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Dissolution Testing of Veterinary Products

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Dissolution Testing of Tetracycline Boluses

Background

Antibiotics such as tetracycline may be indicated in animals for the treatment of bacterial pneumonia or bacterial enteritis caused by E. Coli and Salmonella organisms susceptible to tetracycline. USP 24 (1) includes monographs on tetracycline and tetracycline hydrochloride drug substance as well as several dosage forms, including a monograph for Tetracycline Boluses. The monograph for these boluses does not include a dissolution test; however, a dissolution test is provided in the USP that uses water as the medium for tetracycline hydrochloride capsules and tablets.

While the general objectives of this work are addressed in the preceding articles on Sulfa and Aspirin boluses, the intent of this study is to demonstrate that existing methods may be appropriate and to consider when water may be suitable as the dissolution medium.

Calf Scour Bolus Antibiotic examined in this study (NADA 65-004, Lot# 30DRB manufactured for Durvet, Inc., Blue Springs, MO by Pharmacia & Upjohn Company, Kalamazoo, MI) contains 500 mg of tetracycline hydrochloride in a bolus that weighs approximately 5.8 g. While the bolus is quite large, the amount of the active ingredient in this dosage form is not that much larger than what is often found in capsules and tablets for human use.

Solubility

A considerable amount of information related to the solubility of tetracycline and its hydrochloride salt is readily available in the literature. The approximate solubility of tetracycline in water at 25 ° C is 1 g in 2500 mL (0.0040 g/mL; 0.00090 M), while the solubility of the hydrochloride salt is 1 g in 10 mL (0.100 g/mL; 0.208 M) (1). Also, the pH of a 2% W/V (0.0416 M) solution of tetracycline hydrochloride in water is stated to be approximately 2.1 – 2.3 (2). At least one classical textbook in pharmaceutical chemistry presents a detailed discussion of the amphoteric nature of this drug (3).

For the tetracycline hydrochloride bolus dissolution, 0.500 g of drug in 900 mL corresponds to a concentration of 0.000556 g/mL (0.001 16 M). Thus, the concentration of drug in water at the end of complete dissolution is 180 times less than the solubility of the salt in water. This value far exceeds the 10 fold criterion for sink conditions and clearly suggests that water is a suitable medium.

Perhaps the main concern when considering water as the medium is that there is essentially no buffer capacity, and the pH of the medium at any time will be determined by the acidic and/or basic character of the dissolved drug and soluble excipients in the formulation. In this case, if the pH of the system is elevated such that the solid phase governing the solubility relationship is changed from the HCl salt to the much less soluble neutral amphoteric form, a precipitous decrease in solubility could occur. While the salt might dissolve quickly, it could precipitate from solution because of the pH of the environment.

Tetracycline [Tetra] is a complex electrolyte with multiple pK values: pK1 = 3.30; pK2 = 7.68; and pK3 = 9.69 (4). This complex character may be represented by the following equilibria:

$$H^+T \overset{K_1}{\leftrightarrow} H^+ + H^+T^- \qquad \text{Eq. (1)}$$

$$H^+T^- \overset{K_2}{\leftrightarrow} H^+ + H^+T^{-2} \qquad \text{Eq. (2)}$$

$$H^+T^{-2} \overset{K_3}{\leftrightarrow} H^+ + T^{-2} \qquad \text{Eq. (3)}$$

where H^+T, H^+T^-, H^+T^{-2}, T^{-2} are the various forms of tetracycline that can exist in solution with net charges of +1, 0, -1, and –2 respectively. It is particularly interesting that acid salts are formed through protonation of the third pK_a which is associated with the dimethylamino group on tetracycline. However, for the dissolution of drug products containing the hydrochloride salt, it is the equilibrium represented by Eq. 1 that is deterministic because of the low pH encountered and the expectation that the neutral amphoteric form H^+T^- is least soluble.

Assuming complete dissociation of the salt, a proton balance equation (5) for the titration of an aqueous solution of tetracycline hydrochloride with base would be:

$$(H^+) = (OH^-) + (H^+T^-) + 2\,(H^+T^{-2}) + 3\,(T^{-2}) - b \qquad \text{Eq. (4)}$$

where the parenthetical expressions represent molar concentrations of the respective species, and b refers to the molar concentration of added base which could be used to adjust pH. To simplify

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things, it is reasonable to assume that the concentrations of $H^{+}T^{-2}$ and T^{-2} are negligible in the pH region encountered in this study (e.g., pH values below 5). Thus, a "one-point" estimation of a functional dissociation constant may be made by using the pH of 2.2 cited above for the 2% W/V solution (where b = 0). With this information, an estimate of $pK_1' = 2.95$ is obtained. This appears to be a low, but certainly reasonable value, as the aforementioned value of 3.30 reported in the literature is presumably for a dilute solution. In fact, since the concentration of tetracycline in this solution is relatively low, and the overall ionic strength is much lower than that encountered in the previous articles where highly concentrated buffers were used, the literature value for pK1 will be used in subsequent calculations.

With complete dissolution of 0.500 g in 900 mL of water, using a value of pK1 = 3.30, the calculated pH at the end of dissolution would be 3.26. A preliminary experiment was performed by placing a bolus in a flask containing 900 mL of water, followed by sonication and vigorous agitation. The resulting system, which was yellow and somewhat cloudy, produced a pH of 3.32 at room temperature. These numbers compare reasonably well, and there is no indication that a formulation component is responsible for a significant elevation in pH.

At a pH value of 3.32, and an overall tetracycline concentration of 0.00116 M, the relative species concentrations would be (H+T) = 0.000564 and (H+T-) = 0.000591. While additional information is necessary to complete the solubility analysis, it can be noted that both of these values are less than the respective intrinsic solubilities for tetracycline hydrochloride (0.208 M) and tetracycline (0.0009 M) cited earlier.

The resulting solution pH of 3.32 is very close to the pK1 value. A model of the pH vs solubility relationship, assuming that the solid form present is H+T- with an intrinsic solubility of 0.0009 M, yields an estimated solubility at pH 3.32 of 0.00179 M. In this case, one would not expect the dissolved tetracycline in the dissolution test to precipitate.

Since the actual concentration and predicted solubility values are so close, an additional test was done to see if precipitation could be induced. The pH of a sample of the solution previously prepared by putting a bolus in 900 mL of water was elevated to a value of 5 by the dropwise addition of 0.1 N sodium hydroxide solution. No precipitation was observed over a 2 hour period. This is an indication that the nucleation and crystal growth process takes more time, or that the estimate of the intrinsic solubility of H+T- is not accurate.

Finally, tetracycline is unstable in aqueous solutions and the analysis of the dissolution samples should be completed as quickly after collection as possible.

Dissolution of Tetracycline Boluses

Dissolution testing of the tetracycline boluses was conducted using the method described for Tetracycline Capsules in the USP: Apparatus 2 at 37°C, 75 RPM, water as the medium, and UV analysis at 276 nm. Samples were collected at 5, 10, and 15 minutes using an automatic sampler with 0.45 micron in-line filters. To permit analysis without dilution, a cell with a path length of 0.01 cm was employed. The results are presented in Figure 1.

The boluses disintegrated within the first three minutes, and a large quantity of insoluble excipient was observed in dispersion in the vessels. As seen previously, some of the in-line filters became partially clogged, although manual sampling was not necessary. In some cases, fine powder was observed in the collected samples, indicating that the filters were not completely effective. Because of this observation, all samples were filtered again using 0.45 micron disposable syringe filters before spectrophotometric analysis. The entire analysis was completed within 1 hour of sampling, and there was no evidence of precipitation of tetracycline in any of the samples.

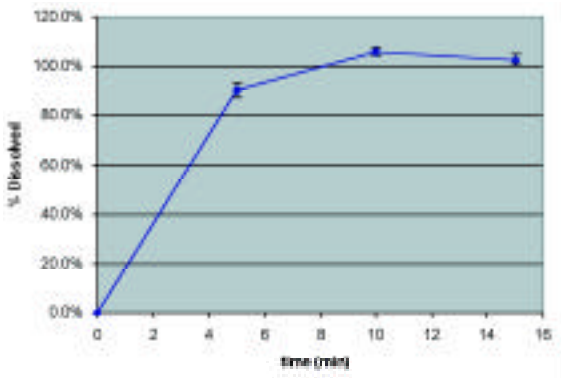

Figure 1. *Dissolution of Tetracycline Boluses in water at 37 ° C.*

Conclusions

Dissolution testing of veterinary boluses containing 500 mg of tetracycline HCl was accomplished using USP Apparatus 2 with conventional volume and stirring rates, with water as the dissolution medium. Based on the preliminary studies performed here, the tetracycline bolus dosage

forms dissolve quickly with apparently complete dissolution at 10 minutes with water as the medium at a stirring rate of 75 RPM.

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References

1. USP 24/NF 19, United States Pharmacopeial Convention, Rockville, MD (2000)
2. The Merck Index, 12th Edition, S. Budavari (ed), Merck Research Laboratories, Whitehouse Station, NJ (1996)
3. White, AW: Antibiotics, in Wilson, CO, Gisvold, O, and Doerge, RF: Textbook of Organic Medicinal and Pharmaceutical Chemistry, 6th Ed., J.B. Lippincott Company, Philadelphia, PA (1971)
4. Dean, JA, Lange's Handbook of Chemistry, 14th Ed, McGraw-Hill, Inc., New York, NY (1992)
5. Martin, A, Physical Pharmacy, 4th Ed., Lea and Febiger, Philadelphia, PA (1993)