Correlation of Absorption of Sulfamethazine Boluses with Dissolution Using a New Dissolution Apparatus for Veterinary Tablets

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Abstract □ A rotating-basket apparatus for dissolution testing of veterinary bolus tablets was designed and constructed. Sulfamethazine boluses containing different disintegrating agents were evaluated *in vitro* and by blood level data following administration to cattle. The dissolution t_{50} and various pharmacokinetic parameters showed directly compressible starch and carboxymethylstarch to be the most effective disintegrants in the concentrations employed while magnesium aluminum silicate and microcrystalline cellulose were about equal but less effective than the previous disintegrants. A bolus formulation containing no disintegrant gave even less satisfactory results. A correlation was established between the dissolution t_{50} and the time to peak plasma level and also between the t_{50} and the area under the plasma–time curve for the first 36 hr.

Keyphrases □ Sulfamethazine—veterinary bolus tablets, dissolution testing apparatus described, various disintegrating agents evaluated and compared to *in vivo* absorption, cattle □ Dissolution testing apparatus—designed for veterinary bolus tablets, various disintegrating agents evaluated, compared to *in vivo* absorption, cattle □ Disintegrating agents, various—in veterinary bolus tablets, evaluated using dissolution testing apparatus, compared to *in vivo* absorption, cattle □ Absorption—sulfamethazine veterinary bolus tablets, various disintegrating agents evaluated *in vitro*, compared, cattle □ Veterinary bolus tablets—dissolution testing apparatus described, various disintegrating agents evaluated and compared to *in vivo* absorption, cattle □ Tablets, veterinary bolus—dissolution testing apparatus described, various disintegrating agents evaluated and compared to *in vivo* absorption, cattle

Dissolution rate testing has become an increasingly more important part of the pharmaceutical sciences, as evidenced by the volume of research articles reviewed by Wagner (1). These tests may detect potential absorption problems which can be caused by various excipients in the dosage form. Dissolution tests also can become important quality control procedures once the final formulation is established.

Since all apparatus developed for dissolution testing have been designed to accept conventional human dosage forms, the purpose of this work was to develop a larger dissolution apparatus capable of accepting veterinary bolus tablets. The device was used to determine the dissolution of sulfamethazine from bolus tablets containing various disintegrating agents. The boluses also were administered to cattle, and plasma sulfamethazine levels were determined. Various pharmacokinetic parameters were calculated to show an *in vitro–in vivo* correlation.

EXPERIMENTAL

Materials—Sulfamethazine[1] USP, gelatin[2] USP, magnesium stearate[3] USP, reduced iron[4] NF, directly compressible starch[5], carboxymethylstarch[6], magnesium aluminum silicate[7], microcrystalline cellulose[8], and sodium hydroxide USP were used.

Table I—Sulfamethazine Bolus Formulas[a]

Ingredients	Formula A	B	C	D	E
Sulfamethazine USP	67.5	60.75	64.13	65.82	64.13
Reduced iron NF	30.0	27.0	28.5	29.25	28.5
Gelatin USP	2.3	2.07	2.18	2.24	2.18
Magnesium stearate USP	0.2	0.18	0.19	0.19	0.19
Directly compressible starch	—	10.0	—	—	—
Carboxymethyl-starch	—	—	5.0	—	—
Magnesium aluminum silicate	—	—	—	2.5	—
Microcrystalline cellulose	—	—	—	—	5.0
Total	100.0	100.0	100.0	100.0	100.0

[a] Values are in percent (w/w).

Bolus Preparation—A single lot of sulfamethazine granulation containing finely powdered iron was prepared by the wet granulation method, using gelatin solution as the binder. Magnesium stearate was mixed with the dried granulation to serve as the lubricant, and the master lot was divided into five sublots. One sublot was tableted without the addition of a disintegrant. Each disintegrating agent was mixed with one of the four remaining sublots in a tumbler blender[9] for 10 min.

A single-punch tablet press[10] equipped with a bolus-shaped punch and die set, 7.8 cm long × 2.2 cm wide, was used to compress the bolus tablets. Boluses were tableted by manually filling the die with a preweighed portion of granulation and bringing the upper punch to the top of its compression cycle, followed by a power stroke of the machine. Sulfamethazine bolus formulas are given in Table I. The concentration of disintegrant in each formulation was within the concentration range recommended by the manufacturer.

Finely powdered iron was included in all formulations to increase the density of the bolus, since this factor is reported (2) to cause the bolus to remain in the rumeno-reticular sac until disintegration is complete. Since Formulation A contained no disintegrant, it was deemed advisable to include the iron. Bolus tablets were assayed for sulfamethazine content by the Bratton–Marshall (3) method after suitable preparation. Average weight, hardness, and disintegration times also were determined.

Dissolution Basket—The rotating dissolution basket for veterinary boluses (Fig. 1) is an adaptation of the device specified in the official compendia (4, 5). The top plate is a flat Plexiglas disk, 0.6 cm thick, 15 cm in diameter, with four 0.3-cm vent holes. A 25-cm stainless steel shaft is attached at the center. The tube is a Plexiglas pipe, 12.5 cm long, 0.5 cm thick, and 12.5 cm in diameter. Two windows, 8.8 cm high and 17.2 cm long, are cut in the walls of the tube. A 1.9-cm lip remains at each end of the tube, with two 2.5-cm supports between

[1] Napp-Lemke, Lodi, N.J.
[2] Wilson Laboratories, Chicago, Ill.
[3] Mallinckrodt, St. Louis, Mo.
[4] National Pulverized Metals, Chicago, Ill.
[5] STA-Rx 1500, Colorcon, Inc., West Point, Pa.
[6] Primojel, Edward Mendell Co., Yonkers, N.Y.
[7] Veegum WG, Vanderbilt, Inc., New York, N.Y.
[8] Avicel PH-102, FMC Corp., Marcus Hook, Pa.
[9] Twin Shell blender, Patterson Kelly Co., East Stroudsburg, Pa.
[10] Stokes model R, F. J. Stokes Machine Co., Philadelphia, Pa.

Table II—Bolus Properties

	Formula				
	A	B	C	D	E
Sulfamethazine, % (w/w)[a]	66.1	59.8	62.4	64.0	63.2
Weight, g[b]	26.55 (0.047)	29.53 (0.025)	27.95 (0.023)	27.31 (0.028)	27.91 (0.038)
Hardness, kg/cm²[c]	22.6 (0.17)	22.1 (0.62)	20.0 (0.82)	24.1 (0.24)	24.3 (0.37)
Disintegration time[d], min	>90[e]	1.96 (0.08)	1.44 (0.06)	>90[e]	>90[e]

[a] Average of three determinations. [b] USP XVIII, average of 20 tablets ± *SD*. [c] Carver laboratory press, model C, average of five tablets ± *SD*. [d] USP XVIII, average of six tablets ± *SD*. [e] Test stopped at 90 min.

the windows. The bottom plate is a flat Plexiglas disk, 0.6 cm thick, 15 cm in diameter, with a 8.8-cm hole located at the center.

The tube and bottom plate are lined with 40-mesh woven stainless steel cloth. The top and bottom plates are held in place on either end of the tube by four bolts, which pass on the outside of the tube through matching holes in each plate.

***In Vitro* Dissolution Tests**—A 20-liter tank[11] with a tight fitting lid containing a hole for the shaft and sample removal was used. The tank was filled with 18.0 kg of dissolution medium, which was allowed to equilibrate with the room temperature (controlled at 25 ± 1°). When the dissolution medium reached the desired temperature, the weight was rechecked and an addition was made if necessary. Then the bolus tablet to be tested was placed in the basket, and the top plate was attached. The cover was put in place, over the shaft, which was then attached to the constant-speed stirring motor[12].

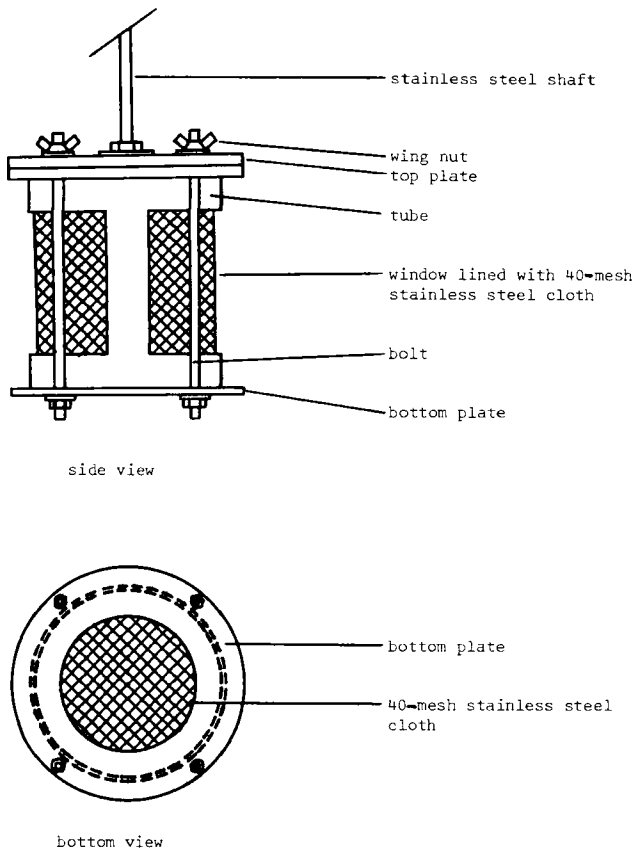

Figure 1—*Veterinary bolus rotating basket.*

[11] United States Plastic Corp., Lima, Ohio.

Table III—Dissolution (Percent) of Sulfamethazine from Bolus Tablets[a]

	Formula				
Minutes	A	B	C	D	E
0	0.0	0.0	0.0	0.0	0.0
5	2.86 (1.28)	58.80 (3.6)	71.25 (7.85)	2.40 (0.51)	2.12 (0.85)
10	4.93 (1.23)	69.75 (1.6)	80.92 (4.72)	5.20 (0.53)	3.86 (0.99)
15	6.22 (0.80)	74.55 (2.06)	84.82 (3.74)	6.79 (0.74)	6.19 (0.94)
30	9.03 (0.94)	80.50 (2.40)	93.07 (4.08)	10.23 (0.87)	8.21 (0.89)
60	12.51 (1.74)	86.15 (2.76)	97.90 (2.80)	16.19 (1.17)	13.47 (2.16)
120	17.83 (1.39)	92.91 (2.83)	99.84 (1.48)	23.12 (1.13)	21.19 (0.73)
180	21.62 (0.84)	95.17 (2.42)	100.18 (0.96)	27.96 (1.14)	27.60 (1.29)
240	25.06 (1.91)	96.66 (1.68)	101.39 (1.06)	32.18 (0.48)	32.76 (0.65)
300	28.61 (1.65)	96.83 (0.67)	101.72 (2.03)	36.16 (1.10)	37.11 (1.04)
360	32.08 (1.56)	97.16 (1.24)	101.98 (1.64)	39.37 (0.70)	39.93 (1.86)

[a] Average of five determinations ± *RSD*.

The basket was lowered into the dissolution medium, a height adjustment was made so that the clearance between the bottom of the tank and the bottom of the basket was 2.5 cm, and rotation was started. Samples were obtained by pipet, followed by replacement of an equal volume of dissolution medium. Sulfamethazine concentration was determined spectrophotometrically at 257 nm. Sodium hydroxide solution, 0.1 *N*, was selected as the dissolution medium to assure sink conditions if complete drug release occurred, and the rotational speed was arbitrarily set at 50 rpm. Five boluses of each formula were tested in the described apparatus.

***In Vivo* Absorption Tests**—Three healthy Black Angus cattle (two heifers and one steer) were kept in an indoor stock pen, 8 × 12 m, allowing for moderate exercise; the building temperature was maintained at approximately 16°. The animals were allowed free access to food[13] and water, except during a 4-hr period on the morning of each dosage administration. All three animals received the same formulation at the same time. Two days after drug could not be detected in their plasma, they were given the next formulation. In this manner, all animals received all five formulations in the order B, C, D, E, and A. The time between the peak blood level and the administration of the next formulation was always in excess of 10 half-lives (elimination), assuring essentially complete "washout" of the drug. It was assumed that no "period" effects occurred.

The dose of sulfamethazine was 21 mg/kg; when necessary, a portion of bolus was cut off to maintain a uniform dose. This procedure avoided different dosages for each animal and kept constant the weight of drug per unit weight of tablet. While there was some increase in surface area of tablet per unit weight of drug, it was minimal since each animal received a minimum of two complete boluses and part of a third bolus and a maximum of three complete boluses and part of a fourth bolus.

Boluses were administered with the aid of a balling gun[14]. Blood samples were taken from the jugular vein with heparinized vacuum tubes[15]. Plasma sulfamethazine was determined by the Bratton–Marshall (3) method after preparation of the samples by hemolysis, deproteinization, and filtration.

RESULTS AND DISCUSSION

The results of the assay for sulfamethazine content of tablets of the different formulations are given in Table II. Also presented are the average weight, hardness, and disintegration times. *In vitro* dissolu-

[12] Hi-Torque stirrer, VWR Scientific, Kansas City, Kans.
[13] Purina Preconditioning Chow V, Ralston-Purina, St. Louis, Mo.
[14] Jensen-Salsbery Laboratories, Kansas City, Mo.
[15] Becton-Dickinson, Rutherford, N.J.

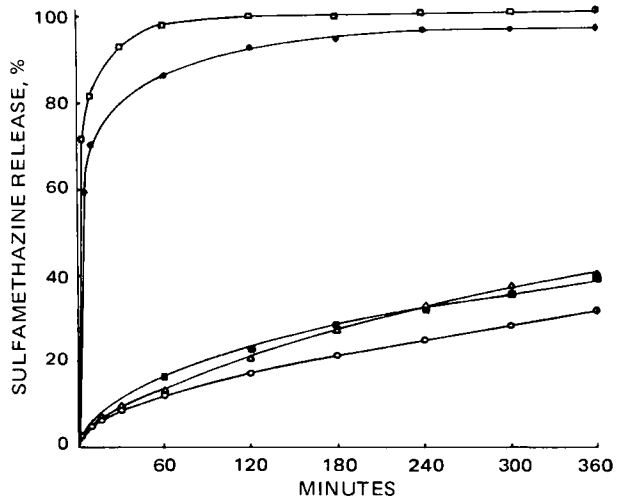

Figure 2—*Dissolution profiles of sulfamethazine from bolus formulations. Key: ○, Formula A; ●, Formula B; □, Formula C; ■, Formula D; and △, Formula E.*

tion tests of five experimental sulfamethazine bolus formulations were conducted in the specially designed rotating-basket apparatus. Concentrations of sulfamethazine in solution are given in Table III, and dissolution profiles are shown in Fig. 2.

Boluses containing directly compressible starch and carboxymethylstarch literally fell apart in the apparatus while those of the other formulas were intact at completion of the test. The time required for 50% of the sulfamethazine to go into solution (t_{50}) was estimated by two different methods. The log of percent drug undissolved plotted against time (6) for the slow dissolving formulations, A, D, and E, was linear, especially for data at 2–6 hr. Calculation of linear regression

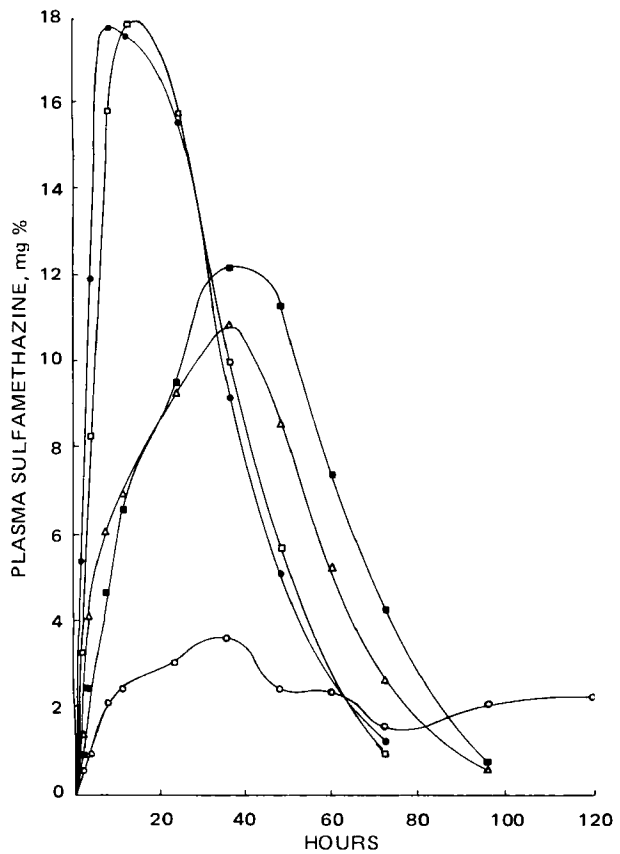

Figure 3—*Plasma levels of sulfamethazine from bolus formulations. Key: ○, Formula A; ●, Formula B; □, Formula C; ■, Formula D; and △, Formula E.*

Table IV—Plasma Sulfamethazine Levels (Milligrams Percent) following Administration of Sulfamethazine Boluses[a]

Hours Post-administration	Formula				
	A	B	C	D	E
0	0.0	0.0	0.0	0.0	0.0
2	0.51 (0.12)	5.46 (0.77)	3.32 (1.55)	0.86 (0.18)	1.46 (0.22)
4	0.87 (0.29)	12.02 (2.35)	8.35 (0.73)	2.46 (1.00)	4.14 (0.86)
8	2.12 (0.51)	17.79 (2.29)	15.91 (0.53)	4.67 (1.47)	6.09 (0.90)
12	2.43 (0.30)	17.57 (0.74)	17.90 (0.59)	6.61 (1.73)	6.97 (1.13)
24	2.97 (0.07)	15.57 (0.89)	15.84 (0.74)	9.46 (1.72)	9.29 (0.39)
36	3.58 (0.21)	9.24 (1.53)	10.10 (0.37)	12.19 (0.44)	10.90 (0.93)
48	2.43 (0.06)	5.19 (1.82)	5.83 (0.48)	11.27 (0.72)	8.57 (1.55)
60	2.35 (0.04)	—	—	7.39 (0.39)	5.32 (1.25)
72	1.57 (0.41)	1.25 (0.92)	1.04 (0.16)	4.31 (0.92)	2.74 (0.72)
96	2.07 (0.42)	—	—	0.78 (0.11)	0.56 (0.20)
120	2.26 (0.79)	—	—	—	—
144	1.44 (0.98)	—	0.0	—	—
168	0.75 (0.90)	0.0	—	0.0	—
192	0.31 (0.44)	—	—	—	0.0
240	0.22 (0.32)	—	—	—	—
288	0.0	—	—	—	—

[a] Average of three cattle ± *SD*.

for the 2–6-hr data for individual tablets was used to predict the t_{50}. The intercepts of linear regressions were consistently close to the theoretical value of 2.0. The fast dissolving formulations, B and C, dissolved so rapidly that the data did not fit either the log percent undissolved–time plot or the probit percent dissolved–log time plot (6). The t_{50} values for individual tablets were thus estimated by proportion from the amount dissolved at 5 min. The mean t_{50} and standard deviations of the five tablet values for each formulation are given in Table V.

Mean plasma sulfamethazine levels determined in the *in vivo* absorption studies are given in Table IV, and plasma sulfamethazine curves are shown in Fig. 3. Various pharmacokinetic parameters determined from these data are given in Table V along with t_{50} dissolution values. The times to reach peak concentrations were estimated from the plasma curves, and absorption half-lives were calculated by the method of residuals (7). Elimination half-lives were calculated from log plasma concentration–time plots. Areas under the plasma

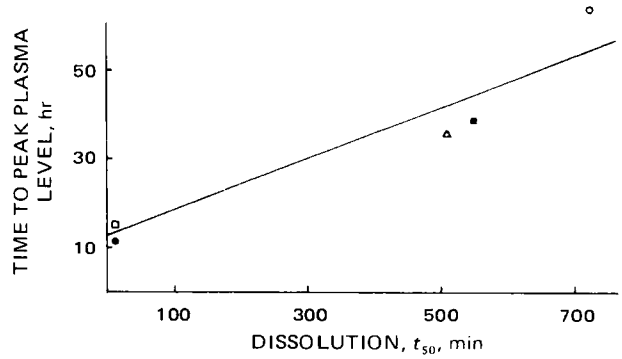

Figure 4—*Correlation of dissolution with peak plasma levels. Key: ○, Formula A; ●, Formula B; □, Formula C; ■, Formula D; and △, Formula E.*

Table V—Pharmacokinetic Parameters

Parameter	Formula A	B	C	D	E
Area under plasma–time curve, mg% × hr	377[a]	680	679	658	571
	(124)[b]	(69.4)	(2.57)	(25.5)	(49.0)
Area under plasma–time curve, 0–36 hr, mg% × hr	88.7	501	490	268	272
	(9.22)	(21.1)	(7.91)	(53.4)	(24.1)
Estimated time to peak plasma concentration, hr	64.0	11.7	15.3	38.7	36.0
	(48.5)	(4.73)	(1.15)	(2.31)	(0.0)
Absorption half-life, hr	19.3	6.6	6.3	9.1	10.7
	(14.6)	(1.07)	(0.52)	(1.71)	(1.76)
Elimination half-life, hr	23.2	10.9	8.6	10.0	12.6
	(20.7)	(3.07)	(1.08)	(1.99)	(1.25)
Estimated t_{50} dissolution, min	722	4.26	3.54	545	509
	(85.7)	(0.285)	(0.425)	(12.2)	(44.4)

[a]Mean of three values. [b]Standard deviation.

curves were calculated for individual animals by the trapezoidal rule and include the area to 288 hr for Formulation A. These areas were subjected to an analysis of variance for single-factor experiments having repeated measures on the same elements, and this analysis showed statistically significant differences among the five formulations at $p = 0.01$. Application of the Tukey t test showed Formulation A to be different from the other four at less than the 0.05 level of confidence; at less than the 0.01 level of confidence, the results showed no significant difference between A and E or between E, D, C, and B.

Two characteristics of the plasma level curve that should be noted are the low concentrations and prolonged time for complete elimination of the drug from Formulation A. An early investigation set a minimum therapeutic blood level at 5 mg% (8). While it is recognized that the minimum therapeutic concentration cannot be set in such broad terms but depends on several factors, one can question whether Formulation A released the drug at a rate to produce an effective antibacterial level. The prolonged blood levels caused by this formulation would result in a longer time between the cessation of treatment and the marketing of the animal for human consumption since tissues of such animals must be free of drug prior to harvesting.

Absorption half-lives were used to evaluate the disintegrating agents. A statistical comparison of absorption half-lives at less than the 0.05 level of confidence revealed that there was no significant difference between half-lives for boluses containing directly compressible starch and carboxymethylstarch but that these values were significantly lower than for the other boluses. There was no significant difference between boluses containing magnesium aluminum silicate and microcrystalline cellulose; the bolus containing no disintegrating agent produced the slowest absorption. Carboxymethylstarch was the most effective disintegrating agent tested since it produced results comparable with directly compressible starch and was used at a lower concentration.

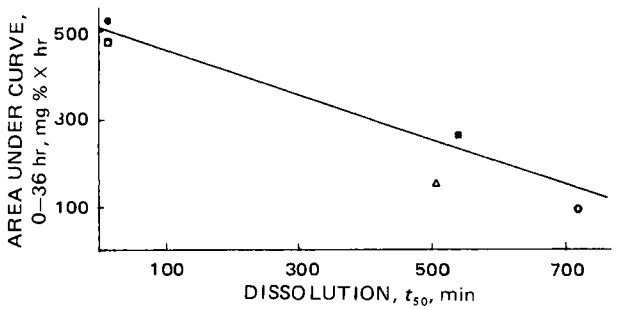

Figure 5—*Correlation of dissolution with area under the plasma level–time curve, 0–36 hr. Key:* ○, *Formula A;* ●, *Formula B;* □, *Formula C;* ■, *Formula D; and* △, *Formula E.*

Comparison of the mean dissolution t_{50} values with the mean pharmacokinetic parameters indicated correlations with the time to peak plasma levels and with the area under the plasma–time curve for the first 36 hr. These correlations are shown in Figs. 4 and 5. Calculations showed Pearson's r to be 0.953 for the t_{50}–time to peak plasma concentration correlation, which was significant at less than the 0.02 level of confidence; $r = -0.980$ for the t_{50}–area under the plasma curve, 0–36 hr, which was significant at less than the 0.01 level of confidence.

In conclusion, the rotating basket gave consistent results and could serve as a useful tool in the development and quality control of veterinary bolus tablets.

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