A Physicochemical Approach to the Investigation of the Stability of Trimethoprim-Sulfamethoxazole (Co–Trimoxazole) Mixtures for Injectables

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Abstract □ The stability of the trimethoprim/sulfamethoxazole (1:5, w/w) combination suitable for administration by injection was investigated to determine the nature of solid phases that can separate after dilution with infusion fluids. Phase-solubility analysis was performed on the binary system in water and in buffered aqueous media (pH 7 and 9), thus allowing a comprehensive picture of solid–solution compositions. Commercial samples of this combination were tested for solid phases separating after dilution with various infusion fluids. The interaction between trimethoprim and sulfamethoxazole, forming a 1:1 molecular compound with low solubility in water, is mainly responsible for the physicochemical properties of mixtures of these drugs in solution. Other solid phases (i.e., trimethoprim monohydrate and sulfamethoxazole emihydrate) can separate on long-term standing of solutions, depending on the value of the pH of the medium and the fluid used for dilution.

The combination of the antifolate drug 2,4-diamino-5-(3,4,5-trimethoxybenzyl)pyrimidine (trimethoprim, TMP) with the sulfa drug 3-sulfanilamido-5-methylisooxazole (sulfamethoxazole, SMZ) in a 1:5 (w/w) ratio (co-trimoxazole) represents a very popular chemoterapeutic agent that has been widely used in the last two decades and lately has been receiving considerable attention for the therapy of opportunistic infections in patients with HIV; namely, pneumonia by *Pneumocystis carinii*, shigellosis, and urinary tract infections. When diluted before administration, the intravenous (iv) form of co-trimoxazole is known to have stability problems,[1–4] as underlined by manufacturers (see package inserts: Roche, Bactrim; Burroughs Wellcome, Septrin) who recommend administration within 6 h after dilution at 1:25 and a maximum infusion time of 1.5 h. In particular, it has been reported that depending on both the dilution ratio and some of the suggested injection vehicles, a precipitate can form in ampuls or iv tubings. No attempt has been made to determine the exact nature of the crystalline precipitate, but when diluted 1:10 with normal saline, TMP concentration dropped to 50% after 48 h[2] and to 38% of the initial concentration after 120 h,[1] whereas SMZ concentration was reported to remain unaltered in some cases and decreased in others.

Moreover, the pH value of the diluted solution has been recognized to play a definite major role in the stability profile of this formulation. The commercial product before dilution has a pH value of ~10, whereas after dilution with normal saline, the pH is lowered to 9.5–9.1, depending on the final volume reached. On the other hand, a 1:10 dilution of the commercial product with phosphate buffer (pH 7.4) leads to a massive precipitation of both TMP and SMZ from a solution with final value of pH 7.8.[1] Other authors of papers regarding the evaluation of solubility or dissolution rates of TMP and SMZ alone or in combination repeatedly claim that TMP solubility is strongly depressed by the presence of SMZ, but they fail to give any conclusive interpretation.[5–7]

In aqueous solutions, TMP and SMZ form a crystalline molecular compound with 1:1 mol:mol stoichiometry and low water solubility whose physicochemical and technological properties have been determined.[8,9] Also, when TMP is recrystallized from alkaline aqueous media, it forms a crystalline hydrate of peculiar morphology.[10] Unfortunately none of these possible phenomena was considered when investigating the stability problems of co-trimoxazole drug products for injection. Therefore, it seems necessary to explore this topic with the aim of describing and defining the nature of both the solid and liquid phases present at equilibrium in aqueous media at different pH values for the TMP–SMZ binary system. Investigations were performed on solid phases isolated from solutions that obtained after dilution with injection fluids of commercial samples of co-trimoxazole (Bactrim, Roche). Thermal analyses [differential scanning calorimetry (DSC) and thermogravimetric analysis (TGA)], together with hot-stage microscopy, were used to assess the composition of solid phases. High-performance liquid chromatography (HPLC) allowed the simultaneous determination of TMP and SMZ in solution.

Experimental Section

Materials—TMP (form I; mp, 199–200 °C) and SMZ (form I; mp, 169–170 °C) were obtained by recrystallization of commercial samples (Roche, Milan, Italy) from water:ethanol mixtures (1:9, v/v). Commercial phosphate and borate buffers, (Normex, Carlo Erba, Milan, Italy), were used for the preparation of standard solutions at two pH values (7 and 9). No correction was adopted for ionic strength. Double-distilled, CO_2-free water was used throughout all the solubility experiments. Commercial Bactrim ampuls (TMP, 80 mg; SMZ, 400 mg; ethanolamine, 11 mg; ethanol, 500 mg; propylene glycol, 2.05 g; sodium hydroxide, 65 mg; water for injection, q.s. to 5 mL) and the infusion fluids recommended by the manufacturer [5% dextrose, 10% dextrose, Hartmann's solution, Ringer's solution, 6% dextrose (Macrodex, Pharmacia AB, Uppsala, Sweden), 0.9% sodium chloride, 0.45% sodium chloride + 2.5% dextrose] were purchased commercially.

Apparatus—DSC measurements were obtained with a Mettler TA3000 thermal analyzer equipped with DSC-25 and TG-50 cells. Samples (3–4 mg) were weighed and scanned in aluminum pans from ambient temperature to 230 °C at a heating rate of 10 K min^{-1}. Hot-stage microscopy (HSM) was performed with a Mettler FP80HT hot stage equipped with an FP82HT cell and a Reichert Microstar IV microscope. Solutions were analyzed for TMP and SMZ contents by a previously published HPLC (Varian 9010) method,[1] with a μ-Bondapak C_{18} column (Waters, Millford, MA; 3.9 × 300 mm; 10-μm particle size). Measurements of pH were made with a Mettler DL40GP pH meter.

Methods—Phase-solubility analysis[11] of the binary system TMP–SMZ in water and in buffered solutions was achieved by accurately weighing a fixed amount (≈100 mg) of TMP with increasing quantities of SMZ in screw-capped glass bottles (50-mL capacity), so that in the last containers, stoichiometric excess of SMZ, with respect to TMP and calculated according to the 1:1 (mol:mol) interaction between components, was always present. Thirty milliliters of water or buffered solution were then added to the bottles that were mechani-

[⊗] Abstract published in *Advance ACS Abstracts*, July 15, 1995.

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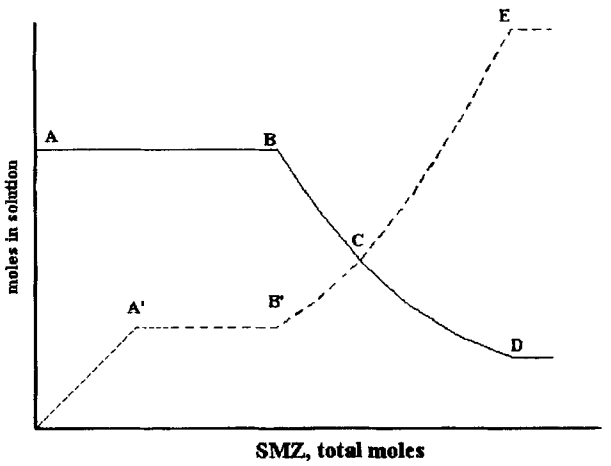

Figure 1—Theoretical phase-solubility diagram of the TMP–SMZ system. Key: (- - -) SMZ; (—), TMP.

cally agitated at constant temperature (25 ± 0.5 °C). After equilibrium was attained (48–72 h), the suspension was filtered (Millipore, 0.22 μm), and the filtrate, after proper dilution, was assayed for TMP and SMZ contents by HPLC. The solid residue was dried under a stream of air and tested for thermal behavior. When using water, the pH of the filtrate was determined.

Bactrim ampuls were diluted 1:10 with infusion fluids in 50-mL flasks. After 24 h, crystals were isolated by filtration and tested for thermal behavior (DSC and HSM), and the filtrate was assayed for TMP and SMZ contents by HPLC.

Results and Discussion

Phase Solubility Analysis—According to previous results,[8] the 1:1 interaction compound between TMP and SMZ, hereafter referred to as TSC, readily precipitates in aqueous media as thin, long needles and shows an aqueous solubility lower than its components. In contrast, in solution, TSC is completely separated, as demonstrated by solution-state NMR spectral.[12]

Given these results and taking into account the experimental conditions outlined in the previous section, the theoretical phase-solubility diagram of this ternary system (water, TMP, and SMZ), at constant pressure and temperature, can be drawn (Figure 1).

In Figure 1, the length AB depends on the initial amount of solid TMP present at equilibrium. The concentration of TMP in solution (i.e., TMP solubility) along AB does not change, whereas that of SMZ rises from zero to A′ (slope = 1) and then remains constant. Starting from A′, there is a progressive transformation of solid TMP into TSC that is caused by the addition of SMZ, and the system is invariant (three phases and three independent components, TMP, SMZ, and water). The relevant equilibria are as follows:

$$\text{TMP (s)} \leftrightarrow \text{TMP (s)} \quad \text{s = solid; s = solution} \qquad (1)$$

$$\text{TMP (s)} + \text{SMZ (s)} \leftrightarrow \text{TSC (s)} \qquad (2)$$

$$\text{TMP (s)} + \text{SMZ (s)} \leftrightarrow \text{TSC (s)} \qquad (3)$$

When all solid TMP is converted into TSC, the addition of SMZ lowers the concentration of TMP and increases that of SMZ (BD and B′E, respectively, in Figure 1). In C, the system contains equivalent amounts of TMP and SMZ and, therefore, the solution at equilibrium contains equivalent amounts of both components. Further addition of SMZ depresses TSC solubility down to D and increases the concentration of SMZ up to E, where SMZ in solution reaches its solubility value; at this point, solid SMZ separates as a new solid phase and the system becomes once more invariant. Solubilities of TMP, SMZ, and TSC are the only parameters needed for describing the entire system with simple calculations, provided that the amount of initially present TMP is known and the dissociation of TMP and SMZ, as a function of pH, is neglected.

Table 1—Solubilities (mol/L × 1000) of TMP, SMZ, and TSC in Aqueous Media

	Water	pH 7 Buffer	pH 9 Buffer
TMP	2.11	2.23	1.41
SMZ	1.37	12.11	>100
TSC	1.082	1.42	unstable

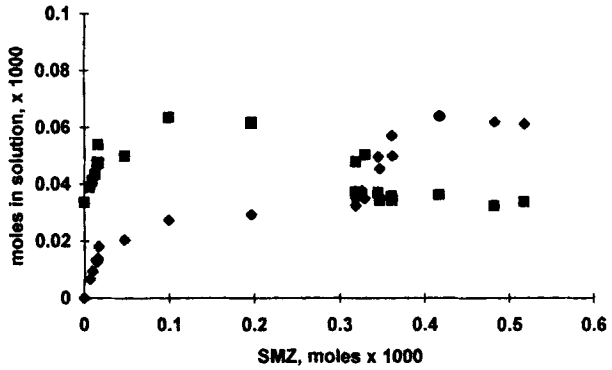

Figure 2—Experimental phase-solubility diagram of the TMP-SMZ system in water (30 mL). Key: (◆) SMZ; (■) TMP.

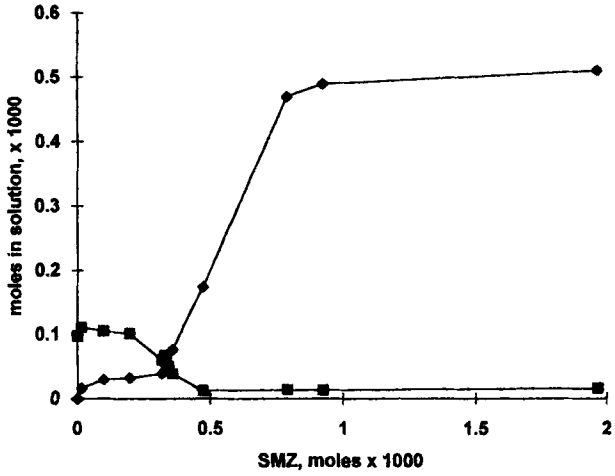

Figure 3—Experimental phase-solubility diagram of the TMP-SMZ system in pH 7 buffer (30 mL). Key: (◆) SMZ; (■) TMP.

Solubility data for TMP, SMZ, and TSC in the aqueous media investigated are reported in Table 1. The experimental phase-solubility diagrams (in water and in buffers) are reported in Figures 2–4. In all cases, the amounts of TMP and SMZ in solution refer to the experimental setup (i.e., 30 mL of aqueous medium and 100 mg of solid TMP). From a general inspection of Figures 2 and 3, the experimental curves are in good agreement with the assumed theoretical model; this may seem less true when phase-solubility studies were carried out in water, but this can be explained by the lack of pH control. In fact, CO_2-free water saturated with TMP shows an initial pH value of ~8, which is progressively shifted toward acidity by the addition of SMZ (Figure 5); the initial portion of the TMP curve drawn in Figure 2 is therefore not

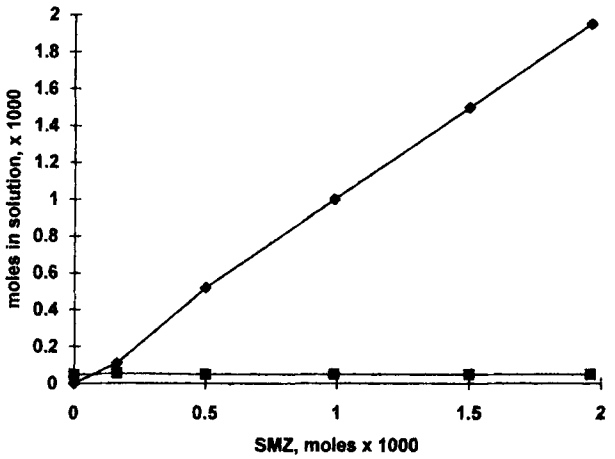

Figure 4—Experimental phase-solubility diagram of the TMP-SMZ system in pH 9 buffer (30 mL). Key: (◆) SMZ; (■) TMP.

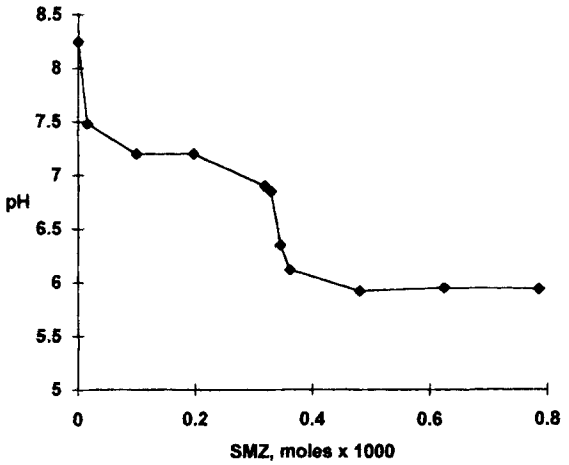

Figure 5—pH profile for phase-solubility analysis in water (see Figure 2).

linear (as in the corresponding AB tract in Figure 1) because of the increase in TMP solubility.

Examination of the data obtained in aqueous alkaline media (Figure 4) indicates that because the high value of the apparent solubility of SMZ prevents the formation of TSC, one solid phase is missing; therefore, the system cannot be invariant both for TMP and SMZ.

Analysis of Solid Phases—All solid phases isolated at equilibrium, corresponding to each experimental point reported in the graph, were tested for thermal behavior and crystal habit. To this aim, the phase diagram of the binary system TMP–SMZ, drawn by the method already discussed by Giordano et al.,[8] is reported in Figure 6 to facilitate the interpretation of endotherms in DSC curves. This phase diagram shows two stable eutectic fusions, E1 and E2, at 158 °C (x_1 = 0.8), and 178 °C (x_1 = 0.4), respectively, and a metastable eutectic fusion, T_e, at 128 °C. This fusion is characterized in the DSC traces by an endo–exo effect (not shown by any of the recovered solid samples) and is ascribed to the formation of the 1:1 molecular compound on the basis of direct observation at HSM.

Thermal traces relevant to isolated solid phases containing TMP + TSC, SMZ + TSC, and TSC are shown in Figures 7, 8, and 9, respectively. These traces represent the only possible combinations of solid phases when TMP and SMZ are suspended together in aqueous media (water and pH 7 buffer). Actually, in our experiments and in some other cases, other solid phases separated on long-term standing of aqueous suspensions; for examples, SMZ emihydrate[13,14] (when excess

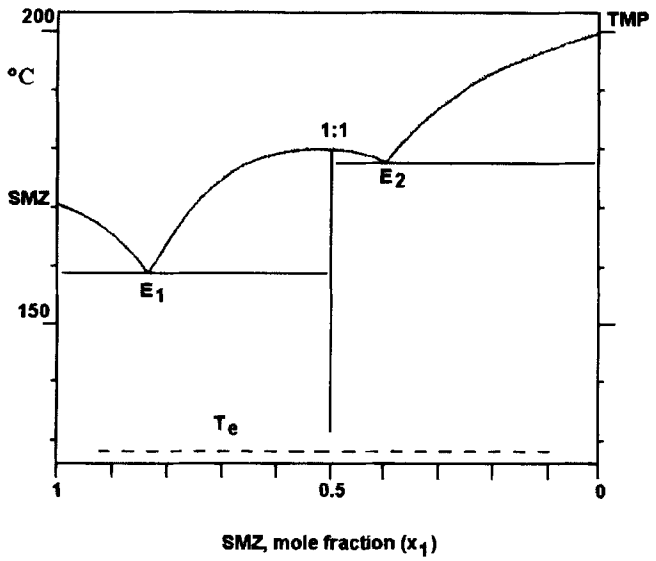

Figure 6—Phase diagram of the TMP-SMZ binary system.

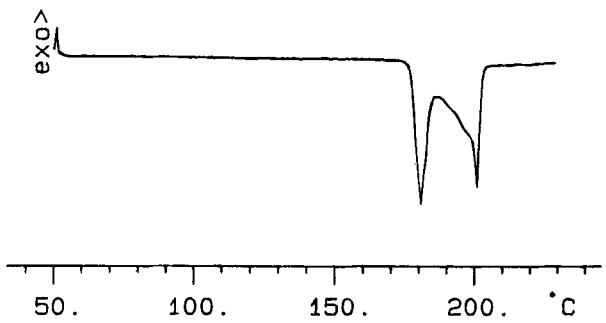

Figure 7—DSC trace of TSC + TMP mixture.

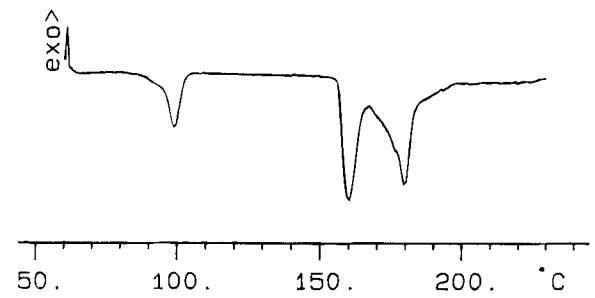

Figure 8—DSC trace of TSC + SMZ mixture.

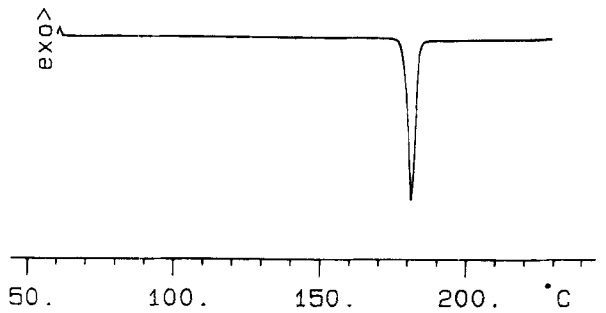

Figure 9—DSC trace of TSC.

SMZ is present, as shown in Figure 8) and TMP monohydrate[10] (after dilution of Bactrim ampuls with Hartmann's solution, see next section).

Dilution of Bactrim with Infusion Fluids—Data concerning values of pH, SMZ:TMP ratios in solution, and the

Table 2—Solid Phases and Compositions of Solutions of Bactrim after Dilution (1:1 v:v)

Infusion Fluid	pH[a]	SMZ:TMP[b]	Solid Phases
5% Dextrose	8.9	8.7	TMP
10% Dextrose	8.9	10.6	TMP
Hartmann's solution	9.1	14.8	TMP + TMP·H_2O
Ringer's solution	9.2	15.1	TMP
6% Dextrose (Macrodex)	9.3	12.5	TMP
0.9% Sodium chloride	9.4	16.1	TMP
0.45% NaCl + 2.5% Dextrose	9.1	11.6	TMP

[a] After dilution and standing for 24 h. [b] Weight to weight ratio in solution.

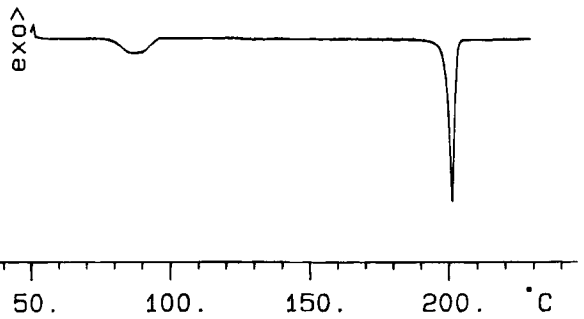

Figure 10—DSC trace of TMP monohydrate.

nature of isolated solid phases 24 h after dilution are reported in Table 2. In all cases, SMZ concentration was within ±5% of the expected value, whereas that of TMP dropped to 30% of the initial value in those media showing the highest values of pH, as a consequence of solubility lowering.

Crystalline TMP was always recovered in these experiments, irrespective of the infusion fluid used for dilution; a mixture of TMP and TMP monohydrate (as long, thin, hairy crystals) formed when Hartmann's solution was used. The thermal behavior of TMP monohydrate, whose crystal habit was already reported,[10] is shown in Figure 10. Dehydration occurs in the 70–80 °C range and final fusion is as TMP form I.

At this point, interpretation of some results previously reported by McDonald and Faridah[1] and our results regarding the progressive decrease of TMP and SMZ contents in solution at different time intervals when the injection was diluted 1:10 with phosphate buffer (pH 7.4) or normal saline is possible. In Figure 11, the calculated percentages of each component remaining in solution with respect to the initial expected concentrations (when starting with a 1:5, w/w, mixture of TMP and SMZ) were plotted (straight line) assuming that the loss of both components is due to the formation of solid TSC (TMP:SMZ, 1:1 in moles), which subtracts, by precipitation, equivalent amounts of both TMP and SMZ from the solution. TMP, being the minor component, readily drops as TSC precipitation goes on to a very low percent of the initial amount; on the other hand, SMZ, assuming that TSC is completely formed, will diminish to ~83% of the initial amount. The experimental data at different time intervals taken from another report[1] that are relevant to dilution with phosphate buffer fit rather well with the calculated straight line because solid TSC can separate in this medium; in one case (32.8% of the initial amount of TMP and 100% of the initial amount of SMZ), 120 h after dilution with normal saline and final value of pH 9.5, there is a significant difference due to the instability of TSC in alkaline media and separation of solid TMP.

Conclusions

To reach a reasonable explanation of the different and in some cases conflicting results reported in the literature, the

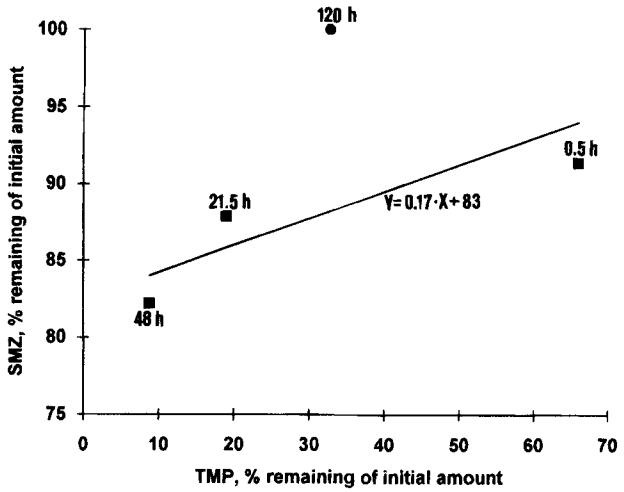

Figure 11—Percentages of the initial amounts (1:5, w/w, TMP:SMZ) for TMP and SMZ remaining in solution after dilution with normal saline (●) and with phosphate buffer (■) at different time intervals (experimental values taken from ref 1); the straight line was calculated assuming a 1:1 (mol:mol) interaction and separation of solid TSC from a solution containing TMP and SMZ in a 1:5 (w/w) ratio.

formation of TSC should be primarily taken into account. Phase-solubility analysis of the TMP–SMZ binary system in different aqueous media allowed a comprehensive picture of this peculiar combination. The solubility profile of this system, because of the formation of TSC, can be correctly assessed only by exploring it in a wide range of solid mixtures containing, at equilibrium, both excess TMP and TSC or excess SMZ and TSC. Some of the reported studies seem to have focused simply on 5:1 (w/w) SMZ:TMP solid mixtures and therefore lack essential information. When investigating the solubilities of TMP and SMZ separately and together, it is necessary to know the exact composition of the binary system: in fact, if water is saturated both with TMP and SMZ, which react rapidly to form TSC, the solid phases at equilibrium can be TSC (if saturated with equimolecular amounts of TMP and SMZ), TSC + SMZ (if excess SMZ, with respect to TSC stoichiometry, was used to saturate the water solution), or TSC + TMP (in the other case). In the first situation, the solution composition will be given by TSC solubility, and TMP and SMZ will be in the same ratio, 1:1 (mol:mol), as in the solid phase. In both other cases, the solution composition will be given by excess drug solubility plus TSC solubility, which is of course lowered according to the mass law.

The system described here represents a peculiar example of noncompatibility induced by dilution of a drug product for injections. Three main effects can be ascribed to the unusual (from a clinical point of view) high alkaline pH of this drug product for iv infusion: they are, the solubilization of SMZ, the consequent instability of TSC, and the precipitation of TMP (and in some cases of TMP monohydrate). In this respect, the possibility of obtaining stable systems around neutral pH values by the addition of cyclodextrins[1,15] appears very promising.

References and Notes

1. McDonald, C.; Faridah, *J. Parent. Sci. Technol.* **1991**, *45*, 147–151.
2. Jarosinski, P. F.; Kennedy, P. E.; Gallelli, J. F. *Am. J. Hosp. Pharm.* **1989**, *46*, 732–737.
3. Lesko, L. J.; Marion, A.; Ericson, J. *Am. J. Hosp. Pharm.* **1981**, *38*, 1004–1006.
4. Deans, K. W.; Lang, J. R.; Smith, D. E. J. *Am. J. Hosp. Pharm.* **1982**, *39*, 1681–1684.
5. Meshali, M.; El-Sabbagh, H.; Ghanem, A.; Foda, A. *Pharmazie* **1983**, *38*, 403–406.

6. Meshali, M.; El-Sabbagh, H.; Ramadan, I. *Pharmazie* **1984**, *39*, 407–408.
7. Dahlan, R.; McDonald, C.; Sunderland, B. *J. Pharm. Pharmacol.* **1987**, *39*, 246–251.
8. Giordano, F.; Bettinetti, G.; La Manna, A.; Ferloni, P. *Il Farmaco, Ed. Sci.* **1977**, *32*, 889–896.
9. Caramella, C.; Giordano, F.; Bettinetti, G.; Colombo, P.; Conte, U.; La Manna, A. *Il Farmaco Ed. Pr.* **1980**, *35*, 277–285.
10. Bettinetti, G.; Giordano, F.; La Manna, A.; Giuseppetti, G. *Boll. Chim. Farm.* **1978**, *117*, 522–529.
11. Higuchi, T.; Connors, K. A. *Adv. Anal. Chem. Instr.* **1965**, 117–212.
12. Fruttero, R.; Hawkes, G. E.; Randall, E. W.; Gasco, A.; Groombridge, C. J. *J. Chem. Soc., Perkin Trans. II* **1988**, 1863–1866.
13. Graf, E.; Beyer, C.; Abdallah, O. *Pharm. Ind.* **1982**, *44*, 1071–1074.
14. Abdallah, O.; Abd El-Fattah, S. *Pharm. Ind.* **1984**, *46*, 970–971.
15. Muñoz de la Pena, A.; Salinas, F.; Durán-Merás, I.; Moreno, M. D. *Anal. Lett.* **1994**, *27*, 1893–1906.

Acknowledgments

We thank Prof. Amedeo Marini for helpful discussion and criticism and Dr. Paola Perugini for skillful technical assistance in performing HPLC determinations. The financial support of MURST (60% funds) is also gratefully acknowledged.

JS940653B