Technical Problems of the USP/NF Dissolution Test

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Abstract □ The USP/NF dissolution test (Method I or rotating-basket method) was applied to different commercial tablet formulations of sulfisoxazole, sulfamethoxazole, and tolbutamide. It was found that the hydrochloric acid solution required for testing the sulfa drug tablets corroded the basket so that it could not be used after 40 hr. of exposure to the acid. Because the dissolution time of a sulfamethoxazole formulation in phosphate buffer (pH 7.2) was close to that obtained using compendial conditions, it is suggested that the official dissolution medium could be replaced, with advantage, by the phosphate buffer. The dissolution times of nine different sulfisoxazole formulations tested in a less acidic medium were not comparable in rank order and magnitude to those obtained with the compendial test. Dissolution times of eight different tolbutamide formulations in phosphate buffer (pH 7.6) were similar in rank order and magnitude to those obtained with the compendial test. It is suggested that this buffer replace the tromethamine [tris-(hydroxymethyl)aminomethane] buffer, currently official, which has a large temperature coefficient of pH.

Keyphrases □ Dissolution testing, USP/NF method—corrosion of basket apparatus, replacement of official fluids with phosphate buffer □ Rotating-basket dissolution method—corrosion of basket, replacement of official fluids with phosphate buffer □ Sulfisoxazole tablet dissolution—corrosion of rotating basket, proposed use of phosphate buffer □ Sulfamethoxazole tablet dissolution—corrosion of rotating basket, proposed use of phosphate buffer □ Sulfa drug formulations dissolution—corrosion of rotating basket, proposed use of phosphate buffer □ Tolbutamide tablet dissolution—use of phosphate buffer in rotating-basket method

The USP (1) and NF (2) have established dissolution requirements for 13 drugs. As with many innovations, there are technical problems in the application of the present tests. During a survey of the dissolution characteristics of Canadian drug products, several problems with the practical aspects of the USP XVIII and NF XIII Method I became apparent. These problems are described in this paper, together with some suggestions for alternative methods that attempt to retain the same rank order and magnitude of dissolution times as the official tests. In this way, any existing *in vivo–in vitro* correlation would not be disturbed.

EXPERIMENTAL

Tablets—Sulfamethoxazole, sulfisoxazole, and tolbutamide tablets were obtained from various manufacturers.

Dissolution Tests—The official test (1, 2) (rotating-basket method) was applied to the various drug products. In this paper, t_x indicates the time for x% of the labeled drug content of a single tablet to dissolve; $\bar{t}_x$ is the mean of six successive t_x determinations.

Additional Measurements—Sulfisoxazole and sulfamethoxazole tablets were tested in a less concentrated (1 in 100) hydrochloric acid solution and in phosphate buffer (0.05 *M*, pH 7.2). Stirring rates of 100 and 150 r.p.m. were used. In one experiment, 16 tablets (Product D) were tested consecutively in the same basket using the official method (1). The basket was weighed and photographed after each test (2.5 hr.). The loss in weight of the basket after exposure to a 1 in 100 hydrochloric acid solution for a total of 40 hr. was recorded.

Tromethamine (1 in 40) and phosphate (0.05 *M*) buffers at pH 7.2 and 7.6 were used as solvents in the dissolution studies of tolbutamide tablets.

RESULTS AND DISCUSSION

Sulfisoxazole and Sulfamethoxazole—Initial studies with the official dissolution fluids resulted in considerable basket destruction (Fig. 1). To determine if increased abrasion of tablets in mutilated baskets progressively affected dissolution times of tablets, one sulfisoxazole formulation (Product D), with a $\bar{t}_{60}$ value of 153 min. and a low coefficient of variation (5.3) in its t_{60} value, was selected for a detailed study. There was no marked effect on either the overall mean t_{60} (159 min.) or the coefficient of variation (7.6) for the 16 tablets tested. The corresponding values for three successive groups of six tablets in the 16-tablet series were: Tablets 1–6: $\bar{t}_{60}$, 153 min., and *CV*, 5.3; Tablets 6–11: $\bar{t}_{60}$, 166 min., and *CV*, 5.1; Tablets 11–16: $\bar{t}_{60}$, 158 min., and *CV*, 9.1.

During the experiment the basket weight decreased in a regular manner (about 18 mg./hr.) (Fig. 2). After 42 hr. of exposure to the acid, the basket could not retain a tablet. The major areas of destruction were different for the two baskets used. Deterioration of the first basket occurred mainly on the 40-mesh wire screen sides, while the second basket was mainly corroded on the bottom (Fig. 1). Since there was no deterioration of the basket in hydrochlorothiazide studies using a less concentrated (1 in 100) hydrochloric acid solution, five sulfisoxazole products (A–E) representing a wide range of t_{60} values (under compendial conditions) were tested in the less concentrated hydrochloric acid. While there was no noticeable corrosion or loss in weight of the basket, dissolution times were increased considerably (Table I). Although there was no change in the rank order t_{30} values in the 1 in 100 hydrochloric acid, the slow dissolution of the drug makes this solvent inconvenient.

Dissolution times (t_{30}) were increased 25–40% when the stirring rate was raised to 150 r.p.m. (Table I). However, the t_{30} values were

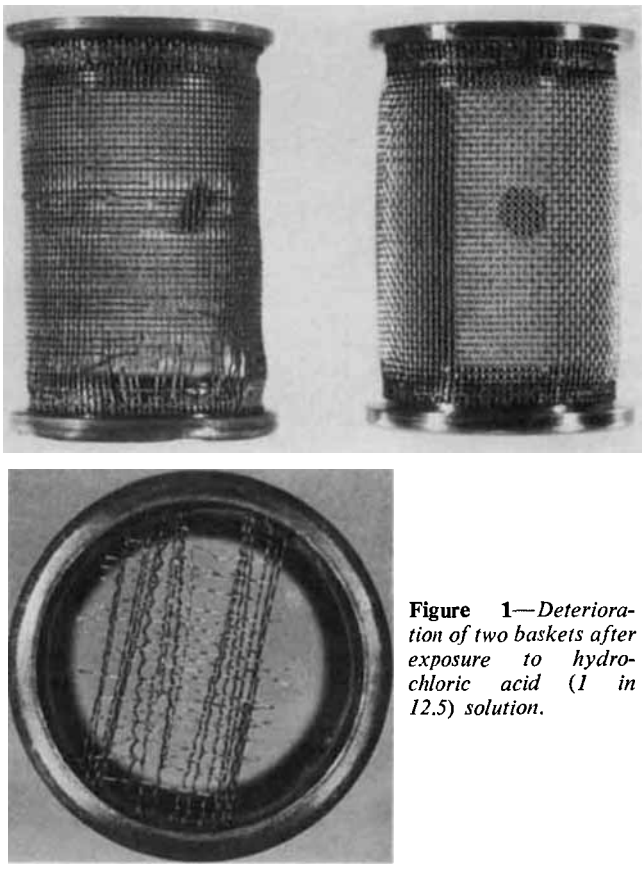

Figure 1—*Deterioration of two baskets after exposure to hydrochloric acid (1 in 12.5) solution.*

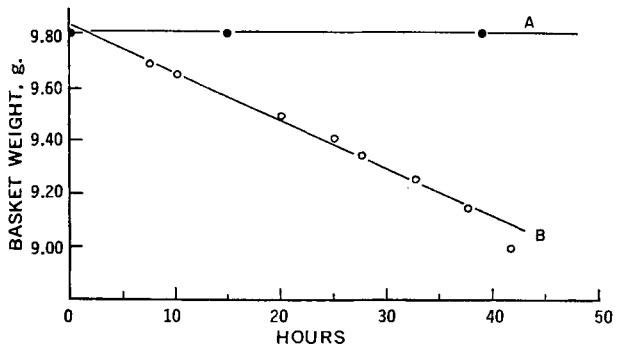

Figure 2—*Effect of exposure to hydrochloric acid solution (A, 1 in 100; B, 1 in 12.5) on basket weight.*

still generally much longer than those obtained with the official procedure. Estimations of sulfisoxazole concentrations in the various tests were based on comparisons with standard solutions of the drug prepared at the same final acid strength as the test solution. This is important since the absorptivity of sulfisoxazole in dilute hydrochloric acid solutions varies with the acid strength (*viz.*, 1 in 100, *a* 20.0; 1 in 2500, *a* 55.7). When a phosphate buffer (pH 7.2) was substituted for the hydrochloric acid solution, dissolution times (t_{60}) for the faster dissolving formulations were comparable to those obtained with the compendial test conditions; however, slower dissolving formulations (D and E) showed increased dissolution times (Table I). Four additional formulations (F, G, H, and J) which met compendial requirements were then tested in the phosphate buffer. Two of these (F and H) showed no major change in t_{60} values, but the other two (G and J) gave longer t_{60} times in the alkaline dissolution medium and would not meet the current official limits.

The variety of test conditions used yielded dissolution times that were not comparable in either rank order or magnitude to those obtained with the current compendial test (1). Some 50 compendial tests could be done on sulfisoxazole tablets with each basket if the average useful life of a basket is about 40 hr. In the absence of an *in vivo–in vitro* correlation and since deterioration of the basket does not appear to affect dissolution times, the advantage of a shorter testing time with the current compendial conditions (1) appears to outweigh that of an increased life of a basket which would be obtained with any of the conditions examined in this study.

Table I—Dissolution Times of Sulfisoxazole from Different Formulations

Formulation	Solvent[a]	t_{60}, min.	t_{30}, min.	t_{15}, min.
A	I	14, 11	10, 8	2, 2
	II	28, 35	16, 15	7, 7
	III	19, 15	8, 7	3, 2
B	I	39, 31	16, 15	8, 7
	II	96, 114	31, 31	17, 17
	II[b]	74, 57	20, 22	11, 12
	III	38, 25	17, 13	8, 7
C	I	120, 121	53, 53	24, 22
	II	—	—	144, 133
	II[b]	—	—	83, 83
	III	—	126, 141	45, 48
D	I	160, 145	71, 76	34, 28
	II	—	—	164, 167[c]
	II[b]	—	—	136, 140
	III	195, 192	88, 87	44, 42
E	I	164, 207	87, 108	42, 50
	II	—	402, 309	225, 194
	III	294, 270	105, 99	57, 54
F	I	16, 14	8, 5	4, 2
	III	13, 11	5, 4	2, 1
G	I	18, 12	9, 8	5, 5
	III	30, 46	16, 20	7, 8
H	I	12, 13	6, 6	2, 3
	III	17, 20	9, 11	5, 5
J	I	13, 15	7, 8	4, 5
	III	64, 43	40, 40	17, 17

[a] I, hydrochloric acid (1 in 12.5); II, hydrochloric acid (1 in 100); and III, phosphate buffer, 0.05 *M*, pH 7.2. [b] Stirring rate 150 r.p.m. instead of the usual 100 r.p.m. [c] t_{10} values.

Table II—Dissolution Times of Tolbutamide from Different Tablet Formulations in Tromethamine and Phosphate Buffers

Formulation	Buffer	pH	t_{60}, min.	t_{50}, min.
L	Tromethamine	7.2	3	1
		7.6	2	1
	Phosphate	7.2	3	1
		7.6	2	2
M	Tromethamine	7.2	13	6
		7.6	6	4
	Phosphate	7.2	7	5
		7.6	9	6
N	Tromethamine	7.2	42	27
		7.6	53	39
	Phosphate	7.2	36	16
		7.6	41	26
O	Tromethamine	7.2	205	79
		7.6	87	44
	Phosphate	7.2	59	20
		7.6	59	25
P	Tromethamine	7.2	821	557
		7.6	389	351
	Phosphate	7.2	902	556
		7.6	514	382
Q	Tromethamine	7.6	35	28
	Phosphate	7.6	37	28
R	Tromethamine	7.6	56	41
	Phosphate	7.6	70	57
S	Tromethamine	7.6	31	23
	Phosphate	7.6	23	18

The minor difference in acid strength (7 in 100 instead of 1 in 12.5) required by the compendial test for sulfamethoxazole determinations (2) did not reduce the extent of corrosion of the basket. The dissolution of sulfamethoxazole from the one formulation tested was, like sulfisoxazole, slower in the less concentrated (1 in 100) hydrochloric acid solution than the official (7 in 100) hydrochloric acid solution (t_{50} 8.2 and 5.0 min., respectively). The dissolution time (t_{60} 6.3 min.) in phosphate buffer (pH 7.2) was close to that obtained with the compendial conditions (5.0 min.). Either the phosphate buffer or the less concentrated hydrochloric acid could then replace the present official dissolution fluid (hydrochloric acid, 1 in 12.5) without change in present limits.

Tolbutamide—While the original (1) lack of definition of the tromethamine buffer required for dissolution measurements of tolbutamide tablets has been corrected (3), the temperature-dependent (about 0.024 pH unit/°C) tromethamine buffer was retained. Data obtained under the original (1) and modified (3) conditions indicate that t_{60} values for some formulations vary appreciably in the pH range 7.2–7.6 (Table II); care must be exercised to ensure that the solution is at the required pH at 37°. Because a less temperature-dependent buffer would be simpler to prepare, dissolution times in phosphate buffer (pH 7.2 and 7.6) were obtained. A similar rank order to that found in tromethamine buffer at the same pH value was obtained, although the t_{50} and t_{60} values were, with one exception, slightly lower in phosphate buffer (Table II). In the absence of any precise *in vivo–in vitro* correlation, the differences in dissolution times in the tromethamine and phosphate buffers are probably not significant; therefore, a change of dissolution medium to phosphate buffer (pH 7.2) should be considered.

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NOTES

Turbidimetric Method for Assay of Nitrofuran Compounds

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Abstract □ A turbidimetric method for the assay of nitrofuran compounds in urine and serum is described. The results of the assay were subjected to statistical analysis. The extent of inhibition and the form of regression of responses were affected by the concentration of indicator organism, time of incubation, and quantity of serum or urine. Optimal conditions for the assay of nitrofurantoin and furazolidone were determined.

Keyphrases □ Nitrofuran compounds — turbidimetric assay in urine and serum, using *S. faecalis* as test organism □ Turbidimetric assay—nitrofuran compounds, in urine and serum, using *S. faecalis* as test organism

Nitrofurantoin and other 5-nitrofuran derivatives are used extensively as antimicrobial agents, and extensive work is being conducted on new nitrofuran derivatives. This work has emphasized a need for the determination of low levels of such compounds in biological fluids. There are chemical methods (1–6) for the determination of nitrofuran compounds. However, microbiological methods are preferable for the potency determination of active compounds because these assays are more sensitive. Furthermore, compounds possessing analogous chemical structure or even the breakdown products can interfere with the chemical methods of assay, while the microbiological methods can help to assess the antimicrobial activity available.

Colorimetric and other chemical methods (1–5) involve conversion of 5-nitrofuran derivatives to 5-nitrofurfuraldehyde phenylhydrazone, followed by extraction in solvent and column chromatography or by extraction in nitromethane and color development with hyamine (6). These methods are elaborate and time consuming.

The purpose of this study was to develop a turbidimetric method for the estimation of nitrofuran compounds in biological fluids.

MATERIALS AND METHODS

Assay Medium—The medium contained 2% (w/v) glucose, 0.9% (w/v) casitone (Difco), 0.5% (w/v) yeast extract (Difco), 1.0% (w/v) sodium citrate, 0.1% (w/v) potassium phosphate monobasic, and 0.1% (w/v) potassium phosphate dibasic. The pH was adjusted to 6.8 ± 0.1. The assay medium, double strength, was prepared and sterilized at 15 lb./in.^{2} for 15 min.

Inoculation Medium—The inoculation medium was the same as the assay medium.

Indicator Strain and Inoculum—After preliminary investigation, *Streptococcus faecalis* (ATCC 10541) was selected as the test microorganism for the assay procedure. Material from the stock agar stab culture was inoculated into the inoculation medium and incubated overnight at 37°. This culture was then adjusted to a reading of 80 on a colorimeter[1] prior to inoculation into the assay medium.

Preparation of Standard Stock Solution—Stock solutions of nitrofurantoin and furazolidone were prepared by dissolving 20 mg. of each compound into 2 ml. of dimethylformamide and were preserved in the refrigerator at 4–5° in brown bottles.

Assay Procedure—The stock solutions were further diluted with sterile water to contain 10 and 5 mcg./ml. of nitrofurantoin and furazolidone, respectively. To the clean dry sterilized test tubes were added, in triplicate, 0.0, 0.2, 0.4, 0.6, 0.8, 1.0, and 1.2 ml. of the standard solution, 0.5 ml. of serum or urine, and sterilized water to a total volume of 2.5 ml. Then 2.5 ml. of the inoculated double-strength assay medium was added to each tube and mixed. The tubes were suitably covered with cotton plugs and incubated at 37 ± 0.5° in a constant-temperature water bath. The incubation period was best adjudged by visual observation after 4 hr.

The control serum and urine added to the standard tubes were passed through a filter (Seitz).

Recording of Results—At the end of the incubation period, two drops of 40% formalin solution was added to each tube and thoroughly mixed to arrest further growth. Turbidity measurements were made in the colorimeter[1], using a 640 filter after adjusting to zero with proper blanks of the standard and the test samples. Averages of the standard readings were plotted against the dose on ordinary graph paper.

Statistical Treatment of Results—A statistical analysis of the assays was carried out according to the method described by Kirshbaum *et al.* (7); an analysis of variance was made and tabular and calculated F values of linearity and regression were compared to establish a dose–response relationship. Standard deviation and mean percentage were determined whenever necessary.

RESULTS AND DISCUSSION

Figures 1 and 2 present a typical dose–response curve of the test organism to nitrofurantoin in rat and human serum and urine and of furazolidone in rat serum and urine, respectively. Table I shows the analysis of variance of these data to test for the possible departure from linearity and the effect of regression. The calculated F values are significantly higher for regression and lower for linearity than those of the tabular F values. This finding shows the existence

[1] Klett–Summerson.