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Exothermic-endothermic heat of solution shift of cyclosporin A related to poloxamer 188 behavior in aqueous solutions

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Abstract

The solubility of cyclosporin A was determined in water and aqueous solutions of a surface active poly (oxyethylene)-poly(oxypropylene)-poly(oxyethylene) block copolymer (poloxamer 188 or pluronic^{R} F68 (PF68)) at concentrations and temperatures ranging from 4 to 22 g/l and from 10 to 50°C, respectively. The solubility behaviour was different between PF68 solutions and water. Solubility values indicated an exothermic heat of solution in each case except for PF68 solutions above 10 g/l at 37–50°C, where a change to endothermic heat of solution was detected. The slopes corresponding to water samples or 4g/l PF68 solutions at temperatures above 37°C do not statistically differ from zero. Aqueous solutions of the poloxamer 188 were also studied using frequency spectrum analysis (dynamic light scattering) and size exclusion chromatography. Below 40°C and 16 g/l essentially invariant values for the hydrodynamic radius were found with broad polydispersities associated. Increasing temperature and poloxamer concentration, the hydrodynamic radius also increased and the systems showed a narrower size distribution, possibly due to micelle formation according to the closed association model.

Keywords: Cyclosporin A; Solubility; Poloxamer 188; Nanoparticle; Bioavailability

1. Introduction

Cyclosporin A (CyA) is an immunosuppressive agent commonly used to prevent organ rejection and graft-versus-host disease after transplantation and for autoimmune diseases. Ismailos et al. (1991) studied the solubility of CyA in aqueous medium between 5 and 37°C and suggested that the unusual exothermic heat of solution for CyA could be, among many other factors, one of the reasons for the low and highly variable bioavailability after oral dosing. The pharmacokinetic problems associated to the use of CyA (Wood et al., 1983) under conventional pharmaceutical dosage forms could be solved including the CyA in a colloidal carrier, i.e., nanoparticles, due to their advantages over conventional formulations, as have been proven for other drugs (Kreuter, 1991). The formulations of these systems include a surface active agent, i.e., polox-

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Table 1
Solubility of cyclosporin (μg/ml) in water and Poloxamer 188-water mixtures at several temperatures[a]

Temperature (°C)	Poloxamer 188 conc. (g/l)				
	0	4	10	16	22
10	134.84 ± 6.95	177.76 ± 10.65	174.26 ± 2.62	161.12 ± 15.93	174.26 ± 5.74
20	41.56 ± 0.18	46.97 ± 0.31	50.55 ± 1.59	52.24 ± 2.17	54.68 ± 0.62
25	19.92 ± 2.50	5.45 ± 0.41	5.34 ± 0.51	6.86 ± 1.81	9.04 ± 2.41
30	8.08 ± 4.58	3.12 ± 1.17	3.19 ± 0.95	2.66 ± 0.46	2.67 ± 0.12
37	3.69 ± 1.27	3.03 ± 1.24	2.45 ± 0.31	2.21 ± 0.19	2.37 ± 0.12
40	3.93 ± 2.15	3.10 ± 0.41	3.12 ± 0.51	3.57 ± 0.91	4.58 ± 0.71
50	4.39 ± 3.01	2.47 ± 0.71	5.12 ± 0.61	7.44 ± 0.61	12.55 ± 2.51

[a]Mean values ± S.D.

amer 188 (Pluronic^R F68 (PF68)) at concentrations around 10 g/l to prevent particle aggregation during storage (Michel et al., 1991). PF68 is a surface active block copolymer (molecular weight, 8350 kDa) showing low toxicity and able to solubilize drugs such as indomethacin at room temperature (Lin and Kawashima, 1985). Due to its low toxicity it is included in the formulation of intravenous fat emulsions, such as Infonutrol^R, for parenteral administration. The association behavior of poloxamer 188 in aqueous solution has been thoroughly studied but this question seems to remain open for discussion since variable results have been obtained (Prasad et al., 1979; Al-Saden et al., 1982; Zhou and Chu, 1988). Previous to nanoparticle preparation it is recommended to establish the drug solubility so as to approach the maximum encapsulation efficiency (Illum et al., 1986). In this work, conclusive results concerning the unusual solubility profile of CyA in water and PF68-water mixtures at the temperature range 10–50°C are reported. This range covers those temperatures that may occur under storage conditions of pharmaceutical dosage forms and also the fever periods, which are very common after organ transplantation.

2. Materials and methods

The solubility of CyA (Sandoz, Basle, Switzerland) in ultrapure water and aqueous solutions of PF68 (0.48×10^{-3} to 2.6×10^{-3} M) (Fluka, Switzerland) was measured. Excess amounts of CyA in solvent were placed in sealed glass vials and were shaken in a thermostated bath at 10, 20, 25, 30, 37, 40 and 50 ± 0.2°C. The equilibrium saturation was studied during 78 days. Samples were taken at several times and centrifuged for 15 min at 11 000 × g in a thermostated centrifuge (Beckman, USA). Centrifugation was chosen to separate the nondissolved solute because CyA adsorbs to membrane filters (Molpeceres, 1994). One hundred-μl samples of the clear solution were withdrawn very carefully to avoid contamination with solid particles, due to the tendency of CyA to float. The samples were assayed for drug content by HPLC (Guzman et al., 1993). The stability of CyA during the study was tested by comparing the chromatograms obtained during the experiment to those for drug standards. The solubility results (Table 1) are the average of three or four measurements. The differences between mean concentrations were tested by ANOVA and least significant difference test.

The size of poloxamer aggregates was measured using a frequency spectrum analyzer (Microtrac Series 9200, Leeds and Northrup, Ireland). The qualitative changes in the molecular size of poloxamer as a function of temperature or concentration were also evaluated by using size exclusion chromatography (SEC). Fifty-μl samples were injected into the chromatograph at 25, 40 and 50°C using ultrapure water as mobile phase. The analysis was carried out on a Progel^{TM}-TSK GMPWXL (30 × 0.78 cm) (Supelco Inc., USA) lineal

column at 1 ml/mn. The system had been previously calibrated at each temperature with polyethylene-glycol (PEG) and polyethylene-oxide (PEO) standards (molecular weights from 400 to 855 000 kDa).

3. Results and discussion

Fig. 1 shows a plot of dissolved drug as a function of time. Full saturations were achieved in 21 days in all cases, although the dissolution rate for CyA in water was slower than in pluronic solutions. Times longer than 24 days did not show statistical differences among mean concentrations of dissolved drug ($P \geq 0.05$). The stability of the drug in solution was assessed by comparing each chromatogram to those obtained from the begining of the study and to drug standards in MeCN/water used for external calibration. Very few references in the literature deal with CyA instability. It has been reported that even when administered to the organism, the molecular ring of CyA remains intact (Mahmoud and Mohammed, 1987). When dissolved in commonly used intravenous solutions only 7% of drug loss was detected during 24 h probably due to adsorption to the polymeric materials involved in the study, so glass containers are recommended for CyA storage (Ptachcinski et al., 1986). Flores et al. (1994) also studied the stability of CyA in hydroalcoholic solutions and no significant degradation was detected for a 7-day period. In our study, neither significant changes in retention times or peak shape nor significant drug loss from 24 to 78 days were found. These results may be explained taking into account the studies by Bundgaard and Friis (1992), Friis and Bundgaard (1992) and Oliyai and Stella (1992). They reported a slow conversion of CyA into isoCyA below neutrality at different temperatures, but the reversible conversion of IsoCyA into native CyA at pH around neutrality (as in our samples) is a faster process.

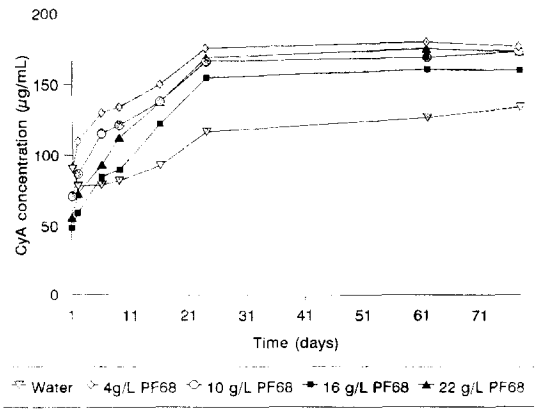

Fig. 1. Concentration of cyclosporin A in solution as a function of time in water and poloxamer solutions. The coefficients of variation associated to experimental data were around 5%. ▼, water; ◆, 4 g/l PF68; ●, 10 g/l PF68; ■ 16g/l PF68; ▲, 22 g/l PF68.

Increasing concentrations of pluronic did not affect the dissolution rate ($P \geq 0.05$) as shown in Fig. 1. The long period of incubation needed to ensure saturation is not usual, but it is well known that poorly soluble hydrophobic compounds, such as CyA (Loosli et al., 1985), dissolve slowly. Yalkowsky and Banerjee (1992) cited an extreme case where 2 months were required to achieve full saturation for Aroclor 1254, a commercial formulation of polychlorinated biphenyls.

Table 1 shows the dependency of solubility of CyA on temperature and pluronic concentration. Pluronic increased the aqueous solubility of CyA at 10 and 20°C, but this effect was not dependent on surfactant concentration. It is remarkable that the different solubility behavior of CyA between 25 and 50°C depends on the concentration of pluronic. Between 25 and 37°C, the surfactant decreased CyA water solubility ($P < 0.05$), and at 25°C the effect was inversely related to pluronic concentration. While solubility was not affected by surfactant at 40°C it was increased at 50°C when pluronic concentration increased. Pluronic concentrations less than 1.2×10^{-3} M did not significantly ($P > 0.05$) affect the aqueous solubility of CyA. These findings suggest that micellar solubilization of CyA is strongly dependent on temperature; solubilization only occurs at high temperature (50°C) and for pluronic concentrations larger than 1.9×10^{-3} M. For other drugs such as indomethacin, solubilization by PF68 starts at room temperature, the process being favored by raising temperature (Lin and Kawashima, 1985).

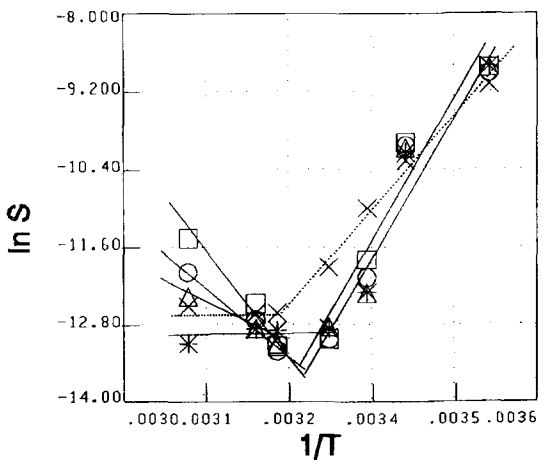

Fig. 2. Van't Hoff plots of cyclosporin A in water and water-poloxamer 188 mixtures. The coefficients of variation can be deduced from Table 1. ×, water; * 4 g/l PF68; △, 10 g/l PF68; ○ 16g/l PF68; □ 22 g/l PF68.

Fig. 2 shows van't Hoff plots of ln S against $1/T$ of CyA in water and PF68-water solvent mixtures, where S stands for molarity. The heat of solution of CyA in water is exothermic between 10 and 37°C, in agreement with the result of Ismailos et al. (1991). The heat of solution in pluronic solutions is also exothermic and larger than that found for water at the same temperature range (Table 2). Above 37°C, the aqueous solubility of CyA is not significantly affected by temperature ($P \geq 0.05$). However, the heat of solution of CyA shifts from negative to positive values for concentrations of pluronic larger than 1.2×10^{-3} M and temperature above 37°C (Table 2).

Exothermic enthalpies are usually associated with favorable solute-solvent interactions (hydrogen bonding) and/or hydrophobic solvation of nonpolar solutes by water (Hermann, 1972) which involves a strong entropy loss due to the ordering of water molecules. Since CyA is very hydrophobic, the negative enthalpies and entropies of solution found below 37°C (Table 2) are likely the result of hydrophobic solvation rather than from solute-solvent hydrogen bonding that should increase solubility. The low aqueous solubility is entropy controlled since the enthalpy change is favorable below 37°C. Hydrophobic solvation decreases by raising temperature (Ross and Subramanian, 1981) and this may explain the shift of the heat of solution in water from strongly exothermic to a value that does not statistically differ from zero ($P \geq 0.05$) above physiological temperature (Fig. 2).

The increased solubility of CyA in PF68 solutions at high temperature is associated with positive heat and entropies of solution (Tables 1 and 2). This suggests that hydrophobic interaction is the driving force of micellar solubilization of CyA. Hydrophobic interaction squeezes out the ordered layer of water molecules surrounding the nonpolar parts of pluronic and CyA. The breaking of hydrogen bonds of water increases both, enthalpy and entropy to positive values and solubilization is entropy controlled (Table 2). Thus, the hydrophobic effect of water may explain the opposite sign for the heat of solution at low temperatures (hydrophobic hydration by water, exothermic) and high temperature (hydrophobic interaction of CyA and PF68, endothermic).

The finding that solubilization of CyA by PF68 only takes place at high temperature may be related to the ability to form micelles as a function of concentration and temperature. The formation of micelles by PF68 has been controversial; Prasad et al. (1979) suggested polymolecular aggregation at concentrations larger than 1.5×10^{-3} M. Zhou and Chu (1988) reported three temperature regions for the unimer (21–30°C), transition (unimers and micelles at 30–50°C) and micelle (50–78°C). The results in Table 3 show that the hydrodynamic radius (r_h) is essentially invariant between 10 and 40°C. In contrast, r_h is markedly concentration-dependent at 50°C. The two-fold increase of r_h and the lower associated polydispersity for the largest PF68 concentrations, suggests polymolecular aggregation. According to Zhou and Chu (1988), above 50°C the only species of PF68 present are multimolecular micelles. Those pluronic concentrations showing larger r_h solubilized CyA, so it can be postulated that polymolecular micelles are the only species with solubilizing capacity.

Although solubilization of CyA does not occur below 50°C, the shift of the heat of solution to endothermic values above 37°C suggests that hydrophobic interaction of CyA and PF68 begins at

Table 2
Apparent enthalpies (kJ/mol) and entropies of solution[a] (J K^{-1} mol^{-1}) for cyclosporin in water and water-poloxamer 188 mixtures[b] (mean values ± S.D.)

Conc. poloxamer (g/l)	10–30°C		37–50°C	
	ΔH_2	ΔS_2	ΔH_2	ΔS_2
0[c]	−98.1 ± 5	−421 ± 96	—	—
4	−151.9 ± 28	−607 ± 96	—	—
10	−151.0 ± 31	−604 ± 104	45.9 ± 4	39.3 ± 14
16	−150.2 ± 29	−602 ± 100	73.9 ± 13	129.2 ± 42
22	−149.7 ± 28	−102 ± 95	101.4 ± 18	219.0 ± 56

[a]ΔH_2 (soln.) = $-R \times$ slope and ΔS_2(soln.) = $R \times$ intercept, where R is the gas constant, 8.3143 J K^{-1} mol^{-1}.
[b]Values of ΔH_2 and ΔS_2 for poloxamer 188 mixtures between 10 and 20°C do not statistically differ from those for water.
[c]Values of ΔH_2 and ΔS_2 correspond to the interval 10–37°C.

this temperature. At room temperature, the surfactant is highly hydrated due to the interaction of the ether oxygen of PF68 with water molecules (Zhou and Chu, 1988), a fact that may disfavour the interaction with a hydrophobic peptide such as CyA. The motion of the hydrophilic chains may also contribute to prevent interaction on pluronic micelles. A similar explanation has been suggested for some proteins which do not adsorb on microparticles coated with pluronic (Lee et al., 1989).

Table 3
The effect of temperature and concentration on the hydrodynamic radius of poloxamer 188 in aqueous solution (n = 4)

Temperature (°C)	Concentration (g/l)	r_h (nm)	CV (%)
10	4	3.42	16
	10	4.51	23
	16	3.90	13
	22	3.57	18
25	4	3.80	17
	10	4.17	20
	16	4.86	21
	22	4.54	23
40	4	5.45	21
	10	5.65	24
	16	5.38	25
	22	4.63	26
50	4	4.81	23
	10	5.91	20
	16	8.87	13
	22	9.90	9

Size exclusion chromatography (SEC) was used here to study desolvation of poloxamer as a function of temperature (Table 4). It should be noted that the molecular weights and polydispersities given in Table 4 are only indicative of the relative desolvation and folding of poloxamer molecules. According to our results the size of poloxamer molecules sligthly decreases (dehydration) as temperature or concentration increases from 25 to 40°C and 4 to 22 g/l, respectively, but the polydispersity indexes remain invariant. These results suggest a molecular folding without any detectable changes in surfactant aggregation; above 40°C and 0.0013 M poloxamer concentration, the size dramatically decreases for the unaggregated molecules. Furthermore, a significant increase in polydispersity index for the lower molecular weight peaks accounts for a transition stage in molecular aggregation at this temperature which is very close to the transition region reported by Zhou and Chu (1988) where unimers and micelles coexist. A second monodisperse peak of high molecular weight above 10 g/l at 50°C corresponded to the association of the dehydrated unimers to form multimolecular micelles. These results correlate very well with those found by frequency spectrum analysis.

From the above discussion, hydrophobic interaction of CyA with poloxamer micelles is the suggested solubilization mechanism. Two factors, increasing desolvation and number of micelles with temperature may favor hydrophobic interac-

Table 4
Size exclusion chromatography parameters for poloxamer solutions at several temperatures

Poloxamer 188 conc. (g/l)	Parameter	Temperature (°C)		
		30	40	50
4	R_t(min)[a]	8.80	8.90	9.43
	M_w(Da)[b]	8000	6918	2092
	M_n(Da)[c]	4948	4269	294
	P.I.[d]	1.61	1.62	7.12
10	R_t	8.82	8.95	9.44 (6.30)[e]
	M_w	7678	6346	2111 (64 299)
	M_n	4594	3890	347 (64 151)
	P.I.	1.67	1.63	6.07 (1.00)
16	R_t	8.85	8.98	9.45 (6.30)
	M_w	7282	5986	2108 (65 852)
	M_n	4344	3681	356 (65 506)
	P.I.	1.67	1.62	5.91 (1.00)
22	R_t	8.88	9.00	9.45 (6.30)
	M_w	6805	5742	2077 (68 928)
	M_n	3999	3532	347 (68 406)
	P.I.	1.70	1.62	5.98 (1.00)

[a]Retention time.
[b]Mean molecular weight by weight.
[c]Mean molecular weight by number
[d]Polydispersity index.
[e]Data between parentheses correspond to the aggregation of poloxamer 188.

tion of CyA with PF68. Increasing temperature produces dehydration of surfactant molecules and increase in aggregation number. The solubility of CyA is minimum at about 37°C or higher temperatures in aqueous media, which constitutes a serious restriction for drug absorption in the gastrointestinal tract. Furthermore, small variation in body temperature (very common after transplantation) can produce significant changes in drug solubility (2.37 μg/ml at 37°C and 4.58 μg/ml at 40°C, both at 22 g/l poloxamer concentration), affecting the bioavailability of CyA when administered in pharmaceutical dosage forms containing this type of surfactant, such as nanoparticles or microparticles.

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References

Al-Saden, A.A., Whateley, T.L. and Florence, A.T., Poloxamer association in aqueous solution. *J. Colloid Interface Sci.*, 90 (1982) 303–309.

Bundgaard, H. and Friis, G.J., Prodrugs of peptides. 16. Isocyclosporin A as a potential prodrug of cyclosporin A. *Int. J. Pharm.*, 82 (1992) 85–90.

Flores, G., Pascual, B., Pou, L. and Sabín, P., Ciclosporina inhalada: ¿Una alternativa en el transplante de pulmón?. Estabilidad de la solución hidroalcohólica. *Farm. Hosp.*, 18(5) (1994) 270–272.

Friis, G.J. and Bundgaard, H., Kinetics of degradation of cyclosporin A in acidic aqueous solution and its implication in its oral absorption. *Int. J. Pharm.*, 82 (1992) 79–83.

Guzman, M., Molpeceres, J., Garcia, F. and Aberturas, M.R., The formation and characterization of cyclosporin-A loaded nanoparticles. *J. Pharm. Sci.*, 82 (1993) 498–502.

Hermann, R.B., Theory of hydrophobic bonding. II. The correlation of hydrocarbon solubility in water with solvent cavity surface area. *J. Phys. Chem.*, 76 (1972) 2754–2759.

Illum, L., Khan, M.A., Mark, E. and Davis, S.S., Evaluation of carrier capacity and release characteristics for poly (butyl-2-cyanoacrylate) nanoparticles. *Int. J. Pharm.*, 30 (1986) 17–28.

Ismailos, G., Reppas, C., Dressman, J.B. and Macheras, P., Unusual solubility behavior of cyclosporin A in aqueous media. *J. Pharm. Pharmacol.*, 43 (1991) 287–289.

Kreuter, J., Peroral administration of nanoparticles. *Adv. Drug Del. Rev.*, 7 (1991) 71–86.

Lee, J., Martic, P.A. and Tan, J.S., Protein adsorption on Pluronic^{R} copolymer-coated polystyrene particles. *J. Colloid Interface Sci.*, 131 (1989) 252–266.

Lin, S. and Kawashima, Y., The influence of three poly(-oxyethylene) poly(oxypropylene) surface active block copolymers on the solubility behavior of indomethacin. *Pharm. Acta Helv.*, 60 (1985) 339–343.

Loosli, H.R., Kessler, H., Oschkinat, H., Weber, H.P., Petcher, T.J. and Widmer, A., The conformation of cyclosporin A in the crystal and in solution. *Helv. Chim. Acta*, 68 (1985) 683–704.

Mahmoud, M.A. and Mohammed, A., Cyclosporin. In Florey, K. (Ed.), *Analytical Profiles of Drug Substances*, Vol. 16, Academic Press, New York, 1987, pp. 145–206.

Michel, C., Aprahamian, M., Defontaine, L., Couvreur, P. and Damgé, C., The effect of site of administration in the gastrointestinal tract on the absorption of insulin from nanocapsules in diabetic rats. *J. Pharm. Pharmacol.*, 43 (1991) 1–5.

Molpeceres, J., *Novel drug delivery systems: Cyclosporin A loaded polycaprolactone nanoparticles.* Ph.D. Thesis, University of Alcalá de Henares, Spain (1994).

Oliyai, R. and Stella, V.J., Kinetics and mechanism of isomerization of cyclosporin A. *Pharm. Res.*, 9(5) (1992) 617–622.

Prasad, K.N., Luong, T.T., Florence, A.T., Paris, J., Vaution, C., Seiller, M. and Puisieux, F., Surface activity and association of ABA polyoxyethylene-polyoxypropylene block copolymers in aqueous solution. *J. Colloid Interface Sci.*, 96 (1979) 225–232.

Ptachcinski, R.J., Logue, L.W., Burckart, G.J. and Venkataramanan, R., Stability and availability of cyclosporine in 5% dextrose injection or 0.9% sodium chloride injection. *Am. J. Hosp. Pharm.*, 43(1) (1986) 94–97.

Ross, P.D. and Subramanian, S., Thermodynamics of protein association reactions: forces contributing to stability. *Biochemistry*, 20 (1981) 3096–3102.

Wood, A.J., Maurer, G., Niederberger, W. and Beveridge, T., Cyclosporine: Pharmacokinetics, metabolism and drug interactions. *Transplant Proc.*, 15 (Suppl. 1) (1983) 2409–2412.

Yalkowsky, S.H. and Banerjee, S., *Aqueous Solubility. Methods of Estimation for Organic Compounds*, Marcel Dekker, New York, 1992, p. 4.

Zhou, Z. and Chu, B.J., Ligth scattering study on the association behavior of triblock polymers of ethylene oxide and propylene oxide in aqueous solution *J. Colloid Interface Sci.*, 126 (1988) 171–180.