Investigations into the physical and chemical stability of concentrated co-trimoxazole intravenous infusions

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ABSTRACT

Objectives High dose of intravenous sulfamethoxazole and trimethoprim (co-trimoxazole) is often used in immunocompromised patients for the treatment of *Pneumocystis jiroveci* pneumonia. Current manufacturer's dilution recommendation for intravenous co-trimoxazole (1:25 v/v) requires the administration of 2 L of additional fluid per day causing serious complications including pulmonary oedema. Intravenous administration of concentrated solution of co-trimoxazole may minimise the risk of fluid overload associated side effects.

Therefore, the objective of the study was to investigate the physicochemical stability of concentrated intravenous co-trimoxazole solutions.

Methods Four ampoules of intravenous co-trimoxazole were injected into an infusion bag containing either 480 (1:25 v/v), 380 (1:20 v/v), 280 (1:15 v/v) or 180 (1:10 v/v) mL of glucose 5% solution. Three bags for each dilution (total 12 bags) were prepared and stored at room temperature. An aliquot was withdrawn immediately (at 0 hour) and after 0.5, 1, 2 and 4 hours of storage for high-performance liquid-chromatography (HPLC) analysis, and additional samples were withdrawn every half an hour for microscopic examination. Each sample was analysed for the concentration of trimethoprim and sulfamethoxazole using a stability indicating HPLC method. Samples were assessed for pH, change in colour (visually) and for particle content (microscopically) immediately after preparation and on each time of analysis.

Results Intravenous co-trimoxazole at 1:25, 1:20, 1:15 and 1:10 v/v retained more than 98% of the initial concentration of trimethoprim and sulfamethoxazole for 4 hours. There was no major change in pH at time zero and at various time points. Microscopically, no particles were detected for at least 4 hours and 2 hours when intravenous co-trimoxazole was diluted at 1:25 or 1:20 and 1:15 v/v, respectively. More than 1200 particles/mL were detected after 2.5 hours of storage when intravenous co-trimoxazole was diluted at 1:15 v/v.

Conclusions Intravenous co-trimoxazole is stable over a period of 4 hours when diluted with 380 mL of glucose 5% solution (1:20 v/v) and for 2 hours when diluted with 280 mL glucose 5% solution (1:15 v/v).

INTRODUCTION

Intravenous sulfamethoxazole and trimethoprim (co-trimoxazole) is the first-line treatment for *Pneumocystis jiroveci* pneumonia (PJP).^{1 2} PJP is uncommon in general population; however, immunosuppressed patients such as those receiving chemotherapy, patients with immune deficiency syndrome or post-transplant patients are at

high risk.^{3 4} The recommended dose of intravenous co-trimoxazole for PJP is 15–20 mg/kg/day of trimethoprim given every 6 or 8 hours.⁵ The recommended dilution of intravenous co-trimoxazole is 1:25 v/v,⁶ and therefore a typical patient can receive up to 2.5 L of excessive fluid per day.⁷ Given the concurrent bacterial infections in immunocompromised patients, additional fluid volume from antibiotics may lead to fluid overload causing life-threatening complications such as acute pulmonary oedema.⁸

Each 5 mL ampule of intravenous co-trimoxazole contains 80 mg of trimethoprim and 400 mg of sulfamethoxazole. Previous studies investigating the stability of such formulation indicated that intravenous co-trimoxazole undergoes precipitation when diluted at the ratio of 1:1 with various types of infusion fluids. At this dilution ratio, intravenous co-trimoxazole exceeds its solubility limit resulting in the formation of crystals.⁹ Furthermore, the commercial formulation of intravenous co-trimoxazole is prepared in a solubilising vehicle containing 45% propylene glycol. Infusion of a solution containing a high concentration of propylene glycol could increase the risk of severe hypotension, cardiac arrhythmias and renal toxicity.^{10 11}

Studying the physical and chemical stability of concentrated solution of intravenous co-trimoxazole may provide an alternative to the currently recommended 1:25 v/v solution. It is imperative to know the physicochemical stability of intravenous co-trimoxazole when diluted to less than 480 mL of diluent to avoid the risk of administration of precipitated or degradation products.⁶ Although the stability data of intravenous co-trimoxazole infusion has been available for the last 30 years,^{12–14} the current practice is limited to 1:25 v/v dilution ratio. This is because the reported studies did not mimic the current clinical practice as well as the reported results are conflicting. For example, diluted intravenous co-trimoxazole solutions were prepared and stored in glass or cellulose-based containers and the use such containers has been phased out in clinical practice with various plastic materials.¹⁵ Second, earlier studies have reported conflicting results. Dean *et al* found that diluted intravenous co-trimoxazole infusion (1:10 v/v) is chemically and physically stable for 24 hours at room temperature.¹³ However, Lesko *et al* reported that the physicochemical stability of intravenous co-trimoxazole infusion (1:10 v/v) is up to 2 hours.¹² However, Jarosinski *et al* only investigated the chemical stability of intravenous co-trimoxazole and reported that at 1:10 v/v infusion ratio it is stable for 1 hour

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Table 1 List of studies investigating the physical and chemical stability of intravenous co-trimoxazole

Authors (year)	Type of container	Temperature (C°)	Type of analysis				Diluent, dilution ratio used and the reported stability
			HPLC	Microscopy	Visual	pH	
Lesko <i>et al</i> ¹²	Volume control set (cellulose propionate)	22±1	✓		✓	✓	1:25 D5W, NS: stable for 4 hours 1:10 D5W: stable for 2 hours 1:10 NS: stable for 1 hour 1:5 D5W: stable for 0.5 hour 1:5 NS: unstable
Dean <i>et al</i> ¹³	Evacuated glass container	24±1	✓	✓	✓	✓	1:25, 1:20, 1:15 and 1:10 with D5W, ½ NS, NS and D5/ ½ NS: stable for 24 hours 1:10 with RL: stable for 12 hours
Jarosinski <i>et al</i> ¹⁴	Evacuated glass container	24±1	✓		✓	✓	1:25 D5W, NS: stable for 48 hours 1:20 D5W: stable for 24 hours 1:20 NS: stable for 12 hours 1:15 D5W: stable for 4 hours 1:15 NS: stable for 2 hours 1:10 D5W: stable for 2 hours 1:10 NS: stable for 1 hour
Curtis <i>et al</i> ¹⁶	PVC bag and glass container	23±2	✓	On selected samples	✓	✓	1:15, 1:10 D5W, NS: stable for 24 hours in PVC container 1:15, 1:10 D5W, NS: stable for 12 hours in glass container
Current study	PVC bag	21±1	✓	✓	✓	✓	1:25, 1:20 D5W: stable for 4 hours 1:15 D5W: stable for 2.5 hours 1:10 D5W: unstable

½ NS, 0.45% normal saline; D5W, 5% dextrose; D5/½ NS, 5% dextrose, 0.45% normal saline; HPLC, high-performance liquid chromatography; NS, 0.9% normal saline; PVC, polyvinyl chloride; RL, Ringer lactate.

only.¹⁴ While Curtis *et al.* reported that the stability of 1:10 v/v depends on the type of the container.¹⁶ Therefore, this study investigated the physiochemical stability of concentrated intravenous co-trimoxazole prepared using the materials and conditions currently used in clinical practice (table 1).

METHODS

Preparation of intravenous co-trimoxazole infusion bags

Intravenous co-trimoxazole infusion bags were prepared aseptically by injecting four ampoules (20 mL) of commercially available DBL sulfamethoxazole 400 mg and trimethoprim 80 mg concentrated injection BP (Hospira Limited, Melbourne, Australia) in either 480 mL (1:25 v/v), 380 mL (1:20 v/v), 280 mL (1:15 v/v) or 180 mL (1:10) of glucose solution 5% BP (Baxter Healthcare, Sydney, Australia). In total, 12 bags (three bags for each of the four dilution ratios) were prepared. Infusion bags were then stored at room temperature (20°C–22°C) for up to 4 hours. Control samples (n=3) were prepared and stored under identical conditions except that intravenous co-trimoxazole was omitted. An aliquot (5 mL) was withdrawn from each infusion bag immediately after preparation (0 hour) and after 0.5, 1, 2 and 4 hours and analysed in duplicate for the concentration of trimethoprim and sulfamethoxazole using a stability indicating high-performance liquid chromatography (HPLC) method. The sample was defined as chemically stable if retained at least 90% of its original concentration at time 0. Samples were also assessed for pH (with a pH metre), colour (visually) and particle content (microscopically) immediately after preparation and on each time of analysis.

HPLC assay

Chromatographic analyses of intravenous co-trimoxazole samples were performed on a Dionex UltiMate HPLC system

(Dionex UltiMate HPLC system, Thermo Fisher Scientific, Melbourne, Australia). The separation of analytes was performed on an ACE C₁₈ column (ACE C₁₈ column, Advanced Chromatography Technologies, Edinburgh, UK, 150×4.6 mm). The mobile phases were composed of 0.1% trifluoroacetic acid (TFA) in Milli-Q water (mobile phase A) and 0.1% TFA in acetonitrile (mobile phase B). The gradient flow for mobile phase B was as follows: 10%–15% for 4 min, 15%–35% for 1 min, 35% for 1 min, 35%–10% for 1 min and 10% for another 3 min. Then the column was re-equilibrated with 10% of mobile phase B for 3 min. The temperatures of the column and sample compartments were set at 45°C and 25°C, respectively. The injection volume was 10 µL, and the detection was carried out at 242 nm.

HPLC assay performance

Standard solutions containing 6.25, 12.5, 25, 50, 100 and 200 µg/mL of trimethoprim USP micronised (PCCA, Baltimore, Maryland, USA) or 31.25, 62.5, 125, 250, 500 and 1000 µg/mL sulfamethoxazole (Sigma Aldrich, Sydney, Australia) were prepared in methanol as a solvent (because of poor solubility in glucose 5%) and used for linearity determination. Linearity was determined by constructing a calibration curve for the trimethoprim or sulfamethoxazole peak area using a linear regression equation. Interday (five consecutive days) precision values were investigated using peak areas with repeat analysis (n=6) of 100 µg/mL of trimethoprim and sulfamethoxazole standard solution. The intraday precision of different concentrations of trimethoprim (6.25, 12.5 and 25 µg/mL, n=6) and sulfamethoxazole (31.25, 62.5 and 125 µg/mL, n=6) was also determined. Mean intraday and interday accuracy values for trimethoprim (100 µg/mL) and sulfamethoxazole (500 µg/mL) peaks (n=6) were investigated by the regression equation as follows: (observed concentration

–expected concentration)/expected concentration $\times 100$. Mean intraday and interday retention times were determined based on peak retention time with repeat analysis of 6.25, 12.5 and 25 $\mu\text{g/mL}$ of trimethoprim and 31.5, 62.5 and 125 $\mu\text{g/mL}$ of sulfamethoxazole.

Forced degradation

The stability indicating capability of the developed HPLC method was investigated by subjecting trimethoprim and sulfamethoxazole solutions to acidic, basic or oxidative stress in capped glass vials. Trimethoprim and sulfamethoxazole solutions were mixed with equal volume of either 1N hydrochloric acid, 1N sodium hydroxide or 3% hydrogen peroxide and stored at 50°C for 100 min. Each sample was prepared in triplicate and analysed in duplicate.

Sample preparation for HPLC analysis

The stock solution (16 mg/mL) was prepared by dissolving trimethoprim and sulfamethoxazole in glucose 5% solution. The stock solution was diluted to obtain standards containing various concentration of trimethoprim (6.25, 12.5, 25, 50, 100 and 200 $\mu\text{g/mL}$) and sulfamethoxazole (31.25, 62.5, 125, 250, 500 and 1000 $\mu\text{g/mL}$). The standards were injected into the HPLC system on each day of sample analysis, and a calibration curve was generated by plotting the peak areas of trimethoprim and sulfamethoxazole against their concentrations. An aliquot withdrawn from each infusion bag was diluted with Milli-Q water to obtain a concentration that fits within the linear range of calibration curve (6.25–200 $\mu\text{g/mL}$ for trimethoprim and 31.25–1000 $\mu\text{g/mL}$ for sulfamethoxazole). Each sample was analysed in duplicate.

Analysis of particle content

Samples withdrawn from each infusion bags were thoroughly shaken and an aliquot (50 μL) was placed onto a clean glass slide (75 $\times$ 25 mm, Dako Limited, Sydney, Australia) before covering with a cover slip (24 $\times$ 50 mm). Samples were observed using light microscopy (Nikon microscope ECLIPSE 50i, Adelaide, Australia) at 5 $\times$ and 10 $\times$ optical zoom using NIS Elements software. For each sample, different fields were chosen for counting, the corners and at the centre of the cover slip. Particles were counted using the Image J software (NIH, Baltimore, Maryland, USA). Positive control (n=3) prepared by mixing 1 mL of glucose 5% with 1 mL of intravenous co-trimoxazole and negative control (n=3) prepared by mixing 1 part of glucose 5% with 1 mL of Milli-Q water were analysed the same way. Each sample was analysed in duplicate.

RESULTS

Stability indicating HPLC assay

Different chromatographic profiles were obtained for trimethoprim or sulfamethoxazole samples when stressed under acidic, basic or oxidative conditions (figure 1). With the unstressed sample, the peak of trimethoprim and sulfamethoxazole were eluted at 6.63 and 7.25 min, respectively. Exposure to hydrochloric acid 0.5N, sodium hydroxide 0.5N and 1.5% hydrogen peroxide resulted in the loss of 10%, 35% and 55% of trimethoprim and 20%, 75% and 80% of sulfamethoxazole, respectively. More importantly, the degradation products of trimethoprim or sulfamethoxazole did not interfere with the peaks of trimethoprim and sulfamethoxazole, suggesting the developed HPLC method is suitable for the stability analysis of trimethoprim and sulfamethoxazole.

HPLC assay validation

The HPLC assay performance results are shown in online supplementary table and supplementary figure. Linearity (estimated by r^2) was greater than 0.999 with six different concentrations of trimethoprim (6.25, 12.5, 25, 50, 100 and 200 $\mu\text{g/mL}$) and sulfamethoxazole (31.25, 62.5, 125, 250, 500 and 1000 $\mu\text{g/mL}$) over 5 days of analysis. The intraday precision relative standard deviation (RSD) was 0.83% for trimethoprim (100 $\mu\text{g/mL}$) and 0.58% for sulfamethoxazole (500 $\mu\text{g/mL}$). The interday precision RSD was 0.35% for trimethoprim (100 $\mu\text{g/mL}$) and 0.57% for sulfamethoxazole (500 $\mu\text{g/mL}$). The intraday precision RSDs for the trimethoprim peak at the levels of 6.25, 12 and 25 $\mu\text{g/mL}$ were less than 0.1%, 0.17% and 0.06%, respectively. The intraday precision RSDs for the sulfamethoxazole peak at the levels of 31.25, 62.5 and 125 $\mu\text{g/mL}$ were less than 0.60%, 0.63% and 0.15%, respectively. The interday accuracy RSDs for the trimethoprim peak at the levels of (200, 100 and 50 $\mu\text{g/mL}$) were less than 1.19%, 0.9% and 0.46%, respectively. The interday accuracy RSDs for the sulfamethoxazole peak at the levels of (1000, 500 and 250 $\mu\text{g/mL}$) were less than 0.6%, 0.47% and 0.23%, respectively. The intraday accuracy RSDs for the trimethoprim peak at the levels of (200, 100 and 50 $\mu\text{g/mL}$) were less than 0.1%, 0.36% and 0.17%, respectively. The intraday accuracy RSDs for the sulfamethoxazole peak at the levels of (1000, 500 and 250 $\mu\text{g/mL}$) were less than 0.15%, 0.32% and 0.20%, respectively. Interday retention times RSDs for trimethoprim at the levels of 6.25, 12.5 and 25 $\mu\text{g/mL}$ were less than 0.27%, 0.65% and 0.63% and for sulfamethoxazole at the levels 31.5, 62.5 and 125 $\mu\text{g/mL}$ were less than 0.056%, 0.17% and 0.048%. Intraday retention times RSDs for trimethoprim at the levels of 6.25, 12.5 and 25 $\mu\text{g/mL}$ were less than 0.52%, 0.85% and 0.48% and for sulfamethoxazole at the levels 31.5, 62.5 and 125 $\mu\text{g/mL}$ were less than 0.46%, 0.28% and 0.42%.

Chemical stability of co-trimoxazole

The percentage of trimethoprim and sulfamethoxazole remaining in intravenous co-trimoxazole infusion before and after storage at room temperature for various time points is shown in table 2. Sulfamethoxazole retained more than 98% of its initial concentration for 4 hours at dilution ratios of 1:25 v/v, 1:20 v/v, 1:15 v/v and 1:10 v/v. The concentration of trimethoprim was found to be more than 98% at dilution ratios of 1:25 v/v, 1:20 v/v, 1:15 v/v and 1:10 v/v.

Physical stability of co-trimoxazole

The baseline pH of intravenous co-trimoxazole at 1:25 v/v, 1:20 v/v, 1:15 and 1:10 v/v dilution ratios were 9.09 ± 0.06 (mean $\pm$ SD), 9.31 ± 0.01 , 9.27 ± 0.02 and 9.37 ± 0.07 , respectively. There was no major change in the pH at time zero and at various time points. The maximum change in pH at any dilution ratio was found to be less than ± 0.4 . Visual examination of the samples under fluorescent light and against white/black background showed no evidence of colour change at any time point.

Microscopic images of the samples stored at room temperature for 4 hours are shown in figure 2. Total number of particles in the positive control were found to be more than 2000/mL. At time zero, intravenous co-trimoxazole samples at 1:25 v/v, 1:20 v/v and 1:15 v/v did not show the presence of particles. Microscopic analysis showed the absence of particles after 4 hours of storage when intravenous co-trimoxazole was diluted at 1:25 v/v or 1:20 v/v ratio. While the number of particles after 2, 3 and 4 hours of storage at 1:15 v/v dilution were estimated to

Figure 1 HPLC chromatograms of intravenous co-trimoxazole (trimethoprim and sulfamethoxazole) (A), trimethoprim before (B) and after stressing under acidic, basic or oxidative conditions at 50°C for 1 hour (C–E), sulfamethoxazole before (F) and after stressing under acidic, basic or oxidative conditions at 50°C for 1 hour (G–I). Each sample was prepared in triplicate and analysed in duplicate.

contain greater than 1200/mL, 1400/mL and 1700/mL, respectively. Intravenous co-trimoxazole at 1:10 v/v dilution was not subjected to microscopic analysis after 2 hours since particles were attached to walls of the infusion bag as early as 1.5 hours of storage at room temperature. However, the physical mechanism

behind attachment of observed particles to the surface of the PVC bag requires further investigation. Visual analysis did not reveal the presence of particles in any of the solutions except in a 1:10 v/v diluted solution, which was stored at room temperature for 1.5 hours.

Table 2 The percentage of intravenous co-trimoxazole remaining in the glucose 5% infusion stored at room temperature for various time points. Results are expressed as mean±SD. Each intravenous co-trimoxazole infusion bag was prepared in triplicate and each sample analysed in duplicate

Dilution ratio	Drug	Initial concentration	% Initial drug concentration remaining			
		0 hour (mg/mL)	0.5 hour	1 hour	2 hours	4 hours
1:25 v/v	Trimethoprim	0.63	101±0.8	100±0.6	100±1.0	100±1.5
	Sulfamethoxazole	3.18	100±6.2	100±2.2	100±4.5	100±6.3
1:20 v/v	Trimethoprim	0.8	100±0.8	100±0.4	99±0.5	99±1.2
	Sulfamethoxazole	3.99	100±2.8	100±1.9	100±2.2	99±6.3
1:15 v/v	Trimethoprim	1.06	99±0.5	99±0.4	99±0.5	98±0.4
	Sulfamethoxazole	5.29	99±2.2	100±4.7	99±2.6	98±2.8
1:10 v/v	Trimethoprim	1.64	100±0.5	99±1.0	100±1.6	100±0.7
	Sulfamethoxazole	8.12	100±2.7	99±5.2	100±5.9	100±4.7

DISCUSSION

In the current study, HPLC analysis showed that intravenous co-trimoxazole retained more than 98% of its trimethoprim and sulfamethoxazole concentration over a period of 4 hours at 1:25 v/v, 1:20 v/v, 1:15 v/v and 1:10 v/v dilutions. However, microscopic analysis, contrary to HPLC analysis, showed that intravenous co-trimoxazole is physically stable for 4, 2 and 0 hours at 1:25 and 1:20 v/v, 1:15 v/v and 1:10 v/v dilution ratios. Intravenous co-trimoxazole infusion at 1:15 v/v dilution contained more than 1700 particles/mL (greater than 500 000 particles/infusion bag) after 4 hours of storage and at 1:10 v/v contained more than 180 particles/mL (greater than 30 000 particles/infusion bag) at 0 hour. The discrepancy was likely to be due to the detection sensitivity of the HPLC method, where the sample was required to be diluted considerably. For example, an aliquot (50 µL) withdrawn from 1:15 v/v diluted intravenous co-trimoxazole was mixed with 950 µL of Milli-Q water before injecting it into the HPLC system. Also, dilution was made with water, which may have increased the solubility of the particles not visible through unaided eye. There are advantages and disadvantages of using light microscopy in particle count. Instruments such as Coulter Multisizer can be used to

determine particle content present in intravenous co-trimoxazole solution. However, the instrument required considerable dilution of the sample increasing the possibility of dissolving the particles present in the sample. Unlike Coulter Multisizer, in microscopy analysis, the sample is observed without any dilution avoiding the possible risk of particle dissolution. Dissolution of the particles was confirmed by mixing intravenous co-trimoxazole (1 mL, n=3) with 4 mL of glucose 5% (1:5 v/v) in a plastic phial. Visual inspection of the sample showed the presence of particles after 30 min of storage at 4°C. The sample was then (50 µL) diluted 100 times with Milli-Q water before subjecting to microscopic and HPLC analyses. Microscopic analysis did not show the presence of particles. HPLC analysis showed that the sample contained more than 99% of its initial trimethoprim and sulfamethoxazole concentration.

Intravenous injections of particles larger than 5 µm can block the healthy blood vessels of vital organs. Blockage of healthy blood vessels may decrease the required blood supply resulting in ischaemic episodes.¹⁷ Injection of particles smaller than 5 µm can cause cardiac and vascular damage including development of atherosclerotic lesions.¹⁸ At 1:15 v/v dilution, more than 60% of the observed particles were found to be between 10 µm and 20 µm. Approximately 25% were larger than 20 µm and 15% were smaller than 5 µm. At 1:10 v/v dilution, 75% of the particles were found to be larger than 20 µm. The size of approximately 25% of the particles was found to be in the range of 1–20 µm. The intravenous co-trimoxazole solution diluted at 1:10 and 1:15 v/v ratio did not meet the USP criteria¹⁹ in terms of accepted range of particles after 0 and 2.5 hours of storage respectively at room temperature. As shown in table 3, when diluted at 1:10 v/v, the number of particles lower than 10 µm, between 10 µm and 25 µm and greater than 25 µm were found to be more than 15, 40 and 130/mL, respectively, at time zero. Similarly, when diluted at 1:15 v/v, the number of particles lower than 10 µm, between 10 µm and 25 µm and greater than 25 µm were found to be more than 170, 700 and 130/mL, respectively after its storage at room temperature for 2.5 hours. Through unaided eyes, a bag containing intravenous co-trimoxazole (1:10 v/v) stored at room temperature for 1.5 hour showed visible particles (figure 3A). However, a bag containing intravenous co-trimoxazole (1:15 v/v) stored at room temperature for 2.5 hours did not show visible particles as shown in figure 3B. However, microscopic analysis revealed the presence of particle in 1:15 v/v diluted bag stored at room temperature for 2.5 hours (figure 3D). In light of such findings, we recommend that future studies investigating the stability of an extemporaneously prepared infusion should include microscopic analysis to confirm the results obtained through HPLC analysis. The

Figure 2 Microscopic images (10× magnification) of positive control prepared by mixing 1 mL of glucose 5% with 1 mL of intravenous co-trimoxazole at time 0, 2 and 4 hours (A1, A2 and A3), intravenous co-trimoxazole infusion (1:20 v/v) stored at room temperature for 0, 2 and 4 hours (B1, B2 and B3) and intravenous co-trimoxazole infusion (1:15 v/v) stored at room temperature after 0, 2 and 4 hours (C1, C2 and C3). Each sample was prepared in triplicate and analysed in duplicate.

Table 3 Number and size of particles determined by light microscopy in intravenous co-trimoxazole samples diluted at various ratios. For each dilution ratio, three infusion bags were prepared and kept at room temperature up to 4 hours. An aliquot was withdrawn from each bag at various time points and was analysed in duplicate. Results are shown as $\pm$ SD. ‘–’ means no particle was present in the sample. ‘#’ means the sample was not analysed at that particular time point

Dilution ratio (v/v)	Particle size (μ m)	Number of particles/mL					
		0 hour	1 hour	2 hours	2.5 hours	3 hours	4 hours
1:10	<10	15 $\pm$ 2	#	#	#	#	#
	10–25	40 $\pm$ 5	#	#	#	#	#
	>25	130 $\pm$ 17	#	#	#	#	#
1:15	<10	–	–	170 $\pm$ 9	#	#	#
	10–25	–	–	700 $\pm$ 13	#	#	#
	>25	–	–	300 $\pm$ 6	#	#	#
1:20	<10	–	–	–	–	–	–
	10–25	–	–	–	–	–	–
	>25	–	–	–	–	–	–
1:25	<10	–	–	–	–	–	–
	10–25	–	–	–	–	–	–
	>25	–	–	–	–	–	–

microscopic analysis is especially important when the samples required further dilution prior to HPLC analysis.

Intravenous co-trimoxazole infusions at 1:15 v/v and 1:10 v/v dilutions were found to be stable for 2 hours and 0 hour, respectively. Our results are not consistent with the previously reported results.^{12–14} One possible explanation could be the use of a different type of container. Deans *et al*¹³ and Lesko *et al*¹² prepared the infusion solution (1:10 v/v) by mixing the 10 mL of intravenous co-trimoxazole with 90 mL of diluent in a glass container. However, Jarosinski *et al*¹⁴ prepared the solution (1:10 v/v) by mixing the 5 mL of intravenous co-trimoxazole with 45 mL of diluent in a plastic container composed of cellulose propionate. It has been reported that the stability of an antibiotic can be greatly affected depending on the type and

material of the storage container.^{20,21} In this study, intravenous co-trimoxazole infusions were prepared using the techniques and materials (eg, 20 mL of intravenous co-trimoxazole mixed with 480 mL of glucose 5% solution in a PVC infusion bag) used in current practice, making the results more clinically relevant.

Excessive fluid overload has been linked to several physiological adverse effects such as acute lung injury, acute pulmonary oedema and acute kidney injury.²² Patients receiving intravenous co-trimoxazole infusion are often immunocompromised and susceptible to adverse drug events from medications and other medical interventions. Adding an unnecessary complication of fluid overload may potentially harm this patient population. Usual duration for intravenous co-trimoxazole infusion is 60–90 min.⁶ However, the information on the physical and chemical stability of intravenous co-trimoxazole infusion solution longer than 90 min would provide flexibility to the hospital healthcare professionals for the preparation of extemporaneous intravenous co-trimoxazole infusion bag and administration of such infusion solution. Therefore, the current study investigated the stability of intravenous co-trimoxazole infusion for up to 4 hours rather than for 90 min. The important finding of the current study is that intravenous co-trimoxazole at 1:15 v/v dilution (60% of the recommended dilution volume) is stable for up to 2 hours. Administration of intravenous co-trimoxazole infusion (1:15 v/v) will reduce the required intravenous volume by 1000 mL/day and therefore can decrease the risk of fluid overload in patients with severe PJP. Based on our findings, we recommend the minimum dilution ratio for intravenous co-trimoxazole infusion should be 1:15 v/v, and the infusion should be administered within 2 hours of its preparation because of the risk of formation after that time.

In the current study, PVC bag containing 250 mL of glucose 5% was used for the preparation of intravenous co-trimoxazole infusion at 1:10 v/v dilution ratio. The final volume of glucose 5% present in 1:10 v/v solution was 180 mL. However, PVC bag containing 500 mL of glucose 5% was used to prepare intravenous co-trimoxazole infusion at 1:25 v/v, 1:20 v/v or 1:15 v/v dilution ratio. The final volume of glucose 5% present in 1:25 v/v, 1:20 v/v or 1:15 v/v solution was 480 mL, 380 mL or 280 mL. Therefore, the ratio of bag surface area to infusion volume was lower with 1:10 v/v diluted intravenous co-trimoxazole than the other dilution ratios. The precipitation of

Figure 3 Photographs of infusion bag A and infusion bag B. Infusion bag A was prepared by mixing 20 mL of intravenous co-trimoxazole with 180 mL of glucose 5% and stored at room temperature for 1.5 hours. Infusion bag B was prepared by mixing 20 mL of intravenous co-trimoxazole with 280 mL of glucose 5% and stored at room temperature for 2.5 hours. Microscopic images (10 $\times$ magnification) of an aliquot (50 μ L) withdrawn from the infusion bag A (C) and infusion bag (B) after being stored at room temperature for 1.5 and 2.5 hours at room temperature (C and D).

What this paper adds

What is already known on this subject

- The dose of intravenous co-trimoxazole for *Pneumocystis jirovecii* pneumonia is 15–20 mg/kg/day of trimethoprim given every 6 or 8 hours.
- The recommended dilution of intravenous co-trimoxazole is 1:25 v/v, and therefore a typical patient can receive up to 2.5 L of excessive fluid per day.
- Given the concurrent bacterial infections in immunocompromised patients, additional fluid volume from antibiotics may lead to fluid overload causing life-threatening complications such as acute pulmonary oedema.

What this study adds

- Intravenous co-trimoxazole at 1:20 v/v and 1:15 v/v dilution (60% of the recommended dilution volume) is physically and chemically stable for up to 4 and 2 hours, respectively.
- Administration of intravenous co-trimoxazole infusion (1:15 v/v) will reduce the required intravenous volume by 1000 mL/day and therefore can decrease the risk of fluid overload in patients with severe *P. jirovecii* pneumonia.

intravenous co-trimoxazole was observed at time zero when diluted at 1:10 v/v. However, the precipitation of intravenous co-trimoxazole was not observed for at least for 4 hours when diluted at 1:20 v/v or 1:25 ratio. Therefore, increasing ratio of bag surface area to solution volume is less likely to be responsible for the precipitation of intravenous co-trimoxazole. However, the current study did not examine the effect of bag surface area on the physical stability of intravenous co-trimoxazole solution. Trimethoprim and sulfamethoxazole are administered intravenously to the patient as a combination formulation, and the presence of particles in the infusion solution can have severe medical consequences. The current study did not attempt to determine the composition of the observed particles. However, future studies should examine whether the formation of particles was due to sulfamethoxazole, trimethoprim, combination of sulfamethoxazole and trimethoprim or adducts from the active drug ingredients and the PVC infusion bag material.

We did not examine if 1:15 v/v diluted solution of intravenous co-trimoxazole can be safely administered using peripheral line access or will require central line administration. This issue was beyond the scope of our pharmaceutical study and should be investigated in a clinical study of intravenous co-trimoxazole administration in real-life settings. In light of our experimental results, we suggest that the 1:15 v/v diluted solution should be administered via a large vein and preferably via a central venous catheter as a precautionary measure. This is because intravenous administration of intravenous co-trimoxazole has been associated with localised irritation and thrombophlebitis in a small number of patients.

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