AAPS PharmSciTech

AAPS PharmSciTech 2001; 2 (1) article 2
(http://www.pharmscitech.com)

Solubilization of Cyclosporin A

Submitted: November 20, 2000; Accepted: January 15, 2001

Yingqing Ran,[1] Luwei Zhao,[2] Qing Xu,[3] Samuel H. Yalkowsky[1]

[1]Department of Pharmaceutical Sciences, College of Pharmacy, The University of Arizona, Tucson, AZ 85721

[2]Aventis Pharmaceuticals Company, Bridgewater, NJ 08807

[3]Department of Chemistry, The University of Arizona, Tucson, AZ 85721

ABSTRACT This study investigated the solubilization of cyclosporin A (CsA), a neutral undecapeptide, by cosolvency, micellization, and complexation. Cosolvents (ethanol, propylene glycol, polyethylene glycol, tetrahydrofurfuryl alcohol polyethyleneglycol ether, and glycerin), surfactants (polyoxyethylene sorbitan monooleate [(Tween 80)], polyoxyethylene sorbitan monolaurate [(Tween 20)], and Cremophor EL), and cyclodextrins (α-cyclodextrin [(αCD)] and hydroxypropyl-β-cyclodextrin[(HPβCD)] were used as solubilizing agents in this study.

Surfactants had a noticeable effect in increasing CsA solubility. Twenty percent solutions of Tween 20, Tween 80, and Cremophor EL increased the solubility by 60 to 160 fold. Cyclodextrins can increase the CsA solubility, but αCD was more effective than HPβCD. Cosolvents on the other hand did not increase the solubility of CsA as much as expected from the LOGP (logrithm of water-octanol partition coefficent) value of CsA.

KeyWords: Cyclosporin A, Conformation, Solubility, Solubilzation

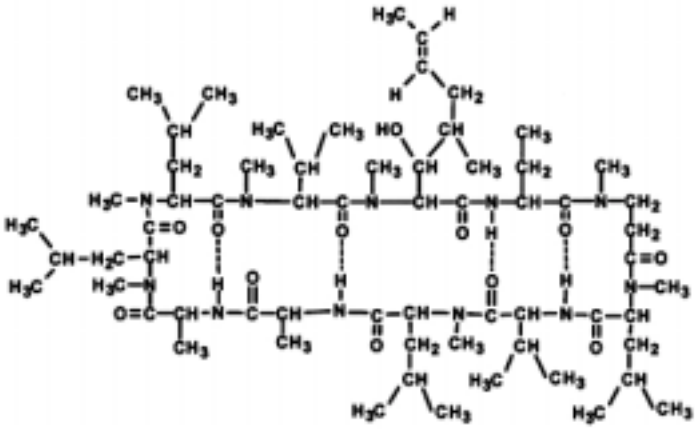

Figure 1. Structure of cyclosporin A as established by Rügger and Petcher [1].

***Corresponding Author:** Yingqing Ran, PhD; Department of Pharmaceutical Sciences; College of Pharmacy, The University of Arizona; 1703 Mabel Street; Tucson, AZ 85721; Telephone: 520-626-3847; Fascimile: 520-626-4063; E-mail: ran@pharmacy.arizona.edu

INTRODUCTION

Cyclosporin A (CsA; see **Figure 1**) [1] is an effective immunosupressive agent used in transplantation to prevent organ rejection. The aqueous solubility of CsA is 27.67 μg/mL at 25°C [2, 3]. This study investigated the use of the traditional solubilization approaches to increase the solubility of CsA.

The solubility of the drug is determined by the interaction of solute with solvents and the crystallinity of the solute. Solvent alteration is the most effective means to produce a thermodynamically stable increase in solubility [2]. As discussed by Yalkowsky [4], the 3 most commonly used approaches for solubilizing nonionizable drugs such as CsA are cosolvency, micellization, and complexation.

Cosolvency

Cosolvents are organic compounds that are substantially miscible with water. Cosolvents have small hydrocarbon regions. Since these regions are nonpolar and they do not interact strongly with water, they can reduce the ability of the aqueous system to squeeze out nonpolar solutes. The logarithmic relationship between total drug solubility (S_{mix}) in a mixed solvent and cosolvent concentration (C_{cosol}) can be described by equation 1 [4-6].

$$\log S_{tot}^{mix} = \log S_w + \sigma C_{cosol} \qquad (1)$$

where Sw is drug solubility in water and σ is cosolvent solubilization power. The σ value depends on the polarity of both the solvent and the solute. The more nopolar the solvent and the solute, the larger the σ value.

Micellization

Organic solutes can be solubilized by incorporation into the micelles formed by surfactants. The more nonpolar the solute, the more likely it is to be incorparated near the core or center of the micelle. The

relationship between the drug solubility in a micellar solution and surfactant concentration is described by equation 2a [4, 7, 8].

$$S_{tot}^{mic} = S_w + \kappa(C_{surf} - CMC) \quad (2a)$$

where C_{surf} is the concentration of micellar surfactant (ie, the total surfactant concentration minus the critical micellar concentration) and κ is the molar solubilization capacity, the number of moles of solute that can be solubilized by 1 mole of micellar surfactant. If the critical micellar concentration (CMC) is much lower than C_{surf}, equation 2a can be approximated by

$$S_{tot}^{mic} \approx S_w + \kappa C_{surf} \quad (2b)$$

Complexation

Cyclodextrins are cylic oligosaccharides that have been recently used as complexation ligands. Cyclodextrins can interact with appropriately sized molecules to result in the formation of inclusion complexes. The effects of various cyclodextrins can be approximately described by equations 3 and 4 [4, 7, 9, 10].

$$S_{tot} \approx S_w + K_{1:1} S_w L$$
$$S_{tot} \approx S_w + K_{1:1} S_w L + K_{1:1} K_{1:2} S_w L^2 \quad (3)$$

where L is the total ligand concentration. Equation 3 is applicable to 1:1 (drug:ligand) complexation, and equation 4 is applicable to 1:1 and 1:2 (drug:ligand) complexation. K1:1 is the complexation constant for 1:1 drug-ligand complex; K1:2 is the complexation constant for 1:2 drug-ligand complex. Higher-order complexes are also possible.

Since cyclosporin A does not have ionizable groups, we did not consider pH control to increase its solubility. But the other 3 approaches have the potential to be used to solubilize CsA.

MATERIALS AND METHODS

Materials

Cyclosporin A was a gift from the Institute of Microbiology, Fujian, China. α-Cyclodextrin (αCD) and hydroxypropyl-β-cyclodextrin (HPβCD) were obtained from Cyclodextrin Technologies Development Inc (Gainesville, FL). All other chemicals were of analytical or high-performance liquid chromatography (HPLC) grade, purchased from Sigma and Aldrich (St. Louis, MO).

Solubility Measurement

The CsA powder was added to vials containing various percentages of cosolvents, surfactants, or complexation agents. Duplicate sample vials were prepared for each solubilizing agent at its particular concentration and were placed on an end-over-end mechanical rotator at 25 rpm at room temperature for 7 days. Samples with drug crystals present were considered to have reached the equilibrium and were removed from the rotator. The samples were then centrifuged on the Micro16 centrifuge (Fisher Scientific, Pittsburgh, PA) at 12 000 rpm for 30 minutes. Supernatant was diluted before injection into the HPLC system.

The cosolvents used were ethanol (EtOH), propylene glycol (PG) and polyethylene glycol (PEG400), tetrahydrofurfuryl alcohol polyethyleneglycol ether (glycofurol), and glycerin. The surfactants were polyoxyethylene sorbitan monooleate (Tween 80), polyoxyethylene sorbitan monolaurate (Tween 20), and Cremophor EL. The complexation ligands were αCD and HPβCD. The concentration ranges are given in **Table 1.**

HPLC Assay

A Beckman Gold (Fullerton, CA) system equipped with a model No. 168 detector at 215 nm was used for HPLC assay in this study. A pinnacle octylamine column 150 cm × 4.6 mm, (Restek, Bellefonte, PA) was used with a mobile phase composed of 70% acetonitrile and 30% water [11]. The flow rate is 1 mL/min. The temperature was elevated to 70°C by immersing the column in a waterbath. The retention time of CsA was 5.6 minutes. The injection volume was 50 μL. The CsA standard curve at concentrations ranging from 5 μg/mL to 100 μg/mL was used to evaluate all of the assays.

RESULTS AND DISCUSSION

The solubility values of CsA as functions of the cosolvent, surfactant, and cyclodextrin concentrations are presented in **Figures 2**, **3**, and **4**, respectively. The solubilization parameters described can be determined by fitting the data in these figures to equations 1 to 4. The resulting parameters are presented in **Table 1**, which gives the range of concentration of the solubilization agents used in this study and the solubilization parameters for CsA.

Cosolvency

Figure 2 shows the exponential increase in CsA solubility with concentration of the cosolvent. The

value of σ here depends only on the polarity of the cosolvents. **Table 1** shows that the less polar the cosolvent, the more effective the increase in the solubility of CsA and the larger value of σ. EtOH has the largest σ value and glycerin has the smallest. Although cosolvents can increase the solubility of CsA, their solubilization power is much lower than expected from the MLOGP value of CsA (MLOGP = 2.92) [12].

Micellization

Figure 3 shows the effect of some surfactants on CsA solubility. Cremophor EL has the largest κ value. Tween 80 and Tween 20 produce similar effects on CsA solubility; if less than 10% is used, the solubility enhancement of CsA is relatively small. The slightly higher κ value of Tween 80 may the result of its longer alkyl chains.

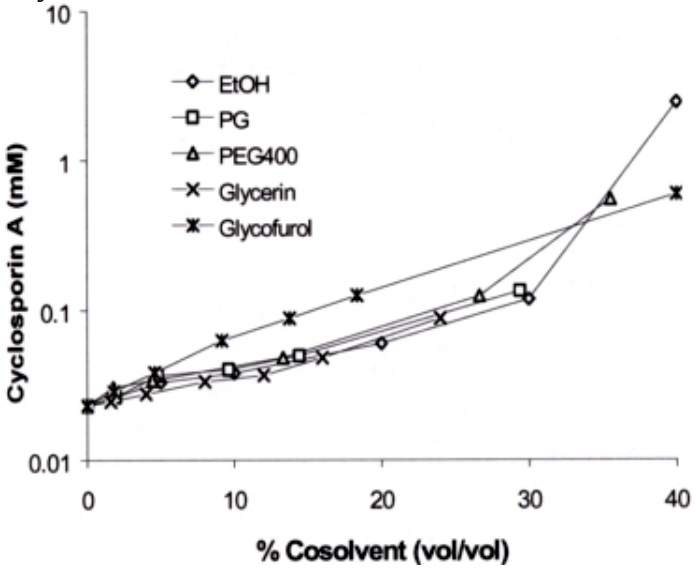

Figure 2. Effects of cosolvents on cyclosporin A solubility.

Table 1. Solubilization Parameters for Cyclosporin A

Solubilization Agents	Concentration Range, %	Parameters
EtOH	0-40 (vol/vol)	σ= 0.044 $\%^{-1}$
PG	0-40 (wt/vol)	σ= 0.025 $\%^{-1}$
PEG400	0-40 (wt/vol)	σ= 0.035 $\%^{-1}$
Glycofurol	0-40 (wt/vol)	σ=0.031 $\%^{-1}$
Glycerin	0-30 (wt/vol)	σ=0.023 $\%^{-1}$
Tween 20	0-20 (wt/vol)	κ=0.0079
Tween 80	0-20 (wt/vol)	κ=0.0119
Cremophor EL	0-20 (wt/vol) 0-20 (wt/vol)	κ=0.0427
αCD	0-20 (wt/vol)	K1:1=47.8 M^{-1}, K1:2=18.22 M^{-1}
HPβCD	0-20 (wt/vol)	K1:1=21.7 M^{-1} , K1:2=6 M^{-1}

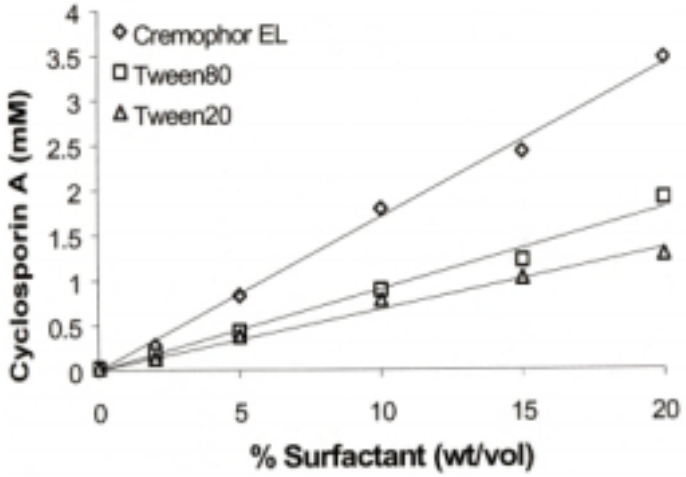

Figure 3. Effects of surfactants on cyclosporin A solubility.

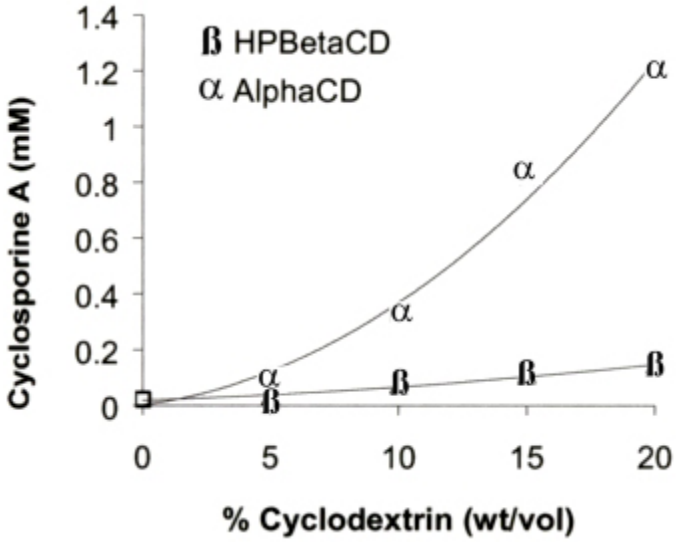

Figure 4. Effects of cyclodextrins on cyclosporin A solubility.

Complexation

Figure 4 shows the effects of αCD and HPβCD on cyclosporine solubility. The CsA solubility does not show a linear rise as the function of the ligand concentration. It did not fit equation 3 but fit equation 4, suggesting that it forms both 1:1 and 1:2 complexes with both of the cyclodextrins. The bigger K value of αCD is possibly a result of the smaller cavity (5Å), which is the more appropriate size for the nonpolar aliphatic parts of CsA to be held.

CONCLUSIONS

This study investigated and compared 3 different approaches to solubilize CsA: cosolvents, surfactants, and cyclodextrins. The effectiveness of these approaches on CsA is not comparable to that of these approaches on most other nonpolar drugs. This may be the result of a conformational change in CsA structures

with solvent polarity (ie, the hydrogen bonds in **Figure 1** would be favored by nonpolar media but they are not likely to exist in strongly hydrogen bonding media such as water). The NMR spectrum of CsA13 in CDCl3 shows 4 doublets from 7 ppm to 8 ppm. This corresponds to the 4 intramolecular H-bonds that stabilize the conformation of CsA molecules in nonpolar media. The solubility of CsA in water is not sufficient to allow the study of the conformation of CsA in water, but the NMR spectrum of CsA in CD3OD does not contain the 4 doublets with high chemical shifts indicating the absence of intramolecular H-bonds of CsA in CD3OD. El Tayar et al [12] provided evidence of a conformational change of CsA in polar and nonpolar media from the structural information derived from both partitioning and simulation studies.

The structure in **Figure 5** schematically illustrates the type of CsA conformation that may exist in water, whereas the structure depicted in **Figure 1** illustrates its conformation in a nonpolar media. In water, the nonpolar parts of CsA are likely to associate with the polar parts pointed to the water molecules. This structure may make CsA behave somewhat like an unimolecular micelle, which has a higher affinity for water than would be expected from its structural composition.

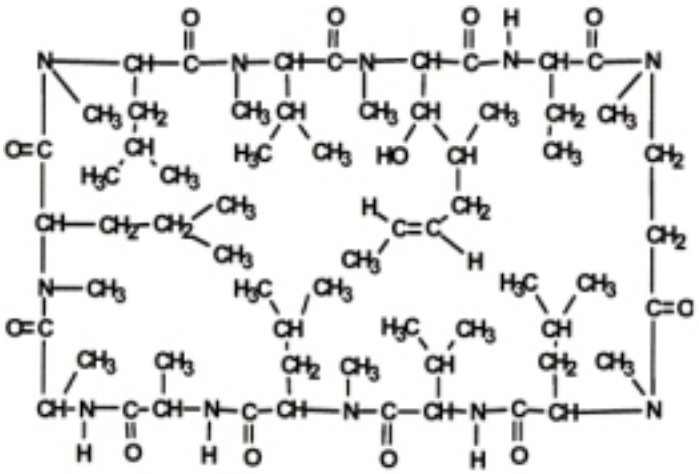

Figure 5. Schematic structure of cyclosporin A in water.

REFERENCES

1. Petcher TJ, Weder HP, Rüegger A. Crystal and molecular structure of an iodo derivative of the cyclic undecapeptide cyclosporin A. *Helvetica Chimica Acta*. 1976;59:1480-1488.

2. Alfred F. Cyclosporin clinical pharmacokinetics. *Drug Dispos.* 1993;24:472-495.

3. Ismailos G, Peppas C, Dressman J, Macheras P. Unusual solubility behavior of cyclosporin A in aqueous media. *J Pharm Pharmacol.* 1991;43:287-289.

4. Yalkowsky SH. *Solubility and Solubilization in Aqueous Media.* New York, NY: Oxford University Press; 1999.

5. Yalkowsky SH, Roseman TJ. Solubilization of drugs by cosolvents. In: *Techniques of Solubilization of Drugs*. New York, NY: Dekker; 1981.

6. Yalkowsky SH, Rubino JT. Solubilization of cosolvents 1: organic solutes in propylene glyco-water mixtures. *J Pharm Sci*. 1985;74:416-421.

7. Zhao L, Li P, Yalkowsky SH. Solubilization of fluasterone. *J Pharm Sci*. 1999;88:967-969.

8. Attwood D, Florence AT. *Surfactant Systems.* New York, NY: Chapman and Hall; 1983.

9. Connors KA, Mollica JA. Theoretical analysis of comparative studies of complex formation: solubility, spectral, and kinetic techniques. *J Pharm Sci.* 1966;55:772-780.

10. Loftsson T, Brewster ME. Pharmaceutical application of cyclodextrins. 1. Drug solubilization and stabilization. *J Pharm Sci.* 1996;85:1017-1025.

11. Gilbert BE, Wilson SZ, Garcon NM, Wyde PR, Knight V. Characterization and administration of cyclosporine liposomes as a small-particle aerosol. *Transplantation.* 1993;56:974-977.

12. El Tayar N, Mark AE, Vallat P, Brunne RM, Testa B, van Gunsteren WF. Solvent-dependent conformation and hydrogen-bonding capacity of cyclosporin A: evidence from partition coefficients and molecular dynamics simulations. *J Med Chem.* 1993;36:3757-3764.

13. Kessler H, Gehrke M, Lautz J, Kock M, Seebach D, Thaler A. Complexation and medium effects on the conformation of cyclosporin A studied by NMR spectroscopy and molecular dynamics calculations. *Biochem Pharmacol*. 1990;40:169-173.