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Enhanced stability of sulfamethoxazole and trimethoprim against oxidation using hydroxypropyl-β-cyclodextrin

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The effect of hydroxypropyl-β-cyclodextrin (HPβCD) on the chemical stability of sulfamethoxazole and trimethoprim (co-trimoxazole) under oxidation stress at $50 \pm 2\,^{\circ}C$ was investigated. The concentrations of sulfamethoxazole and trimethoprim in aqueous solutions (pH 5.4) containing 0, 1%, 2%, 5%, 10% and 15% w/v hydroxypropyl-β-cyclodextrin were measured by HPLC. Both sulfamethoxazole and trimethoprim degradation appeared to follow pseudo-first order kinetics in the presence and in the absence of hydroxypropyl-β-cyclodextrin. The observed half-lives for sulfamethoxazole and trimethoprim in 15% w/v hydroxypropyl-β-cyclodextrin were 910 h and 609 h respectively, 11.8 and 3.4 times greater than in solutions without hydroxypropyl-β-cyclodextrin. Using a Lineweaver-Burk equation, the half-lives for sulfamethoxazole and trimethoprim outside the complex in a solution containing 15% w/v hydroxypropyl-β-cyclodextrin were estimated at 77 h and 193 h respectively, whereas inside the complex the half-lives were estimated at 850 h and 821 h. In terms of relative increases in stability under oxidation stress the half-lives for sulfamethoxazole and trimethoprim inside the complex were 11.0 times and 4.2 times greater than their half-lives outside the complex. In conclusion, chemical stability of sulfamethoxazole and trimethoprim in co-trimoxazole aqueous solutions under oxidation stress at $50 \pm 2\,^{\circ}C$ can be increased using hydroxypropyl-β-cyclodextrin as a molecular inclusion excipient.

1. Introduction

Cyclodextrins have many applications in pharmaceutical technology. In addition to their use as complexing agents to increase aqueous solubility, cyclodextrins can increase dissolution rate and bioavailability of drugs, and improve the stability of labile ingredients. While the enhanced stability of drugs susceptible to hydrolysis has been well described in the literature and has been attributed to susceptible molecules residing in the hydrophobic cavity of the cyclodextrin molecule (Jumaa et al. 2004; Kim et al. 2004; Tirucherai and Mitra 2003; Uekama et al. 2001) there have been very few reports of protection against oxidation (Abe et al. 1995; Kim et al. 2000; Park et al. 2002).

Co-trimoxazole, a 5 : 1 combination of sulfamethoxazole with trimethoprim, is used in the management of a variety of infections such as urinary tract infections, upper respiratory tract infections, and is very effective for *Pneumocystis pneumonia* prophylaxis in immunocompromised patients (Kovacs et al. 2001). Sulfamethoxazole and trimethoprim exhibit low aqueous solubility in parenteral solutions which can be improved with the addition of hydroxypropyl β-cyclodextrin (HPβCD) to the infusion fluid (McDonald and Faridah 1991). There is growing interest in improving dosage forms of co-trimoxazole for applications where the intravenous administration of large doses and volumes (due to poor solubility and cosolvents) is problematic such as in severely ill immunocompromised patients.

Preliminary investigations in our laboratory have revealed that sulfamethoxazole and trimethoprim also exhibit considerable decomposition under oxidative stress testing relative to acid and base hydrolysis. The objective of this study was to examine the stability of co-trimoxazole solution using HPβCD to enhance solubility under oxidation stress with hydrogen peroxide.

2. Investigations, results and discussion

The stability of sulfamethoxazole and trimethoprim was studied at pH 5.4 in aqueous buffer solutions containing 0–15% HPβCD, under oxidation stress at $50 \pm 2\,^{\circ}C$. Both sulphamethoxazole decomposition (Fig. 1) and trimethoprim decomposition (Fig. 2) appeared to follow pseudo-first order kinetics in the presence and in the absence of HPβCD.

Sulfamethoxazole and trimethoprim were found to decompose at much slower rates in the presence of HPβCD and the decomposition specific rate constant decreased with increasing concentration of HPβCD. The Table shows the effect of cyclodextrin concentration on the half-lives of sulfamethoxazole and trimethoprim at pH 5.4 and $50 \pm 2\,^{\circ}C$ under oxidation stress. The observed half-lives

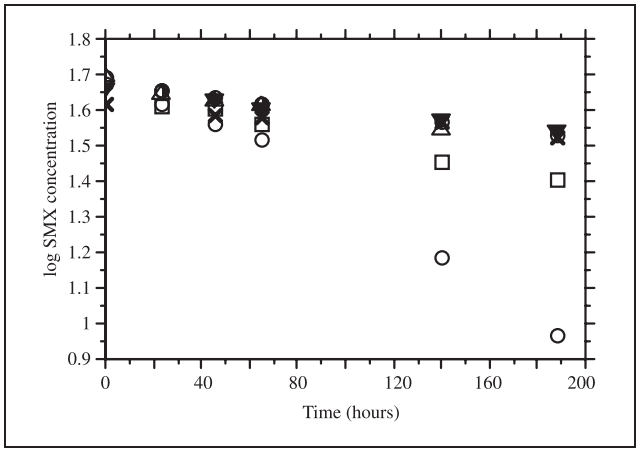


Fig. 1: The effect of hydroxypropyl-β-cyclodextrin (HPβCD) on the degradation of sulfamethoxazole (SMX) in aqueous solutions at pH 5.4 and 50 ± 2 °C under oxidation stress; (○) 0% HPβCD, (□) 1% mM HPβCD, (△) 2% HPβCD, (◑) 5% HPβCD, (▼) 10% HPβCD, (×) 15% HPβCD

for sulfamethoxazole and trimethoprim in an aqueous solution with 15% HPβCD were 910 h and 609 h respectively, 11.8 and 3.4 times greater than solutions without HPβCD.

Specific rate constants for oxidation of complexed sulfamethoxazole ($k_c = 8.154 \times 10^{-4}$) and complexed trimethoprim ($k_c = 8.440 \times 10^{-4}$) were calculated from the y-intercept values ($1/(k_o - k_c)$) from the linear regression equations shown in Figs. 3 and 4, respectively. The half-lives for sulfamethoxazole and trimethoprim outside the complex in a solution containing approximately 15% HPβCD were estimated at 77 h and 193 h respectively, whereas inside the complex the half-lives were estimated at 850 h and 821 h, respectively. An observed half-life longer than inclusion complex half-life may be attributed to limitations of the Lineweaver-Burk curve fitting and drug-cyclodextrin stoichiometry that may not be a straightforward 1 : 1 relationship.

In terms of relative increases in stability under oxidation stress the half-lives for sulfamethoxazole and trimethoprim inside the complex are 11.0 times and 4.2 times greater than their half-lives outside the complex. The observed half-lives of trimethoprim (shown in the Table) appear to

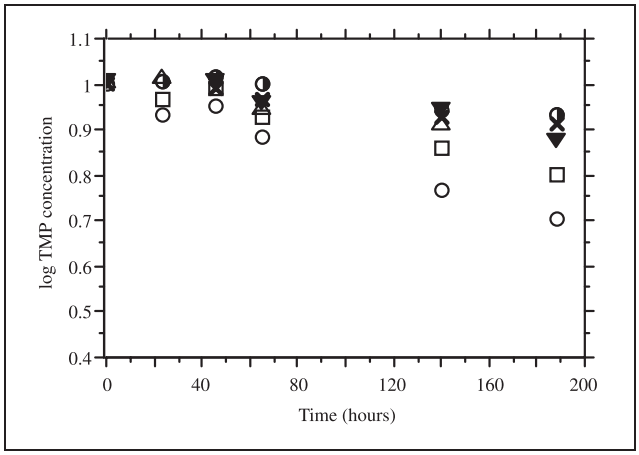


Fig. 2: The effect of hydroxypropyl-β-cyclodextrin (HPβCD) on the degradation of trimethoprim (TMP) in aqueous solutions at pH 5.4 and 50 ± 2 °C under oxidation stress; (○) 0% HPβCD, (□) 1% HPβCD, (△) 2% HPβCD, (◑) 5% HPβCD, (▼) 10% HPβCD, (×) 15% HPβCD

Table: The effect of HPβCD concentration on the half-life of sulfamethoxazole and trimethoprim at pH 5.4 and 50 ± 2 °C under oxidation stress.

HPβCD (mM)	Sulfamethoxazole		Trimethoprim	
	k_{obs} (hr^{-1})	$t_{1/2}$ (h)	k_{obs} (hr^{-1})	$t_{1/2}$ (h)
0.00 (0%)	8.992×10^{-3}	77.1	3.577×10^{-3}	193.4
7.25 (1%)	3.351×10^{-3}	206.9	2.436×10^{-3}	284.6
14.50 (2%)	2.098×10^{-3}	330.4	1.682×10^{-3}	412.2
36.23 (5%)	1.698×10^{-3}	408.3	1.078×10^{-3}	642.7
72.46 (10%)	1.346×10^{-3}	514.9	1.551×10^{-3}	446.8
108.70 (15%)	0.762×10^{-3}	909.9	1.139×10^{-3}	608.8

plateau between 2% and 15% which may be indicative of non-linear complexation with cyclodextrin. This is also seen in Fig. 4 with a weaker regression fit ($r^2 = 0.886$) compared to sulfamethoxazole ($r^2 = 0.965$).

Both sulfamethoxazole and trimethoprim were relatively stable in aqueous solution without oxidative stress conditions and retained greater than 95% original concentration after 6 days. Given the stability of sulfamethoxazole and trimethoprim in aqueous solution without the addition of hydrogen peroxide, any enhanced stability due to HPβCD was difficult to observe over the time frame of this investigation.

Sulfamethoxazole and trimethoprim have previously been shown to form pH dependent inclusion complexes (McDonald and Faridah 1991) and it would not be surprising that

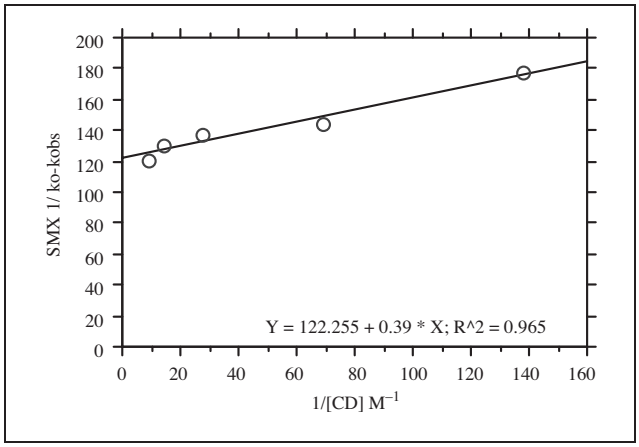


Fig. 3: A Lineweaver-Burk plot for the oxidation of sulfamethoxazole (SMX) with varying cyclodextrin (CD) concentration

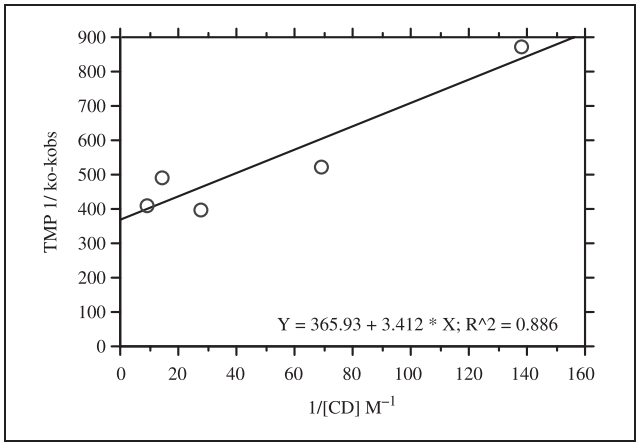


Fig. 4: A Lineweaver-Burk plot for the oxidation of trimethoprim (TMP) with varying cyclodextrin (CD) concentration

some protection against hydrolysis in aqueous solutions would be afforded by the inclusion nature of the drug-cyclodextrin complex. There has been considerable work showing the stability advantages of formulating hydrolysis susceptible drug molecules with cyclodextrins (Jumaa et al. 2004; Kim et al. 2004; Tirucherai and Mitra 2003; Uekama et al. 2001). The limited work examining cyclodextrins and stability against oxidation has not included drug products such as sulfamethoxazole and trimethoprim (Abe et al. 1995; Kim et al. 2000; Park et al. 2002). These results indicate that cyclodextrins can also offer protection against oxidation under stress testing with hydrogen peroxide.

In conclusion, chemical stability of sulfamethoxazole and trimethoprim in co-trimoxazole aqueous solutions under oxidation stress at 50 ± 2 °C, can be increased using HPβCD as a molecular inclusion excipient.

3. Experimental

3.1. Materials

Hydroxypropyl-β-cyclodextrin (HPβCD; MW 1380, molar substitution 0.6), sulfamethoxazole and trimethoprim were obtained from Sigma-Aldrich (Sydney, Australia). All solvents were of HPLC grade and other reagents were of analytical grade.

3.2. HPLC analysis

Quantitative determinations of trimethoprim and sulfamethoxazole were carried out by HPLC using a Varian instrument (Melbourne, Australia) consisting of a ProStar 230 solvent delivery module, a ProStar 330 Photodiode array detector (with detection at 253 nm), a ProStar 410 autosampler and Varian Star® chromatography software (version 5.52). A 5 µm Nova-pak® C_{18} HPLC column (3.9 mm × 15 cm; Waters Australia Pty. Ltd., Sydney, Australia) was used, with a mobile phase consisting of 13% acetonitrile, 1% acetic acid, and 0.1% triethylamine in water. The flow rate was 1.7 ml/min and the injection volume was 10 µl. The analysis was conducted at room temperature, and the retention times were 2.7 min and 6.0 min for trimethoprim and sulfamethoxazole respectively. Analytical performance was within acceptable limits with inter-day and intra-day relative standard deviations of less than 5% with external standardisation.

3.3. Stability studies

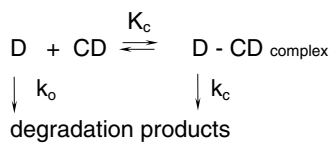
Complexation was carried out using the method adopted from Ma et al. (2000). Sulfamethoxazole (50 mg) and trimethoprim (10 mg) were accurately weighed and dissolved in a 1000 ml acetonitrile-water (13 : 87) bulk solution to ensure drug solubility. Aliquots of this solution (5 ml) were taken and HPβCD was added (50, 100, 250, 500 and 750 mg) to give solutions of approximately 0, 1%, 2%, 5%, 10% and 15% HPβCD given that there were small but not easily measured volume increases due to the addition of the cyclodextrin solid. Two samples were prepared for each HPβCD concentration. Complexes were prepared by 5 min sonication of HPβCD solutions followed by mixing for 24 h at room temperature using an orbital shaking water bath. After complexation, the initial concentrations of sulfamethoxazole and trimethoprim were determined by HPLC. It is recommended that oxidation stress testing should be carried out using solutions of hydrogen peroxide (Bakshi and Singh 2002). Hydrogen peroxide (5%, 500 µl) was added to each solution which were then stored in 2 ml ampoules and placed in oven at 50 ± 2 °C. Decomposition kinetics were followed by removing samples at appropriate intervals (23, 45, 65, 140 and 188 h) and analysing each sample by HPLC.

3.4. Mathematical model

Shown in the scheme is a simple model which has previously been used to describe the overall decomposition of drug (D) forming a 1 : 1 inclusion complex (D-CD) with cyclodextrins (CD) where k_o and k_c are the rate constants for degradation of free and complexed drug respectively (Loftsson and Brewster 1996).

The stability afforded by the addition of cyclodextrin is dependent on both the rate of drug decomposition within the complex and the fraction of drug within the complex (Loftsson and Brewster 1996). The observed first-order rate constant (k_{obs}) is the weighted average of the two rate constants:

Scheme Model to describe degradation of drugs forming 1 : 1 inclusion complexes with cyclodextrins.



$$\frac{d[D]_t}{dt} = -k_{obs} \cdot [D]_t \quad (1)$$

$$k_{obs} = k_o \cdot f_f + k_c \cdot (1 - f_f) \quad (2)$$

The fraction of free drug f_f in solution can be written as:

$$f_f = \frac{1}{1 + K_c \cdot [CD]} \quad (3)$$

Total drug and drug-cyclodextrin complex can also be determined from f_f as follows:

$$[D]_t \cdot f_f = [D] \quad \text{and} \quad [D]_t \cdot (1 - f_f) = [D - CD] \quad (4)$$

These equations can then be further arranged in a Lineweaver-Burk equation:

$$\frac{1}{k_o - k_{obs}} = \frac{1}{K_c \cdot (k_o - k_c)} \cdot \frac{1}{[CD]} + \frac{1}{k_o - k_c} \quad (5)$$

Assuming a low concentration 1 : 1 drug-cyclodextrin stoichiometry, k_c and K_c can be determined from a plot of $1/(k_o - k_{obs})$ versus 1/[CD] (Loftsson and Brewster 1996). A comparison between drug stability of free drug (for both sulfamethoxazole and trimethoprim) and stability of drug in cyclodextrin complex was made from the rate constants k_o and k_c. The half-life under each cyclodextrin concentration was determined from ln $2/k_c$.

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