The 3'-Keto-Diol Equilibrium of Trospectomycin Sulfate Bulk Drug and Freeze-Dried Formulation: Solid-State Carbon-13 Cross-Polarization Magic Angle Spinning (CP/MAS) and High-Resolution Carbon-13 Nuclear Magnetic Resonance (NMR) Spectroscopy Studies

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Understanding how moisture interacts with a drug or formulation is a critical component of product development. This study demonstrates how water affects the 3'-gem-diol ↔ 3'-keto equilibrium in trospectomycin sulfate bulk drug and freeze-dried formulation, as probed by solid-state carbon-13 cross-polarization magic angle spinning (CP/MAS) and high-resolution nuclear magnetic resonance (NMR) spectroscopy. Drying the bulk drug or formulation to low water levels dehydrates trospectomycin sulfate from the diol to the keto form. Carbon-13 CP/MAS NMR spectroscopy measures the keto drug concentration in solid samples directly. The bulk drug, which contains approximately 16% water, is more than 90% in the 3'-diol form. Oven drying to <3% water converts approximately 75% of the drug to the 3'-keto form. The drug is formulated as a freeze-dried, sterile powder that can contain up to 12% water depending on the freeze-drying conditions. These studies show that the 3'-keto concentration rises uniformly (up to 75%) with decreasing residual water in the freeze-dried cake. The keto-diol equilibrium was also studied in solution by high-resolution carbon-13 NMR experiments, and it was found that raising the temperature or using dimethyl sulfoxide (DMSO) as a solvent also dehydrates the drug. For example, in aqueous solution at 25°C, nearly all (>95%) of the drug is in the 3'-diol form. After equilibration at 60°C, however, the 3'-keto content increases to 7%, and in D2O-DMSO solvent at 25°C the drug is mostly (60%) in the 3'-keto form.

KEY WORDS: nuclear magnetic resonance spectroscopy; carbon-13 cross-polarization magic angle spinning spectroscopy; water; antibiotic.

INTRODUCTION

Water generally affects the chemical, physical, and manufacturing properties of pharmaceutical solids including bulk drugs, excipients, and formulations. A significant effort has been made to understand how moisture interacts with these solids and how it alters drug and product stability.

crystal structure, dissolution rate, tableting behavior, and solid-state equilibria (1,2). As an example of how water can affect an equilibrium and take part in a chemical reaction, it is known that the drug spectinomycin can exist as the diol or keto form, depending on the amount of water present, and that the keto form of the drug reacts to produce the ring-opened spectinoic acid under base hydrolysis conditions (2). In this paper, we examine how water affects the equilibrium between the keto and the diol forms of a related drug, trospectomycin sulfate, both in solution and in the solid state. Trospectomycin sulfate (6'-n-propylspectinomycin) is an aminocyclitol antibiotic under development as a broad-spectrum antibacterial for the treatment of sexually transmitted diseases and anaerobic infections. The bulk drug is highly crystalline and contains approximately 16% water by weight. This is equivalent to five water molecules per drug molecule, with one molecule involved in specific hydration of the drug at the 3' position (Fig. 1). This antibiotic will be marketed as a lyophilized, amorphous sterile powder that contains mostly drug and that will be reconstituted with aqueous diluents before use.

As Fig. 1 indicates, water is intimately involved in the equilibrium between the keto and the gem-diol forms of the drug. For example, dehydration results in conversion of the 3'-gem-diol form to the corresponding 3'-keto form; therefore, the equilibrium concentrations of these two forms are directly related to the amount of water present. We have used solid-state carbon-13 cross-polarization magic angle spinning nuclear magnetic resonance (CP/MAS NMR) spectroscopy to study this equilibrium in solid samples. In addition, high-resolution carbon-13 NMR measurements demonstrate how temperature and solvent influence this equilibrium in solution.

MATERIALS AND METHODS

Trospectomycin sulfate bulk drug, manufactured by The Upjohn Company, was used without further purification. The drug contains 16.1% water, as determined by Karl Fischer methods, and X-ray diffraction measurements indicate that the bulk drug is highly crystalline and exists predominantly as one crystal form. Carbon-13 CP/MAS experiments analyze the solid bulk drug directly. For high-resolution NMR measurements, solutions were prepared by dissolving 25–30 mg of solid in 0.5 ml of D2O or D6-DMSO (dimethyl sulfoxide).

Freeze-dried powders containing 3–12% residual water were prepared by varying shelf temperatures and drying times during freeze-drying (3). The resulting cakes are amorphous, giving nearly featureless X-ray diffraction patterns. The amount of water in each sample was determined by thermogravimetric analysis (TGA). In these measurements, the samples were heated from room temperature to 120°C and the total weight loss was recorded. This procedure measures the total amount of water in the sample, including water obtained from dehydration of the drug to the keto form. To reduce sample heterogeneities, the freeze-dried cakes were crushed and mixed. The solid-state NMR rotors were quickly packed (within 90 sec) to minimize changes in sample water content; loss of water during data collection was insignificant. For solution state measurements, the freeze-
dried samples were dissolved in \( d_6 \)-DMSO under a dry nitrogen atmosphere to exclude extraneous moisture.

Carbon-13 CP/MAS experiments yield high-resolution NMR spectra of solids directly. This technique has been extensively used to study bulk synthetic polymers (4) and to probe tautomerism and hydrogen bonding in organic solids (5). Recently, carbon-13 CP/MAS spectroscopy has been used to study polymorphism, to probe conformational states of drugs, and to distinguish crystalline hydrates of drugs (6). In these experiments, the carbon signal is enhanced by transferring magnetization from the proton spins to the carbons, a process known as cross-polarization. The time during which the two spin reservoirs are in magnetic contact is called the contact time. Spectral resolution is enhanced by magic angle spinning, which reduces line broadening from chemical shift anisotropies. Spectral resolution is further increased by high-power proton decoupling, which reduces broadening arising from proton–carbon dipolar interactions. The solid-state carbon-13 CP/MAS spectra were collected using a Bruker MSL-200 NMR spectrometer. The 4.7-T Oxford magnet gives a proton resonance frequency of 200 MHz and a carbon-13 resonance frequency of 50 MHz. A Doty Scientific CP/MAS probe was used, and the angle between the sample rotation axis and the magnetic field was adjusted to 54.7° (the “magic angle”) using KBr (7). The samples were spun about the magic angle at 4.7 kHz in 7-mm standard sapphire rotors. The proton 90° pulse time was 5.8 \( \mu \)sec, and the contact time was 3.0 msec. The applied field strength for protons, \( B_1(H) \), was 10 G and that for carbon was 40 G. The spectral width and acquisition time used for these experiments were 20 kHz and 51 msec, respectively, and each free induction decay was digitized into 2048 points. The recycle delay between scans was 4.0 sec.

When processing the free induction decay from the sterile powders, a Lorentzian multiplication of 50 Hz was used to improve the signal-to-noise ratio; the free induction decay signals from the bulk drug samples, however, were Fourier transformed directly, without line broadening. Chemical shifts are relative to tetramethylsilane, using adamantane as an external standard.

High-resolution carbon-13 NMR spectra were recorded using a Varian XL-400 spectrometer with a 9.4-T Oxford magnet operating at 100 MHz for carbon-13. Data were acquired using a Varian 5-mm broadband probe with proton decoupling into 32,000 or 16,000 points, and a Lorentzian multiplication of 2 Hz was applied prior to Fourier transfor-

\[ B_1(H) \] is related to the tip angle (\( \theta = 90° \)) and the 90° pulse time (\( \tau = 5.8 \mu \)sec) by \( B_1(H) = \theta(\tau) \). The magnetic field \( B_1(C) \) is calculated as \( B_1(C) = B_1(H)\gamma(H)\gamma(C) = 3.98B_1(H) \), where \( \gamma(H) \) and \( \gamma(C) \) are the magnetogyric ratios for proton and carbon nuclei, respectively.

**Quantitation Procedure**

The 3'-keto drug concentrations are calculated as follows: Since both the 3'-diol and the 3'-keto forms of the drug contain the 9' methyl group, the ratio of the 3'-keto intensity to 9' methyl intensity gives the fraction of drug in the 3'-keto form. For this quantitation procedure to be valid, however, the cross-polarization efficiencies of the 3'-keto and 9'-methyl carbons must be identical. Since protonated carbons tend to cross-polarize faster and to a greater extent for short contact times, the 3'-keto and 9' methyl carbons are not expected a priori to have similar cross-polarization rates. Grant and co-workers, however, have examined the quantitative reliability of CP/MAS, and have found that carbons with protons within two or three bonds will give relative signal intensities that agree with atomic ratios (8). Since the 4' carbon of the drug is protonated, quantitative measurements based on the 3' carbon signal might not be unreasonable. For example, it was found that the sum of the signal intensities from the 1', 2', and 3' (in both 3'-diol and 3'-keto forms) carbons was 2.5 to 3.1 times that from the 9' carbon; this figure is in reasonable agreement with the 3:1 ratio expected from the number of nuclei present. (Because of spectral overlap, it is necessary to measure the total signal from these three carbons.) Furthermore, if cross-polarization rates were vastly different, one would expect signal intensities to vary greatly with contact time. It was found, how-

![Fig. 1. The 3'-keto-diol equilibrium of trospenemycin sulfate.](image)

![Fig. 2. Carbon-13 NMR spectrum of trospenemycin sulfate bulk drug dissolved (a) in \( D_2O \) at 25°C and (b) in \( d_6 \)-DMSO.](image)