly sericoside-arjunglucoside/CD complexes ($K_{SA/CD}$) and the apparent stability constant (K_C) were also estimated from the phase solubility diagrams (see Section 2.6). After including the solubility of arjunglucoside in the calculation, lower ratios between 1:1.6 (γ-CD) and 1:2.1 (M-β-CD) were obtained. Due to 1:1 displacement, a 1:1 ratio of complex formation of sericoside/CD and arjunglucoside/CD was suggested. Furthermore, it could not be excluded that other components of TSE like sericic acid [1], the aglycon of sericoside, complexed with CDs. The apparent stability values (K_C) (Tables 1 and 2) were estimated from the phase solubility diagrams, although 1:1 formations were never found. The calculated stability constants of sericoside with γ-CD derivatives were similar to those with β-CD derivatives. In addition, the calculated stability constant of sericoside-arjunglucoside differed only slightly from that of sericoside. Referring to the data obtained, two statements could be formulated: complex stability did not depend on the cavity size of the different CDs; furthermore, sericoside and arjunglucoside seemed to have similar stability constants. It must be taken into consideration that the true complex formation ratio of sericoside was only calculated, since it was not directly measurable.

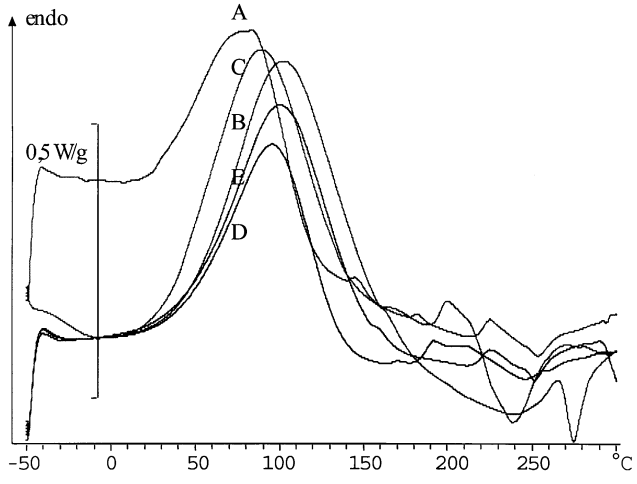

Fig. 5. DSC curves of (A) pure TSE, (B) TSE/γ-CD complex, (C) TSE/HP-γ-CD complex, (D) TSE/M-β-CD complex, and (E) TSE/HP-β-CD complex.

3.5. Stability studies in topical formulations

The stability of the TSE/CD complex in topical formulations was determined through screening for sericoside crystals by microscopy. Unfortunately, crystallization

Table 1
Complex formation constants of sericoside/CD derivatives ($K_{S/CD}$) and apparent stability constants (K_C) of TSE-derived sericoside complexed with γ-CD, HP-γ-CD, M-β-CD and HP-β-CD

TSE/CD complex	Complex formation ($K_{S/CD}$) (sericoside/CD)	Apparent stability constant (K_C) (M^{-1})
γ-CD	1.0:2.0	233
HP-γ-CD	1.0:2.0	254
M-β-CD	1.0:2.6	241
HP-β-CD	1.0:2.2	355

Table 2
Complex formation constants ($K_{SA/CD}$) and apparent stability constants (K_C) of complexes formed by TSE-derived sericoside and arjunglucoside with γ-CD, HP-γ-CD, M-β-CD and HP-β-CD

TSE/CD complex	Complex formation ($K_{SA/CD}$) (sericoside + arjunglucoside/CD)	Apparent stability constant (K_C) (M^{-1})
γ-CD	1.0:1.6	210
HP-γ-CD	1.0:1.5	305
M-β-CD	1.0:2.1	103
HP-β-CD	1.0:1.6	260

of sericoside occurred in all formulations tested containing more than 0.05% TSE. Fig. 6B shows crystallization after incorporation of 0.1% TSE in o/w formulation no. 3 (see Table 3). After their isolation from the o/w formulation and analysis with IR-Raman spectroscopy, the crystals could be identified as sericoside. In contrast, after complex formation of 0.3% TSE with HP-γ-CD and γ-CD, neither crystallization nor a change in the degree of dispersity was evident during 6 months storage at temperatures of +6 and +40 °C (Fig. 6A); however, incorporation of TSE/β-complexes into o/w formulation no. 3 resulted in immediate crystallization of sericoside. Crystallization of sericoside out of TSE/β-complexes was noticed in all o/w and w/o formulations tested, suggesting interactions between β-CD and raw materials therein.

3.6. In vitro percutaneous penetration

Finally, the influence of the sericoside/CD complex on percutaneous penetration was determined. The TSE/γ-CD complex was used because the incorporation of TSE/M-β-CD and TSE/HP-β-CD complexes resulted in a recrystallization of sericoside (see Section 3.5). The penetration of pure TSE and that of TSE complexed with γ-CD was compared. The apparent stability constant of the sericoside/γ-CD complex was nearly the same as that of sericoside/HP-γ-CD (233 M^{-1}:254 M^{-1}, see Table 1). Furthermore, the required concentration of sericoside for penetration studies was obtainable; it did not matter which γ-CD derivatives were used, since the solubility of sericoside/γ-CD was sufficient in all cases. The results of the penetration studies are shown in Fig. 7. TSE (0.25%) was incorporated in o/w formulation no. 2 with 5% ethanol to guarantee the solubility of sericoside in TSE. Furthermore, a 0.25% TSE/γ-CD complex with a molar ratio of 1.0:1.25 in o/w formulation no. 2 without ethanol was tested. The TSE/CD concentration was equivalent to 0.25% pure TSE. We found that 6.6% (ethanol formulation) and 5.2% (CD formulation) of total sericoside were lost and this was attributed to analytical aberration ('loss').

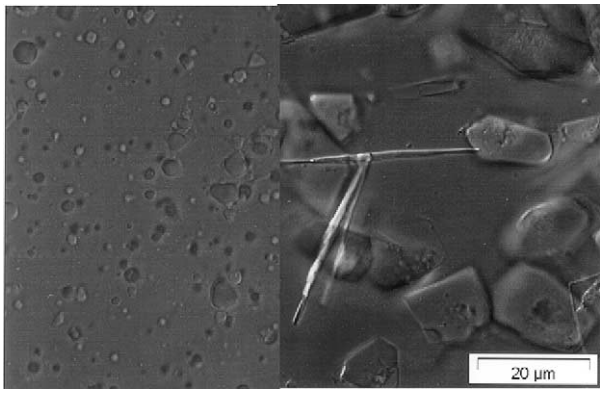

Fig. 6. (A) O/w formulation no. 2 with 0.3% TSE/γ-CD complex and 0.1% of pure TSE (B) at a magnification of 1:1250.

- 67.7 ± 3.3% of the sericoside in the ethanol formulation remained in the formulation, and thus did not penetrate into skin layers ('non-absorbed', Fig. 7); 24.3 ± 4.1% was found in the lifeless stratum corneum and also had no anti-inflammatory effect. Only 1.1 ± 0.4% penetrated the epidermis and 0.3 ± 0.1% penetrated the dermis. No sericoside was found in the receptor phase.
- In contrast, 60.5 ± 8.7% of the sericoside in the TSE/CD complex penetrated the stratum corneum and only 27.0 ± 1.6% remained in the formulation. In epidermis and dermis 6.2 ± 2.3% and 0.7% sericoside were found. It is assumed that not more than 0.1% penetrated the receptor phase.

Complex formation between TSE and γ-CD resulted in a 2.6-fold higher percutaneous penetration compared to that

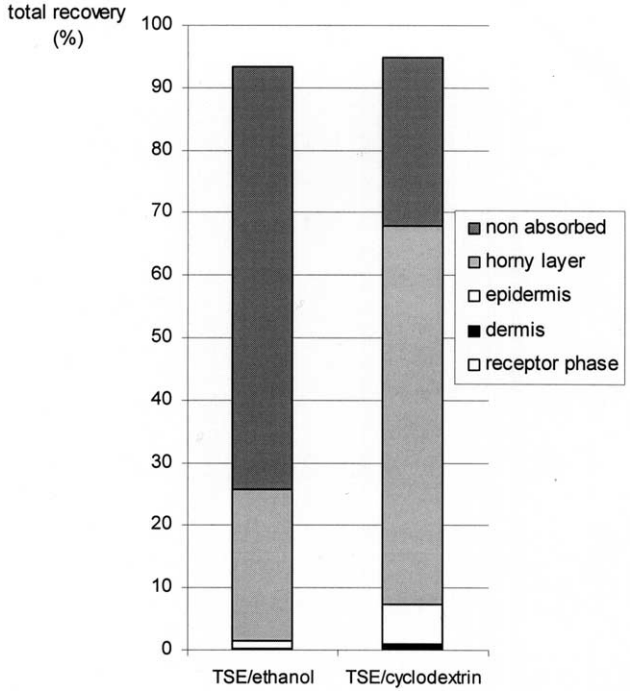

Fig. 7. Percutaneous penetration of sericoside from pure TSE containing 5% ethanol and of a TSE/γ-CD (1.0:1.25) complex in o/w formulation no. 2.

Table 3
List of w/o and o/w formulations referred to in this paper

Formulation no.	Type	Emulsifier systems
1	w/o	Polyglyceryl-2 dipolyhydroxystearate, polyglyceryl-3 diisostearate
2	o/w	Glyceryl stearate citrate, dipentaerythrityl hexacaprylate/hexacaprate
3	o/w	Glyceryl lanolate, triacetearet-4 phosphate

of pure sericoside in an ethanol formulation. In contrast, the percentage distribution of sericoside in skin layers which were treated with the TSE and ethanol formulation did not change significantly in comparison to skin layers which were treated with the formulation containing the TSE/γ-CD complex. The ratio of sericoside in sericoside/γ-CD to sericoside/ethanol formulations was 2.5 in the stratum corneum and 2.3 in the dermis. In the epidermis a higher ratio of 5.6 was found. This could be disregarded because of the naturally occurring inconsistency of the skin layers. Variant influences of formulations on skin microstructure could be excluded because the same formulations were used for both samples. Therefore, it seems that when γ-CD is complexed to TSE the equilibrium between complexed and pure sericoside is altered resulting in significantly higher penetration. If γ-CD were to interact with the stratum corneum, the percentage sericoside distribution would be different from that of the formulation without γ-CD. The concentration of sericoside in the TSE/γ-CD formulation would probably be higher in the stratum corneum than in the TSE/ethanol formulation. These results indicate that γ-CD had no influence on the stratum corneum. One must also take into account that the studies on pig skin only provide a clue as to the percutaneous penetration in human skin. Additionally, the distribution of sericoside may be altered in human skin upon repeated application of the formulation, as opposed to the single application which was tested in this study.

4. Conclusion

The complex formation of TSE containing 86% sericoside with β- and γ-CD derivatives such as M-β-, HP-β-CD and γ-, HP-γ-CD significantly improves the solubility of sericoside in water. After equilibration of TSE with all utilized CD derivatives, complex formation could be confirmed by DSC, TG and XRD. After incorporation into topical o/w and w/o formulations, only complexes with γ-CD were stable upon storage. The incorporation of β-CD complexes in topical o/w and w/o formulations resulted in immediate crystallization of sericoside. After incorporating the dehydrated TSE/γ-CD complex into an o/w formulation, the percutaneous in vitro penetration of sericoside using a Franz cell with excised pig skin increased 2.6-fold compared to that of pure TSE in an ethanol o/w formulation. The percentage sericoside distribution over the various isolated skin layers was not significantly different between the TSE/γ-CD and γ-CD-free formulation. This indicated that γ-CD does not affect the stratum corneum of pig skin layers.

These investigations led to the achievement of increased stability and excellent skin penetration of sericoside. The technique described may serve as a flexible instrument for the further incorporation of relatively insoluble components into topical emulsion based formulations.

Acknowledgements

Thanks to N. Rasenack (Christian-Albrechts-University Kiel) for help in obtaining the XRD spectra. Additionally, thanks to M. Akihani, F. Hetzel and V.S. Schlesiger (Beiersdorf AG, Hamburg) for their help with the penetration studies and the DSC and TG curves.

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