Table 2. Recovery of standard solution added to pharmaceutical preparations*.

	Added ($\mu g\ mL^{-1}$)	Found ($\mu g\ mL^{-1}$)	Recovery (%)
Salbutamol sulphate tablets			
Sample			
Commercial	4	3·93	98·2
	8	7·86	98·2
	20	20·05	100·2
Simulated	4	3·98	99·5
	8	7·95	99·4
	20	20·28	101·4
Simulated two-component sample			
Compound			
Salbutamol	24	23·84	99·3
Beclomethasone	12	11·93	99·4

*All the results are an average of 3 assays.

Precision of analytical results. The precision of the amplitude measurements under the instrumental conditions was determined by recording the first-derivative spectra of ten drug solutions at their analytical concentrations. The relative standard deviations of the amplitudes of salbutamol sulphate, salbutamol and beclomethasone dipropionate were all in the range of 0·7–1·1%.

Comparison with the official method. To verify the efficiency of the proposed method, the concentrations of salbutamol sulphate in commercial and simulated tablets were determined by the derivative procedure and also by the procedure described in the British Pharmacopoeia 1988. The good agreement of the results obtained (Table 1) confirmed that the proposed procedure is accurate and suitable as a rapid alternative to the official method for salbutamol tablets.

No official procedure has been described and published for assay of the aerosol with two components but the results obtained by the application of the derivative procedure in simulated samples demonstrates its efficiency (Table 3).

Table 3. Data obtained in the analysis of samples using the first-derivative spectrophotometry and the official method.

Formulation	Declared strength	Found (% declared strength) BP	New method
Salbutamol sulphate tablets	2·0 mg	102·8	101·6
Salbutamol sulphate tablets	2·0 mg	100·2	101·5
Salbutamol (two-component) aerosol	0·1 mg/dose	—	101·2
Beclomethasone dipropionate (two-component) aerosol	0·05 mg/dose	—	98·3

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Unusual solubility behaviour of cyclosporin A in aqueous media

GEORGE ISMAILOS, CHRISTOS REPPAS, JENNIFER B. DRESSMAN*, PANAYOTIS MACHERAS, *Department of Pharmacy, University of Athens, Athens 106 80, Greece and* *College of Pharmacy, The University of Michigan, Ann Arbor, MI 48109–1065, USA*

Abstract—The solubility of cyclosporin A was determined in water and in Sorensen buffers at pH 1·2 and 6·6 at temperatures ranging from 5 to 37°C. No differences in solubility behaviour were observed among the three aqueous media. Solubility was found to be inversely proportional to the temperature in each medium, indicating that the heat of solution was exothermic in each case.

The only oral dosage form of cyclosporin A (CyA) currently available consists of olive oil, ethanol, and polyoxyethylated oleic glycerides (Labrafil) (40:18:42) with a CyA concentration of 100 mg mL^{-1} (Tarr & Yalkowsky 1989). It is recommended that this formulation (Sandimmune) be diluted with milk, chocolate milk or orange juice immediately before administration. However, the bioavailability of CyA from this dosage form is incomplete and erratic. Problems which can be specifically associated with the dosage form include precipitation of the drug upon dilution, inaccuracy of the dose administered and lack of patient compliance. Dissolution limitations, problematic intestinal permeability and first pass metabolism during transfer through the gut wall and liver (Ueda et al 1984; Humphrey 1986; Ptachcinski et al 1986) have also been identified as potential sources of the erratic absorption behaviour. One factor which has not been considered and which may play a role in reproducibility of absorption is the solubility profile of CyA as a function of temperature. Large changes in CyA solubility with

Correspondence: P. Macheras, Department of Pharmacy, University of Athens, 104 Solonos St, 106 80 Athens, Greece.

temperature could influence the choice of diluent temperature as well as determining the degree to which CyA can be solubilized by the gastrointestinal fluids. In this communication, we report on the effect of temperature on the solubility of CyA in aqueous media.

Materials and methods

Solubility of CyA (Sandoz Inc, NJ and Athens, Greece) was studied in distilled water and in Sorensen buffer (disodium citrate-NaOH/HCl, 0·05 M) at pH 1·2 and 6·6. Experiments were performed in a shaking water bath (Julabo SW1, Schwarzwald, Germany) at 5, 10, 20, 30, and 37°C.

The equilibration time was established in water at 37°C. No significant differences were observed (as determined by paired *t*-test) when samples were collected at 6, 12, 16 and 24 h following the beginning of the experiment. In subsequent experiments, samples were taken over 18–24 h to ensure equilibration.

Each sample was filtered through 2 μm pore diameter Nuclepore filters (Nuclepore Corp., Pleasanton, CA) and assayed with HPLC using cyclosporin D (CyD) as internal standard. The filtrate (1·0 mL) was diluted in analytical grade methanol and vortexed with the appropriate amount of CyD to obtain a peak of similar magnitude to that of the CyA in the sample. The injection volume was 20 μL (Rheodyne model 7125 injector, Cotati, CA). The HPLC system consisted of a Spectra-Physics model SP 8800/8810 LC pump (San Jose, CA), a model SP 100 UV detector set at 215 nm coupled with an SP 4400 Integrator (San Jose, CA) and a Spherisorb S10 ODS2 column (25 cm × 4·6 mm, Spherisorb, Phase Separation Ltd, Queensferry, UK) thermostated at 65 ± 0·1°C (Jones Chromatography, Model 7960). The mobile phase consisted of 64% acetonitrile, 34% water and 2% methanol. The flow rate was 2·3 mL min^{-1} which resulted in retention times of 7·4 and 10·0 min for CyA and CyD, respectively.

Solubility at each temperature was determined as the average value from three experiments except in the case of 10°C where only two experiments in each medium were performed. Furthermore, due to the wide scatter of the data at 5°C in water, a fourth experiment was performed under these conditions. An unpaired *t*-test was used to test differences between values at two subsequent temperatures. Differences were considered significant for $P < 0.05$.

Results

Solubility data are presented in Table 1. The solubility in water was found to be in accordance with previously reported values at 37 and 25°C (Reymond & Sucker 1988; Hahn & Sucker 1989). As temperature was decreased, there was an increase in the solubility, with the solubility at 5°C being at least 10 times higher than at 37°C. The solubility of CyA was found to be inversely proportional to the temperature, as shown in Fig. 1.

Table 1. Effect of temperature on the solubility of cyclosporin A (μg mL^{-1}) in aqueous media[1].

Temp °C	Water	pH 1·2	pH 6·6
5	101·5 (37·7)	87·1 (21·5)	78·4 (12·6)
10	[38·8, 48·5]^{NS}	[30·1, 34·9]	[36·5,31·1]
20	32·9 (6·7)	32·9 (2·0)^{NS}	25·4 (1·2)
30	12·2 (0·6)	12·0 (0·8)	9·6 (0·9)
37	7·3 (1·3)	7·6 (0·6)	6·2 (0·7)

[1]Data are presented as mean ± s.d. except for 10°C, where numbers represent the values of each individual experiment. NS indicates that there is no significant difference between the solubility at the temperature indicated from that at the next lower temperature.

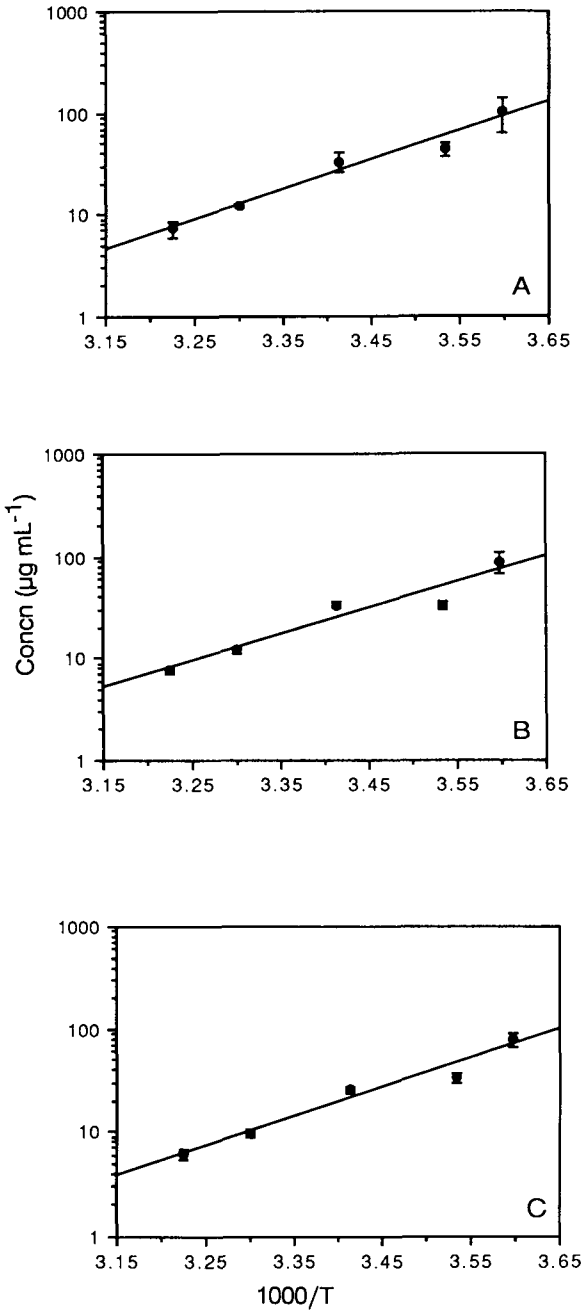

FIG. 1. Typical Van't Hoff plots (mean ± s.d.) for the solubility of cyclosporin A in aqueous media. (A) water, (B) buffer pH 1·2, (C) buffer pH 6·6. In all cases linear regression analysis resulted in significant correlations with $0.957 < r < 0.982$.

The two buffered media were chosen to represent the pH conditions in the stomach and the small intestine. For any given temperature, there were no differences in CyA solubility among the three media. These results suggest that no changes in solubility of CyA would be expected as a result of pH changes along the upper gastrointestinal tract after the dosage form has been administered.

For any given medium, the solubilities at two different temperatures were always statistically different, except for the two cases noted in Table 1. The high degree of scatter in the data

at 5°C was attributable to inability to precisely control the temperature during the filtration process.

Discussion

Typical Van't Hoff plots, shown in Fig. 1, reveal significant positive slopes for the solubility behaviour of CyA in each medium. Heats of solution, ΔH, of CyA were calculated (Table 2) using the following formula (Sokoloski 1970):

$$\ln C_s = -\frac{\Delta H}{RT} + J$$

where C_s is the solubility, ΔH is the heat of solution (kJ mol^{-1}), R is the molar gas constant (0·008314 kJ mol^{-1} $Kelvin^{-1}$), T is the temperature (K) and J is a constant.

As seen from Table 2, the values of ΔH are large (Martin et al 1970) and the dissolution process is exothermic. The high negative value for the enthalpy change implies an extensive solute-solvent interaction (Sokoloski 1970). In addition, the entropy change due to the solution process was calculated to be $-0{\cdot}1609 \pm 0{\cdot}0016$ KJ mol^{-1} deg^{-1} (mean ± s.d.) for the temperature range used. This significant decrease of CyA entropy in solution suggests that this process is enthalpically driven. This behaviour is in contrast to that observed for most drugs, where a rise in temperature results in a corresponding increase in the solubility of the drug (Sokoloski 1970; Macheras et al 1990) resulting in an endothermic heat of solution. Typical examples include chlorothiazide and hydrochlorothiazide, which have heats of solution of 44·2 and 32·6 KJ mol^{-1}, respectively, measured in phosphate buffer (pH 6·5) over the same range of temperatures used for the CyA experiments (Macheras et al 1989).

Table 2. Heat of solution, ΔH, of cyclosporin A in aqueous media.

Medium	ΔH (kJ mol^{-1})
Water	−55·1
pH 1·2*	−49·1
pH 6·6*	−53·4

*Sorensen buffer, 0·05 M.

The exothermic heat of solution for CyA is in accord with previous work regarding the effect of temperature on the conformation of CyA in solution. Loosli et al (1985) suggested that at higher temperatures the intramolecular H-bonds are stronger and the molecule adopts a conformation corresponding to an isolated rather than a solvated molecule. In other words, intramolecular H-bonds (produced by the NH groups) which result in a more rigid configuration, become weaker as temperature decreases, with a subsequent increase in solubility.

Data from the literature regarding effects of temperature on the solubility of other peptides and related compounds are limited. Macritchie (1973) concluded that the presence of lysine, proline and alanine residues tend to produce a protein whose solubility is decreased by increasing temperature. In the case of CyA, though, there are only two alanine residues and no lysine or proline. On the other hand, Conio et al (1973) reported that the solubility behaviour of glycine, diglycine and triglycine in water-ethanol mixtures (ethanol content: 0–50%) was directly proportional to increases in the temperature over the range 20 to 60°C.

A further example in which the physical behaviour of CyA has proved to be anomalous lies in its protein binding behaviour as a function of temperature. Legg & Rowland (1987) found that the binding of CyA to lipoproteins was endothermic. Therefore, binding increases with an increase in temperature, in contrast to the usual situation in which the binding of drugs to lipoproteins decreases at elevated temperatures.

It is clear that temperature has a dramatic effect on the solubility of CyA, with the process being highly exothermic. The data indicate that the temperature of the diluent fluid used to mix the oral solution before administration would have a significant bearing on the solubility of CyA. Diluents maintained under refrigeration until immediately before dilution should be more effective in keeping the CyA in solution than fluids at room temperature. Additional data regarding the effects of temperature on the solubility in various diluents and in physiologically relevant media containing bile salts would be therefore of great interest.

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