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Enhancement of the release of azelaic acid through the synthetic membranes by inclusion complex formation with hydroxypropyl-β-cyclodextrin

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Abstract

The aim of this study was to investigate the release rates of azelaic acid and azelaic acid-hydroxypropyl-β-cyclodextrin (HPβCD) inclusion complex through three types of synthetic membranes, namely cellophane, silicone and elastomer membranes. Solid inclusion complexes of azelaic acid-HPβCD at the molar ratio of 1:1 were prepared by coevaporation and freeze-drying methods, subsequently characterized by differential scanning calorimetry, X-ray diffractometry and dissolution studies. Solid inclusion complex obtained by coevaporation method which exhibited the inclusion of azelaic acid in the HPβCD cavity and gave the highest dissolution rate of azelaic acid was selected for the release study. Release studies of azelaic acid and this complex through the synthetic membranes were conducted using vertical Franz diffusion cells at 30 °C for 6 days. The release rates of azelaic acid through the synthetic membranes were enhanced by the formation of inclusion complex with HPβCD at the molar ratio of 1:1, with the increasing fluxes of about 41, 81 and 28 times of the uncomplexed system in cellophane, silicone and elastomer membranes, respectively. The result from this study can be applied for the development of azelaic acid for topical use.

Keywords: Azelaic acid; Franz diffusion cells; Hydroxypropyl-β-cyclodextrin; Inclusion complex; Release study; Synthetic membranes

1. Introduction

Cyclodextrins are cyclic oligosaccharides, containing at least 6 D-(+)-glucopyranose units attached by α-1,4-linkage. Three types of cyclodextrins exist in the nature, namely α-, β- and γ-cyclodextrins, containing 6, 7 and 8 D-(+)-glucopyranose units, respectively. β-Cyclodextrin appears to be the best natural cyclodextrin due to its cavity size, efficient drug complexation, availability in pure form, and relatively low cost (Le Bas and Rysanek, 1987; Beker et al., 1991; Loftsson, 1999). Natural cyclodextrins can be modified

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for many purposes, for example, to improve the low aqueous solubility. One of the pharmaceutically important cyclodextrin derivatives is 2-hydroxypropyl-β-cyclodextrin (HPβCD), which is a powerful solubilizer of several drugs (Beker et al., 1991). The cyclodextrin molecule has torus shape, with the hydrophilic outside and hydrophobic inside the cavity. If any molecule entirely or at least partially enters into the cavity, an inclusion complex may be formed (Le Bas and Rysanek, 1987; Beker et al., 1991).

An inclusion compound is a unique form of chemical complex, in which one molecule is enclosed within another molecule (Frank, 1975). The formation of inclusion complexes of several drugs and cyclodextrin has been investigated to improve the solubility and dissolution rate (Blanco et al., 1991; Palmieri et al., 1997; Castillo et al., 1999), physical and chemical stability (Le Bas and Rysanek, 1987; Beker et al., 1991) and the bioavailability of poorly water soluble drugs (Castillo et al., 1999). Cyclodextrin may improve the efficacy of dermally applied drugs by increasing the apparent solubility or stability of the drug as well as an enhancement of membrane permeability (Rajewski and Stella, 1996).

Azelaic acid is a naturally occurring saturated dicarboxylic acid, and has been used for the treatment of acne (Fig. 1). At high concentration, azelaic acid is bactericidal against *Propionibacterium acnes* and *Staphylococcus epidermis*, and possesses bacteriostatic properties against a variety of aerobic microorganisms (Gollnick and Schramm, 1998). The poor water solubility of azelaic acid results in difficulties in the formulation of this substance for topical application.

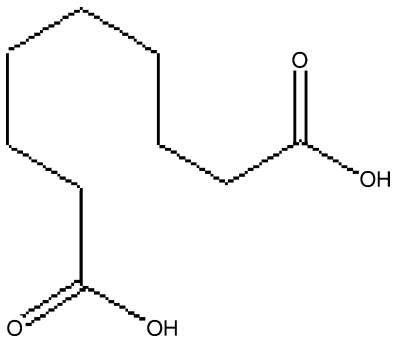

Fig. 1. Chemical structure of azelaic acid (1,7-heptanedicarboxylic acid).

In this study, the release of uncomplexed and complexed azelaic acid with HPβCD through three types of synthetic membranes (elastomer, silicone and cellophane) was investigated.

2. Materials and methods

2.1. Materials

Azelaic acid was from Sigma Chemical Company (St. Louis, MO, USA). HPβCD (Kleptose®HPB, average MW = 1400, molar substitution = 0.630) was obtained from Roquette-Freres (Lestrem Cedex, France). Silicone elastomer membrane (7-4107) was obtained from Dow Corning Corporation (Midland, MI, USA). Silicone membrane was obtained from Silex (Lindford, Bordon, Hants, UK). Cellophane membrane (Cellu-Sep®; MWCO 12,000–14,000) was obtained from Membrane Filtration Products, Inc. (TX, USA). All other chemicals were of analytical reagent grade and obtained from commercial sources.

2.2. Preparation of binary systems

Two types of binary systems were prepared, namely physical mixture (PM) and solid inclusion complexes, prepared by coevaporation (coevaporated systems, COE) and freeze-drying (colyophilized systems, FD) methods. In all systems, azelaic acid to HPβCD molar ratios were 1:1. PM was prepared by blending of the weighed and previously sieved individual components through a 315-μm mesh in a mortar for 5 min. In COE method, azelaic acid and HPβCD were dissolved in the minimum amount of 80% (v/v) ethanol to obtain a solution, and stirred for 30 minutes. Then, the solutions were evaporated under vacuum at 50 °C by a rotary evaporator (Büchi Rotavapor Model R-124, Switzerland). In FD method, azelaic acid and HPβCD were dissolved in the minimum amount of absolute ethanol and water, respectively. The two solutions were mixed for 24 h and lyophilized at −30 °C (Christ FOC-1 Model K-40 equipment, Balzers-Pfeiffer GmbH, Asslar, Germany). All dried products were crushed, sieved through a 315-μm mesh, and stored under vacuum in a desiccator prior to use. The azelaic acid contents were determined by HPLC following derivatization with

p-bromophenacyl (Passi, 1983; Bojar, 1993; Ferioli, 1994).

2.3. Differential scanning calorimetry

Approximately 5 mg of azelaic acid, PM and inclusion complexes (COE and FD systems) were subjected to DSC analysis, using a Perkin-Elmer DSC-7 Model. Alumina was used as a reference material and the scanning rate was 5 °C min^{-1}, with the scanning temperature range of 20 and 200 °C.

2.4. X-ray diffractometry

Powder X-ray diffraction patterns were obtained from a Siemens D-500 diffractometer. Samples were irradiated with monochromatized Cu Kα radiation and analyzed between 2θ angles of 5 and 60° at a scan rate of 1 min^{-1}.

2.5. Dissolution studies

Dissolution studies were performed using the USP XXIII type 2 paddle method (50 rpm) in 900 mL of distilled water, and maintained at 37 ± 0.5 °C for 90 min. The previously sieved through a 315-μm mesh azelaic acid, PM and solid inclusion complexes (COE and FD systems), equivalent to 25 mg of the drug were used. At predetermined time intervals, 3 mL-sample was withdrawn with a filter-syringe (pore size: 0.45 μm) and assayed for azelaic acid content by HPLC after derivatizing with *p*-bromophenacyl. All experiments were performed in triplicates.

2.6. Release studies

Uncomplexed azelaic acid and azelaic acid-HPβCD inclusion complex (COE system) dispersed in water, equivalent to 0.2 mg/mL of azelaic acid, were used as samples. Three types of synthetic membranes namely cellophane, silicone, elastomer were used. Vertical Franz diffusion cells (Crown Bio Scientific, Inc., Sommerville, NJ, USA) were set at 30 ± 1 °C, and the receiver chamber was filled with 12 mL of pH 7.4 phosphate buffer solution. The synthetic membranes were placed in the diffusion cells with the top side in contact with the donor chamber, and the bottom side in contact with the receiver medium (contact area, 1.77 cm^2). The cell was clamped and the receiver medium was stirred continuously for 6 days by a magnetic bar. The 1 mL-sample was loaded into the donor chamber. During the study, the donor chamber and the sampling port of receiver chamber were covered by parafilm. Half milliliter of sample was withdrawn from the receiver chamber at predetermined time intervals. The azelaic acid contents were determined by HPLC following derivatization with *p*-bromophenacyl. All experiments were performed in triplicates.

3. Results and discussion

3.1. Preparation and characterization of the solid inclusion complexes

Fig. 2 shows the DSC thermograms of azelaic acid, HPβCD, PM, COE and FD systems. The thermogram of azelaic acid revealed an endothermic peak at around 105 °C, corresponding to its melting point. The thermogram of PM still demonstrated the melting point of azelaic acid, indicating that an inclusion complex could not be obtained by simply blending the drug and HPβCD. The COE system did not exhibit the melting endothermic peak of azelaic acid, indicating that azelaic acid was incorporated in the HPβCD cavity. This demonstrated that an inclusion complex could be obtained by coevaporation method, in agreement with previous studies reported by Blanco et al. (1991) and Castillo et al. (1999).

Further evidence of inclusion complex formation was observed from X-ray powder diffractograms (Fig. 3). The diffraction pattern of azelaic acid displayed crystallinity, whereas HPβCD was amorphous in the solid state. The diffraction pattern of PM system was the sum of those of pure azelaic acid and HPβCD. The diffractograms of COE and FD systems did not exhibit peaks corresponding to azelaic acid and were practically identical to those of the amorphous HPβCD. The amorphous diffractogram of FD system might be attributed to the freeze-drying process, and not due to the formation of an inclusion complex of azelaic acid in the HPβCD cavity. Thus, the X-ray diffraction analysis confirmed the DSC results, showing that an inclusion complex was formed in the COE system.

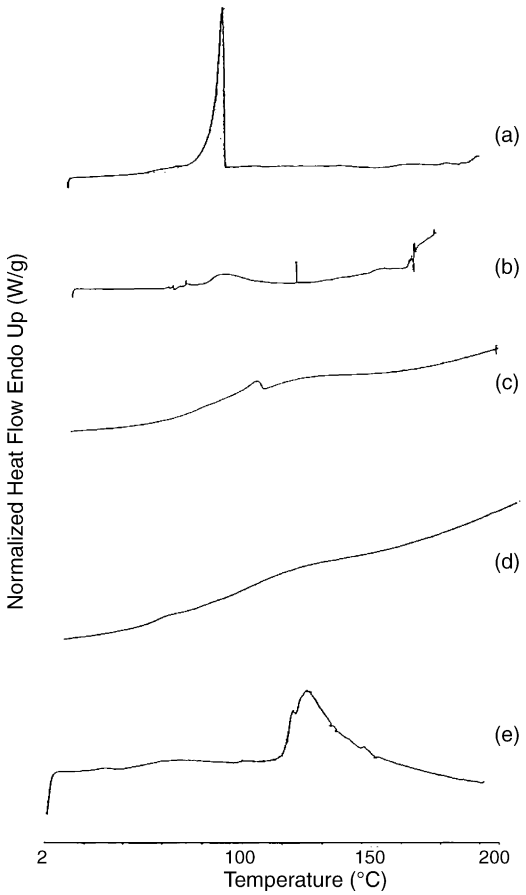

Fig. 2. Differential scanning calorimetry thermograms of (a) azelaic acid, (b) HPβCD, (c) physical mixture (PM), (d) coevaporated system (COE), and (e) colyophilized system (FD).

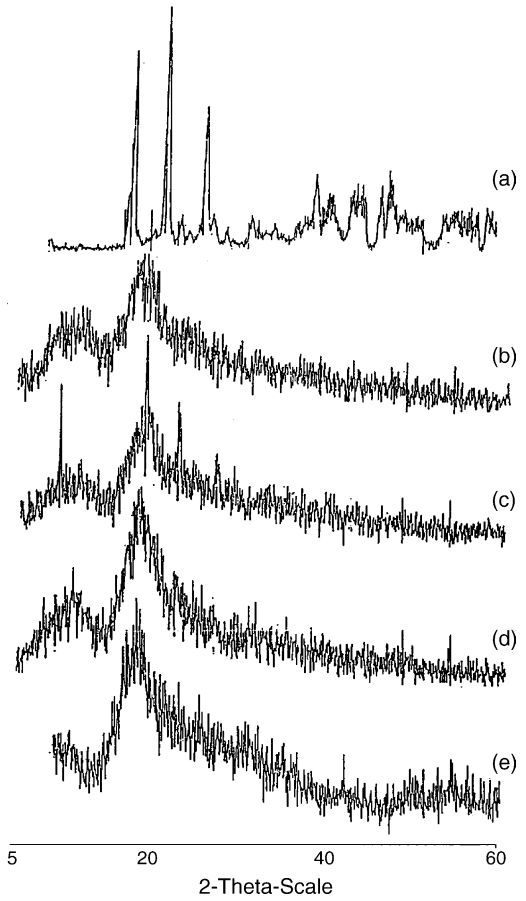

Fig. 3. Powder X-ray diffractograms of (a) azelaic acid, (b) HPβCD, (c) physical mixture (PM), (d) coevaporated system (COE), and (e) colyophilized system (FD).

All of the solid systems exhibited enhanced dissolution rates of azelaic acid in water. This was due to the more hydrophilic characteristic of the solid systems of azelaic acid when complexed with HPβCD than the azelaic acid. The COE system exhibited the highest dissolution rate of azelaic acid. Dissolution profiles of all solid systems exhibited a descending portion (Fig. 4). These phenomena were probably due to a partial dissociation of the complex with the formation of an equilibrium in water solution (Palmieri et al., 1997). The maximum concentrations of azelaic acid dissolved in COE and FD systems in water at $37 \pm 0.5\,^{\circ}C$ were 22.2 and 11.1 mg/L, respectively, in comparison to the corresponding value of the uncomplexed azelaic acid, i.e. 9.7 mg/L (Fig. 4).

3.2. Release studies through the synthetic membranes

Three types of synthetic membranes were selected as an in vitro model for simulating the skin. Thus, maintaining the skin tissues alive is not required in this experiment. Silicone membrane may adequately function as an epidermis. Silicone and silicone elastomer membranes differ on the porosity and lipophilicity properties. Unlike silicone membrane, silicone elastomer is a non-porous membrane, permeable to organic species, but impermeable to inorganic materials such as inorganic and organic salts. Both sides of the surface of silicone membrane are lipophilic in nature, whereas one side of the surface of silicone elastomer membrane is

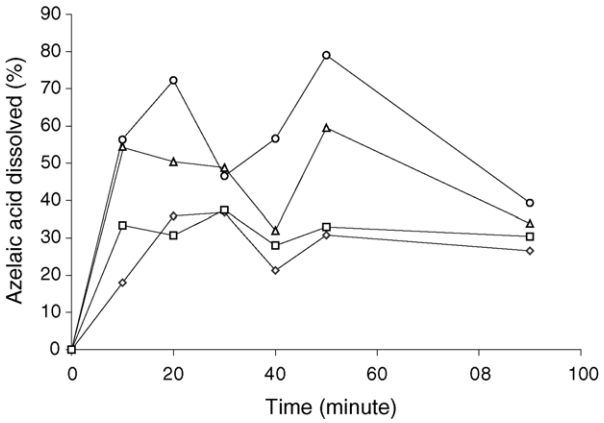

Fig. 4. Dissolution profiles of (◊) azelaic acid, (Δ) physical mixture (PM), (◯) coevaporated system (COE), and (□) colyophilized system (FD). Values denote the mean of three determinations.

lipophilic and hydrophilic on another side. Cellophane is selective, but unlike living membranes, its selectivity does not vary. Water and other small molecules can pass through a cellophane membrane, but large molecules are blocked. This membrane is commonly used in a haemodialysis process.

The COE system was selected as a sample in the release study, since it exhibited the highest dissolution rate of azelaic acid. The fluxes of uncomplexed azelaic acid through cellophane, silicone, and elastomer membranes were 0.0066, 0.0031, and 0.0062 mg cm^{-2} h^{-1}, respectively. In comparison, the respective fluxes of complexed azelaic acid prepared by COE method through cellophane, silicone, and elastomer membranes were 0.2706, 0.2516, and 0.1737 mg cm^{-2} h^{-1}, approximately 41, 81 and 28 times greater than the uncomplexed drug (Fig. 5 and Table 1).

Table 1
Comparison of the fluxes and times required to reach steady state (h)[a] of azelaic acid and coevaporated system (COE) through cellophane, silicone and elastomer membranes

Membrane	Flux (mg cm^{-2} h^{-1})[b]	
	Azelaic acid	COE system
Cellophane	0.0066 ± 0.0028 (4.60)	0.2706 ± 0.0875 (0.42)
Silicone	0.0031 ± 0.0021 (5.03)	0.2516 ± 0.1492 (0.56)
Elastomer	0.0062 ± 0.0027 (3.53)	0.1737 ± 0.1176 (0.77)

[a] Time required to reach steady state is expressed in parentheses.
[b] Experimental data represent the mean ± S.D. of three determinations.

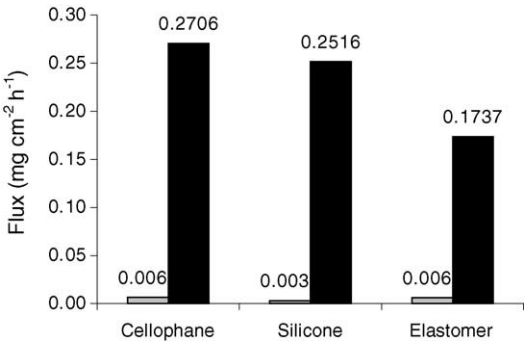

Fig. 5. Fluxes of (▒) azelaic acid and (■) coevaporated system (COE) through cellophane, silicone and elastomer membranes.

The complexation of azelaic acid with HPβCD resulted in the enhancement of the azelaic acid release. The maximum amounts of azelaic acid released across three types of synthetic membranes from COE system (0.16–0.20 mg) were higher than those from the uncomplexed drug (0.02–0.07 mg, Fig. 6). In addition, times required to reach steady state conditions for COE system were shorter than those for the uncomplexed azelaic acid (Fig. 6 and Table 1). Based on the fluxes data, times required to reach steady state for both uncomplexed and complexed azelaic acid through three types of synthetic membranes were less than 6 h (Table 1). The steady state conditions were maintained throughout the study. All samples in the donor chamber, either uncomplexed or complexed azelaic azid, were present in a suspension form. The amount of azelaic acid incorporated in the donor chamber (200 mg/L) exceeded the apparent solubilities of un-

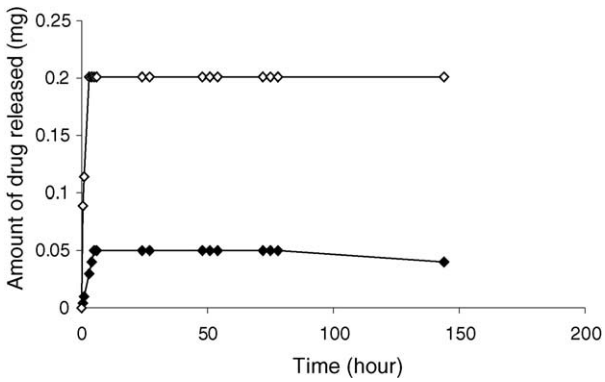

Fig. 6. Typical flux patterns of (♦) azelaic acid and (◊) coevaporated system (COE) through a silicone membrane.

complexed and complexed azelaic acid (COE system) in water at $37 \pm 0.5\,^{\circ}C$, i.e. 9.7 and 22.2 mg/L, respectively. A species available in molecular dispersion is capable of passing through a semipermeable membrane. The amount of azelaic acid present in solution form for COE system in the donor chamber was 2.3-fold greater than that for the uncomplexed drug. The higher fluxes of azelaic acid observed in the COE system through three types of synthetic membranes might be attributed to the higher percentages of drug available in solution in the donor chamber. The enhanced release rate of azelaic acid via the formation of an inclusion complex with HPβCD might be due to the increased dissolution rate of azelaic acid in water. Thus, the release patterns of azelaic acid through three synthetic membranes might be a dissolution-controlled process.

The fluxes of hydrocortisone from suspensions are increased with an increase of HPβCD concentration up to approximately 10–13% HPβCD, as a result of an increased apparent solubility of the drug. However, when all hydrocortisone are available initially in solution, additional increase in HPβCD concentration decreases the observed flux of the drug, as a result of competition between the cyclodextrin and the skin, as well as the lack of absorption of the cyclodextrin complex of hydrocortisone (Rajewski and Stella, 1996).

Silicone and cellophane are semipermeable membranes with hydrophobic character on both surfaces, while silicone elastomer membrane is hydrophobic on one side and hydrophilic character on another side. The cellophane membrane exhibited the highest flux of azelaic acid from COE system. This was probably due to the lowest binding affinity of the complex to the membrane. On the contrary, the silicone elastomer membrane exhibited the lowest flux of azelaic acid from COE system. The more hydrophilic characteristic of the complex probably has high binding affinity to the hydrophilic side of an elastomer membrane. In fact, silicone elastomer is an ideal membrane that can represent a skin character with the outside hydrophobic and inside hydrophilic properties.

In conclusion, the release rates of azelaic acid through three types of synthetic membranes were enhanced via complexation with HPβCD at a molar ratio of 1:1, with an increasing flux in the order of elastomer, silicone and cellophane membranes. Results from this study can be applied for the development of azelaic acid for topical application.

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