System for Automated Determination of Dissolution Rate

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Abstract □ An automated dissolution rate apparatus meeting requirements of the USP–NF dissolution test and applicable to various other agitation systems in common usage is described. The equipment allows simultaneous determination of the dissolution rate of six unit doses, enabling evaluation of a statistically significant number of samples. The system, composed of readily available standardized components, has a disposable filter unit and can measure the concentration of dissolution solutions in the 0.020–3.000 absorbance range. Dose-to-dose uniformity and stability of dissolution characteristics are shown for several products. Data relating the USP–NF agitation system to the beaker–stirrer (Levy) method are presented. Dissolution rates of sulfisoxazole formulations are correlated with *in vivo* availability, and the adverse effects of inadequate packaging and severe environmental storage conditions on dissolution rate are demonstrated.

Keyphrases □ Dissolution rate determination—automated analysis system described, applications discussed □ Automated analysis—dissolution rate testing, equipment described, applications discussed

It is generally accepted that the rate at which a drug dissolves from the dosage form can be the limiting factor governing its bioavailability in many instances. The correlation between *in vitro* dissolution rates and *in vivo* efficacy remains to be established for most drugs.

The current issues of the official compendia describe an *in vitro* dissolution test and apparatus (1, 2) which, while probably not applicable to all dosage formulations, do constitute the basis for a standardized procedure. Based on data obtained over 2 years in this laboratory, the test does provide discrimination among solid dosages of differing bioavailability when agitation speed and dissolving solvent are properly defined.

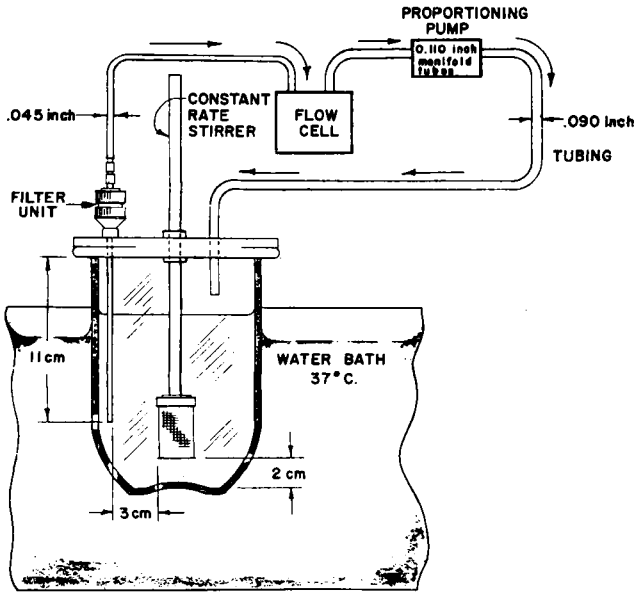

Figure 1—*Flow diagram of apparatus and filter unit assembly with USP–NF Method I dissolution vessel.*

A comparison of the USP–NF system with that described by Levy and Hayes (3), a method widely quoted in the literature, shows that meaningful *in vitro–in vivo* correlations can be established using either test procedure. Both afford a gentle agitation of the dosage unit and ensure minimum abrasion of the sample; however, the USP apparatus appears more widely adaptable since it can better handle the less dense forms such as floating tablets and capsules.

To obtain dissolution profiles on statistically significant samplings of each batch, an automated system was developed with the following significant features:

1. Direct printout (as percent dissolved) of six simultaneous dissolution rate determinations using a completely automated measuring system.
2. Monitoring system containing a minimum volume of dissolving solvent to allow low flow rates, thus eliminating extraneous agitation of the samples.
3. Instrumentation utilizing standard, commercially available, low-cost components.

The instrumentation is adaptable to the USP–NF and Levy agitation systems as well as many others (4, 5). It is adaptable to all single drugs that exhibit a sufficient visible or UV spectrum and to combinations of drugs where the individual spectra lend themselves to simultaneous measurements at different wavelengths. Dissolution rate determinations of combination drugs and single-component dosages whose assay requires measurement at more than one wavelength are made possible by the use of a spectrophotometer equipped with an automatic wavelength programmer.

A major obstacle to be overcome in any continuous flow system is that of assuring the measurement of a clear solution, free of extraneous particles which would cause turbidity in the solution and cloud the surfaces of the flow cells. This was accomplished by the incorporation of filter units into the system which utilize disposable Teflon or polyvinyl inserts and allow the selection of a filter to provide adequate filtration of the solution while minimizing difficulties that could be caused by a poor flow rate through the measuring system.

The entire system as described maintains conditions of agitation exactly as specified in the official test method (USP–NF Method I). All sample agitation is provided by precise, mechanically controlled stirring of the sample basket.

Elimination of any extraneous turbulence within the dissolution vessel is assured by maintaining a low

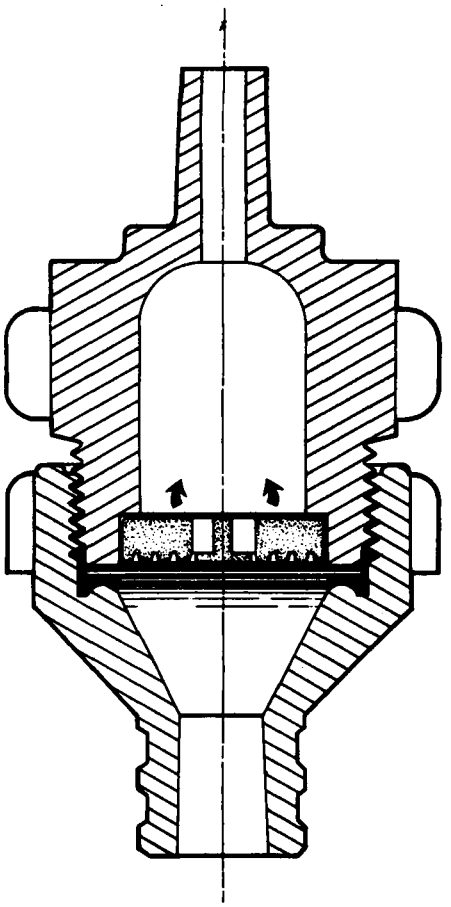

Figure 2—*Cross-sectional drawing of filter unit assembly.*

volume of fluid in the measuring system so as to allow a pumping rate through the flow cell of approximately 2 ml/min. This also minimizes the chances of error caused by eddy currents in the solution being measured in the flow cell itself.

EXPERIMENTAL

Apparatus—Instrumentation for the simultaneous monitoring of six dissolution rate determinations was assembled from commercially available components consisting of a spectrophotometer[1] with automatic wavelength programmer and turret assembly[2], a proportioning pump[3], and a multispeed dissolution tester[4]. A constant temperature of 37° is maintained by a water bath[5]. A digital display and printer[6] and a linear recorder[7] were incorporated to provide data directly as "percent dissolved." The dissolution solvent is filtered through Teflon or polyvinyl filters[8]. The proportioning pump is incorporated into the system on the return side of each of the six flow cells[9] utilized in the turret assembly (Fig. 1).

The filter unit employed in the system (Fig. 2) has the advantages that the Teflon or polyvinyl filter inserts can be discarded after each test, eliminating cleaning, and that various porosities can be selected for each application to eliminate clogging and maximize solvent flow. An 11-cm glass tube having an inside diameter of 2.5 mm is attached to the sampling orifice of the filter unit. Alternative agitation systems, such as described in NF XIII, Method II, and the beaker–stirrer assembly of Levy and Hayes were studied in place of the multidissolution tester (USP–NF) by simply inserting the filter units in the respective dissolution vessels specified for those systems in place of the USP dissolution flask.

Agitation speeds between 25 and 250 rpm are provided by a variable speed multidissolution tester, which incorporates a tachometer to facilitate precise speed adjustment. A standard model spectrophotometer was modified to allow direct digital printout in percent drug dissolved. The use of standard, commercially available components both for the agitation and measuring systems minimizes the cost of the unit; however, modifications of both the printer and spectrophotometer are necessary.

Circuitry changes in the spectrophotometer were made so that a single "print" impulse is transmitted to the printer, which corresponds to the maximum absorbance obtained while a particular cell is in the light path. Modifications were also made in the printer circuitry to allow for recycling after each series of six printouts. Light path dwell times for the individual cells were adjusted to give 2.5- and 5-min cycles, allowing a measurement for each of the six dissolution tests to be recorded at any multiple of 2.5 or 5 min.

Method—Normally, 900 ml of simulated gastric, intestinal fluid or other dissolving solvent is heated to 37° and placed in the glass vessels specified in the the USP–NF test. Filter units are placed in position through the vessel covers parallel to and approximately 3 cm from the side of the sample basket. The solutions are drawn from the flask through the filters and the flow cells by a proportioning pump and are returned to the vessels at a flow rate of approximately 2 ml/min. The total volume in the measuring system is 4.8 ml.

At the appropriate wavelength setting, each cell is adjusted to zero absorbance using variable beam attenuators, modified by enlarging the bore to allow more reliable positioning. To set the concentration mode, the spectrophotometer is set in the single-beam position with cell 1 in the light path. The absorbance representing

Table I—Tubing Selection

Configuration	Delivery Tubing Diameter, in.	Pump Tubing Diameter, in.	Seconds to Cell
1	0.045	0.073	30–35
2	0.073	0.073	>60
3	0.073	0.090	60
4	0.045	0.110	20

Table II—Manual and Automated Dissolution Rates Using 50-mg Chlorprothixene Tablets

Minutes	Percent Dissolved	
	Manual	Automated
5	2	1
10	19	17
15	84	85
20	97	97

Table III—Diffusion Rate within the Dissolution Vessel (100 rpm)

Station	Time to Indicate 100% Diffusion, sec	Percent of Theory at Maximum
1	110	102
2	140	100
3	130	99
4	110	100
5	90	102
6	120	99
Average	117	100
ts: 95%	±43	±2.8

[1] Beckman model DB GT spectrophotometer.
[2] Spectrophotometer model DB GT P/N 1405 and turret assembly P/N 565810, Beckman Instruments, Mountainside, N.J.
[3] Autoanalyzer proportioning pump PP2 105-A200-1, Technicon Instrument Corp., Tarrytown, N.Y.
[4] Model T1044-20, Hanson Research Corp., North Ridge, Calif.
[5] Lab-Line Instruments, Melrose Park, Ill.
[6] Digital display P/N 573230 and printer model 3115, Beckman Instruments, Mountainside, N.J.
[7] Model 100500, Beckman Instruments, Mountainside, N.J.
[8] Swinnex-13 filter units (polypropylene) SX00-01300, Millipore Corp., Bedford, Mass.
[9] Using 0.1- and 1-cm flowthrough cells QS170 and QS175, Hellma Cells Inc., Jamaica, N.Y.

Table IV—Cell-to-Cell Variation

Cell	Percent of Assay Value					
	1	2	3	4	5	6
Cycle 1	101	101	101	100	100	101
Cycle 2	101	102	100	100	101	101
Cycle 3	101	102	100	100	101	101
Average: 101 *ts:* 95% ± 0.9						

100% theoretical dissolution is set on the digital display using the dynode voltage control, and the digital display is switched to the "conc" mode. A concentration count of 100 or 1000 is then fixed, which corresponds to 100%. The spectrophotometer is reset to double-beam operation. Six tablets are placed in individual 40-mesh stainless steel baskets and attached to the spindles of the agitator. The baskets are immersed in sequence at the appropriate time intervals into the dissolving medium and properly positioned in the flask.

To obtain optimum flow conditions the minimum volume within the system, various tubing configurations were studied (Table I). Configuration 4 was chosen, because it provides a minimum lag time in delivering the dissolving solution to the flow cell.

RESULTS

Dissolution rates obtained by the automated system and those obtained by manual measurement agree. Dissolution of sugar-coated tablets containing 50 mg of chlorprothixene was studied both manually and in the automated apparatus (Table II).

The time for the entire system to reach equilibrium and the reproducibility of the actual measurement were studied by introducing 10 ml of a standard solution containing 250 mg of levodopa to each of the six stations. The time for the system to indicate complete diffusion and the accuracy of six determinations were evaluated (Table III).

Considering the accuracy and reproducibility of the instrumentation, real differences in the dissolution rates of unit doses within a batch can be measured if these exceed ±2.8% of the average assay. The small variations shown in Column 3 of Table III are indicative of limitations in the mixing action within the USP–NF dissolution vessel. Actual variation caused by the instrumentation is negligible, as demonstrated by the values obtained when a single solution was passed through each of the six flow cell channels (Table IV). Here no significant variations (less than 1%) due to cell positioning or electrical imbalance were found within three repetitive cycles.

The intertablet variations obtained with a typical batch of 500-mg levodopa tablets and with a 2-mg benzodiazepine tablet are shown in Table V.

Variations in this range present no problems, especially with levodopa, since the drug is given in large doses, each dose containing several tablets. However, excessive tablet-to-tablet variations could present problems with other drugs given as a single low dose where a rapid therapeutic response is required, as exemplified by benzodiazepine.

A comparison of dissolution rates of levodopa tablets, using the USP–NF agitation system and the Levy and Hayes system, shows rank-order correlation between the two methods based on the average of six tablet determinations. The range of the individual tests was much greater with the Levy agitation system, undoubtedly because of the less defined parameters it provides for positioning of the dose and specific design of the vessel (6). Data obtained at a moderate agitation speed of 60 rpm by both methods are shown in Table VI.

Table V—USP–NF *In Vitro* Dissolution Rate in Simulated Gastric Fluid

Minutes	500-mg Levodopa Tablet			2-mg Benzodiazepine Tablet		
	Average, %	Range, %	*ts:* 95%	Average, %	Range, %	*ts:* 95%
2.5	48	40–57	15	22	15–36	17
5	85	78–89	10	69	63–74	11
10	97	90–101	10	83	78–87	7
15	99	94–101	7	91	85–97	11
20	100	97–102	4	100	94–105	11
30	100	98–102	4	—	—	—

Table VI—USP–NF and Levy Agitation (60 rpm) Using 500-mg Levodopa Tablets

Minutes	USP–NF		Levy	
	Average, %	Range[a], %	Average, %	Range[a], %
2.5	17	9–26	16	9–26
5	38	21–64	30	20–42
10	78	57–84	53	31–74
15	87	79–91	67	39–90
20	90	86–94	73	46–96
30	94	91–96	81	70–100

[a] Six determinations.

APPLICATIONS

As typical examples illustrating the use of the methodology described, the following are considered: *(a)* a packaging problem relating to sulfisoxazole tablets, and *(b)* a more sophisticated metabolic problem that attempts to relate the complex metabolic behavior of levodopa, which is extensively and rapidly biotransformed in the body, to the conditions of *in vitro* dissolution.

Correlation of *in vitro* dissolution rates and blood levels in the dog was examined using the USP–NF method of agitation (Method I) with sulfisoxazole tablets that had been packaged inadequately in polyvinyl chloride unit dose blisters and exposed to elevated conditions of temperature and relative humidity (37°, 85% relative humidity) for 1 month. These tablets showed considerable moisture pickup and greatly reduced *in vitro* rates of dissolution (Sample B). The reduction in dissolution rate was apparent when either 0.84 *N* HCl or simulated intestinal fluid was used as the dis-

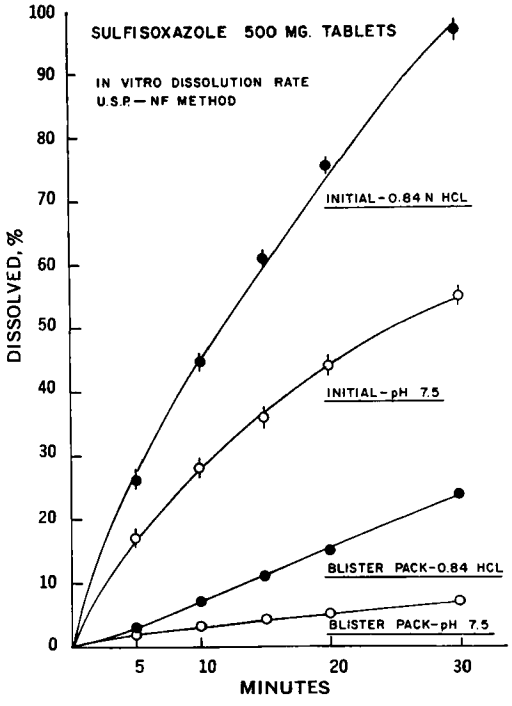

Figure 3—In vitro *dissolution rate of 500-mg sulfisoxazole tablets using USP–NF Method I. Key: ● and ○, Sample A, control tablets; and ● and ○, Sample B, tablets exposed to 37° and 85% relative humidity in inadequate packaging.*

Table VII—Plasma Levodopa Micrograms per Milliliter at 30–300 min

	Minutes						
	30	60	90	120	160	240	300
Formulation A	0.58	0.52	0.69	0.69	0.48	0.27	0.14
Mean ± *SE*	±0.12	±0.08	±0.13	±0.20	±0.08	±0.04	±0.02
Formulation B	0.44	0.56	0.61	0.52	0.47	0.34	0.15
Mean ± *SE*	+0.14	±0.12	±0.15	±0.10	±0.08	±0.07	±0.01

solution solvent, both of which provided sink conditions for the test (Fig. 3).

Samples of these tablets along with a control sample (Sample A) were administered to two beagle dogs in a crossover study[10], and blood levels were determined over 24 hr (Fig. 4). The measurements suggest some rank-order correlation, as shown by a plot of the dissolution rate $T_{25\%}$ *versus* the reciprocal of the area under the blood level curve (Fig. 5). However, the radical reduction in *in vitro* dissolution (Method I) was not reflected in any drastic change in bioavailability.

The importance of providing gentle agitation of the dosage form has been demonstrated (7); however, caution must be exercised in reducing the degree of agitation to a level where observed differences do reflect significant differences in bioavailability. To illustrate, dissolution rates of two different solid dosage formulations of levodopa were determined, using agitation speeds of 50 or 100 rpm with either 0.01 or 0.1 *N* HCl, respectively, as the dissolving solvents. Dissolution rates determined using a 50-rpm agitation speed in 0.01 *N* HCl showed differences between the formulations (Fig. 6), depending upon use of $T_{50\%}$ as contrasted with use of $T_{80\%}$ as the critical points. At a speed of 100 rpm in 0.1 *N* HCl, no such differences were observed.

Withey (8) observed wide variations when dissolving a single sodium chloride crystal at an agitation speed of 50 rpm. The large coefficient of variation reported reflects the nondisintegrating nature of the sample and the small percentage of sodium chloride dissolved at the low agitation speed of 50 rpm and illustrates the importance of selecting the proper speed for each dosage form based on correlations with *in vivo* data.

Biologically, crossover blood level studies in humans failed to show any significant differences between these two formulations, corroborating the results of the second test (Table VII).

Further evidence of the validity of the second set of test parameters was provided by correlations with human blood levels obtained on experimental formulations (C and D), which showed marked *in vitro* differences using the higher agitation speed and 0.1 *N* HCl as the dissolving solvent (Fig. 7). Correlation of $T_{50\%}$ *in vitro* dissolution data and the time required to reach peak blood levels is shown in Fig. 8[11]. This example serves to emphasize the importance of having critical, unique biological data available before choosing a suitable dissolution test for control purposes.

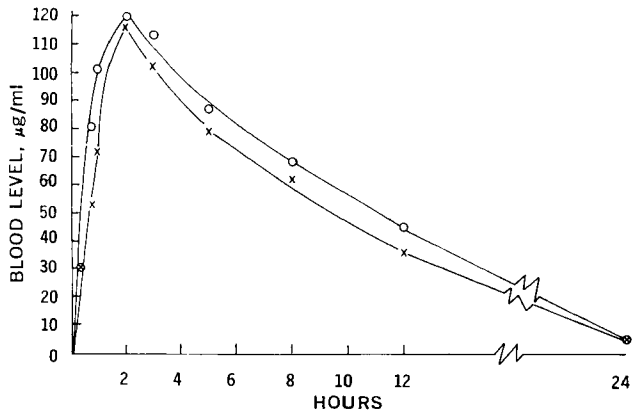

Figure 4—*Comparative drug blood levels in dogs using 500-mg sulfisoxazole tablets. Key: ○, Sample A; and ×, Sample B.*

DISCUSSION

An automated system that will determine the dissolution rate of six unit doses simultaneously was designed and assembled from commercially available, moderately priced components. The instrumentation has been used routinely over 2 years with good utility. High precision and reproducibility within the measuring system are achieved by maintaining low volume and flow rates, thus allowing measurement of real differences in dissolution characteristics between dosage forms, if these exceed ±3%. The Levy method was shown to give results in the same rank order as the USP–NF Method I, although with poorer reproducibility due to the lack of precise definition within the dissolution vessel.

The filter units allow selection of the proper filter porosity that provides adequate flow rates within the system commensurate with solution clarity. The need for extensive cleaning between sample runs is eliminated by the use of disposable inserts. The low volume measuring system minimizes the amount of solution required to be drawn through the filter units, thus eliminating the need for elaborating anticlogging systems (9), and makes exact absorbance measurement possible by preventing the formation of eddy currents within the flow cells.

Dissolution rates of dosage forms can be markedly affected by exposure to improper storage conditions. The official test currently used for sulfisoxazole appears to present a poor measure of the true bioavailability of the drug, since dosages that fail the test specification provide adequate therapeutic blood levels in dogs. The differences between the control and package sample are in the same order as those reported (10) in a study comparing oral solutions of sulfisoxazole to acceptable tablet formulations.

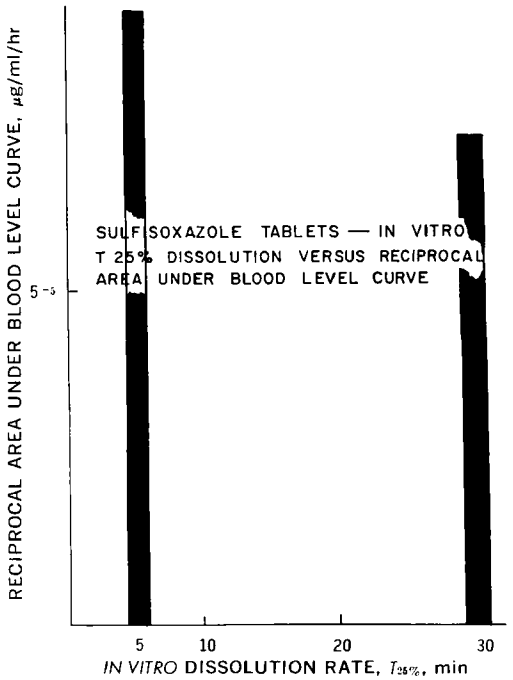

Figure 5—In vitro $T_{25\%}$ *dissolution* versus *reciprocal of the area under blood level curve for 500-mg sulfisoxazole tablets. Key: left, Sample A; and right, Sample B.*

[10] S. Kaplan, Hoffmann-La Roche Inc., Nutley, N.J., personal communication.

[11] C. Joseph, Hoffmann-La Roche Inc., Nutley, N.J., personal communication.

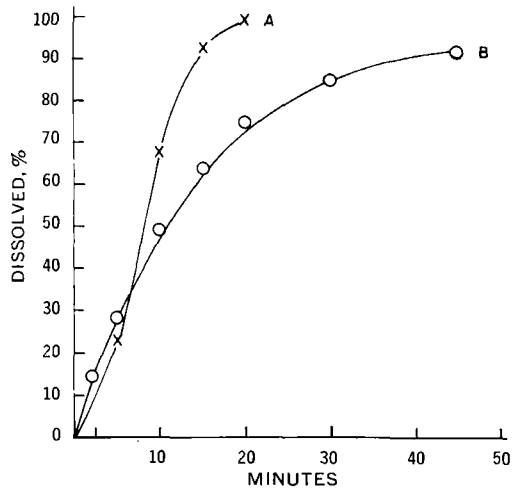

Figure 6—In vitro *dissolution rate of 500-mg levodopa Formulations A and B using USP–NF Method I, 50 rpm, and 0.01* N *HCl.*

The adequacy of a final package configuration should always be evaluated with regard to maintaining a drug's bioavailability. In the case of sulfisoxazole tablets, it was shown that polyvinyl chloride blister packages will not provide adequate protection to ensure compliance with current USP specification on exposure to 37° and 85% relative humidity conditions of storage. However, in this instance the biological significance of the specification is questionable.

It is also apparent that overly discriminatory *in vitro* tests can be designed that indicate differences in dissolution rates between formulations that have no biological significance, as illustrated by the first test described for levodopa dosages. This is not to say that a significant *in vitro* test is not possible, but only that its selection should be based soundly on *in vivo* correlation and not established arbitrarily. The second set of test parameters described for levodopa provide such a correlation and represent a meaningful evaluation.

SUMMARY

An automated dissolution rate apparatus that meets the requirements of the USP–NF dissolution test and is applicable to various other agitation systems in common usage has been assembled. The equipment allows simultaneous determination of the dissolution rate of six unit doses, enabling evaluation of a statistically significant number of samples. Dose-to-dose uniformity and stability of dissolution characteristics are shown for several products.

Data relating the USP–NF agitation to the beaker–stirrer (Levy) method are presented. The authors prefer the current USP–NF method because of its better defined parameters and concur with the findings of other investigators (11) that it is the simplest and most versatile of the available methods. Dissolution rates of levodopa formulations and, to some extent, sulfisoxazole formulations were found to correlate with *in vivo* availability when the *in vitro* test was properly designed.

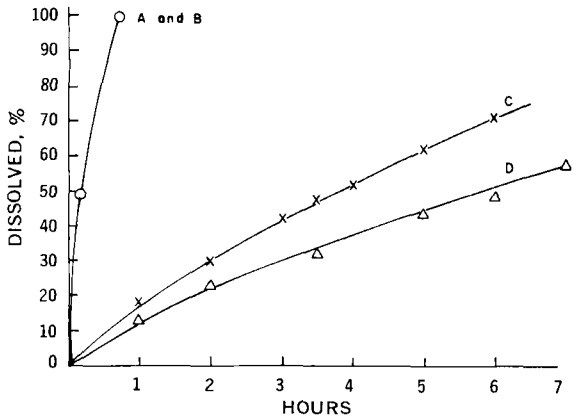

Figure 7—In vitro *dissolution rate of 500-mg levodopa formulations using USP–NF Method I, 100 rpm, and 0.1* N *HCl. Key:* ○, *Samples A and B;* ×, *Sample C; and* △, *Sample D.*

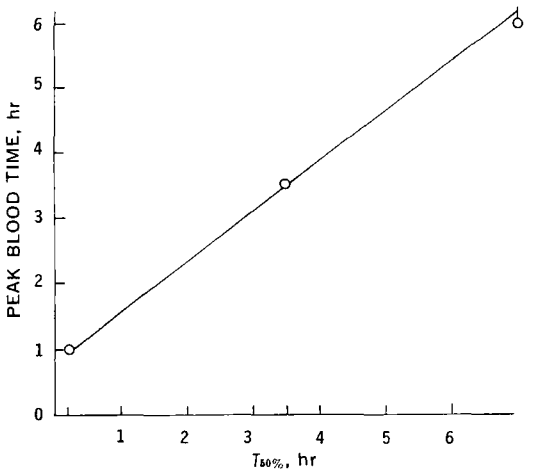

Figure 8—*Levodopa tablets,* in vitro $T_{50\%}$ *dissolution value* versus *time in hours to reach peak blood level.*

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