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Dissolution Testing of Veterinary Products

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Dissolution Testing of Sulfa Boluses

Introduction:

The Food and Drug Administration (FDA) and the U.S. Pharmacopoeia (USP) recognize the importance of dissolution testing for the quality control of solid oral dosage forms. The Center for Veterinary Medicine (CVM) within the FDA approves and regulates animal drug products, including solid oral dosage forms unique to animal drugs, for example, boluses (large tablets or oblets for use in animals such as cattle). Currently, most of the approved bolus dosage forms are not quality control tested using a dissolution test; instead a disintegration test is used.

In 1997, the U.S. Congress enacted a new law, the Food and Drug Administration Modernization Act (FDAMA), that requires, in part, that both the human and animal drug manufacturers validate or assess the effects of any manufacturing change on their drug products. FDA has published a number of guidance documents that describe the documentation required to validate the effects of manufacturing changes, for example, Scale-Up Post-Approval Changes for Immediate Release Solid Oral Dosage Forms (SUPAC-IR). For many of the manufacturing changes, comparative dissolution studies on the drug product before and after the change was made is recommended. In these instances, dissolution testing is used to determine whether or not additional bioequivalency data are needed to support the manufacturing change.

Because there is no established discriminatory dissolution method for bolus dosage forms, comparative dissolution testing cannot be performed on boluses before and after a manufacturing change. Therefore, additional costly bioequivalence testing may be required in order to obtain CVM approval of a manufacturing change. CVM and the University of Maryland have initiated studies to develop a discriminatory dissolution method for boluses using existing USP methodology (i.e., General Chapter For Dissolution). The results of these studies may lessen the regulatory burden on the animal drug industry to validate the effects of a manufacturing change on bolus dosage forms.

Background

In the context of this work, a "bolus" is a solid oral dosage form for administration of one or more therapeutic agents to a large animal. A bolus can contain a large amount of drug relative to the typical content of a dosage form for humans. The development of an in vitro dissolution test for a bolus presents several challenges: selection or design of an appropriate dissolution apparatus and identification or formulation of a dissolution medium capable of providing sink conditions and sufficient buffer capacity.

The objective of this research paper is to explore the development of a potentially discriminating in vitro dissolution test for veterinary boluses using USP Apparatus 2. The widespread availability of this apparatus makes it a compelling choice, particularly when contrasted with the expense of both equipment and chemicals for larger volume testing. Thus, the objective was to use 900 mL or less of an aqueous dissolution media at 37 ° C, with a stirring rate less than or equal to 100 RPM.

Sulfa drugs were chosen for examination of the issues associated with in vitro testing of boluses: Sulfadimethoxine where each bolus contains 5 g of sulfadimethoxine; Sulfachlorpyradazine, where each bolus contains 2 g of sulfachlorpyradazine; and Sulfamethazine, where each bolus contains 5 g of sulfamethazine.

Solubility

It is generally desirable to study dissolution under sink conditions. For the purpose of this analysis, the assumption is made that sink conditions are met when the solubility of the drug, under the conditions of the test, is 10 times the maximum concentration that would occur if the dosage of drug were to completely dissolve. When using USP Apparatus 2, the typical volume is 900 mL. The initial step in design of the method is an assessment of aqueous solubility. As a general rule, the default selection for the medium is deionized water. In most situations the solubility of the drug in water alone will not be sufficient. Whenever possible, it is preferable to avoid the use of solubilizers and cosolvents.

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For a drug with one or more weakly acidic and/or basic functional group(s), which is not subject to rapid specific acid or base catalysis, the pH of the medium may be adjusted to accommodate the large quantity being dissolved. However, it is important to recognize that the dissolution of the drug itself may lead to a significant pH change if the dissolution medium does not have sufficient buffer capacity. Therefore it is critical to identify the pH at which sufficient solubility can be obtained, and then design a buffer system such that it will maintain that pH.

Standard solubility relationships may be used to identify the appropriate pH for the dissolution test. However, substantial deviations from ideal behavior should be expected given the high drug and buffer concentrations that will be present. These deviations should be accounted for, and this first step in developing a suitable method depends upon the availability of "real" solubility data.

Generally, deviations from ideal behavior can be adequately dealt with by using a "functional" dissociation constant rather than one that may have been determined in dilute solution. A functional dissociation constant is one that includes the overall effect of the activity coefficients of the chemical species participating in an equilibrium relationship without explicit determination of these activity coefficients.

For example, consider the drug sulfadimethoxine [SDMH] which has a single weakly acidic functional group:

$$SDMH \leftrightarrow SDM^- + H^+ \qquad \text{Eq. (1)}$$

$$St = (SDMH) + (SDM^-) \qquad \text{Eq. (2)}$$

$$K_{a.SDMH} = [\gamma_{SDM^-} (SDM^-) a_{H^+}] / [\gamma_{SDMH} (SDMH)] \qquad \text{Eq. (3)}$$

$$K_a'_{.SDMH} = [\gamma_{SDMH} K_{a.SDMH}] / \gamma_{SDM^-}] = [(SDM^-) a_{H^+}] / (SDMH) \qquad \text{Eq. (4)}$$

$$St = So [1 + K_a'_{.SDMH} / a_{H^+}] \qquad \text{Eq. (5)}$$

where SDMH and SDM- refer to the acidic (undissociated) and ionized (dissociated) forms of the drug, respectively. The designations (SDMH) and (SDM-) refer to the molar concentrations of these species, and St = total drug solubility. The dissociative equilibrium is characterized in Eq 3, where $K_{a.SDMH}$ = dissociation constant for the drug, and a_{H+} refers to the activity of the hydronium ion in solution as assessed by potentiometric measurement with a combination pH electrode. The terms γ_{SDMH} and γ_{SDM-} are the activity coefficients for the acidic and ionized forms of the drug, respectively. $K_a'_{.SDMH}$ = the functional dissociation constant for the drug which governs the relative concentrations of sulfadimethoxine chemical species in solution as a function of pH. In this analysis, an assumption is made that $K_a'_{SDMH}$ is indeed constant; an equivalent assumption is that the activity coefficient ratio $\gamma_{SDMH}/\gamma_{SDM-}$ is constant. From a theoretical point of view, one would expect the activity coefficient of a charged species to be more sensitive to changes in the ionic strength of the environment than an uncharged species. However, there is convincing evidence to indicate that the assumption of a constant activity coefficient ratio introduces only a slight bias in this and subsequent analyses (see Buffer System section, page 10).

With regard to Eq. 5, So = intrinsic solubility of the drug, which in this case is (SDMH) and which is constant in the portion of the pH-solubility profile where the solid phase is the weak acid.

Since deviations from ideal behavior are expected, and are significant, it is necessary to "fit" real data to Eq. 5. Usually an approach where So and pKa' are varied sequentially, until the theoretical relationship visually converges with the real data, is adequate. (In this case, the final parameters, expressed to three significant figures, correspond to a minimum sum of the squares of the residuals at the pH values where actual data was available.)

Real data for SDMH were obtained from the Merck Index(1). Figure 1 is a pH-solubility profile for SDMH which includes that data and compares it to a theoretical profile based on Eq. 5, with the

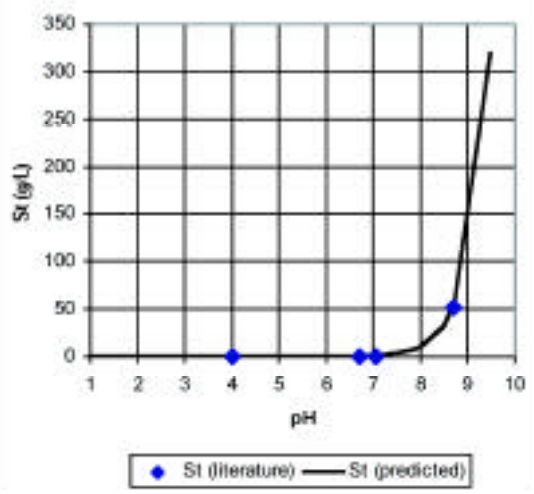

Figure 1. *Solubility of sulfadimethoxine as a function of pH.*

following parameter values: $pK_a'_{.SDMH}$ = 5.54 and So= 0.0349 g/L.

Once the intrinsic solubility and functional dissociation constant have been identified, it is possible to determine the pH where St is 10 times the maximum concentration that will be observed at 100% dissolution. For the SDMH bolus, 5 g of drug in 900 mL corresponds to a concentration of 5.56 g/L (0.0179 M). To achieve sink conditions would require a solubility of 55.6 g/L. Based on Eq 4, a pH of 8.74 would produce a system where the solubility is 55.5 g/L. Thus, the buffer system must be designed so that a pH of no lower than 8.74 will exist at the end of the dissolution process.

Extensive solubility data is not readily available in the literature for the other drugs selected here. However, they are structurally similar weak acids and it was anticipated that the dissolution medium designed for SDMH would also work for the other sulfa drugs. Another aspect of dissolution method development, which is not emphasized here, is to make sure that the drug is sufficiently stable in the dissolution medium to allow for an accurate assessment of the dissolution behavior.

Buffer System

If pH adjustment is the approach taken to increase solubility, then it is essential to make sure that the system's pH is at (or above in this case) that necessary to accommodate the drug. Since the drug itself is an acid, and a large quantity will be dissolved, its dissolution will decrease pH and change the system in a manner that decreases overall solubility. While changes in pH are inevitable, it is desirable to keep the change to a minimum. For this work, an arbitrary maximum change of 0.5 pH units was established as a goal.

A high buffer capacity is essential, and as a general rule one must choose a buffer system that has a pK_a' very close to the target pH. Even so, it will be necessary to utilize relatively high buffer concentrations to achieve the design goal, and once again substantial deviations from ideal behavior are to be expected. If possible, one should also avoid organic buffers that absorb light in the UV range so that simple analysis may be accomplished using spectroscopy.

A borate buffer system was selected for the dissolution medium. The USP Alkaline Borate buffer system was employed as a set of real solution data that allow an estimate of the functional pK_a' of the buffer representing the following equilibrium:

$$H_3Bor \leftrightarrow H+ + H_2Bor^- \qquad \text{Eq. (6)}$$

where H_3Bor and H_2Bor- refer to boric acid and the monobasic borate ion.

A proton balance equation (3) for a borate buffer system made by combining boric acid and sodium hydroxide would be:

$$(H+) = (OH-) + (H_2Bor-) - b \qquad \text{Eq. (7)}$$

where (OH-) = hydroxide ion concentration, (H_2Bor-) = borate ion concentration, and b = concentration of strong base (i.e., NaOH). In this equation, (OH-) and (H_2Bor-) concentrations correspond to the concentrations of hydronium ion contributed by water and boric acid, respectively, while b corresponds to the concentration of hydronium ion neutralized by the addition of the strong base.

Considering the respective equilibrium relationships, Eq. 7 may also be written as:

$$(H+) = K_w/(H+) + Ct\,(H+)/[(H+) + K_a'_{.BOR}] - b \qquad \text{Eq. (8)}$$

with K_w = dissociation constant for water, Ct = buffer concentration, and $K_a'_{.BOR}$ = functional dissociation constant for boric acid. Once again, the real data may be used to estimate $K_a'_{.BOR}$ by sequentially varying parameters in Eq. 7 until data from the theoretical relationship converges with the real data. These variations can be done easily on a spreadsheet, and the following table represents the results of such a process where Ct = 0.05 M.

Table 1. Species concentrations of the USP Borate Buffer System

pH	(H+)	(OH-)	(H_2Bor^-)	b(calc)	b(USP)
8	1.00E-08	1.00E-06	3.38E-03	0.0034	0.0039
8.2	6.31E-09	1.58E-06	5.15E-03	0.0052	0.0060
8.4	3.98E-09	2.51E-06	7.70E-03	0.0077	0.0086
8.6	2.51E-09	3.98E-06	1.12E-02	0.0112	0.0118
8.8	1.58E-09	6.31E-06	1.57E-02	0.0157	0.0158
9	1.00E-09	1.00E-05	2.10E-02	0.0210	0.0208
9.2	6.31E-10	1.58E-05	2.67E-02	0.0267	0.0264
9.4	3.98E-10	2.51E-05	3.23E-02	0.0323	0.0321
9.6	2.51E-10	3.98E-05	3.71E-02	0.0372	0.0369
9.8	1.58E-10	6.31E-05	4.10E-02	0.0411	0.0406
10	1.00E-10	1.00E-04	4.39E-02	0.0440	0.0437

In this case, the pH values used are those specified in the USP. Once pH is stipulated, (OH-) and (H_2Bor^-) can be determined from equilibrium relationships and b can be calculated from Eq. 7. The calculated values for b [i.e., b(calc)] can be compared to values of b determined from the alkaline borate buffer recipes in the USP. The values for

b(calc) in the table were obtained with a $pK_a'_{.BOR}$ = 9.14, and these compare very well with b(USP). Once again, the sum of the squares of the residuals was minimized to obtain the $pK_a'_{.BOR}$ estimate.

Now that functional pK_a' values have been determined for both the drug and the selected buffer, it is possible to proceed to the actual design of the buffer system.

Since the pK_a' for SDMH is 5.54 and the dissolution process will necessarily be conducted at or above pH 8.74, SDMH will be at least 2 pH units above its pKa and will behave like a strong acid, contributing its available protons to the solution. Another way to say this is that the concentration of undissociated SDMH present at pH 8.8 or above will be essentially zero.

Thus, the dissolution of SDMH in the borate buffer system could be represented by the following proton balance equation:

$$(H+) = (OH-) + (Bor-) - b + (SDM-) \qquad \text{Eq. (9)}$$

where, because of the low pK_a, (SDM-) = concentration of SDMH dissolved.

By stipulating the initial pH and concentration of a buffer system, it is then possible to estimate the pH that would result as SDMH dissolves, by solving Eq. 9 for (SDM-) at various pH's. Figure 2 represents a theoretical plot of pH vs amount dissolved in a 0.15 M pH 9.0 Borate buffer system. The graph also includes data from an actual dissolution test described below. It can be seen that the pH resulting after the dissolution of a 5 g bolus will be about 8.75. This corresponds well to the target established in the pH – solubility analysis.

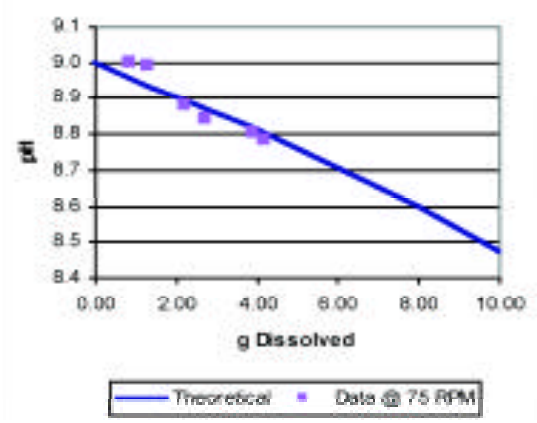

Figure 2. *Effect of dissolution of sulfadimethoxine bolus on the pH of the medium*

Dissolution of Sulfadimethoxine Boluses

Dissolution testing of sulfadimethoxine boluses was initially conducted using USP Apparatus 2 at 37° C and 75 RPM, with the 0.15 M pH 9.0 Borate Buffer as the medium. An automatic sampler was employed, and programmed to collect 3.5 mL at each of the following time points: 5, 10, 20, 30, 45 and 60 minutes. The plan was to analyze by UV spectroscopy at 268 nm, using 0.01 cm path length cells in an attempt to avoid sample dilution. However, analysis of standards indicated that this would not be possible for sulfadimethoxine, and a 1:10 dilution of samples was required. Samples from vessel 3 were arbitrarily selected for pH measurement, and the results of these measurements are included in Figure 2.

The sulfadimethoxine boluses tested disintegrated quickly, and the resulting quantity of solid in dispersion in the dissolution flask was quite large. Initial testing was performed with 0.45 micron in-line filters; however, several lines became clogged during the run and manual sampling was periodically necessary. In all cases, samples were cloudy and were filtered after collection using a 0.45 micron syringe filter. Subsequent attempts without the in-line filters resulted in clogging of the dip tubes, and resolving this issue will require additional work.

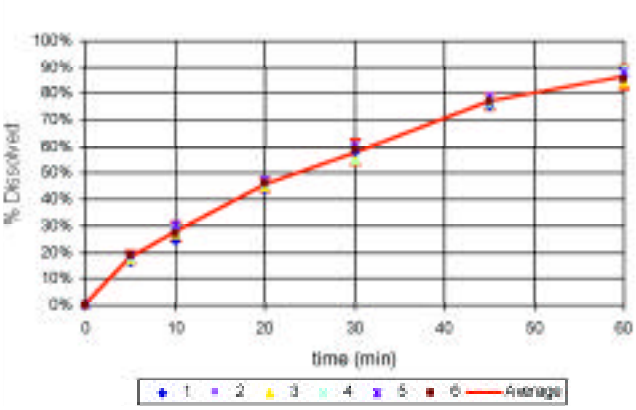

Figure 3. *Dissolution of sulfadimethoxine boluses at 75 RPM in 0.15 M pH 9 Borate Buffer at 37 ° C.*

Dissolution results from this initial test are presented in Figure 3, and coordinate grams Dissolved and pH measurements are included in Figure 2.

These results suggest that the dissolution medium works well, and that the boluses tested display very little variation in drug release at the time points tested. However, it is evident that the 60 minute time frame was not sufficient to obtain

Sulfa Bolus testing … *continued*

100% release with an RPM of 75.

A more comprehensive analysis was conducted at RPM's of 50, 75, and 100 using the same apparatus and conditions. These results are presented in Figure 4, where each profile is based on averages of 6 boluses; the error bars included in the figures are based on ± 2 standard deviations. The dependence of dissolution extent on RPM's suggests that the method has the ability to discriminate and may serve as an acceptable in vitro quality control method.

Dissolution of Sulfachlorpyradazine and Sulfamethazine Boluses

Sulfachlorpyradazine and sulfamethazine boluses were also tested in the 0.15 M pH 9.0 borate buffer medium, and the results are presented in Figures 5 and 6, respectively. The problems previously encountered with plugging of the filters did not occur in either of these dissolution studies. Once again, each of the profiles presented represent the mean of six boluses, and the error bars included are based on ± 2 standard deviations. The 2 g boluses of sulfachlorpyradazine presented complete and relatively rapid dissolution, with little effect of RPM on the profile. On the other hand, the 5 g sulfamethazine boluses presented slower dissolution with greater sensitivity to a change in stirring rate.

Conclusions

Potentially discriminating in vitro dissolution testing of veterinary boluses containing sulfa drugs with dosages up to 5 g can be accomplished using USP Apparatus 2 with conventional volumes and stirring rates in an aqueous medium specially designed to provide and maintain sink conditions. The design of an appropriate buffer system to be used as the dissolution medium for weakly acidic or weakly basic drugs can be accomplished by using standard theoretical relationships fitted to real solubility and buffer data.

The 0.15 M pH 9.0 borate buffer system employed in this analysis admittedly is more concentrated than normal, and the pH of 9.0 is outside of the preferred range for testing of human drug products. While the physiological relevance of testing under these conditions for drugs in animals remains to be determined, the sulfadimethoxine, sulfachlorpyradazine, and sulfamethazine boluses tested all disintegrated rapidly under the test conditions and each would have met a dissolution specification of Q = 85% in 60 minutes at 75 RPM, for example.

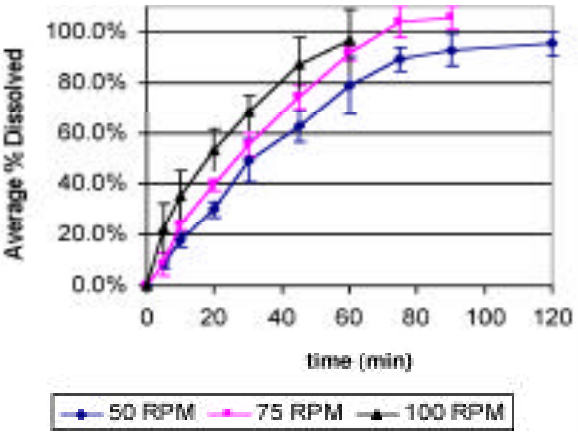

Figure 4. *Dissolution of Sulfadimethoxine Boluses in 0.15 M pH 9.0 Borate Buffer at 37 ° C.*

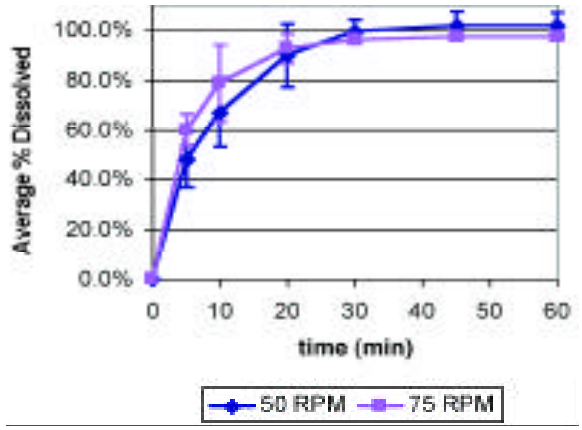

Figure 5. *Dissolution of Sulfachlorpyradazine Boluses in 0.15 M pH 9.0 Borate Buffer at 37 ° C.*

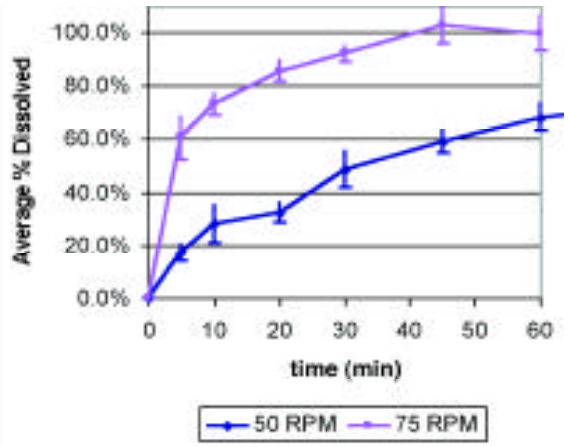

Figure 6. *Dissolution of Sulfamethazine Boluses in 0.15 M pH 9.0 Borate Buffer at 37 ° C.*

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