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Effect of hydroxypropyl-β-cyclodextrin on the solubility, photostability and in-vitro permeability of alkannin/shikonin enantiomers

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Abstract

The influence of hydroxypropyl-β-cyclodextrin (HPCD) on the solubility, photostability, and the in-vitro permeability of alkannin/shikonin enantiomers (A/S) was investigated. Solubility of A/S increased approximately 200 fold at 50% HPCD. The phase-solubility diagram revealed the formation of a 1:1 inclusion complex of A/S-HPCD and a stability constant (k_s) of 260.5 M^{-1}. Addition of HPCD significantly decreased the photochemical decomposition rate of A/S at pH 9.0. The apparent-first-order photochemical decomposition rate constants of free and complexed A/S were calculated from the Lineweaver-Burk plot to be 0.329 and 0.156 h^{-1}, respectively. The in-vitro permeability study showed that the release of A/S from four test bases followed a Higuchi release model. The presence of HPCD enhanced the A/S release rate by 34 and 54 fold in cream and gel form, respectively, at one-tenth A/S concentration of the control preparation-Shiunko ointment.

Keywords: Alkannin/shikonin enantiomers; Hydroxypropyl-β-cyclodextrin; Solubility; Photostability; Permeability

1. Introduction

β-Cyclodextrin (β-CD) is a cyclic oligosaccharide of seven glucose residues with a cavity formed through an α-1,4-linkage cyclization. β-CD as well as its alkylated and hydroxyalkylated derivatives are used extensively as pharmaceutical excipients due to their remarkable molecular complexation property with many drugs (Bekers et al., 1991a; Duchêne and Wouessidjewe, 1990). These complexes exhibit different properties from their

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original free forms and usually result in improved aqueous solubility (Loftsson et al., 1991; Müller and Brauns, 1985), better chemical stability (Backensfeld et al., 1990; Bekers et al., 1991b; Brewster et al., 1992) and increased percutaneous permeability and bioavailabilty (Choudhury and Nelson, 1992; Frijlink et al., 1991a,b; Okamoto et al., 1986). Despite their broad applications in pharmaceutical and cosmetic preparations, β-CDs have never been used in crude pharmacognosy preparations.

Alkannin/shikonin enantiomer (A/S) is the main pharmacologically active component of Shikon (*Macrotomia euchroma*), which is used to prepare Shiunko ointment for the treatment of wounds, skin disease, and burns in the Orient for the past few centuries (Seto et al., 1992). The pharmacological activities of A/S and its derivatives include anti-inflammatory (Hayashi, 1977), anti-bacterial (Tabata et al., 1982), wound-healing (Hayashi, 1977), and anti-tumor effects (Sankawa et al., 1977). In our previous studies, we have demonstrated that a rapid photochemical decomposition occurs at the side chain of A/S when it is exposed to light (Cheng et al., 1995; Chen et al., 1996). Therefore, the photostability of A/S is a major concern in the development of A/S formulation. A/S and its derivatives are sparingly soluble in water. Preparations containing A/S were traditionally restricted to oily base such as Shiunko ointment, which had very limited bioavailability and poor patient compliance. It was our attempt to expand the application of CDs into the traditional oriental pharmacognosy preparations using A/S as our model drug to solve above problems. The influence of HPCD on the solubility, photostability, and the in-vitro permeability of A/S was thus studied. The results indicated that HPCD was able to improve the aqueous solubility of A/S, to reduce the photochemical decomposition rate and to enhance the in-vitro permeability of A/S through complexation.

2. Materials and methods

2.1. Materials

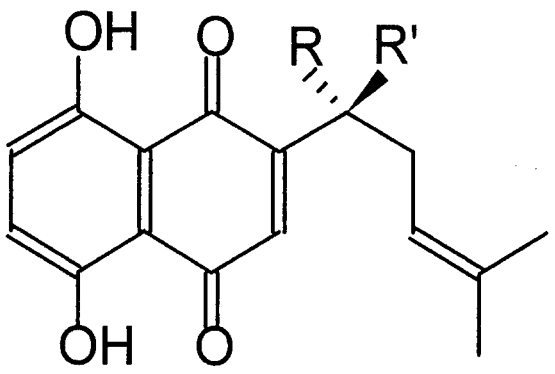
A/S and β,β-dimethylacrylalkannin/shikonin (DMAS) (Fig. 1) was prepared from *M. euchroma* as previously described with minor modification (Tabata et al., 1982). The obtained A/S mixture (purity > 98.5%) has a enantiomeric ratio of 83:17 as previously reported (Cheng et al., 1995). HPCD with Ave. MS 0.8 was purchased from Aldrich (USA), carbopol 940 from B.F. Goodrich (Germany) and methanol (LC grade) from Lab-Scan (Ireland). All other reagents (analytical grade) were purchased from Nakalai Testique (Japan).

2.2. Compounding of A/S preparations

Shiunko ointment, 0.1% A/S cream, 0.01% A/S-HPCD cream and 0.01% A/S-HPCD gel were prepared with the detailed composition shown in Table 1. Shiunko ointment containing 0.1% A/S was prepared as reported (Hayashi, 1977). Each ingredient of Shiunko ointment base was accurately weighed and heated to 75°C. A/S was added to the dissolved base vehicle with constant stirring. The ointment was then cooled to room temperature. Each ingredient of the oil and aqueous phase of 0.1% A/S cream and 0.01% A/S-HPCD cream was accurately weighed, placed into two separate containers and heated to 75°C. Aqueous phase was then slowly added to the oil phase with constant stirring until a homogenous phase was obtained. Each cream was then cooled to room temperature. A/S-HPCD gel was prepared by dropwise addition of 1 N NaOH to the carbopol 940 and A/S-HPCD solution with constant stirring until the gel was formed.

2.3. Solubility studies

Solubility studies were conducted by adding an excessive amount of A/S to various concentrations of HPCD aqueous solution (0–50%), pH 6.7. The solutions were sonicated in an ultrasonic bath (Elma, Japan) for 30 min and placed in a shaking water bath (Fargo Instrument, Taiwan) at 25.0 ± 0.1°C for 48 h. An aliquot of solution was removed and filtered through a 0.45 μm HVLP membrane (Millipore, USA). A/S concentration in each solution was determined spec-



Alkannin (R=H, R'=OH) Shikonin (R=OH, R'=H)

β,β-Dimethylacrylalkannin (R=H, R'=OCOCH=CMe2)

β,β-Dimethylacrylshikonin (R=OCOCH=CMe2, R'=H)

Fig. 1. Chemical structures of alkannin/shikonin and β,β-dimethylacryl alkannin/shikonin.

trophotometrically at 515 nm with a Jasco 7800 spectrophotometer (Japan).

2.4. Photostability studies

The photodegradation rate-pH profile reveals that A/S was most unstable between pH 8 and 10 (Chen et al., 1996). Photostability studies were thus performed under aerobic condition by adding 2 ml of 100 μg/ml A/S methanol solution to an equal volume of various concentration (0–40%) of HPCD phosphate buffer solution, pH 9. Each test solution (3 ml) was transferred to a 10 ml clear glass vial and was further exposed to the fluorescent light (NEC-FL20SSEX-N/18). The distance from the light source to the samples was 30 cm. An aliquot of 200 μl solution was removed at each pre-determined checkpoint. The remaining A/S in the solution was assayed with a stability-indicating HPLC method at 515 nm as previously reported (Chen et al., 1996).

2.5. In-vitro permeability studies

Permeability studies were carried out with a Franz cell apparatus at 32°C and 500 rpm stirring rate (Fargo Instruments, Taiwan). Two grams of the test formulation sample were loaded into a donor cell and normal saline was used as the diffusion medium in the receptor cell. Tuffryn membrane with the pore size of 0.45 μm (Gelman, USA) was used as the diffusion membrane. An aliquot of 0.6-ml diffusion medium was removed from the receptor cell at each pre-determined checkpoint. A/S concentration was measured spectrophotometrically at 515 nm.

The flux from Shiunko ointment, A/S cream, A/S-HPCD cream, and A/S-HPCD gel at 8, 12, 16, and 24 h was used for the statistical analysis. Non-paired Student's *t*-test was performed using PSI-Plot software Version 4.01 from Poly Software International (Salt Lake City, UT, USA).

Table 1
The chemical compositions of four A/S formulations

Ingredient	Shiunko ointment (0.1%)	A/S cream (0.1%)	A/S-HPCD cream (0.01%)	A/S-HPCD gel (0.01%)
A/S (g)	0.10	0.10	—	—
Sesame oil (g)	59.50	—	—	—
Bee wax (g)	9.50	—	—	—
White petrolatum (g)	30.90	3.75	3.75	—
Cetyl alcohol (g)	—	10.00	10.00	—
Mineral oil (g)	—	5.00	5.00	—
Propylene glycol (g)	—	5.00	5.00	—
Sodium lauryl sulfate (g)	—	1.25	1.25	—
Carbopol 940 (g)	—	—	—	0.50
1 N NaOH (ml)	—	—	—	2.50
250 ug/ml A/S-80%HPCD (ml)	—	—	40.00	40.00
Water purified q.s. to	—	100.00	100.00	100.00

3. Results and discussion

3.1. Solubility studies

A/S is sparingly soluble in water. To overcome the solubility problem in formulating a topically applied hydrophilic A/S preparation, various types of CD such as α-, β-, γ-CD and HPCD were used to enhance the aqueous solubility of A/S. Of all these CDs tested, HPCD was the most effective one (data not shown). HPCD was thus selected for all future studies. The phase-solubility diagram of A/S in HPCD aqueous solution (0–50% w/v) is shown in Fig. 2. Solubility of A/S increased approximately 200 fold at 50% HPCD. A linear relationship was observed between A/S solubility and HPCD concentration ($r \geq 0.995$). This result suggests that an 1:1 inclusion complex of A/S-HPCD was formed, a typical A_L complexation (Higuchi and Connors, 1965). The apparent stability constant for complex formation (k_s) was calculated to be 260.5 M^{-1} based on the equation:

$$k_s = \frac{\text{Slope}}{\text{Intercept} \times (1 - \text{Slope})}$$

The phase-solubility diagram of DMAS in HPCD solution is also shown in Fig. 2. The substitute at the C11 of the side chain of A/S has significantly reduced the complexation of A/S with HPCD. The steric hindrance effect of β,β-dimethylacryl moiety apparently blocked the incorporation of A/S side chain into HPCD cavity. The total complexation of A/S with HPCD could be separated into two parts: the basic skeleton and the side chain of A/S, each part capable of being complexed with HPCD. With the slope of DMAS representing the 'intrinsic' complexation of the basic skeleton of A/S (5,8-dihydroxy-1,4-naphthoquinone) with HPCD, the slope difference between A/S and DMAS could be interpreted as the incorporation of the A/S side chain into HPCD cavity. The complexation of A/S basic skeleton with HPCD vs that of A/S side chain is calculated to be approximately 1:1.

3.2. Photostability studies

A/S is a light sensitive compound. The photo degradation of A/S follows an apparent-first-order kinetics (Chen et al., 1996). The effect of HPCD on the photostability of A/S was studied. The data revealed that the photochemical decomposition of A/S could not be completely inhibited by the addition of HPCD (Fig. 3). HPCD decreased the observed apparent-first-order rate of photochemical decomposition of A/S in a non-linear relationship. Maximal stabilization of A/S was achieved at a concentration of 15–20% HPCD. These results are consistent with a kinetic system

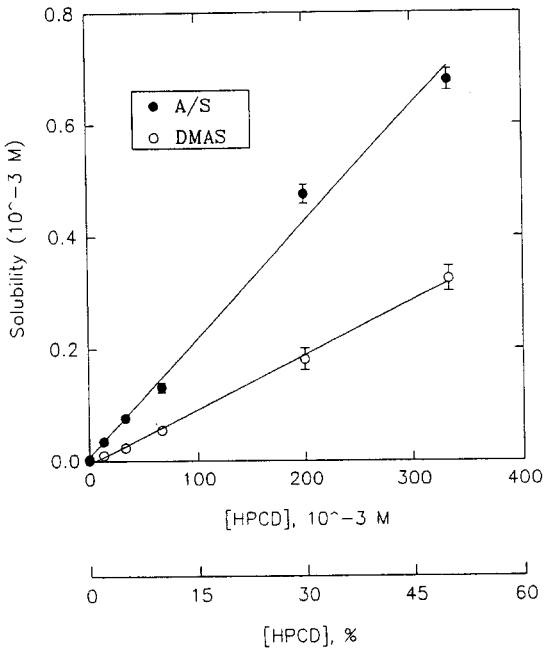


Fig. 2. Phase-solubility diagram of A/S and DMAS in aqueous HPCD solution. All data points are the mean values from three different observations. The error bar represents the standard deviation.

where a free drug degrades at a higher rate than the complex form:

$$\text{A/S} + \text{HPCD} \overset{K_s}{\rightleftharpoons} \text{A/S:HPCD}$$

$$\searrow k_o \qquad\qquad k_c \swarrow$$

Degradation products

where k_o is the observed photo degradation rate constant of free A/S, k_c is the photo degradation rate constant of the complexed A/S-HPCD, and k_s is the apparent stability constant for A/S-HPCD formation.

The k_o, k_c and k_s could be resolved with the Lineweaver-Burk equation from the observed photo degradation rate constants (k_{obs}) obtained at various concentrations of HPCD (Loftsson et al., 1989):

$$\frac{k_o}{k_o - k_{obs}} = \frac{k_o}{k_s(k_o - k_c)[\text{HPCD}]} + \frac{k_o}{(k_o - k_c)}$$

A straight line was observed on the Lineweaver-Burk plot for the A/S photo degradation at pH 9 (Fig. 4). The calculated value of k_o, k_c and k_s were 0.329 h^{-1}, 0.156 h^{-1}, and 15.4 M^{-1}, respectively. The k_s obtained from this stability study is much smaller than that from the phase-solubility study. A/S exists predominantly in its mono-anionic form at pH 9 (Chen et al., 1996). The smaller k_s value is mainly due to the ionized form of A/S that does not form a stable inclusion complex with HPCD as in the cases of famotidine and NSAIDs (Islam and Narurkar, 1991; Orienti et al., 1991). The photo degradation rate of A/S-HPCD complex was approximately half the rate of the free A/S. We have previously demonstrated that the photochemical decomposition of A/S occurred mainly at the side chain, especially at C13 position (Cheng et al., 1995). If we assumed that no photo degradation occurred once the side chain of A/S was within the HPCD cavity, then this reduced k_c value is a strong indication that the side chain and the basic skeleton of A/S were incorporated into the cavity of HPCD in an approximate 1:1 ratio. This result is in agreement with our solubility data.

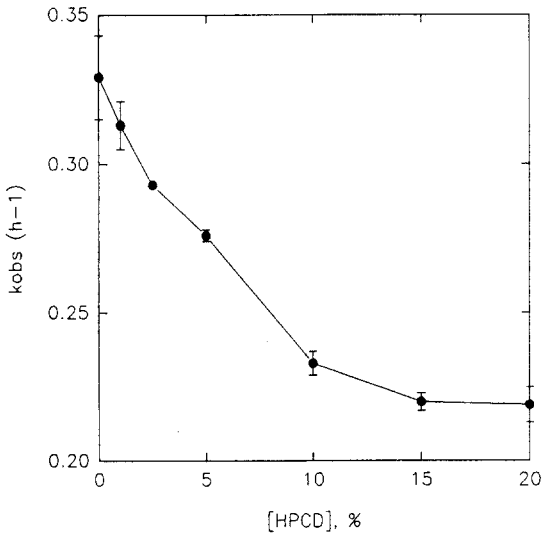


Fig. 3. The photo degradation profile of A/S in aqueous-methanol HPCD solution at pH 9. All data points are the mean values from three different observations. The error bar represents the standard deviation.

3.3. In-vitro permeability studies

The in-vitro permeability study of A/S was performed to evaluate the effect of HPCD on the diffusibility of A/S in different preparations with various degrees of hydrophilicity (Table 1). The flux of released A/S through a synthetic semi-permeable membrane from these test formulations with or without HPCD is presented in Table 2. The release rate of A/S is in the order of A/S-HPCD gel (0.01%) > A/S-HPCD cream (0.01%) > A/S cream (0.1%) > Shiunko ointment (0.1%). The results demonstrated that changing the formulation base to cream form had limited effect on the A/S permeability. The presence of HPCD in either cream or gel, however, had enhanced the permeability of A/S by 34 and 54 fold, respectively, at 1/10 concentration of the authentic Shiunko ointment ($p < 0.01$). The results indicated that increasing the formulation hydrophilicity had greatly enhanced the release rate of A/S with shorter lag time. A linear relationship was observed ($r > 0.981$) when the flux of A/S released was plotted against the square root of time (Fig. 5). The results implied that the release of A/S from these four bases followed the Higuchi release model as described in the following equation (Higuchi, 1967; Takamoto et al., 1990; Rahman et al., 1990):

$$M = 2C_0\sqrt{\frac{Dt}{\pi}}$$

Where M = amount of drug released per unit area of application (mg/cm^2), D = diffusion coefficient (cm^2/s), t = time in seconds, C_0 = initial concentration of the drug in the base (mg/ml) and π = a constant.

The diffusion coefficients were calculated to be 385 000, 196 000, 78.5 and 7.1×10^{-12} cm^2/s for A/S-HPCD gel, A/S-HPCD cream, A/S cream and Shiunko ointment, respectively. The presence of HPCD changed the A/S diffusibility by hundreds of thousand fold while simple dosage form change this merely by ten times. While the ten times increment of A/S diffusibility is due to the faster movement of A/S in the cream formulation, the hundreds of thousand fold of increment of A/S diffusibility in the presence of HPCD could only be explained with the higher hydrophilicity associated with the A/S-HPCD complex.

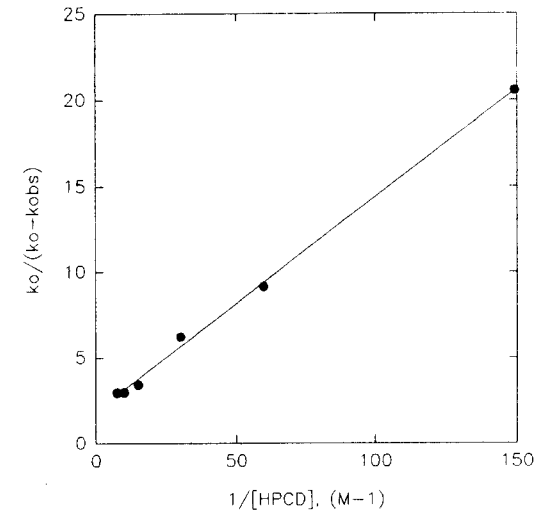


Fig. 4. The Lineweaver-Burk plot of the photo degradation of A/S in aqueous-methanol HPCD solution at pH 9. All data points are the mean values from three different observations.

Table 2
The flux of A/S released from four formulations at various times. The flux from each A/S preparation is expressed as $mg/cm^2 \times 10^3$. Data represent mean ± standard deviation ($n \geq 3$)

Time (h)	Shiunko ointment (0.1%)	A/S cream (0.1%)	A/S-HPCD cream (0.01%)	A/S-HPCD gel (0.01%)
0.5	n.d.	n.d.	n.d.	1.820 ± 0.177
1.0	n.d.	n.d.	0.151 ± 0.028	2.666 ± 0.131
2.0	n.d.	n.d.	1.091 ± 0.047	4.652 ± 0.205
4.0	n.d.	n.d.	1.796 ± 0.210	6.491 ± 0.363
8.0	n.d.	0.045 ± 0.082	3.457 ± 0.224 #	9.513 ± 0.554 #
12.0	0.018 ± 0.171	0.095 ± 0.216	5.282 ± 0.334* #	12.570 ± 1.031* #
16.0	0.058 ± 0.226	0.324 ± 0.501	7.559 ± 0.471* #	15.174 ± 0.697* #
24.0	0.352 ± 0.227	0.887 ± 0.657	12.002 ± 0.973* #	19.028 ± 1.275* #

n.d., not detectable.
[a] There is a significant difference between the flux of Shiunko ointment and that of A/S-HPCD cream or A/S-HPCD gel by Student's t-test ($p < 0.01$).
[b] There is a significant difference between the flux of A/S cream and that of A/S-HPCD cream or A/S-HPCD gel by Student's t-test ($p < 0.01$).

In conclusion, we have demonstrated that HPCD could be used successfully to improve the aqueous solubility, photostability, and in-vitro permeability of A/S. This has not only been the first application of CDs in the pharmacognosy preparations but also has provided a practical means to formulate a more hydrophilic, more stable and better permeable A/S preparation using a smaller amount of A/S without the incorporation of irritating organic co-solvents.

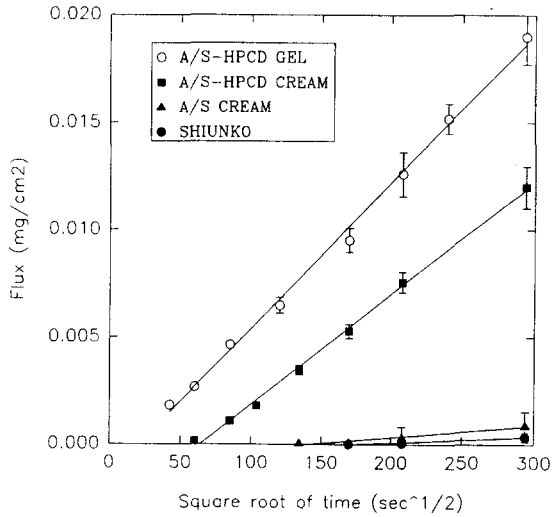


Fig. 5. The in-vitro release profile of A/S from four different formulations. All data points are the mean values from three different observations. The error bar represents the standard deviation.

Acknowledgements

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