

Dissolution Rate Studies II: Modified Column Apparatus and Its Use in Evaluating Isosorbide Dinitrate Tablets

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Abstract □ A modified, continuous flow, column-type dissolution apparatus, using commercially available components, is described. Two different size dissolution chambers are compared. The system is employed to evaluate experimental and commercial tablets of isosorbide dinitrate (oral and sublingual). Continuous dissolution profiles for each product are presented, and some significant differences among products are shown. Results with this method are compared to those obtained with three beaker methods, including the official USP XVIII-NF XIII rotating basket. Correlation of disintegration times and t_{50} 's is made. Specific advantages of the flow method (especially with sublingual tablets) are cited.

Keyphrases □ Dissolution rates, isosorbide dinitrate tablets—modified column apparatus, compared to official methods □ Isosorbide dinitrate tablets—dissolution rates, modified column apparatus, compared to official methods

The inherent advantages of the column-type, continuous flow method of determining dissolution characteristics of solids were discussed at length previously (1), and similar procedures were described by others (2-7). The disadvantages of the static beaker methods, emphasized in that discussion, were clearly shown by Withey (8). He found (as have others) that significantly different dissolution rates for the same solid result when variations in the beaker system are used. With the USP XVIII-NF XIII rotating basket procedure, he demonstrated distinctly different dissolution rates for the same solid simply by varying the depth of sampling the solution. This was also observed in our laboratories.

The potential difficulties inherent in all variations of the beaker method are eliminated or greatly reduced by using the column-type, continuous flow technique to measure the rate of dissolution. This article describes a modified column-type apparatus and reports its use in evaluating the dissolution characteristics of various tablet formulations (including commercial products) of isosorbide dinitrate. Results are compared to those obtained by the beaker system using three types of agitation: the USP XVIII disintegration apparatus, a three-bladed stirrer, and the USP-NF rotating basket.

EXPERIMENTAL

Description of Column-Type Apparatus—A picture of the two dissolution chambers (13- and 25-mm. size) and a schematic drawing of the entire system appear in Figs. 1 and 2, respectively. The basic system is similar to that described previously (1), but a

detailed description of the modified apparatus will be given so others can investigate its capabilities.

The solvent reservoir (A in Fig. 2) consists of a 4-l. flask submerged in a constant-temperature bath. The bath temperature is adjusted to provide a dissolution chamber temperature of $37 \pm 1^\circ$ for any given flow rate. It is not necessary to place the cell itself in a bath, making the system more convenient and useful for routine studies. For closer temperature control, however, a second bath for the chamber is desirable.

A filter holder¹ (B), using a 14- μ size nylon filter, is attached to the flexible tubing (C) in the reservoir. This keeps the dissolution chamber free from extraneous particulate matter. The pump (D) is a variable-speed peristaltic tubing pump with solid-state speed control² with a pump head capable of delivering 7.5-150 ml./min. (other pump heads with less or greater capacity are available). This type of pumping system: (a) allows the use of 0.1 N HCl without causing pump deterioration or solvent contamination, (b) permits the use of different flow rates for different experiments while holding a given flow rate constant during each experiment, (c) eliminates the need for a flowmeter in the system, and (d) allows for more convenient flow control compared to a centrifugal pump, where bypass valves must be used. Lerk and Zuurman (6) cautioned against using anything but a centrifugal pump, but their results were obtained at a relatively high flow rate (120 ml./min.) where pulsation is a problem. At the flow rates of these experiments (12-14 ml./min.), the effects of pulsation are not significant.

The tubing from the pump is connected directly to the bottom of the dissolution chamber, which is a commercially available ultrafiltration cell³. The cell is available in two sizes (with volumes of 3.5 and 15.5 ml.) and consists of a filter holder (13- or 25-mm. size), the two parts (E and J) of which are separated by a polycarbonate cylinder (G) that screws into the two filter holder parts. The system takes standard 13- and 25-mm. filters.

A Teflon-faced stainless steel support screen (F), with a sealing gasket, rests on the bottom half (male part) of the filter holder (E), but no filter is used here. The filter in the solvent reservoir keeps this screen clean; the direction of flow is such that particles in the sample do not fall through the screen, and the absence of any filter at this site helps ensure uniform flow into the chamber. Another support screen and gasket (I), with a 14- μ size nylon filter (H), are placed at the top of the cylinder. The lower screen (F) and the upper filter (H) thus form the boundaries of the system in which undissolved sample material is free to move. The low volume of the chamber reduces homogeneity problems to a minimum, even at low flow rates. Because of its lower volume, the 13-mm. cell is preferred; however, it tends to clog more rapidly because of the small cross-sectional area of the filter.

The top of the cell [*i.e.*, the female part of the filter holder (J)] is connected to flexible tubing by means of a male Luer locking adapter. This facilitates disassembling the cell and also changing the effluent tubing system for different types of experiments. For example, for continuous analysis the effluent solution can be routed [using the appropriate tubing system with a standard t-tube (K) as an air trap] through a spectrophotometric flow cell (L) and

¹ Swinnex-25.

² Cole Parmer, No. 7545.

³ Millipore Corp. No. XX4201300 and No. XX4202500.

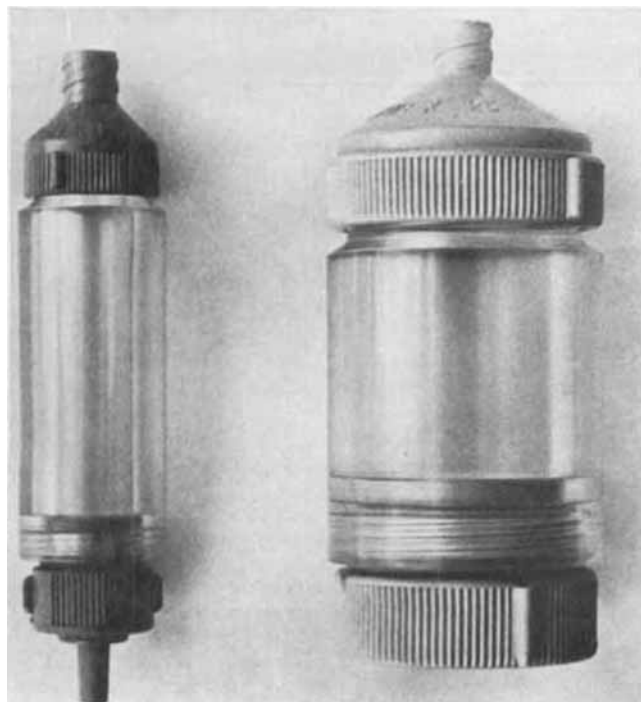


Figure 1—Photograph of (left) 13- and (right) 25-mm. dissolution chambers.

then collected in a receptacle (M) which, in effect, acts as an integrator for subsequent analysis. If continuous monitoring is not needed, this tubing system can be disconnected and a short delivery tube [leading directly into the receptacle (M)] can be used instead.

Procedure for Flow Method—Isosorbide dinitrate in solution was determined at 220 nm. with a recording spectrophotometer⁴. At this wavelength, there was some interference from materials extracted from the filters and tubing; but since this contribution to absorbance reached a constant value within 15 min., the procedure was designed to eliminate this as a variable. Either 0.05% polysorbate 80, which has a surface tension similar to that of gastric juice (9), or 0.1 N HCl was used as the solvent. The flow rate was 13.5 ± 1 ml./min. The 25-mm. chamber was used for oral tablets and the 13-mm. unit for sublingual tablets.

Between determinations, the dissolution chamber was disassembled and cleaned (rinsing with purified water was sufficient). It was then reassembled (including new filters in the cell and solvent reservoir) and the pump was activated. The system was allowed to flush for exactly 15 min. to bring the dissolution cell up to temperature and to reduce the blank absorbance to a constant value for each experiment (0.03–0.06). A 30-ml. sample was then taken for the blank, and the cylinder was unscrewed from the upper part of the filter holder. The cell was dried with absorbent paper, a tablet was placed on the center of the lower support screen, and the chamber was reassembled. The pump was activated and the process was observed until dissolution was complete.

When the spectrophotometric flow cell was used, the dissolution profile was recorded on a strip chart. In all other cases, the effluent solution was continuously collected (directly as it flowed out of the dissolution cell) in graduated cylinders in 5-min. (oral tablets) or 30-sec. (sublingual tablets) intervals. Calculations were corrected for the lag time in the tubing systems. The amount of drug dissolving during any time period was calculated from the volume of that aliquot and its UV absorbance. Each experiment was performed in triplicate.

Procedure for Beaker Methods—The usual procedure, with 800 ml. of 0.1 N HCl at $37 \pm 1^\circ$ in a 1-l. beaker, was followed. Agitation was provided by: (a) the USP disintegration apparatus (official speed) without disks, (b) a three-bladed stirrer (1.5-cm. blades) at 50 r.p.m. (2.0 cm. from the bottom), or (c) the USP-NF rotating basket at 100 r.p.m. (also 2.0 cm. from the bottom). Samples were withdrawn every 5 min. and read at 220 nm. on a spectropho-

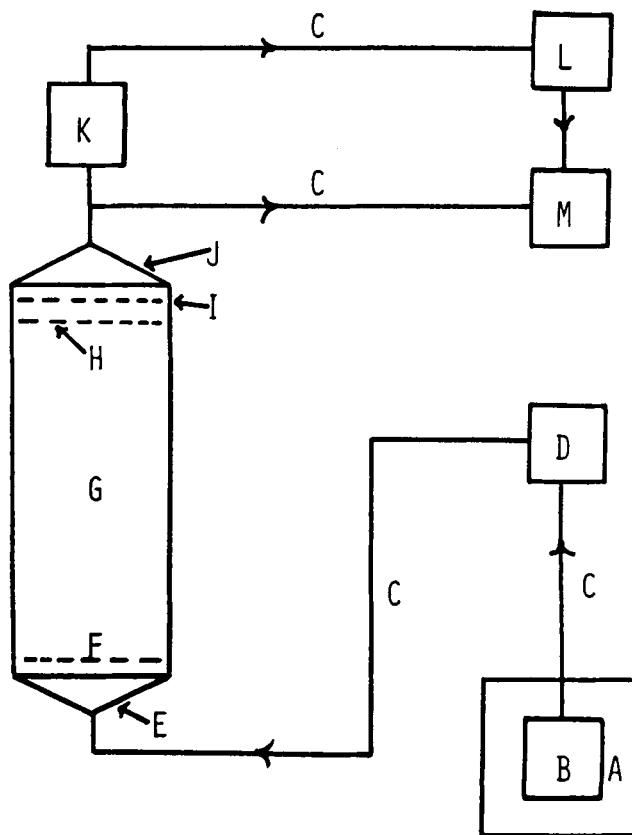


Figure 2—Schematic drawing of modified column-type system. See text for explanation.

tometer⁵. When the rotating basket was used, samples were withdrawn from the same position in the beaker each time. Two 10-mg. or four 5-mg. oral tablets were used for each determination; sublingual tablets were not studied. Each experiment was conducted in duplicate.

Procedure for Determining Disintegration Time—The official procedure was used, except that the solvent was 650 ml. of 0.05% polysorbate 80 and no disks were used. The recorded time was an average of six tablets.

Comparison of 13- and 25-mm. Dissolution Chambers—Dissolution rates of the oral experimental formulations were determined using the 13-mm. cell (under identical conditions) and the results compared to those obtained with the 25-mm. unit.

Preparation of Experimental Formulations—Experimental formulations of 5- and 10-mg. oral isosorbide dinitrate tablets were identically prepared except for drug content. Two formulations of each strength were prepared, the only difference being that in one formula of each strength the trituration (25% drug and 75% lactose) was milled prior to use. One formulation (using milled trituration) of 5-mg. sublingual tablets was prepared.

RESULTS AND DISCUSSION

Validity of Assay—Isosorbide dinitrate has an absorption peak at 200 nm., but 220 nm. was used because of instrument limitations and interference. Since a peak was not used and since 220 nm. is a questionable wavelength for assay purposes, it was necessary to show that this method was a valid experimental technique for detecting isosorbide dinitrate quantitatively. This was done in the following manner.

1. A Beers' law plot was constructed using data from three separate experiments. A good fit was obtained, and an absorptivity of 10.07 ml./mg. (1-cm. cell) was calculated with 95% confidence limits of 0.11 ml./mg.

2. In three separate experiments, accurately weighed 40-mg. samples of isosorbide dinitrate trituration (equivalent to 10 mg.

⁴ Coleman Hitachi EPS-3T.

⁵ Hitachi Perkin-Elmer model 139.

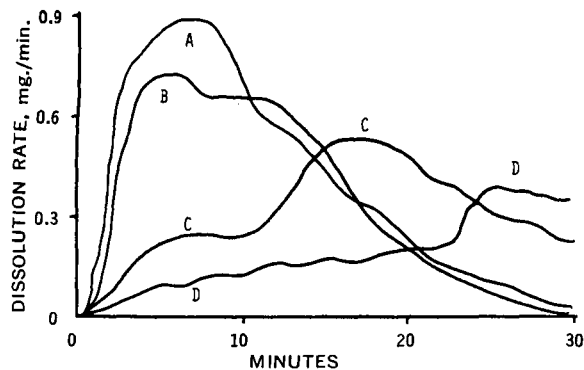


Figure 3—Continuous UV tracing of dissolution rate versus time for 10-mg. oral tablets (A and B are experimental formulations; C and D are commercial products). See text for explanation.

of drug) were subjected to the dissolution procedure. The average recovery was $99.3 \pm 2.9\%$.

3. In 68 determinations of the dissolution rates of isorbide dinitrate preparations, the average recovery was 105%. The high average was primarily due to the fact that no placebos of the commercial products were available.

4. Placebo tablets for the 5- and 10-mg. experimental formulations were subjected to the dissolution procedure; the total absorbance, after correcting for the blank, was zero.

5. Two saturated solutions of isorbide dinitrate were prepared, one involving a 10-fold increase in the amount of trituration used. Both solutions had identical absorbances at 220 nm., indicating that impurities did not contribute significantly to the absorbance.

6. A solution of isorbide dinitrate was prepared and its absorbance was read at 220 nm. After 3 days of storage at room temperature, its absorbance was the same.

Dissolution Rate Comparison of Different Formulations—By using dissolution rate as an *in vitro* criterion of efficacy, the experimental isorbide dinitrate formulations were compared with commercial products (two lot numbers of each) from two manufacturers. In the first series of experiments, the column-type apparatus, with a 1-cm. flow cell in the spectrophotometer, was used to obtain a dissolution profile for each product. The solvent was 0.05% polysorbate 80, the flow rate was 13.5 ml./min., the 25-mm. dissolution chamber was used for oral tablets, and the 13-mm. unit was used for sublingual tablets. A continuous tracing of dissolution rate versus time was obtained for each tablet simply by converting absorbance to dissolution rate (dissolution rate = absorbance \times flow rate/absorptivity). The curves for 10- and 5-mg. oral tablets and 5-mg. sublingual tablets are given in Figs. 3, 4, and 5, respectively. Since these tracings continually monitor dissolution rate, this technique yields accurate and useful information about tablet formulations.

For example, in Fig. 3 the two experimental formulations (A, milled; B, unmilled drug) exhibit similar profiles of a standard disintegrating tablet. The peak dissolution rate occurs in about 3 min. (there is a lag time in the flow system of about 0.6 min.). While these two profiles are similar (A and B have the same formula), profiles obtained on other individual tablets of A and B showed noticeable differences between the two. These discrepancies in results are due to individual tablet variation, which the method is sensitive enough to detect. Occasional similar profiles for A and B are not that surprising, since the isorbide dinitrate trituration is probably made by spraying a solution of the drug on lactose. Thus, the effects of milling on the surface area of isorbide dinitrate would not be as great as where the drug is present as solid particles.

The commercial products have their own distinct profiles. Product C is a wear-away formulation and does not disintegrate in the classic sense; thus, its dissolution rate rises at an even rate until it reaches a maximum in about 9 min. Product D, on the other hand, is a disintegrating tablet which does not disintegrate very well; it reaches a maximum dissolution rate in about 14 min.

While Product D in Fig. 3 exhibits a relatively poor profile, its 5-mg. counterpart (Fig. 4) shows excellent dissolution characteristics, disintegrating almost immediately and reaching its peak rate within 2 min. The 5-mg. experimental formulations (A, milled; B, unmilled) again have somewhat similar profiles, peaking at about

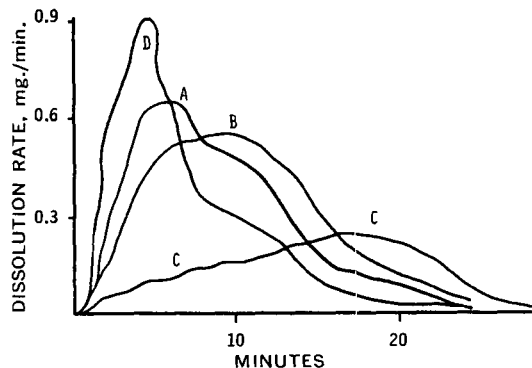


Figure 4—Continuous UV tracing of dissolution rate versus time for 5-mg. oral tablets. (See Fig. 3 and text for further explanation.)

5 min. Product C (5 mg.) again exhibits the profile of a wear-away tablet and reaches a maximum in 7-8 min.

This "profile" technique is especially useful in evaluating sublingual tablets, where dissolution is especially critical. Preliminary experimental sublingual formulations of isorbide dinitrate were quickly and easily screened by this procedure, observing the peak rate and how quickly it was attained. Each sample was evaluated semiquantitatively in less than 10 min. The formulator could vary excipient composition and, in a matter of minutes, determine the effects of that formula change on dissolution. The sensitivity of the profile method allows ready detection of small changes in dissolution behavior.

Figure 5 (sublingual tablets) shows that both the experimental formulation (A) and Product D peak within 2 min. (2.4 mg./min. for D and 2.0 mg./min. for A), with D peaking somewhat earlier but not significantly so. Product C peaks within 2 min. with a relatively low value of 0.7 mg./min.; another peak (0.4 mg./min.) appears as late as 6 min. While none of these *in vitro* results was correlated with *in vivo* data (an extremely difficult task with this class of drugs), it is evident that sublingual Product C could be improved from a dissolution standpoint.

In the second series of experiments, the conditions were the same but the spectrophotometric flow cell was not used. Instead, the solution was collected (directly as it flowed out of the chamber using a short piece of flexible tubing) in 100-ml. graduated cylinders, each receptacle representing the amount of drug dissolved in 5 min. (oral tablets) or 30 sec. (sublingual tablets). The absorbance of each fraction was read at 220 nm. Thus, instead of collecting differential data (dissolution rate) as before, this set of experiments yielded data in the integral form, expressed as amount of drug dissolved in a given time period (amount = volume of fraction \times concentration). Both the profile and the integral analysis could be obtained on the same sample by carefully calibrating the lag time in the tubing system involving the spectrophotometric flow cell.

It is obvious that this type of integral data is identical to that collected in beaker-type experiments. What may be less obvious is the fact that, apart from other advantages, the flow system allows more precise measurements of volume and absorbance. For example, by using the beaker method with a 5-mg. isorbide dinitrate

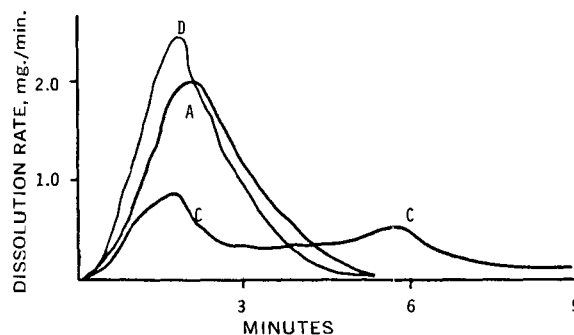


Figure 5—Continuous UV tracing of dissolution rate versus time for 5-mg. sublingual tablets (A is an experimental formulation; C and D are commercial products). See text for explanation.

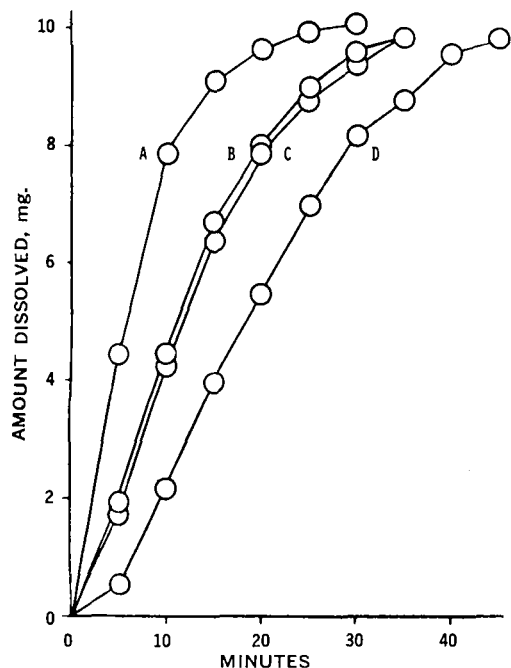


Figure 6—Plot of amount dissolved versus time for 10-mg. oral tablets. (See Fig. 3 and text for explanation.)

tablet in 300 ml. of solvent, 50% dissolution would result in an absorbance reading of 0.084, a relatively inaccurate range. With the flow system, however, 50% dissolution in one 67.5-ml. fraction (13.5 ml./min. \times 5 min.) would give an absorbance of 0.373 and lead to more accurate results. This limitation of the beaker method necessitated using more than one tablet for those experiments.

The experience with the sublingual tablets emphasizes another advantage of the flow method. In this type of procedure, there is no difficulty in collecting fractions as often as every 18 sec. This contrasts with the beaker method, where manual sampling every 18 sec. would be a hectic, if not impossible, procedure. Furthermore, with volumes of 300–1000 ml. and relatively mild agitation, it is doubtful that solute distribution (*i.e.*, equilibrium) would be rapid enough

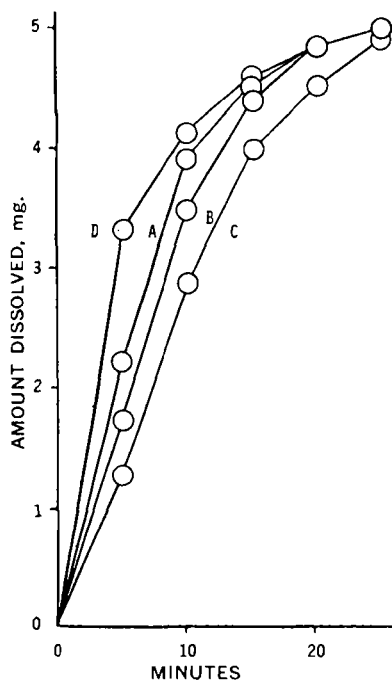


Figure 7—Plot of amount dissolved versus time for 5-mg. oral tablets. (See Fig. 3 and text for explanation.)

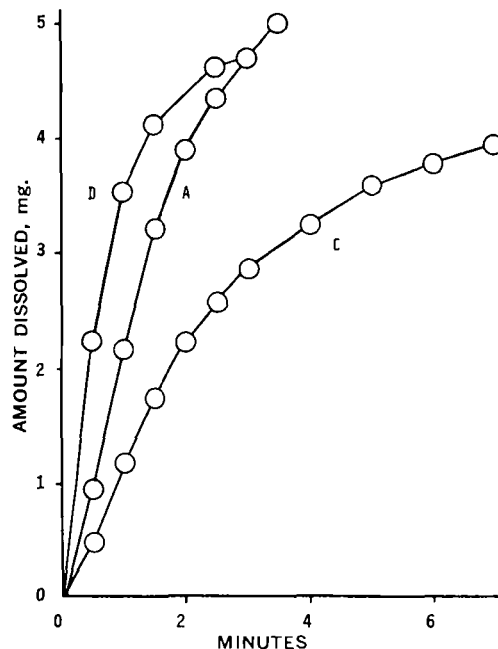


Figure 8—Plot of amount dissolved versus time for 5-mg. sublingual tablets. (See Fig. 5 and text for explanation.)

for such frequent sampling. Without question, Withey (8) showed that a 2-min. sampling interval was not enough time to establish proper solute distribution using the USP–NF rotating basket under his conditions.

Figures 6, 7, and 8 are linear plots of amount dissolved *versus* time for 10- and 5-mg. oral and 5-mg. sublingual tablets, respectively. Comparisons of the different formulations are essentially the same as those found in the profile studies. The observed discrepancies (*e.g.*, A and B have similar profiles in Figs. 3 and 4 but quite different plots in Figs. 6 and 7) are primarily due to individual tablet variation and the fact that profiles represent a single tablet while the plots from integrated data are averages of three determinations.

Dissolution rate data are often plotted with semilog coordinates but were omitted to conserve space. However, the pertinent calculations from such plots (using data presented in Figs. 6–8) are shown in Table I. The slopes are least-squares lines, and the t_{50} and t_{90} values are calculated from the slope. The t_{50}^* values are estimated from the linear plots in Figs. 6–8. Significant differences were calculated using standard methods.

Table II presents similar data except that the solvent was 0.1 *N* HCl; the sublingual tablets were not evaluated in this solvent. These data indicate no appreciable differences between solvents, except that with hydrochloric acid (or without polysorbate 80) the dissolution rates of A and D (10 mg.) were noticeably slower, with the most pronounced effect on D. Since 0.05% polysorbate 80 has a surface tension similar to that of gastric fluid, it seems advisable to use it in combination with 0.1 *N* HCl as an artificial gastric fluid.

In the third series of experiments, dissolution was determined in 0.1 *N* HCl with the beaker method, using three types of agitation. Sublingual tablets were not studied. Table III summarizes the data. Comparisons among formulations using the different methods are expressed as ratios of the semilog slopes (dissolution rates), with the slowest rate used as the common denominator. For example, using the three-bladed stirrer method, the rate of A (10 mg.) is 3.5 times faster than that of D (10 mg.). From the data it is apparent that, while the ratios vary considerably from method to method, the ranking of the formulations within each method is quite consistent.

Comparison of Different Methods—The values presented in Table III (for 0.1 *N* HCl) were rearranged so that the different methods (rather than different formulations) could be compared. These results are shown in Table IV. The numbers were derived in the same manner as those for Table III. Again, there was considerable scatter of the ratios from one formulation to the next; but within each particular formulation, the rank of the methods was quite consistent.

Table I—Semilog Slope, t_{50} , t_{50}^* , and t_{90} for Dissolution of Isosorbide Dinitrate Tablets in 0.05% Polysorbate 80 Using the Flow Method

Product	Slope, min. ⁻¹	t_{50} , min.	t_{50}^* , min.	t_{90} , min.	Significantly Different from
10-mg. oral					
A	-0.0709	4.2	5.8	14.1	B, C, D (0.05)
B	-0.0415	7.2	12.2	24.1	A
C	-0.0382	7.9	11.4	26.2	A
D	-0.0276	10.9	18.6	36.2	A
5-mg. oral					
A	-0.0711	4.2	5.9	14.1	No
D	-0.0708	4.2	3.9	14.1	No
B	-0.0633	4.7	7.2	15.8	No
C	-0.0523	5.7	8.9	19.1	No
5-mg. sublingual					
D	-0.472	0.64	—	2.1	C (0.01)
A	-0.371	0.81	—	2.7	C (0.01)
C	-0.106	2.8	—	9.4	D, A

Table II—Semilog Slope, t_{50} , t_{50}^* , and t_{90} for Dissolution of Isosorbide Dinitrate in 0.1 N HCl Using the Flow Method

Product	Slope, min. ⁻¹	t_{50} , min.	t_{50}^* , min.	t_{90} , min.	Significantly different from
10-mg. oral					
A	-0.0543	5.5	6.7	18.4	D (0.01)
B	-0.464	6.5	9.0	21.6	D (0.01)
C	-0.0412	7.3	12.6	24.3	D (0.01)
D	-0.0110	27.3	28.9	90.9	A, B, C
5-mg. oral					
D	-0.0854	3.5	3.4	11.7	No
A	-0.0742	4.0	5.2	13.5	No
C	-0.0611	4.9	9.2	16.5	No
B	-0.0526	5.7	6.4	19.0	No

Further comparisons among these methods, including various flow rate-chamber size combinations of the flow method, will be the subject of a future article.

Correlation of Dissolution and Disintegration Data—The plot in Fig. 9 shows good correlation between disintegration times and the t_{50}^* 's from Table I. Data from Product C, 10 and 5 mg., are not included because they are wear-away tablets; sublingual tablets were not considered. The correlation between disintegration time and t_{50} 's (calculated from semilog slopes) from Table I is not as good. A discussion of t_{50}^* versus t_{50} appears in the following section.

Validity of Various Methods for Presenting Dissolution Data—There are numerous ways of presenting dissolution data (cube root, semilog, t_{50} , dissolution time, etc.), and an exhaustive discussion of their relative merits is beyond the scope of this paper. However, based on these studies, a few comments seem advisable. With the flow method, t_{90} 's (calculated from semilog slopes) correlated very

well with t_{90} 's (estimated from linear plots). The corresponding t_{50} 's and t_{50}^* 's did not correlate very well, the t_{50}^* values being about 1.4 times greater than the equivalent t_{50} value; this is primarily due to the lag time associated with disintegration. Based upon these findings, it seems best to report t_{50} and t_{90} values as estimates based on linear plots. While t_{50} 's may have more mathematical validity (4), the time it takes for 90% of a drug to dissolve has considerable practical interest from a therapeutic standpoint. A simple and obvious solution is to make a practice of reporting both values.

Table III—Comparison of Different Formulations Using Various Dissolution Methods^a

Product	FPS	FCL	RB	3B	DT	Average
10-mg. oral						
A	2.6	4.9	3.8	3.5	1.7	3.3
B	1.5	4.2	2.3	3.7	1.4	2.6
C	1.4	3.7	1.5	1.4	1.4	1.9
D	1.0	1.0	1.0	1.0	1.0	1.0
5-mg. oral						
D	1.4	1.6	4.1	2.6	2.5	2.4
A	1.4	1.4	1.7	2.7	3.2	2.1
B	1.2	1.0	1.2	2.0	2.2	1.5
C	1.0	1.2	1.0	1.0	1.0	1.0
5-mg. sublingual						
D	4.5	—	—	—	—	—
A	3.5	—	—	—	—	—
C	1.0	—	—	—	—	—

^a FPS = flow method with polysorbate 80, FCL = flow method with hydrochloric acid, RB = rotating basket, 3B = three-bladed stirrer, and DT = USP disintegration basket.

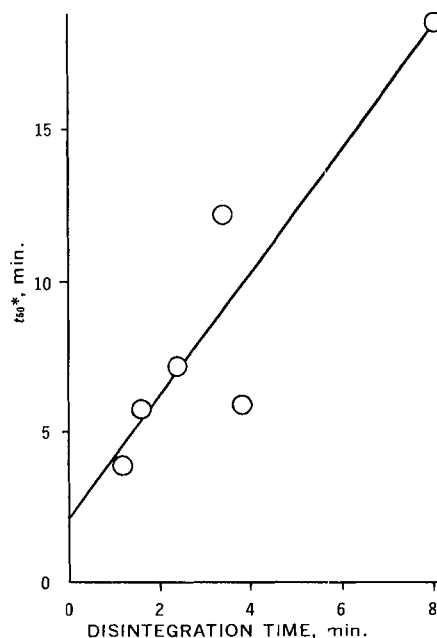


Figure 9—Plot showing correlation of disintegration time and t_{50}^* . (See text for explanation.)

Table IV—Comparison of Different Dissolution Methods^a

Method ^b	10A	10B	10C	10D'	5A	5B	5C	5D	Average
DT	3.0	2.9	3.3	8.7	3.2	3.1	1.5	2.2	3.5
3B	2.9	3.6	1.5	4.1	2.0	2.1	1.1	1.7	2.4
RB	2.8	2.0	1.5	3.7	1.2	1.2	1.0	2.5	2.0
FCL	1.0	1.0	1.0	1.0	1.0	1.0	1.2	1.0	1.0

^a 10A = 10 mg. formulation of A, etc. ^b See footnote to Table III.

Comparison of 25- and 13-mm. Dissolution Chambers—Experience gained in these studies indicates that the 25-mm. chamber is the most useful for routine determinations. It accommodates all tablet sizes, it is easier to handle and use, and its filter seldom clogs during normal operation. Even at low flow rates (12–14 ml./min.), homogeneity of the system appears to be excellent. The 13-mm. chamber is preferred for precise work, especially that involving nondissolving material. It is especially useful in work with sublingual tablets.

Quantitative comparisons between the 13- and 25-mm. chambers can be made using Eq. 11 from Langenbucher's report (4):

$$T = k(Q_A)^{-0.2-0.5} \times (D_{p,0})^{1.5-1.8} \quad (\text{Eq. 1})$$

where T is the dissolution time (minutes), k is a constant, Q_A is the liquid velocity (centimeters per minute), and $D_{p,0}$ is the initial equivalent spherical particle diameter (centimeters). Since the liquid velocity equals the flow rate divided by the cross-sectional area of the chamber, it can be calculated that, using identical samples, the dissolution rates using the 13-mm. cell should be 1.4–2.3 times faster than those using the 25-mm. unit. For the 5- and 10-mg. experimental formulations, the ratios of the semilog slopes (13 mm./25 mm.) are 1.7, 1.8, 2.0, and 2.3—in excellent agreement with the "theoretical" 1.4–2.3 range.

In conclusion, the results of these studies indicate that the modified dissolution chamber, consisting of commercially available

units, is a useful tool in dissolution work. Studies on isosorbide dinitrate formulations, including commercial products, indicate that some differences in dissolution behavior exist between formulations, differences that may or may not be significant from an *in vivo* standpoint.

REFERENCES

- (1) J. E. Tingstad and S. Riegelman, *J. Pharm. Sci.*, **59**, 692(1970).
- (2) E. G. Rippie and J. R. Johnson, *ibid.*, **58**, 428(1969).
- (3) D. C. Baun and G. C. Walker, *ibid.*, **58**, 611(1969).
- (4) F. Langenbucher, *ibid.*, **58**, 1165(1969).
- (5) K. Marshall and D. B. Brook, *J. Pharm. Pharmacol.*, **21**, 790(1969).
- (6) C. F. Lerk and K. Zuurman, *ibid.*, **22**, 319(1970).
- (7) C. Olson, U. S. pat. 3,620,675, The Upjohn Co. (1971).
- (8) R. J. Withey, *J. Pharm. Pharmacol.*, **23**, 573(1971).
- (9) P. Finholt and S. Solvang, *J. Pharm. Sci.*, **57**, 1322(1968).

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NOTES

Immunochemical Studies on Linear Antigenic Polypeptides with a Known Repeating Sequence of Amino Acids

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Abstract □ The specificity of antibody to poly(L-tyrosyl-L-glutamyl-L-valylglycyl)glycine-1-¹⁴C ethyl ester (I) was studied using polypeptides in which the valyl and tyrosyl residues were modified to an alanyl residue and either a phenylalanyl or a OMe-tyrosyl residue, respectively. All of these modified polypeptides cross-reacted with antibodies to I, indicating that they all have a similar

conformation. It was concluded from the diminution of binding abilities of these modified polypeptides that antibodies to I show a greater specificity for the valyl and phenolic hydroxyl group of the tyrosyl residue of the homologous antigen.

Keyphrases □ Polypeptides, linear, antigenic—immunochemical properties □ Antibodies, polypeptide produced—specificity

The specificity of the antibody formed in response to an antigen is dependent on both the antigenic determinants present in the antigen and the genetic capacity of the animal to differentiate between, and respond to, those determinants. Neither the mechanism of specific

antigen recognition nor the nature of its genetic control is well understood. With respect to antibody specificity, we have been studying the antigen poly(L-tyrosyl-L-glutamyl-L-valylglycyl)glycine-1-¹⁴C ethyl ester (I) (1, 2). This article reports some cross-reaction and absorp-