

Crystallization of Toxic Glycol Solvates of Rifampin from Glycerin and Propylene Glycol Contaminated with Ethylene Glycol or Diethylene Glycol

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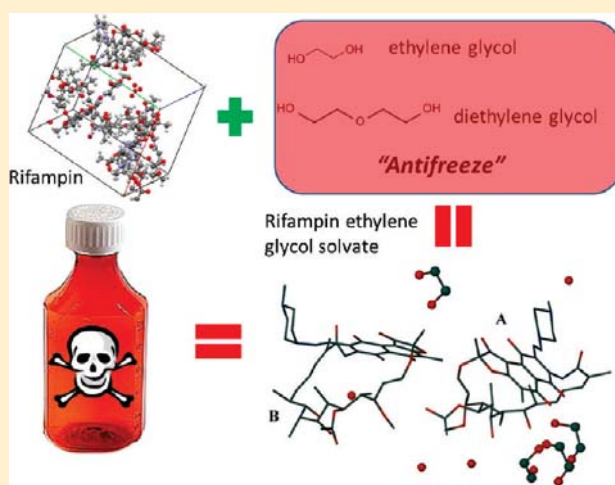
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ABSTRACT: This study was initiated when it was suspected that syringe blockage experienced upon administration of a compounded rifampin suspension was caused by the recrystallization of toxic glycol solvates of the drug. Single crystal X-ray structure analysis, powder X-ray diffraction, thermal analysis and gas chromatography were used to identify the ethylene glycol in the solvate crystals recovered from the suspension. Controlled crystallization and solubility studies were used to determine the ease with which toxic glycol solvates crystallized from glycerin and propylene glycol contaminated with either ethylene or diethylene glycol. The single crystal structures of two distinct ethylene glycol solvates of rifampin were solved while thermal analysis, GC analysis and solubility studies confirmed that diethylene glycol solvates of the drug also crystallized. Controlled crystallization studies showed that crystallization of the rifampin solvates from glycerin and propylene glycol depended on the level of contamination and changes in the solubility of the drug in the contaminated solvents. Although the exact source of the ethylene glycol found in the compounded rifampin suspension is not known, the results of this study show how important it is to ensure that the drug and excipients comply with pharmacopeial or FDA standards.

KEYWORDS: toxic glycols, solvate, rifampin, contaminated solvents



INTRODUCTION

This study was initiated when syringe blockage was experienced upon administration by mouth of an extemporaneously compounded suspension containing rifampin (Figure 1) for treating persistent bacterial pneumonia caused by *Rhodococcus equi* in foals.¹ Syringe blockage started about 10 days after preparation of the suspension. Although rifampin is not specifically approved for veterinary use, the principal indication is in the treatment of *Rhodococcus equi* infections in horses when combined with erythromycin.^{2–5} The commonly prescribed compounded products contain erythromycin ethylsuccinate 46.8% w/v (equivalent to erythromycin 40% w/v) and rifampin 5% w/v in an oral suspension or paste. The sugar-free 5% w/v rifampin suspension prepared here contained polyethylene glycol (PEG) 400 (20% w/v), PEG 3350 (20% w/v) and propylene glycol (PG, 50% v/v). In addition, glycerin (10% w/v) was used to levigate the drug powder before incorporation into the suspension. Evaluation of the 10-day-old suspension showed the presence of large, dark-red almost black, needle-shaped crystals that were easy to resuspend but severely decreased the syringeability of the suspension.

In the meantime, a comprehensive physicochemical analysis of the large rifampin crystals recovered from the suspension was initiated. Thermal analysis (DSC and TGA) showed that the product was a solvate losing about 18–20% of its weight upon heating. However, the X-ray powder diffraction (XRPD) pattern of the crystals was not similar to any reported rifampin crystal forms.^{6–10} Although infrared (IR) spectroscopic analysis indicated that the solvent included in the crystals was not any of the ingredients in the suspension vehicle, it did indicate the presence of an unknown substance. The next step was to look for possible impurities in the solvents, PEG 400, PG or glycerin, used to prepare the suspension. A review of the literature indicated that a major concern with these solvents is contamination with toxic glycols, especially ethylene glycol (EG) and diethylene glycol (DEG) that are widely used in chemical industries as solvents or

Received: December 27, 2010

Accepted: March 14, 2011

Revised: March 11, 2011

Published: March 14, 2011

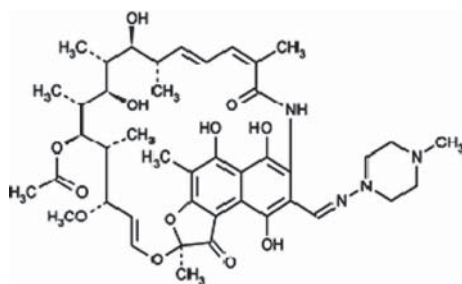


Figure 1. Chemical structure of rifampin (also known as rifampicin, $C_{43}H_{58}N_4O_{12}$, mol wt 822.94).

antifreeze products.^{11,12} Based on this information a re-evaluation of the IR analysis of the crystals identified EG as possibly the solvent in the solvated rifampin crystals. This was later confirmed by single crystal X-ray structure analysis. The source of the contamination is still unknown, but most probably either the glycerin or propylene glycol used during compounding was adulterated with the toxic glycol.¹²

This ability of a drug to form a solvate with a toxic glycol is unique and important since poisoning associated with ingestion of these solvents has caused hundreds, perhaps thousands of deaths.^{11,12} To date poisoning with toxic glycols has been observed mostly in outbreak situations rather than as sporadic cases and has usually been observed in association with contamination of ingestible pharmaceutical products.¹³ The first was the Massengill disaster of 1937 in the United States, when DEG was used as the excipient in a liquid sulfanilamide preparation.^{14,15} One hundred five deaths, predominantly among adults, resulted from ingestion of this product. The emotions aroused by this disaster led directly to the passage of the 1938 Federal Food, Drug and Cosmetic Act.¹⁶ Thirty-two years elapsed before the next significant poisoning was reported in South Africa.¹⁷ This was followed by outbreaks of acute renal failure caused by DEG-contaminated pharmaceutical products in 1986 in India,¹⁸ Nigeria in 1990,¹⁹ Bangladesh in 1990–1992,²⁰ Argentina in 1992²¹ and Haiti in 1996.¹³ All these outbreaks led to another 300–400 deaths, mostly of children.

One of the more recent poisoning outbreaks occurred in September 2006 in Panama. The source of the outbreak was a DEG-contaminated cough syrup. Twelve (57%) of 21 patients died.²² In 2007, the FDA found DEG in toothpastes imported from China, after which the agency warned consumers to avoid using tubes of toothpaste labeled as made in China and, through an import alert, stopped all suspect toothpaste from entering the United States. Between November 2008 and February 2009, 84 Nigerian children were reported to have died after being given “My Pikin”, a teething syrup containing PG that was contaminated with diethylene glycol.²³ The reason for these more recent poisoning epidemics appears financially driven because the toxic glycols are inexpensive solvents that are more profitable to use than the more expensive PG or glycerin.

Glycerin and PG are frequently used in formulations for compounding oral suspensions of drugs such as rifampin that is not available as a liquid dosage form.^{24–27} The unexpected identification of a rifampin:ethylene glycol solvate from a suspension containing glycerin and PG and the continued horrific and fully preventable medical tragedies associated with toxic glycol poisonings prompted this study into the ability of rifampin to form glycol solvates when crystallized from glycerin or PG deliberately or accidentally contaminated with EG or DEG.

MATERIALS AND METHODS

Materials. Rifampin USP (polymorph form II, assay 99.7%) was obtained from Youhan Corporation, Seoul, Korea. Glycerin, PG, EG and DEG were all analytical grade and were obtained from Sigma Aldrich (Milwaukee, WI, USA). The water used was distilled and deionized through a Milli-Q system (Millipore, Milford, MA, USA). For single crystal X-ray structure analysis a crystal from the contaminated suspensions was selected. This solvate, **1**, had a calculated composition of 1:2:2–3 rifampin:ethylene glycol:water. Subsequently, a second solvate **2**, with a composition of 1:2.9:2.8 rifampin:ethylene glycol:water and a different crystal structure from that of **1**, was isolated as follows. Dissolution of 20 mg of rifampin in an ethylene glycol/acetone/water (15:70:15 by volume) at ambient temperature yielded a clear solution that was filtered and placed in a refrigerator at 4 °C to crystallize. After one week, red needles of solvate **2** were isolated. TGA yielded an overall mass loss of 21.0% due to desolvation, in reasonable accord with the 1:2.9:2.8 rifampin:ethylene glycol:water composition (calculated mass loss 21.8%) suggested by the subsequent X-ray analysis.

Physical Characterization of Crystals. Scanning electron microscope (SEM) photomicrographs of the rifampin raw material and the solvates were taken with a field-emission environmental SEM (Quanta 200 ESEM, FEI Corporation, Hillsboro, OR, USA). Samples were mounted on a metal stub with an adhesive layer and then first coated under vacuum with carbon (Emscope TB500 sputter-coater, Cambridge, England) before being coated with a thin gold–platinum film (Eiko Engineering Ion Coater IB-2, Tokyo, Japan).

X-ray powder diffraction (XRPD) patterns were recorded using a Bruker D8 Advanced diffractometer (Bruker, Madison, WI), and the data were analyzed using the Eva software package. The instrumental setup was as follows: voltage, 40 kV; current, 40 mA; radiation source, Cu; divergence slit, 1 mm; antiscatter slit, 0.1 mm; detector slit, 0.1 mm; scan range, from 2° to 40° 2 θ ; scan speed, 0.4° 2 θ /min with a step size of 0.02° 2 θ and a step time of 3.0 s. Approximately 100 mg samples were weighed into aluminum sample holders, taking care not to introduce a preferential orientation of crystals.

Differential scanning calorimetry (DSC) thermograms were recorded with a DSC 2920 modulated DSC (TA Instruments, New Castle, DE). Samples weighing 3–5 mg were heated in closed aluminum crimp cells with a pinhole in the lid at a rate of 10 °C/min under a nitrogen gas flow of 35 mL/min. Thermogravimetric analyses (TGA) were performed with a Hi-Res Modulated TGA 2950 (TA Instruments, New Castle, DE). The sample weight was approximately 5–8 mg, and heating rates of 10 °C/min under a nitrogen gas flow of 35 mL/min were used.

For single crystal structure X-ray analysis intensity data for solvates **1** and **2** were measured on an Enraf-Nonius CAD4 diffractometer and a Bruker Apex Duo diffractometer respectively with Mo K α radiation ($\lambda = 0.71073$ Å), both crystals being cooled in a constant stream of nitrogen vapor using a cryostream cooler (Oxford Cryosystems) to optimize diffraction quality. Inspection of the diffraction patterns revealed Laue symmetries *mmm* (**1**) and 2/*m* (**2**), and the respective space groups $P2_12_12_1$ and $P2_1$ were deduced from systematic absences. Data reduction and structure solution by direct methods employed standard software packages. Atoms of the solvent molecules (ethylene glycol and water) were located in difference Fourier ($\Delta\rho$) syntheses and were added to the models. For solvate **1**, no disorder of the solvent molecules was

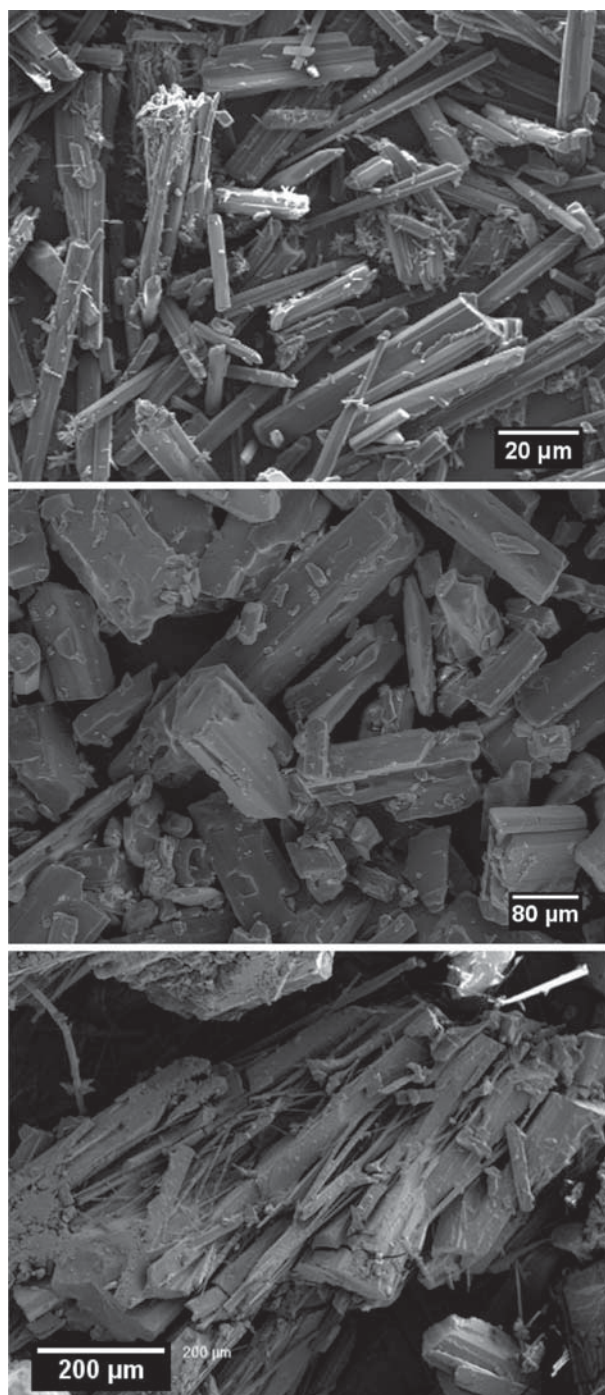


Figure 2. SEM photomicrographs of rifampin USP (top), rifampin ethylene glycol solvate (middle) and rifampin diethylene glycol solvate (bottom).

evident, whereas for solvate 2, both ethylene glycol and water molecules were found to be disordered and were modeled with variable site-occupancy factors, the refined values being consistent with the analytical data for this species.

Attempts were made to locate H atoms unequivocally in $\Delta\rho$ syntheses, especially for the rifampin molecules. These were subsequently included in idealized positions with isotropic temperature factors 1.2–1.3 times those of their parent atoms. In some cases H atoms of water molecules could also be located

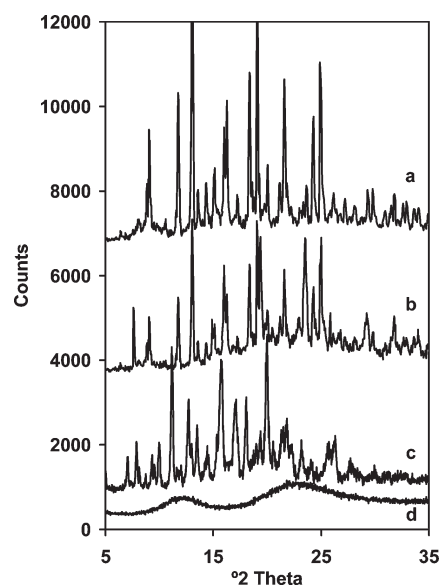


Figure 3. XRPD patterns of (a) rifampin ethylene glycol solvate, (b) rifampin diethylene glycol solvate, (c) rifampin USP, form II, and (d) desolvated diethylene glycol solvate.

and were placed accordingly. Full-matrix least-squares refinement against F^2 (program SHELX-H) was employed with the majority of the non-hydrogen atoms refining anisotropically.²⁸ For some of the solvent molecules, bond length restraints were used to ensure stable refinement. Two rifampin molecules, four ethylene glycol molecules and four water molecules were located in the asymmetric unit of the crystal of solvate 1, while for solvate 2, the corresponding numbers were two, six and six.

GC Analysis. GC analysis of EG and DEG in the solvents and in the crystals was performed using the USP method for diethylene glycol and ethylene glycol impurities in glycerin with some modifications (USP, 2007). A Hewlett-Packard model 5890 gas chromatograph equipped with a flame ionization detector and a HP Chemstation controller were used in this study (Agilent Technologies, Inc., Santa Clara, CA). The column was a 30 m Optima 624 fused silica capillary column with a 3.0 μm film thickness and 0.53 mm i.d. (Macherey-Nagel Inc., Bethlehem, PA). The injection port temperature was maintained at 220 $^{\circ}\text{C}$ and the detector temperature at 250 $^{\circ}\text{C}$. Initial oven temperature was set at 40 $^{\circ}\text{C}$, held for 1 min, and then increased at 70 $^{\circ}\text{C}/\text{min}$ to a final temperature of 260 $^{\circ}\text{C}$. The total run time was 15 min. The injection volume was 1 μL . Gas flow rates were helium 4.5 mL/min, hydrogen carrier 40 mL/min and airflow 400 mL/min. When necessary samples were diluted with or dissolved in methanol.

Solubility, Crystallization and Dissolution Rate Measurements. To determine the rifampin solubility in water, glycerin, PG or solvent contaminant mixtures, excess amounts of rifampin form II were placed in 3 mL vials containing each solvent. These vials were rotated at 10 rpm in a hybridization oven kept at 30 ± 0.1 $^{\circ}\text{C}$ (VWR, West Chester, PA, USA). After 48 h, when equilibrium was reached, aliquots were withdrawn from the vials and centrifuged through a 0.45 μm filter (Spin-X, centrifuge tube filters, Corning, Big Flats, NY, USA). The solutions were suitably diluted with methanol and assayed spectrophotometrically at 333 nm (Beckman DU 640 UV/vis spectrophotometer, Fullerton, CA, USA). Results are the mean of $n = 4$.

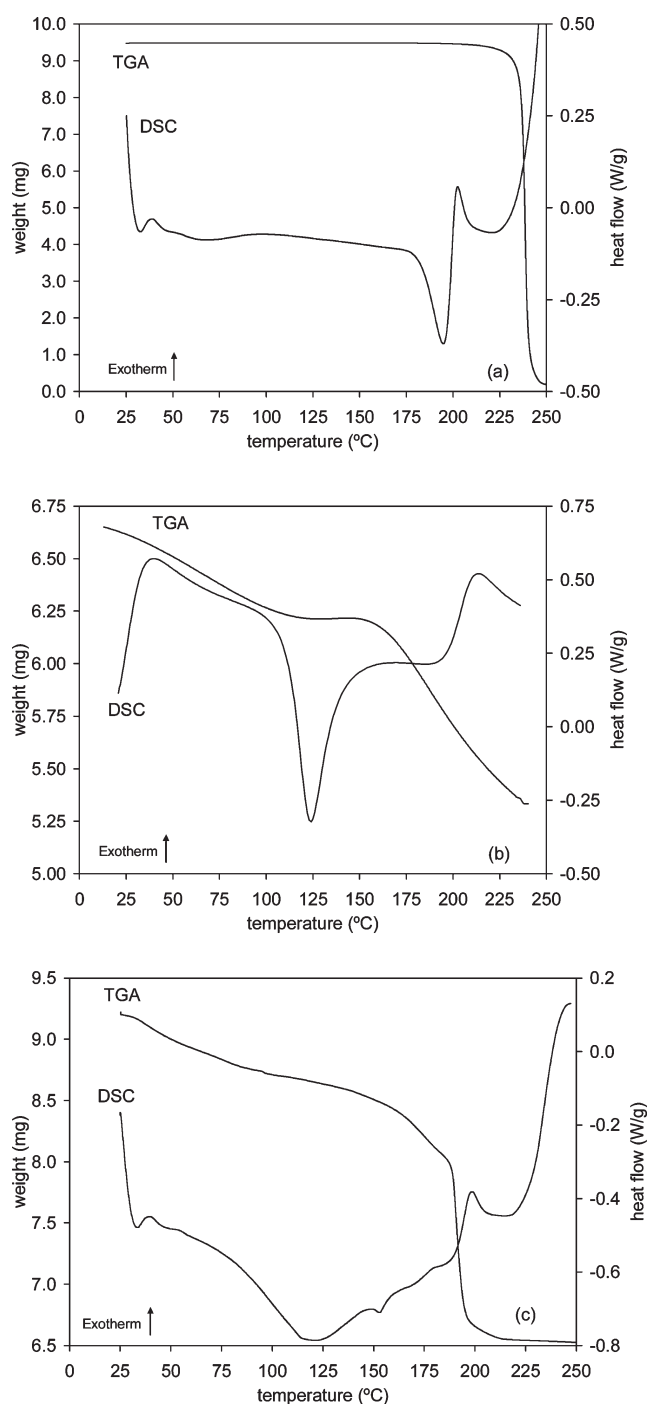


Figure 4. TGA and DSC thermograms of (a) rifampin USP, (b) rifampin ethylene glycol solvate and (c) rifampin diethylene glycol solvate.

Supersaturated solutions of rifampin were prepared by combining the solute and solvent mixtures in a test tube. The drug was completely dissolved by sonicating and heating the test tube in a water bath at 60 °C then and cooling to 30 °C. The solutions were filtered through a 0.45 μm filter before being transferred to crystallization dishes. The filters, crystallization dishes, syringes and syringe needles were placed in an oven at 40 °C beforehand to bring them above the solution temperature to prevent the solution from crystallizing during this process. The supersaturated solutions were

Table 1. Weight Loss Measured by TGA, Contaminant Content Measured by GC Analysis, and Solubility of Solvates in Water at 30 °C

form	TGA			solubility H ₂ O (mg/mL)
	range (°C)	wt loss (%)	GC (%)	
form II	0–200	0	0	1.5 $\pm$ 0.04
EG	30–120	4–6	12 $\pm$ 2.1	0.4 $\pm$ 0.02
	120–200	11–13		
DEG	30–150	3–6	10 $\pm$ 1.6	0.2 $\pm$ 0.04
	150–200	10–11		

carefully prepared to promote homogeneous nucleation, that is, special attention was paid to having clear, nonturbid solutions. Crystallization dishes were placed in an incubation oven at 30 $\pm$ 0.5 °C (Fisher Scientific, Pittsburgh, PA) and were observed every 4 h for 4 days, whereafter it was observed every 12 h. The time when the first crystals were seen on the bottom of the crystallization dish was recorded. As soon as enough crystals collected on the bottom of the dish, they were removed and analyzed by DSC and the EG or DEG content was measured by GC analysis.

Powder dissolution rates were determined according to the method described by Lötter et al.²⁹ The dissolution media used were 900 mL of 0.1 M HCl or 900 mL of water, kept at 37 °C and stirred at 100 rpm. Results are the mean of $n = 6$. The rifampicin content was determined spectrophotometrically at 333 nm after dilution with methanol. The powder or crystals were prescreened to isolate particles between 250 and 500 μm for dissolution testing. Similarity factors were calculated to compare the dissolution profiles.³⁰ The similarity factor (f_2) is a logarithmic reciprocal square root transformation of the sum of squared error and is a measurement of the similarity in the percent (%) dissolution between the two curves. This relationship is described algebraically by

$$f_2 = 50 \times \log \left(\left[1 + \left(\frac{1}{n} \right) \sum_{t=1}^n wt(R_t - T_t)^2 \right]^{-0.5} \times 100 \right) \quad (1)$$

where n is the number of time points, R_t is the dissolution value of the reference sample at time t , and T_t is the dissolution value of the test sample at time t . The value of f_2 is 100 when the test and reference mean profiles are identical. Values of f_2 between 50 and 100 ensure sameness or equivalence of the two curves and, thus, of the performance of the test and reference products.

Raman and IR Analysis. Both Raman and IR spectroscopy were explored as methods to detect ethylene glycol in rifampin solvate crystals. For Raman microscopy analysis, the standard solutions and/or samples were placed on a glass microscope slide and analyzed using a Thermo Scientific DXR Raman microscope (Waltham, MA) with a diode pump solid state (DPSS) laser, with an excitation wavelength of 532 nm and 10 mW laser power. For the infrared (IR) spectroscopic analysis, 20 μL of the standard solutions and/or samples were placed on the ATR sample accessory and analyzed using a Bruker Equinox 55 FT-IR/NIR spectrophotometer (Billerica, MA). Standard solutions of ethylene glycol were prepared by adding varying amounts of ethylene glycol, 0.5 to 20% w/w, to a 15 mg/mL solution of rifampin in methanol. Crystals of the rifampin ethylene glycol solvate were

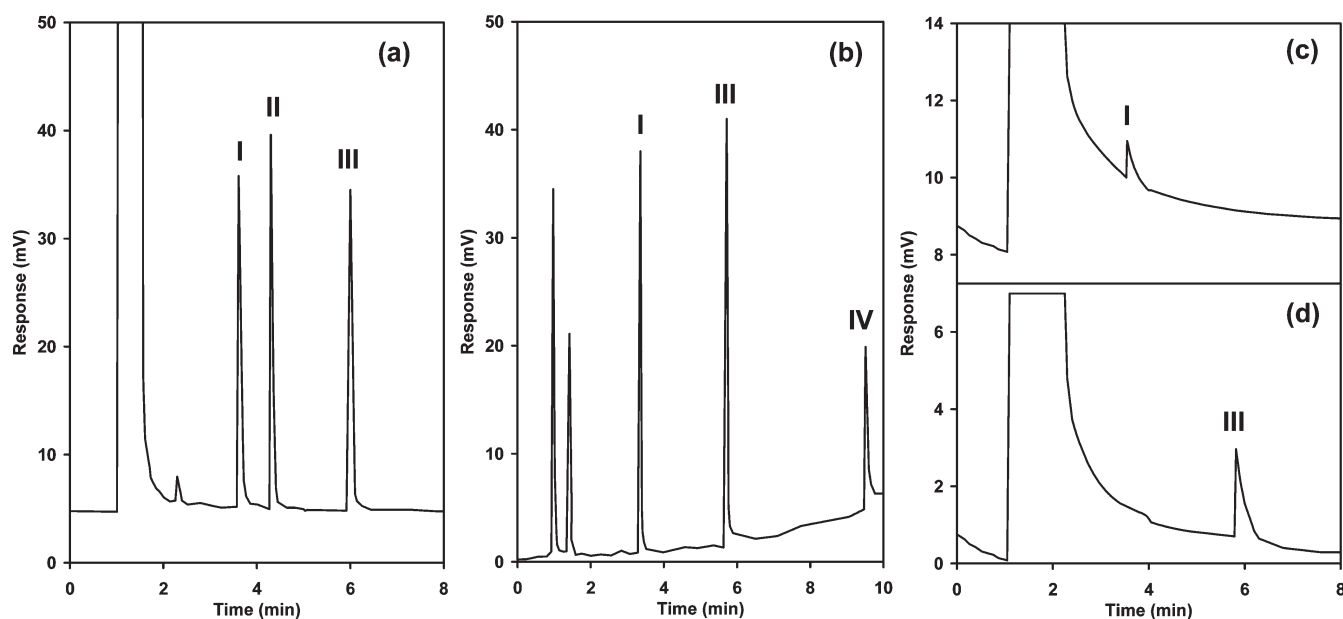


Figure 5. GC chromatograms of standard solutions of (a) ethylene glycol, I, and diethylene glycol, III, in the presence of PG, II, and (b) in the presence of glycerin, IV. Chromatograms of rifampin solvate crystals containing (c) ethylene glycol and (d) diethylene glycol are also shown.

dissolved in methanol before IR analysis. Samples of pure glycerin, ethylene glycol, diethylene glycol, and rifampin were analyzed as controls.

RESULTS AND DISCUSSION

Physical Characterization of the Crystals. In Figure 2, SEM photomicrographs of the solvated crystal forms are shown. For comparison the dark red acicular shaped crystals of rifampin USP, form II, are also shown. The solvate crystals grown from EG were large, dark red, almost black columnar shaped crystals with rough surfaces sparsely decorated with tiny crystals. The crystals grown from DEG were a mixture of dark red, thick and thin tabular crystals growing in a radiating manner. The crystals grown from EG and DEG have distinct XRPD patterns, Figure 3, that were different from each other, and from patterns of form II.

Form II, when heated, showed melting accompanied by decomposition from 180 to 205 °C with no apparent weight loss before melting, Figure 4. DSC analysis of the crystals grown from EG, Figure 4b and Table 1, indicate weight loss of 15–19% before melting and decomposition at 190–200 °C. Weight loss occurred in two stages, 4–5% before 120 °C, and 11–13% between 120 °C and the start of melting/decomposition. Upon heating the crystals grown from DEG show a broad endotherm from 50 to 175 °C before melting and decomposition started at around 200 °C. TGA analysis indicated weight loss of 3–6% before 150 °C and 10–11% between 150 and 200 °C. GC analysis of the crystals dissolved in methanol, Figure 5, indicated the presence of 12% EG in the crystals grown from EG and 10% DEG in the crystals grown from DEG. These values corresponded well with the second stage weight loss observed by TGA. The reason for the higher temperature, 150 versus 120 °C, for the loss of DEG could be because DEG has a higher boiling point, 245 °C, than EG, 197 °C. Since both EG and DEG are very hygroscopic, the additional weight loss observed by TGA is most probably due to water included in the crystal structure. This was confirmed by single crystal X-ray structure analysis of the ethylene glycol solvate.

XRPD, DSC, TGA and GC analysis identified the crystals grown from EG to be a 1:2:2–3 rifampin:ethylene glycol:water solvate. Similarly, the crystals grown from DEG were a 1:1:2–3 rifampin:diethylene glycol:water solvate. Although no crystals suitable for single crystal X-ray structure analysis were obtained for the DEG-solvate, two EG-solvates were isolated. Table 2 lists crystal data and refinement parameters for the two EG-solvates, 1 and 2, both of which contain two crystallographically independent molecules of the drug in their respective asymmetric units. Figure 6 shows the asymmetric unit in the 1:2:2 solvate 1. The symmetry-independent rifampin molecules are labeled A and B. The most notable conformational difference between rifampin molecules A and B involves the orientation of the methylpiperazinyl ring, which in molecule A is equatorial, with the ring plane roughly parallel to the naphthohydroquinone plane (as observed in the structure of rifampin pentahydrate), but in molecule B adopts an axial orientation.³¹ No evidence for the protonation of any of the piperazinyl nitrogen atoms was found in difference Fourier syntheses and the distance range for the six independent naphthohydroquinone C–O bonds is 1.29(1)–1.37(1) Å, very similar to that found in the crystal structure of rifampin pentahydrate [1.273(7)–1.374(7) Å] where these were likewise interpreted as C–O(hydroxyl) bonds.³¹

In Figure 7, the rigid naphthohydroquinone systems of molecules A and B have been superimposed to emphasize the different orientations (equatorial vs axial) of the methylpiperazinyl residues referred to above, as well as the noncoincidence of the *ansa* chains, where the deviation originates from different orientations of the C=C double bond closest to the five-membered ring. Solvate 1 is characterized by extensive hydrogen bonding that involves virtually all possible donor and acceptor atoms of the drug, ethylene glycol and water molecules. A projection of the unit cell contents (Figure 8) shows that the solvent molecules are located in narrow channels or interstices in this crystal.

The crystal asymmetric unit in the 1:2.9:2.8 solvate 2 is shown in Figure 9. The result of superimposing the naphthohydroquinone

Table 2. Crystal and Refinement Parameters for Rifampicin EG-Solvates 1 and 2

	1	2
solvate formula	$C_{43}H_{58}N_4O_{12} \cdot 2(C_2H_6O_2) \cdot 2(H_2O)$	$C_{43}H_{58}N_4O_{12} \cdot 2.9(C_2H_6O_2) \cdot 2.8(H_2O)$
<i>M</i>	983.10	1051.68
crystal system	orthorhombic	monoclinic
space group	$P2_12_12_1$	$P2_1$
<i>a</i> /Å	13.839(4)	13.802(4)
<i>b</i> /Å	20.331(4)	17.517(5)
<i>c</i> /Å	36.791(7)	24.848(8)
α /deg	90.0	90.0
β /deg	90.0	105.803(6)
γ /deg	90.0	90.0
V_{cell} /Å ³	10325(4)	5781(3)
<i>Z</i>	8	4
<i>T</i> /K	173(2)	100(2)
μ /mm ^{−1}	0.097	0.094
<i>F</i> (000)	4224	2263
theta range/deg	1.49–25.47	2.28–24.98
index ranges	$-9 \leq h \leq 16,$ $-7 \leq k \leq 24,$ $-12 \leq l \leq 44$	$-16 \leq h \leq 13,$ $-20 \leq k \leq 20,$ $-29 \leq l \leq 28$
reflns collected	10459	32578
obsd reflns [$I > 2\sigma(I)$]	4127	6192
data/parameters	10459/867	32578/1151
goodness-of-fit on F^2	0.957	1.310
final <i>R</i> indices [$I > 2\sigma(I)$]	$R_1 = 0.0904, wR_2 = 0.2378$	$R_1 = 0.1367, wR_2 = 0.3769$
largest diff peak and hole (e Å ^{−3})	0.66, −0.74	0.91, −0.69
CCDC numbers ^a	788539	788540

^a The CIF files for solvates 1 and 2 have been deposited at the Cambridge Crystallographic Data Centre.

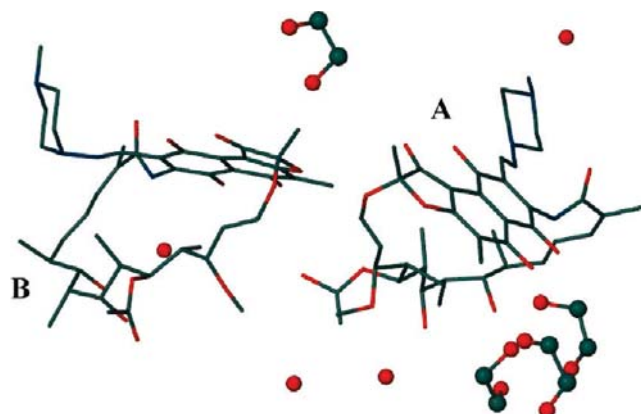


Figure 6. The asymmetric unit in the crystal of solvate 1. H atoms have been omitted for clarity. Rifampicin molecules are represented in stick form while the non-hydrogen atoms of the solvent molecules (water, ethylene glycol) are shown in ball-and-stick mode.

systems of molecules A and B is shown as a stereoview in Figure 10. In contrast to solvate 1, the crystallographically independent molecules in solvate 2 adopt very similar conformations. Evidence for the protonation of the terminal piperazinyl nitrogen atom in molecule B was found, whereas in molecule A, no corresponding electron density was observed. Molecular packing in the crystal of solvate 2 (Figure 11) is significantly different from that in solvate 1.

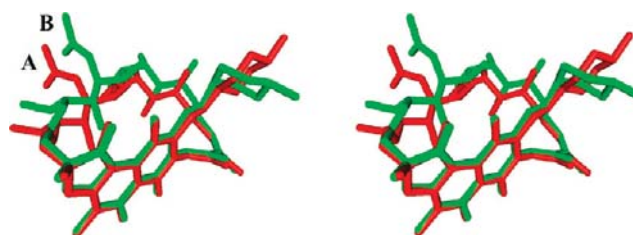


Figure 7. Stereoview of rifampicin molecules A and B in solvate 1 with their naphthohydroquinone systems overlaid.

The crystal of 2 is composed of layers of rifampicin molecules straddling the 002 crystal planes, interleaved by layers of solvent molecules. Extensive hydrogen bonding links the respective layers. PXRD patterns based on the refined single crystal structures of solvates 1 and 2 have been computed (Cu K α radiation, $\lambda = 1.5418$ Å) and are shown in Figure 12 as representative of these phases. The former corresponds to the experimental PXRD shown in Figure 3(a).

In summary, the molecular and crystal structures of two crystallographically distinct, ternary solvates containing rifampicin, ethylene glycol and water molecules have been resolved, revealing interesting conformational differences as well as different crystal packing motifs. Solvate 1 crystallizes in the same space group as rifampicin pentahydrate, but with different unit cell dimensions.³¹ Full details for the new solvates, including

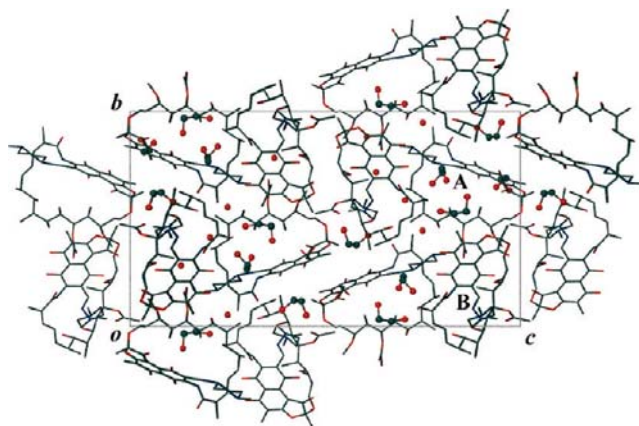


Figure 8. [100] projection of the crystal structure of solvate 1. Rifampin molecules are represented in stick form while the non-hydrogen atoms of the solvent molecules (water, ethylene glycol) are shown in ball-and-stick mode.

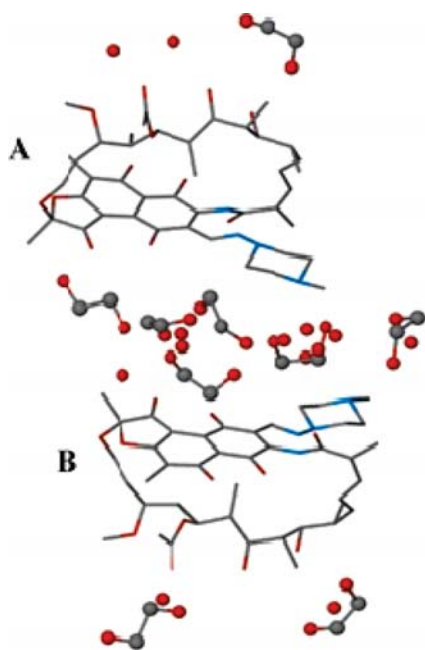


Figure 9. The crystallographic asymmetric unit in solvate 2.

quantitative comparison of the molecular parameters and hydrogen bonding, will be published elsewhere as part of a more extensive account of the variety of structural arrangements we have recently established in a series of rifampin solvates.

Solubility of Rifampin in Mixed Solvent Systems. The solubility of rifampin is pH dependent and ranges from more than 200 mg/mL at pH 1 (0.1 M HCl) to around 3 mg/mL at pH 7.⁷ In practice 1% w/v (mg/mL) rifampin suspensions are usually prepared. The ability to keep the drug in suspension and to prevent dissolution and recrystallization will depend on the solubility of the drug in the vehicle. The solubilities of rifampin at 30 °C in glycerin, PG and the toxic glycols are listed in Table 3. The solubility of rifampin in PG measured here, 50 mg/mL, is higher than that reported in the literature, 0.1 mg/mL.⁷ The solubility of the drug in EG and DEG was not significantly different but was higher than in glycerin and lower

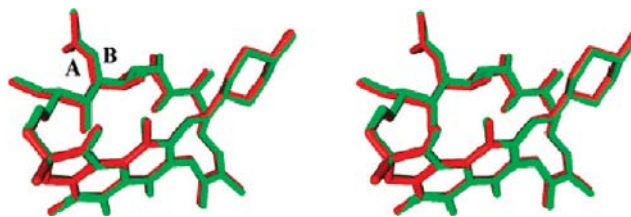


Figure 10. Stereoview of rifampin molecules A and B in solvate 2 with their naphthohydroquinone systems overlaid.

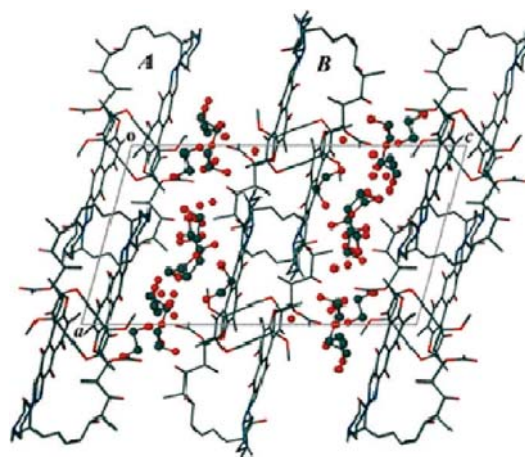


Figure 11. [010] projection of the crystal structure of solvate 2.

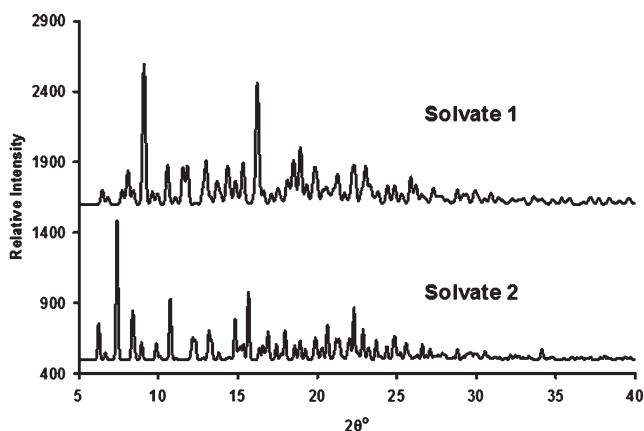


Figure 12. Computed PXRD patterns for the solvates.

than in PG. Both EG and DEG when added to glycerin increased the solubility of rifampin, Figure 13, but the maximum solubility that could be reached was higher in glycerin:EG solutions (55 mg/mL at 40% EG) compared to the glycerin:DEG solutions (24 mg/mL at 20% DEG). Both EG and DEG increased the solubility of rifampin in PG, but the maximum solubility reached was not significantly different. Overall, the drug was more soluble in the PG mixtures.

As shown in Figure 13, after an initial increase in solubility with the addition of the contaminants the solubility decreased after a certain concentration was added. These concentration values are listed in Table 3 and were significantly higher for glycerin compared to PG. The reason could be that the drug is 10 times

Table 3. Solubility of Rifampin in the Solvents and Contaminants and Solubilization Power (Φ) of Contaminants Obtained from the Linear Portions of Log S_{mix} vs ϕ_2 Plots (Figure 5)^a

solvent	contaminant	solubility (mg/mL)	solubilization power (Φ)	
			in glycerin	in PG
glycerin		3.3 ± 0.10		
PG		50.8 ± 0.77		
	EG	31.9 ± 0.01	3.1 (40)	2.7 (5)
	DEG	31.2 ± 0.01	4.6 (20)	3.1 (5)

^a The number in parentheses beside the Φ -value represents the percentage of contaminant where the maximum solubility was observed. Above this concentration, solubility decreased.

less soluble in glycerin compared to EG and DEG while the drug is 1.5 times more soluble in PG compared to the contaminants. The decrease in solubility above the critical concentrations of the contaminants was more gradual when DEG was added to glycerin and EG was added to PG.

The contributions of dielectric constants and of solubility parameters have been used extensively as a measure of solvent polarity and as important parameters in predicting the solubility of drugs in solvent blends.³² Dielectric constants of all the glycerin or PG + toxic glycol mixtures (ϵ_{mix}) were calculated from the relation $\epsilon_{\text{mix}} = \epsilon_1\phi_1 + \epsilon_2\phi_2$, where ϕ is the volume fraction of the solvents and subscript 1 represents glycerin or PG and subscript 2 EG or DEG. The relative permittivities of glycerin, PG, EG and DEG are 47.2, 32, 37 and 31.7, respectively at room temperature. For rifampin, it seems that the solubility decreased with an increase in the polarity of the solvent because the solubility in glycerin, Table 3, was significantly lower than in the other solvents. An evaluation of solubility as a function of ϵ_{mix} values did not show any trends except that for both glycerin + EG and glycerin + DEG mixtures the solubility decreased once ϵ_{mix} fell below 40. Such a trend was not observed for the PG + toxic glycol mixtures. The solvents are all polyhydric alcohols with very similar solubility parameters (δ ranged from 29.9 to 36.1 MPa^{1/2}) that are significantly higher than that of rifampin ($\delta = 20.76$ MPa^{1/2}). Since the difference between δ -values was the greatest between glycerin and rifampin, it could help explain the poor solubility of rifampin in this solvent. However, no trend was observed when changes in solubility parameters of solvent mixtures were compared with changes in solubility. The reason for this could be that the solute was altered by changes in the solvent, i.e. the addition of EG or DEG. This could mean that upon addition of the contaminants solvate formation was promoted which then increased the propensity of the solvated forms of rifampin to crystallize from these solutions.

Several predictive models for cosolvent solubilization have been developed. A simple and accurate one suitable for pre-formulation that requires little or no experimental data is the well-known log-linear model proposed by Yalkowsky and co-workers.^{33–35} This relationship is described algebraically by

$$\log S_{\text{mix}} = \log S + \Phi\phi_2 \quad (2)$$

where S_{mix} and S are the total solute solubilities in the solvent mixtures and solvent (glycerin and PG), respectively, Φ is the cosolvent solubilization power for the particular cosolvent (contaminant), and ϕ_2 is the volume fraction of the contaminant

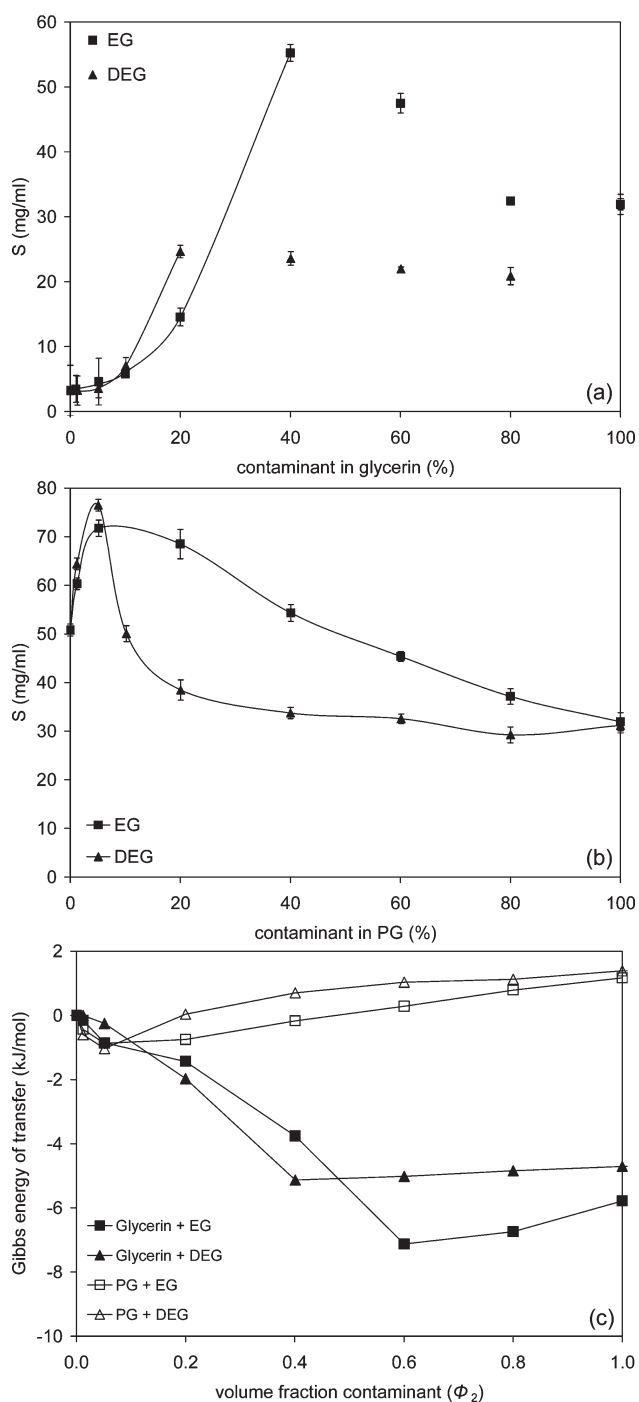


Figure 13. Solubility of rifampin USP at 30 °C in mixed solvent systems composed of (a) glycerin and (b) PG and the contaminants EG or DEG. (c) The change in Gibbs free energy of transfer of rifampin from the solvent to solvent:contaminant mixtures.

in the mixture. The Φ -values were obtained from the slopes of the log(S_{mix}) versus ϕ_2 plots. These values are listed in Table 3 and give a quantitative estimate of the ability of the contaminant to increase the solubility of rifampin in the solvents at 30 °C. For the glycerin mixtures DEG had a significantly higher Φ -value compared to EG. The Φ -values for EG and DEG in the PG mixtures were not significantly different. As shown in Figure 13, above a certain concentration of the contaminants the solubility

Table 4. Contaminant Content of Crystals Recovered from Supersaturated Solutions of Rifampin in Solvent:Contaminant Mixtures^a

contaminant (% v/v)	crystals from glycerin				crystals from PG			
	EG		DEG		EG		DEG	
	<i>t</i> ₀ (days)	%	<i>t</i> ₀ (days)	%	<i>t</i> ₀ (days)	%	<i>t</i> ₀ (days)	%
0	∞	0	∞	0	∞	0	∞	0
1	∞	0	∞	0	∞	0	∞	0
5	10	6.5 ± 1.6	15	5.8 ± 1.4	∞	0	2	3.6 ± 1.7
10	15	9.8 ± 1.8	22	8.7 ± 2.0	3	5.3 ± 1.1	1	5.4 ± 1.5
20	36	12.4 ± 0.8	41	10.5 ± 0.9	4	12.2 ± 1.5	1	9.7 ± 1.4
40	58	12.2 ± 1.2	48	10.4 ± 1.0	3	12.5 ± 2.0	1	10.5 ± 1.9
60	48	12.1 ± 0.8	39	10.6 ± 1.6	4	12.3 ± 1.3	2	10.1 ± 1.7

^aThe time it took for the first solvate crystals to be detected (*t*₀) is also listed. For *t*₀ = ∞ no contaminant solvate crystals were recovered, but crystallization of either the anhydrous, monohydrated or dihydrated form of rifampin was observed.

decreased. Deviations from the log–linear increase in solubility are a result of interactions that take place in the solute–solvent–contaminant mixture which do not take place in the solute–solvent mixture.³⁶ These interactions can also lead to crystal form changes such as the formation of solvates.³⁷ The new solvate might be less soluble than the original crystal form or can even act as seeds that promote the crystallization of even more of the solvate with a resultant drop in solubility. In this study, such a desolubilizing effect was observed for the toxic glycols giving negative Φ -values above certain concentrations. The decrease in solubility was not constant and was faster when EG was added to glycerin ($\Phi = -0.58$) compared to DEG ($\Phi = -0.12$). For EG ($\Phi = -0.39$) added to PG the reverse was true (DEG, $\Phi = -1.87$).

A change in crystal form will result in a difference in the amount of energy required to destroy the crystal lattice in different solvent mixtures. An indication of the process of transfer of rifampin from the crystals to glycerin or PG to the solutions containing EG or DEG may be obtained from the values of Gibbs free energy change. The Gibbs free energy of transfer, ΔG_{tr}° , of rifampin from pure solvent to the mixtures may be calculated as follows:³⁸

$$\Delta G_{tr}^{\circ} = -2.303RT \log \frac{S_{mix}}{S} \quad (3)$$

where S_{mix}/S is the ratio of the molar solubility of rifampin in the mixtures to that in pure solvent. The change in ΔG_{tr}° versus the volume fraction of contaminant is shown in Figure 13c. In the glycerin + EG and glycerin + DEG mixture the ΔG_{tr}° values were all negative indicating the spontaneous nature of drug solubilization. The initial decrease in Gibbs free energy, with an increase in EG and DEG concentration up 40% and 20% respectively, was followed by the ΔG_{tr}° remaining practically constant. The initial decrease demonstrated that the reaction initially became more favorable as the concentration of EG or DEG increased. In the PG solutions, initial decreases in negative ΔG_{tr}° values were followed by positive values indicating a change from spontaneous to nonspontaneous or unfavorable solubilization. In both PG + EG and PG + DEG solutions the solubility decreased above 5% of contaminants added. However, solubilization remains spontaneous, $\Delta G_{tr}^{\circ} < 0$, for up to 40% EG. In the PG + DEG solutions, solubilization became unfavorable, $\Delta G_{tr}^{\circ} > 0$, above 5% DEG.

Crystallization of Rifampin Solvates from Mixed Solvent Systems. Observations and measurements made while studying the crystallization of the toxic glycol solvates from the mixed solvent systems are summarized in Table 4. Overall it took five times longer for solvated crystals to start to appear from glycerin + EG or DEG mixtures compared to mixtures of PG and the toxic glycols. In the glycerin solutions EG and DEG was detected in the recovered crystals from 5% and higher. Above 10% only solvated crystals were recovered from the solutions. For these solutions, an increase in contaminant concentration caused an increase in the solubility of rifampin and this led to an increase in the time it took for crystallization to start. In addition, once crystallization started it took longer for crystallization to proceed to a point where enough crystals could be harvested for analysis. Slower crystallization also meant that the quality of the crystals in terms of size and shape, Figure 2, was better.

The crystallization of the solvates from the PG + toxic glycol solutions started at about 5% of the contaminants added, and from 20% and above the recovered crystals were only the solvate. Crystallization from the PG + DEG solutions was the fastest because crystals were observed after just one day. The time it took for crystallization to be complete was also faster than from the glycerin solutions. The differences seen in the crystallization behavior of rifampin from the glycerin versus PG solutions, Table 4, seem to follow the changes in ΔG_{tr}° because in solutions where solubilization was spontaneous, crystallization was slower than from solutions where solubilization was unfavorable, Figure 13.

Solubility and Dissolution of Rifampin Solvates. Changes in the solubility and dissolution rate of rifampin due to the crystallization of the solvates could change the toxicity and the bioavailability of the drug. For EG death has been reported after ingestion of as little as 30 or 60 mL, although some persons who ingested more than 3000 mL survived. The lethal oral dose is also estimated to be 1.4 mL/kg or 1.56 g/kg.³⁹ The acute lethal dose of DEG for humans has been estimated at approximately 1 mL/kg or 1.18 g/kg. In one case of poisoning the average dose of DEG ingested by patients who died after drinking sulfanilamide with DEG as the vehicle was 1 mL (72% concentration of DEG) per kilogram body weight.¹⁵ One hundred five out of 353 exposed persons died.

These toxic concentrations are low and raise the question: what would happen if patients ingested the rifampin toxic glycol solvates? First, if the suspension vehicle contained EG or DEG

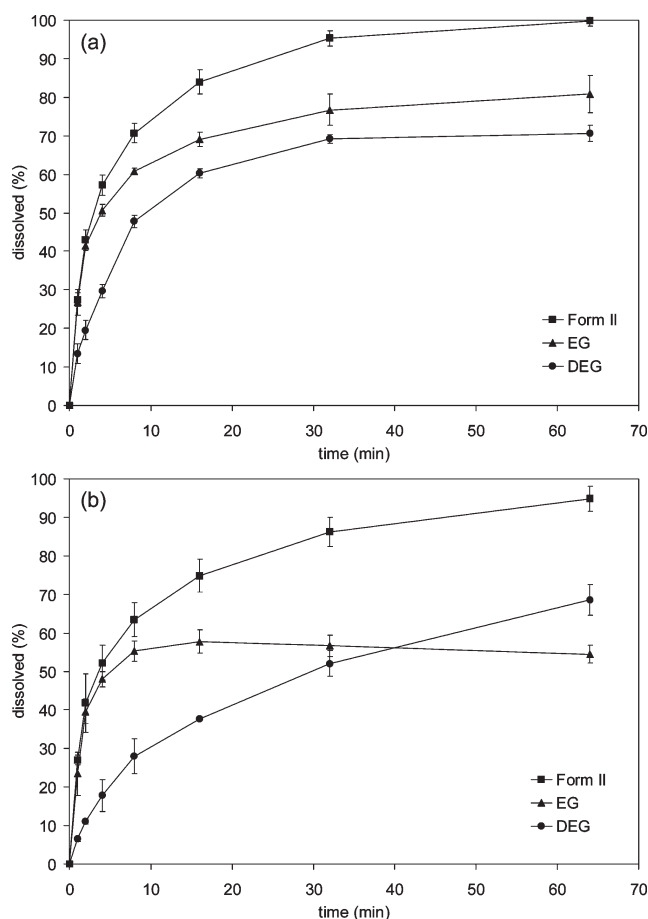


Figure 14. Dissolution profiles of rifampin USP and the toxic glycol solvates in (a) 0.1 M HCl and (b) water.

the history of contaminated pharmaceutical products has shown that patients could ingest toxic quantities of the solvents.^{11–23} Second, if the crystals used to make the suspensions were the toxic solvates, then over time the patients could ingest enough of the toxic crystals to cause poisoning. Additionally changes in the physicochemical properties of the toxic solvates could also reduce the bioavailability of rifampin. As shown in Table 1, the solubilities of both solvates was lower than that of rifampin USP. The solubility of the EG solvate was four times and the DEG solvate eight times less than that of form II. This also meant that the DEG solvate was two times less soluble than the EG solvate.

The reduction in solubility also lowered the dissolution rates of the crystals in simulated gastric fluid, 0.1 M HCl, and water as shown in Figure 14. A similarity factor, f_2 , was used to compare dissolution profiles. The solubility of rifampin in the acid medium is much higher, 200 mg/mL, than in water, 1.5 mg/mL, and this is reflected by a slower dissolution rate, but the dissolution profiles were still similar with an f_2 value = 58. The dissolution rate of the EG solvate in water was slower than in 0.1 M HCl, f_2 = 45. The DEG solvate also dissolved slower in water compared to the acid medium (f_2 = 43). In the acid medium the dissolution profiles of the three crystals forms were different, f_2 < 50, and the order was as follows: form II > EG solvate > DEG solvate. The dissolution order was the same in water but the f_2 < 34.

Infrared Spectroscopic and Raman Microscopic Analysis of the Solvates. The method most often used to identify and

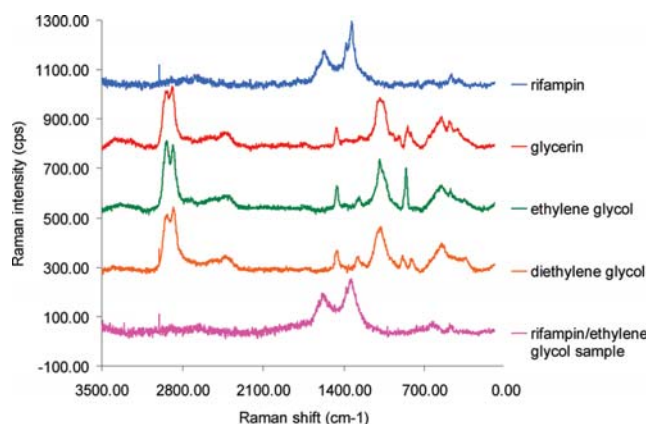


Figure 15. The results from Raman microscopy analysis, which indicates the similarity between the Raman spectra of glycerin, ethylene glycol and diethylene glycol, as well as the lack of detectable ethylene glycol peaks in the rifampin–ethylene glycol solvate sample.

quantify toxic glycols in solutions is GC analysis, the method described by the USP. GC analysis was used in this study, Figure 5, to accurately determine the amount of EG or DEG included in the solvated crystals (Table 4). GC analysis can be time-consuming. As an alternative, due to the requirements of minimal sample preparation and ease of use, Raman microscopy and infrared spectroscopy analyses were investigated as techniques for detecting the presence of the toxic glycols in the crystal structure of the rifampin toxic glycol solvates.⁴⁰ As seen in Figure 15, the Raman spectra of glycerin, ethylene glycol and diethylene glycol are similar, with some differences in spectra in the 1000–700 cm^{−1} range. However, due to the large signal-to-noise ratio in the spectra and the overlap of the peaks between the spectra, it was not possible to detect ethylene glycol or diethylene glycol in the samples containing rifampin. It is also apparent from Figure 15 that none of the unique peaks of ethylene glycol were detected in the rifampin–ethylene glycol solvate sample.

Figure 16a shows the IR spectra of glycerin, ethylene glycol, diethylene glycol, the rifampin–methanol solution and the rifampin–ethylene glycol solvate dissolved in methanol. The absorbance peaks at 882 cm^{−1} and 861 cm^{−1} are unique to ethylene glycol, and there are no significant overlaps between these peaks and those of the other spectra. For this reason, the absolute intensity of the absorbance peaks at both of these positions was used to identify EG. It should be noted that even though standard solutions containing 0.5 and 1% (w/w) ethylene glycol were also analyzed, these concentration values were omitted due to the inability to accurately detect the intensity of the absorbance peaks of ethylene glycol in these solutions. Thus, the lower limit of quantification for this infrared spectroscopic method is 5% (w/w). Although it was not possible to quantify ethylene glycol, it was possible to detect the presence of ethylene glycol at concentrations below 5% (w/w). This is illustrated in Figure 16b, which shows the result from the subtraction of the IR spectra of the rifampin–methanol solution from the spectra of a methanol solution of the rifampin–ethylene glycol solvate. The two characteristic peaks of ethylene glycol are highlighted; however, there is a shift in the positions of these peaks. The theoretical concentration of ethylene glycol in this sample is 2.0% (w/w), and it was possible to detect it in the sample but it was not possible to accurately quantify the amount of ethylene glycol in this sample.

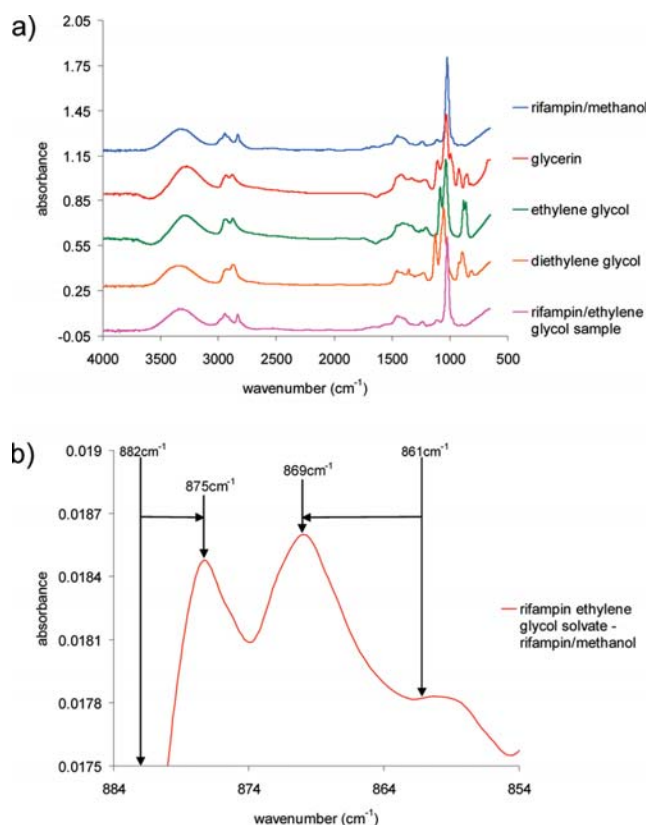


Figure 16. (a) The infrared spectra of glycerin, ethylene glycol, diethylene glycol, the rifampin–methanol solution and the rifampin–ethylene glycol solvate dissolved in methanol. (b) The result from subtraction of the IR spectra of the rifampin methanol solution from the spectra of the rifampin ethylene glycol solvate dissolved in methanol showing the absorbance peaks at 875 and 869 cm^{-1} , indicating the presence of the ethylene glycol in the sample.

These results show that GC analysis was the most accurate method for identifying and quantifying the toxic glycols in the solvated rifampin crystals but that IR analysis might be used to identify the contaminants in adulterated samples.

CONCLUSION

Prescription compounding as a method to prepare individualized medicine for specific patient populations such as children and animals is becoming more and more popular. The results of this study show how important it is to make sure that the drug and excipients comply with pharmacopeial or FDA standards. Although the exact source of the toxic glycol, ethylene glycol, found in the compounded rifampin suspension described here is not known, the results presented demonstrate not only the presence of the toxic glycol but also how adulterated solvents adversely affected the quality of the product.

The CIF files for solvates 1 (CCDC number 788539) and 2 (CCDC number 788540) have been deposited at the Cambridge Crystallographic Data Centre.

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ACKNOWLEDGMENT

M.M.d.V. and S.J.S. thank the University of Wisconsin for financial assistance. W.L. and S.J.S. thank the Northwest University (Potchefstroom) and the NRF (Pretoria) for financial assistance. M.R.C., S.A.B. and J.L. thank the University of Cape Town and the NRF for financial assistance.

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