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RESEARCH PAPER

Interaction of Antimalarial Agent Artemisinin with Cyclodextrins

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ABSTRACT

To obtain an effective solution of the poorly water soluble antimalarial agent artemisinin, the use of several kinds of cyclodextrins (CDs) as solubilizers was examined. The following CDs were used in this study: α-CD, β-CD, γ-CD as parent CDs, 2-hydroxypropyl-β-CD (HP-β-CD), sulfobutyl ether β-CD (SBE7-β-CD), heptakis (2,6-di-O-methyl)-β-CD (DM-β-CD), 2,3,6-partially methylated-β-CD (PM-β-CD) as modified CDs, and glucosyl-β-CD (G1-β-CD), and maltosyl-β-CD (G2-β-CD) as branched CDs. The solubility curves of artemisinin with CDs can all be classified as type A_L. The apparent stability constants for artemisinin–parent CD complexes increased in the order of α- < γ- ≤ β-CD. The constants for artemisinin-β-CD derivative (and β-CD) complexes increased in the order of G2-β-CD ≅ G1-β-CD ≅ PM-β-CD ≅ β-CD < HP-β-CD < SBE7-β-CD < DM-β-CD. These results suggest that the addition of CDs enables the solubilization of artemisinin.
Key Words. *Artemisinin; Cyclodextrins; Solubilization ability.*

INTRODUCTION

Artemisinin is an antimalarial agent that has been in use in China and Southeast Asia. Artemisinin is an extraction product from the herb *Artemisia annua* L. Artemisinin was isolated, and its structure was resolved by Chinese researchers in the early 1970s (1,2). Artemisinin is a so-called sesquiterpene with a trioxane ring and a lactone ring, and the trioxane ring contains a peroxide bridge, the active moiety of the molecule (Fig. 1) (3). The parasite and fever clearance rates with artemisinin are higher than with any other antimalarial drug. Excel-

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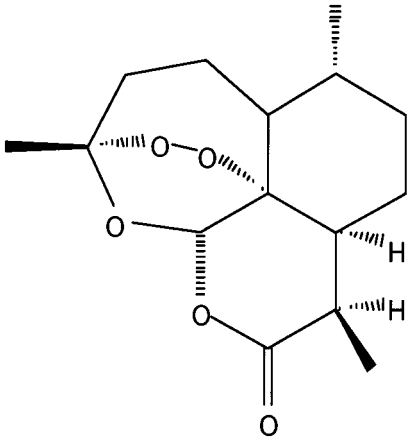

Figure 1. Chemical structure of artemisinin.

lent results have been reported in the treatment of *Plasmodium vivax* and *P. falciparum* infections in patients, including the cerebral form, with both chloroquine-sensitive and chloroquine-resistant strains (4). As dosage forms of artemisinin, four different formulations are used: tablets for oral administration, injectable formulations of oil solutions for intramuscular administration, injectable formulations of oil or water suspensions for intramuscular administration, and suppositories for rectal administration (3,4). However, intravenous injection is not available because of the low solubility in water (0.084 mg/ml) and lack of a suitable solvent for intravenous use (4).

Cyclodextrins (CDs) are cyclomalto-oligosaccharides obtained by the enzymatic conversion of starch. Parent or natural CDs contain 6, 7, or 8 glucopyranose units and are referred to as α-, β-, and γ-CD, respectively. CDs complex with hydrophobic drugs, increasing their aqueous solubility and stability. This property enables the production of formulations for water-insoluble drugs that are typically difficult to formulate and deliver with more traditional additives (5–7). In this study, we investigated the interaction of artemisinin with CDs and assessed their effects on the aqueous solubility of artemisinin. Furthermore, the possibility of new parenteral dosage forms containing artemisinin is discussed.

MATERIALS AND METHODS

Materials

Artemisinin was purchased from Sigma Chemical Company (St. Louis, MO). α-CD, β-CD, and 2-hydroxypropyl-β-CD (HP-β-CD; degree of substitution [DS] value = 4.3) were gifts from Nihon Shokuhin Kako Company, Limited (Tokyo, Japan). γ-CD was purchased from Wacker-Chemie GmbH (Munich, Germany). β-Cyclodextrin sulfobutyl ether, 7 sodium salt (SBE7-β-CD) was donated by CyDex, Incorporated (Overland Park, KS). Heptakis (2,6-di-*O*-methyl)-β-CD (DM-β-CD) was donated by Nihon Shokuhin Kako Company, Limited. 2,3,6-Partially methylated-β-CD (PM-β-CD) was donated by Mercian Corporation (Tokyo, Japan). Glucosyl-β-CD (G1-β-CD) and maltosyl-β-CD (G2-β-CD) were purchased from Bio Research Corporation of Yokohama (BICO, Yokohama, Japan). Polyvinylpyrrolidone (PVP; MW 40,000) and hydroxypropylmethylcellulose (HPMC; 3500–5600 cP) were purchased from Sigma Chemical Company. Sodium carboxymethylcellulose (CMC) was purchased from Maruishi Pharmaceutical Company, Limited (Osaka, Japan). CMC was JP 12 grade. Pullulan was purchased from Wako Pure Chemical Industries, Limited (Osaka, Japan). All other chemicals and solvents were from commercial sources and were used without further purification. Milli-Q water (Millipore Co., Milford, MA) was used in all of the experiments.

Quantitative Determination of Artemisinin

Concentrations of artemisinin were measured according to a slight modification of the method of Zhao and Zeng (8). Samples of 5 ml were alkalinized with 2 ml of 0.56% NaOH. After heating in a water bath at 45°C for 30 min, samples were acidified with 2 ml of 2 mol/L HCl. Samples were then quickly chilled in water and diluted to 10 ml with water. The resulting degradation product had an ultraviolet (UV) maximum at 257 nm and could be analyzed using a Ubest-30 Double Beam spectrophotometer (JASCO Co., Ltd., Tokyo, Japan).

Solubility Studies

Phase solubility diagrams were determined in aqueous cyclodextrin solutions at 25°C. An excess amount of artemisinin was added to aqueous solutions containing CDs. The suspension formed was sonicated for 1 hr and then shaken at 25°C. After equilibration for at least 5 days, the suspension was filtered through a membrane filter (0.45 μm, Kurabo Industries Ltd., Osaka, Japan). The concentration of the filtrates was analyzed as described above. The stability constants K' of the artemisinin-CD complexes were calculated from the slope and intercept of the phase solubility diagrams:

$$K' = \frac{\text{Slope}}{\text{Intercept } (1 - \text{Slope})}$$

according to the method of Higuchi and Connors (9).

Effects of Cyclodextrin on the Alkaline Hydrolysis Rate of Artemisinin

The alkaline hydrolysis rate of artemisinin in the absence and presence of CD was spectrophotometrically monitored by measuring the increase in the absorbance of the degradation product of artemisinin. The reaction was initiated by the addition of 2 ml of 0.0056% (4×10^{-4} M) NaOH into 5 ml of stock solution of artemisinin (2×10^{-4} M) containing β-CD (3×10^{-3} M) at a constant temperature (45°C). The absorbance of the degradation product of artemisinin was measured in the same manner as described above. The residual ratio of artemisinin was calculated according to the following equation:

$$A(t) = \frac{\text{abs.}(\infty) - \text{abs.}(t)}{\text{abs.}(\infty)}$$

where t is the reaction time (hr), $A(t)$ is the residual ratio of artemisinin at t, abs.(t) is the absorbance of degradation product of artemisinin at t, abs.(∞) is the absorbance of a complete degradation product of artemisinin.

Effects of Water-Soluble Polymers on Artemisinin-Cyclodextrin Complexation

Solubility of artemisinin was measured according to the method of Loftsson et al. (10). An excess amount of artemisinin was added to water, aqueous polymer solution, aqueous CD solution, or aqueous solution containing both polymer and CD. The suspension formed was sonicated for 1 hr and then heated in a sealed container to 120°C for 20 min. After equilibration at 25°C for at least 3 days, the suspension was filtered and analyzed in the same manner as described above.

RESULTS AND DISCUSSION

Solubility Studies

The increases in artemisinin solubility after the addition of CDs were examined. Table 1 shows the effects of conventional α-, β-, and γ-CD on the solubility of artemisinin in water at 25°C. The solubilizing effect of β-CD was higher than those of α-CD and γ-CD. However, the solubility of β-CD (1.85 g/100 ml) in water is lower than those of α-CD or γ-CD (14.5, 23.2 g/100 ml, respectively), so the following β-CD derivatives that have higher solubility than β-CD were used for further experiments: HP-β-CD, SBE7-β-CD, DM-β-CD, PM-β-CD as modified CDs, and G1-β-CD and G2-β-CD as branched CDs.

Table 1

Effects of α-, β-, and γ-Cyclodextrins on Solubility of Artemisinin in Water at 25°C

	Solubility of Artemisinin (mg/ml)	Ratio (CD Solution/Water)
Without CD	0.084	—
α-CD	0.142	1.7
β-CD	0.517	6.2
γ-CD	0.427	5.1

CD concentration was 10 mg/ml.

Table 2 shows the effects of β-CD derivatives on the solubility of artemisinin in water at 25°C. With the exception of DM-β-CD, β-CD derivatives were as effective as β-CD, while the solubilizing effect of DM-β-CD was much higher than that of β-CD derivatives or β-CD. This may be because DM-β-CD has high surface activity (6) or increases the hydrophobicity of the CD cavity, possibly by providing an ''extension'' of the cavity by introducing nonpolar methyl groups at the 2- and 6- positions of the glucopyranose units (7).

The interaction of artemisinin with CDs (conventional α-, β-, γ-CD, and β-CD derivatives) in aqueous solution was examined using a solubility method (9). Figure 2 shows the phase solubility diagrams of artemisinin with conventional α-, β-, γ-CD, and pullulan. Except for that with pullulan, all were classified as type A_L. Table 3

Table 2

Effects of Cyclodextrin Derivatives on Solubility of Artemisinin in Water at 25°C

	Solubility of Artemisinin (mg/ml)	Ratio (CD Solution/Water)
Without CD	0.084	—
HP-β-CD	0.457	5.4
SBE7-β-CD	0.506	6.0
G1-β-CD	0.540	6.4
G2-β-CD	0.491	5.9
DM-β-CD	1.086	12.9
PM-β-CD	0.399	4.7

CD concentration was 10 mg/ml.

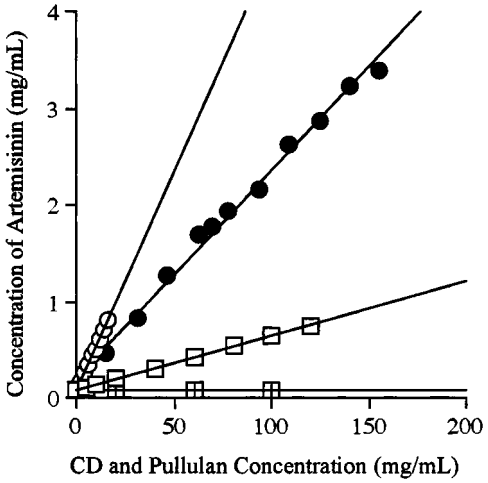

Figure 2. Phase solubility diagrams of artemisinin–conventional CD and pullulan system in water at 25°C: □, α-CD; ○, β-CD; ●, γ-CD; ⊞, pullulan.

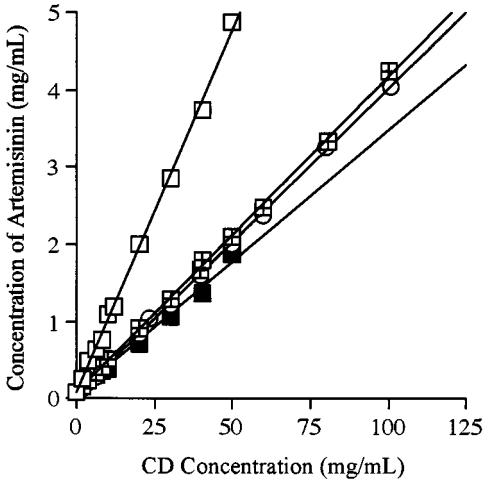

Figure 3. Phase solubility diagrams of artemisinin–modified CD system in water at 25°C: □, DM-β-CD; ⊞, SBE7-β-CD; ○, HP-β-CD; ■, PM-β-CD.

shows the apparent stability constants K' of the artemisinin–conventional CD complexes. The value of K' increased in the order of α-CD < γ-CD < β-CD. This result was consistent with the increasing water solubility shown in Table 1.

Figures 3 and 4 show the phase solubility diagrams of artemisinin with β-CD derivatives. These were all classified as type A_L, and it was suggested that a water-soluble artemisinin-CD complex was formed as well as β-CD. Table 4 shows K' of the artemisinin-β-CD derivative complexes. The value of K' increased in the order G2-β-CD ≅ G1-β-CD ≅ PM-β-CD ≅ β-CD < HP-β-CD < SBE7-β-CD < DM-β-CD.

Effects of Cyclodextrin on the Alkaline Hydrolysis Rate of Artemisinin

Cyclodextrins are known to act as stimulators or inhibitors of various chemical reactions such as hydrolysis, photolysis, and oxidation (6). The suppressive effect of complexation leads to stabilization of pharmaceuticals. Artemisinin is hydrolyzed by alkali because it contains lactone. Accordingly, formation of an artemisinin-CD complex probably has an effect on hydrolysis of artemisinin. Figure 5 shows the time course of the residual ratio of artemisinin on alkaline hydrolysis in the presence or absence of β-CD. The presence of β-CD resulted in the depression of hydrolysis of artemisinin. The first-order rate constant was then calculated from the slope of the line shown in Fig. 6. The rate constant was 0.182 ± 0.0283 and 0.414 ± 0.0616 (hr^{-1}) in the presence and

Table 3

Apparent Stability Constants K′ and Types of Solubility Curves of Artemisinin–Conventional Cyclodextrin Complexes Determined by Solubility Method in Water at 25°C

CDs	K' (M^{-1})	Type
α-CD	63	A_L
β-CD	778	A_L
γ-CD	149	A_L

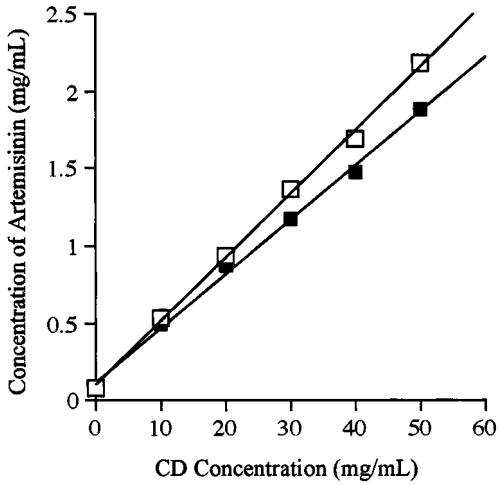

Figure 4. Phase solubility diagrams of artemisinin-branched CD system in water at 25°C: □, G1-β-CD; ■, G2-β-CD.

Table 4

Apparent Stability Constants K′ and Types of Solubility Curves of Artemisinin–Cyclodextrin Derivative Complexes Determined by Solubility Method in Water at 25°C

CDs	K' (M^{-1})	Type
DM-β-CD	2752	A_L
SBE7-β-CD	1536	A_L
HP-β-CD	1218	A_L
PM-β-CD	685	A_L
G1-β-CD	628	A_L
G2-β-CD	514	A_L

absence of β-CD, respectively. These different values also indicated the suppression of hydrolysis of artemisinin. The effects of CDs on organic chemical reactions such as alkaline hydrolysis of esters were examined previously in detail (11–13); in general, the reactions are suppressed strongly when the whole molecule or the active site of the drug is included in the CD cavity (14). In this study, the hydrolysis rate constant in the presence of β-CD was about half that in the absence of β-CD. Accordingly, it was suggested that alkaline attack of the lactone site of artemisinin is suppressed by CD. However, CD may not interact strongly with the lactone site of artemisinin. Artemisinin has hydrophilic areas such as the lactone site and the peroxide site and hydrophobic areas

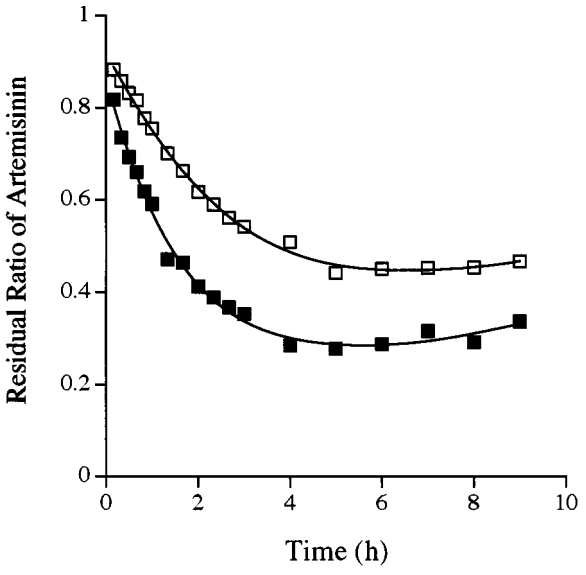

Figure 5. Effects of CD on hydrolysis of artemisinin: □, with β-CD; ■, artemisinin only.

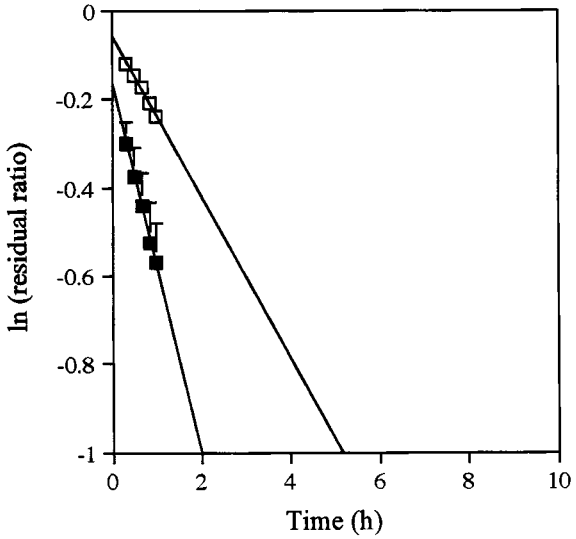

Figure 6. Degradation of artemisinin at 25°C in 4×10^{-4} M NaOH ($N = 3$): □, with β-CD; ■, artemisinin only.

such as the methyl group and the methylene group (Fig. 1). CDs generally include the hydrophobic area of the molecule. Therefore, it is reasonable to assume that CDs interact with the hydrophobic area of artemisinin, not the hydrophilic area containing the lactone site.

Effects of Water-Soluble Polymers on Artemisinin-Cyclodextrin Complexation

Polymers are known to interact with CDs, although the precise nature of polymer-CD interactions is still not known. Some investigators have shown that β-CD interacts with a number of water-soluble cellulose derivatives (15), and that at low concentrations, polymers increase the complexation abilities of CDs (10,16), and that the polymers enhance the availability of drugs in aqueous CD solutions (17). There are various reasons why increased efficiency of CD complexation of drugs is desirable. For example, only a limited amount of CD can be used in many drug formulations, such as in aqueous isotonic solutions and tablets, and increased efficiency will mean that less CD can be used to achieve the same or even greater solubilizing effect. Also, because CDs are still relatively expensive, reduction of the amount of CD in drug formulations will result in lower production costs. Therefore, based on the report that the solubilizing effect of CDs was improved by addition of water-soluble polymers, the effect of water-soluble polymers on the artemisinin-cyclodextrin complex was examined according to the method of Loftsson et al. (10).

Solubility of artemisinin was measured in water and aqueous polymer solution to ascertain the solubilization effect of water-soluble polymer itself on artemisinin. CMC, HPMC, and PVP were used as water-soluble polymers. Figure 7 shows the solubility of artemisinin in the presence or absence of these polymers. The polymers themselves had no solubilization effect on artemisinin because the solubility of artemisinin did not significantly increase in the presence of these polymers. Figure 7 also shows the solubility of artemisinin in CD solution or aqueous solution containing both a polymer and CD. When CMC and PVP were used as polymers, the solubility of artemisinin did not significantly increase. Accordingly, these polymers did not appear to affect the solubilization ability of β-CD and SBE7-β-CD. On the other hand, when HPMC was used, the solubility of artemisinin was decreased, although this effect was not significant. This was probably because the complex is formed between CD and HPMC, resulting in suppression of artemisinin-CD complexation. By these observations, it was suggested that the solubilization ability of CD to artemisinin was not improved by addition of water-soluble polymers under the experimental conditions used in this study.

The Possibility of New Artemisinin Formulation Utilizing CDs

The fundamental problems in the development of new pharmaceuticals are efficacy and safety. Therefore, safety of CD itself must be considered when selecting an optimal CD from among several candidates. As described previously, DM-β-CD has the best solubilizing ability for artemisinin among the CDs used in this study. However, DM-β-CD shows some toxic effects, such as hepatopathy (18), nephropathy (19), and hemolysis (20,21). Thus, DM-β-CD is not suitable as a solubilizing agent for clinical use. On the other hand, SBE7-β-CD and HP-β-CD have better solubilizing ability for artemisinin than other CDs except DM-β-CD, and the safety of these CDs has been confirmed (22–25). Thus, these CDs may be clinically useful.

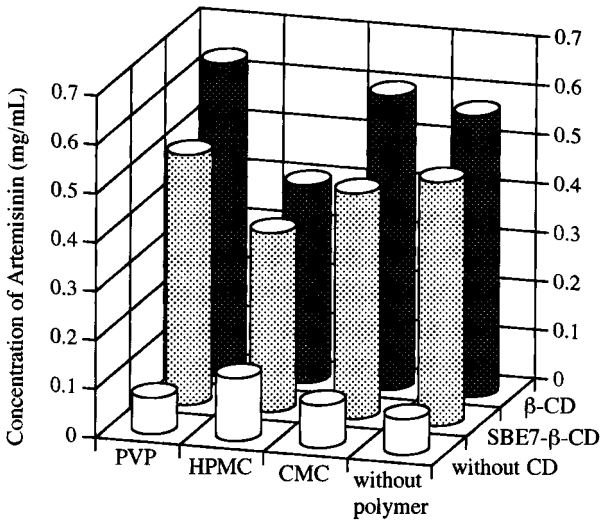

Figure 7. Effects of water-soluble polymers on solubility of artemisinin in water at 25°C ($N = 3$): □, without CD; ▨ with SBE7-β-CD; ■ with β-CD.

Next, the benefits of new artemisinin formulations utilizing CDs were examined. Dissolution is the rate-limiting step in the intestinal absorption of water-insoluble drugs such as artemisinin. Titulaer et al. reported that the bioavailability of oral administration relative to the intramuscular injection of a suspension in oil is only 32% (26). One of the factors responsible for this low bioavailability may be the low solubility in water. Therefore, the efficacy of oral administration is expected to be improved further if artemisinin-CD formulation for oral dosage form can be developed.

Treatment of malaria by intravenous administration of artemisinin is not currently practiced because aqueous solutions for injection cannot be adjusted to practical concentrations due to the low solubility in water. The characteristics of intravenous administration are as follows: Pharmacological effects appear rapidly and are strong because there is no absorption process; bioavailability is approximately 100%; the injection rate can be controlled by intravenous drip infusion. Therefore, if intravenous administration of artemisinin can be achieved, the usefulness of artemisinin will be further enhanced. A primitive prototype of artemisinin solution for injection using CDs is discussed below. SBE7-β-CD was selected due to its low hemolytic activity (25). The necessary dose of artemisinin in intravenous administration was estimated on the basis of pharmacokinetics parameters in oral administration. According to the previously reported blood concentration, distribution volume, and body weight of 260 μg/L, 4.1 L/kg, and 60 kg, respectively, the necessary dose was estimated as 64 mg (26,27). The necessary fluid volume to dissolve 64 mg of artemisinin was about 760 ml in the absence of CD, and this volume is too large for injection. On the other hand, the fluid volume required to dissolve 64 mg of artemisinin was estimated from the phase solubility diagram to be about 10 ml in the presence of 154 mg/ml SBE7-β-CD. This volume is small enough for injection. Thus, SBE7-β-CD enabled treatment for malaria by intravenous administration of artemisinin.

CONCLUSION

In conclusion, the addition of CDs increased the solubilization of artemisinin. The most effective CD for the solubilization of artemisinin was DM-β-CD, but this agent is not suitable for injection because of its high hemolytic activity. Considering the toxicity to humans, SBE7-β-CD or HP-β-CD might be useful for the development of the new parenteral dosage forms containing artemisinin.

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REFERENCES

1. D. L. Klayman, Science, 228, 1049–1055 (1985).
2. X. D. Luo and C. C. Shen, Med. Res. Rev., 7(1), 29–52 (1987).
3. P. J. Devries and T. K. Dien, Drugs, 52(6), 818–836 (1996).
4. H. A. C. Titulaer, J. Zuidema, and C. B. Lugt, Int. J. Pharm., 69, 83–92 (1991).
5. J. Szejtli, *Cyclodextrin Technology*, Kluwer Academic, Dordrecht, 1988.
6. K.-H. Frömming and J. Szejtli, *Cyclodextrins in Pharmacy*, Kluwer Academic, Dordrecht, 1994.
7. D. O. Thompson, Crit. Rev. Ther. Drug Carrier Sys., 14(1), 1–104 (1997).
8. S. Zhao and M. Y. Zeng, Anal. Chem., 58, 289–292 (1986).
9. T. Higuchi and K. A. Connors, Adv. Anal. Chem. Instr., 4, 117–212 (1965).
10. T. Loftsson, H. Frioriksdóttir, A. M. Sigurðardóttir, and H. Ueda, Int. J. Pharm., 110, 169–177 (1994).
11. J. L. Lack and T. F. Chin, J. Pharm. Sci., 53, 924–927 (1964).
12. K. Koizumi, J. Tatsumi, M. Ohae, H. Kumagai, and T. Hayata, Yakugaku Zassi, 89(11), 1594–1600 (1969).
13. R. L. VanEtten, J. F. Sebastian, G. A. Clowes, and M. L. Bender, J. Am. Chem. Soc., 89, 3242–3253 (1967).
14. T. F. Chin, P. H. Chung, and J. L. Lack, J. Pharm. Sci., 57, 44–48 (1968).
15. T. Hladon and B. Cwiertinia, Pharmazie, 49, 497–500 (1994).
16. G. Ganzerli, L. Van Santvliet, E. Verschuren, and A. Ludwig, Pharmazie, 51, 357–362 (1996).
17. A. M. Sigurðardóttir and T. Loftsson, Int. J. Pharm., 126, 73–78 (1995).
18. M. Yamamoto, H. Aritomi, T. Irie, F. Hirayama, and K. Uekama, in *Mins. 5th International Symposium on Cyclodextrins* (D. Duchêne, Ed.), Editions de Santé, Paris, 1990, pp. 541–544.
19. J. Serfõzõ, P. Szabo, T. Ferenczy, and A. Tôth-Jakab, In *Proceedings of the 1st International Symposium on Cyclodextrins* (J. Szejtli, Ed.), Kluwer Academic, Dordrecht, 1982, pp. 123–132.
20. I. Jodál, P. Nánási, and J. Szejtli, in *Proceedings of the 4th International Symposium on Cyclodextrins* (O. Huber and J. Szejtli, Eds.), Kluwer Academic, Dordrecht, 1988, pp. 421–425.
21. A. Yoshida, H. Arima, K. Uekama, and J. Pitha, Int. J. Pharm., 46, 217–222 (1988).
22. W. R. Anderson, M. Calderwood-Mays, M. E. Brewster, and N. Bodor, in *Mins. 6th International Symposium on Cyclodextrins* (A. R. Hedges, Ed.), Editions de Santé, Paris, 1992, pp. 288–291.
23. W. Coussement, H. Van Cauteren, J. Vandenberghe, P. Vanparys, G. Teuns, A. Lampo, and R. Marsboom, in *Mins. 5th International Symposium on Cyclodextrins* (D. Duchêne, Ed.), Editions de Santé, Paris, 1990, pp. 522–527.
24. R. A. Rajewski, G. Traiger, J. Bresnahan, P. Jaberaboansari, V. J. Stella, and D. O. Thompson, J. Pharm. Sci., 84, 927–932 (1995).
25. CyDex, Incorporated, *Captisol™*, Sulfobutylether β-*Cyclodextrin*, product brochure, Author, Overland Park, KS.
26. H. A. C. Titulaer, J. Zuidema, P. A. Kager, J. C. F. M. Wetsteyn, Ch. B. Lugt, and F. W. H. M. Merkus, J. Pharm. Pharmacol., 42, 810–813 (1990).
27. X. Niu, L. Ho, Z. Ren, and Z. Song, Eur. J. Drug Metabol. Pharmacokinet., 10, 55–59 (1985).

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