Nuclear *In Vitro* Method of Continuously Measuring Dissolution Rates

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A new nuclear *in vitro* continuous dissolution-rate measuring method has been developed and evaluated for use in determining the dissolution release rates of labeled materials from solid dosage forms. The results obtained were compared with dissolution rates of similar dosage forms using the U.S.P. disintegration apparatus and the rotating-bottle method. In all three cases the concentration of material released was directly proportional to the square root of time, although the release rates were different for each method. The nuclear method was found to be more precise than either the U.S.P. or rotating-bottle method.

THE PHARMACEUTICAL research scientist in the field of product development, particularly dosage-form development, frequently has need of an *in vitro* method which will provide comparative data on the dissolution of the active constituent or constituents. In addition, such *in vitro* data may correlate with *in vivo* data to provide a basis for appraising the rate of release of the drug from the dosage form. Even in the absence of complete correlation, the *in vitro* method can still be expected to save time, labor, and expense in the preliminary stages of developing the desired *in vivo* dissolution rate characteristics.

The majority of the *in vitro* methods used today to test rapid, standard, or prolonged-release dosage forms involve five basic conditions—namely, a controlled temperature, usually 37°; a solvent, usually simulated gastric or intestinal fluid, or a combination of the two; some degree of agitation; an appropriate quantitative analytical technique; and the constant or periodic attention of an operator to withdraw samples, change simulated fluids, replace samples, and conduct quantitative analyses.

The *in vitro* dissolution-rate characteristics of sustained or prolonged-release dosage forms are at present usually evaluated by means of the rotating-bottle method (1–4) or the modified U.S.P. disintegration-testing basket attached to a Gershberg-Stoll apparatus (5–11). A third method of evaluating *in vitro* dissolution is that employed by the FDA which utilizes a modified U.S.P. disintegration apparatus with a stoppered cylinder containing a coarse porosity fritted glass filter which serves to separate the eluate from the residue (12). In virtually all cases, these methods or their modifications involve the removal of samples periodically from the system followed by some form of quantitative analysis.

There are a few examples in the literature of methods amenable to a form of continuous assay wherein sampling errors, inconvenience, and expense may be reduced. Schroeter and Wagner (13), using the Gershberg-Stoll apparatus, continuously circulated solvent from the release beaker through an ultraviolet spectrophotometer and back into the beaker. At various time intervals the concentration of drug in the eluate was recorded. Other workers (14) have used a similar technique where the fluid passed continuously from a beaker, provided with a mechanical stirrer but no basket apparatus, into a cell in a spectrophotometric analyzer, and back into the beaker. The dissolution-rate constant for this method was shown to be virtually independent of the eluate flow rate between the analyzer and dissolution cell over the range of 60 to 350 ml. of solvent per minute.

Rather surprisingly, not a great deal of attention has been given to the use of radioisotopes in *in vitro* dissolution-rate studies on dosage forms. Rosen and Swintosky (15) used the rotating-bottle method of Souder and Ellenbogen (2) to test for *in vitro* release of a ^{35}S labeled sustained-action product. This method was also used by Rosen (16) in studies designed to establish the relationship between *in vitro* and *in vivo* release data. In another study, Montgomery *et al.* (4) evaluated the dissolution rate of sustained-release tablets containing ^{36}Cl labeled phenylephrine hydrochloride and ^{14}C labeled aspirin. The rotating-bottle method was employed again; the samples were removed at known time intervals, and the concentration of the labeled drugs was determined by dual-channel scintillation counting. Although in all these instances the quantitative estimation of material released from the dosage form by dissolution was facilitated, the retention of the rotating-bottle method necessi-

Received July 6, 1965, from the School of Pharmacy and Pharmacal Sciences, Purdue University, Lafayette, Ind.
Accepted for publication September 15, 1965.
This investigation was supported by fellowship 1-F1-GM-21, 146 from the Institute of General Medical Sciences, National Institutes of Health, U. S. Public Health Service, Bethesda, Md.

tated the removal of samples by an operator who would subsequently be concerned in their analysis.

In this study an automated apparatus has been designed to overcome these disadvantages. The utility of the new apparatus has been demonstrated in a comparative study of the dissolution rates obtained from the new nuclear apparatus, the rotating-bottle method, and the U.S.P. disintegration apparatus.

EXPERIMENTAL

Description and Operation of the Apparatus.—The nuclear apparatus, designed to measure *in vitro* dissolution rates continuously, was composed of two main tubes, the detection section and the sample section, joined by two horizontal flow tubes, as shown in Fig. 1. The all-glass apparatus was contained in a water bath, also made of glass, maintained at 37° ± 0.5°.

The stirring compartment at the base of the sample section contained a Teflon-coated magnetic stirring bar which was situated over a magnetic stirring plate located outside the glass water bath. The spinning bar forced the solvent radially through the bottom connecting tube into the detection section and then up and around the dry well. This flow created a positive pressure head reflected by a rise in the level of the liquid in the detection section of the apparatus so that the fluid now flowed through the top connecting tube back into the sample section and to the stirrer, thus completing the cycle. However, experimentation using a sample holder attached to a Vanderkamp machine rather than using the stirring bar yielded satisfactory fluid mixing; therefore, the stirring bar was not used during the determination of dissolution rates.

Supporting Instruments.—The dry well in the detection section contained a Geiger-Müller tube[1] chosen for its high sensitivity to γ radiation. This glass-walled detector, commonly termed a side-window detector, operates at a potential of 1100 v., has a dead time of 90 microseconds, a bismuth cathode and a background of 83 c.p.m. when shielded by 2 in. of iron. A high level of detection of the γ emitter in the circulating fluid was achieved due to the large surface area of the detector exposed to radiation and its high sensitivity.

A research ratemeter[2] was employed as the source of high voltage required to operate the G–M tube and also transmit any change in current, produced by the presence of a γ emitter, to a strip chart recorder.[3] The count rate was projected on the chart as a curve. The nuclear apparatus and its associated equipment are shown in Fig. 2.

Method of Operation.—The nuclear release apparatus was postioned in a water bath in such a way that the stirring bar was centered 5 cm. above the magnetic stirrer.[4] The 5-gal. bath rested on the magnetic stirrer and two support blocks, and was maintained at a temperature of 37° ± 0.5° by a controlled heater-circulator[5] in the bath. A shield, made of lead sheets to a thickness of 2.5 cm., was suspended between the sample and the detection sections of the release apparatus.

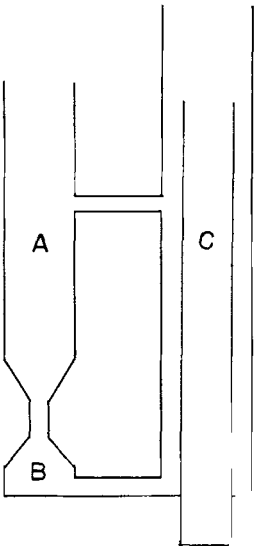

Fig. 1.—The nuclear *in vitro* apparatus. Key: A, sample section; B, stirring compartment; C, dry well for detector.

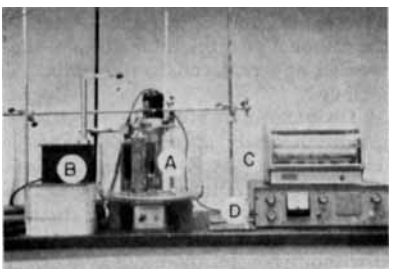

Fig. 2.—The nuclear apparatus with supporting instruments. Key: A, the nuclear apparatus with lead shield; B, disintegration machine; C, recorder; D, ratemeter.

A radioactive tablet was placed in a cage of Monel metal screen, 40-mesh and approximately 0.75 cm. wide at the bottom and 2 cm. in length, fabricated by wiring the corners of an *X* screen section together. The cage, containing the tablet, was then connected to the arm of a Vanderkamp machine[6] by an 18-gauge copper wire.

A solution containing the amount of ^{59}Fe formulated into the tablet was counted in the apparatus, following a background count, and the resultant count-rate curve height was taken to be equivalent to 100% dissolution of the isotope concentration in the dosage form. This procedure was followed before testing each of the tablets.

Following the removal of the ^{59}Fe solution, the tablet and cage were suspended in the dry sample section, and a background count of the radiation from the tablet which penetrated the shield was recorded. The count rate from the dry tablet was recorded as the total background. After the addition of 300 ml. of simulated gastric solution without pepsin, and while the Vanderkamp machine was

[1] Model 10400, Radiation Control Laboratory, Inc., Skokie, Ill.
[2] Model 432A, Baird-Atomic, Inc., Cambridge, Mass.
[3] Model DR1M1N-A16-R-ED, Texas Instruments, Inc., Houston, Tex.
[4] E. H. Sargent & Co., Chicago, Ill.
[5] E. H. Sargent & Co., Chicago, Ill.
[6] Van-Kel Industries, Inc., Livingston, N. J.

operating, the amount of dissolution was measured as the height of the curve above the background base line. The apparatus was allowed to run until sufficient dissolution rate data had been obtained.

The per cent dissolution was calculated from the following equation:

$$D_{tn} = \frac{(C_{tn} - C_{to}) \times 100}{C_{ts}}$$

In this equation D_{tn} is the per cent dissolution at time tn, C_{tn} is the count rate at time tn, C_{to} is the total background count, and C_{ts} is the count-rate equivalent to 100% dissolution.

Rotating-Bottle Method.—The apparatus used was the standard rotating-bottle apparatus operating at 42 r.p.m. in a water bath maintained at 37° ± 1.0°. Sixty-milliliter portions of simulated gastric solution[7] without pepsin were placed in 90-ml. cylindrical bottles on the apparatus, and a standard nonradioactive tablet was added. The bottles were sealed and the test was run over a period of 7 hr., samples being removed at prescribed intervals of time. The iron concentration was determined colorimetrically from a calibration curve prepared using a Bausch & Lomb Spectronic 20[8] set at 480 mμ. After the removal of a milliliter sample, the bottles were resealed and replaced in the bath. The test was continued.

U.S.P. Disintegration Method.—In this technique one standard nonradioactive tablet was placed in one tube of the U.S.P. disintegration basket and was covered with a standard plastic disk. Dissolution was then determined using the apparatus and a liter beaker containing 500 ml. of simulated gastric solution without pepsin, maintained at 37° ± 0.5°. Samples of the solution were removed at specified time intervals and were assayed colorimetrically for iron as described above.

Preparation of the Model Dosage Form.—Tablets of the following formula were prepared in 20-tablet lots:

^{59}Fe (sp. act. 0.027 c./Gm.) as ferric chloride	12	μl.
Ferric chloride	200	mg.
Calcium sulfate	50	mg.
Magnesium stearate	300	mg.
Weight of one tablet	550	mg.

The solution of ^{59}Fe was added to the calcium sulfate powder, mixed, and allowed to dry in an open Petri dish. The dry powder was transferred to a small dry motor and pestle and was mixed with freshly ground ferric chloride (40-mesh) and the required weight of magnesium stearate. Aliquots of the powder mixture equivalent to the weight of one tablet were then compressed with a Carver laboratory press[9] using a $^{7}/_{16}$-in. S. C. punch and die set and an applied load of 2000 lb. Tablets formulated in this way constituted the model solid dosage form comprised of the labeled material representing a soluble drug contained in an insoluble tablet matrix. These tablets provided a satisfactory rate of drug release. The nonlabeled tablets used in the comparative studies with the rotating-bottle method and the U.S.P. disintegration apparatus were prepared in an identical manner.

[7] pH 1.2, simulated gastric fluid U.S.P. without pepsin.
[8] Bausch & Lomb, Inc., Rochester, N. Y.
[9] F. S. Carver, Inc., Summit, N. J.

RESULTS AND DISCUSSION

Presentation of Release Data.—The data presented in Table I show the average per cent release of ferric chloride from the insoluble matrix over a period of 7 hr. using the three different dissolution methods. When these values are plotted against $t^{1/2}$ (Fig. 3), a linear relationship is apparent for all sets of results. Such a relationship is in agreement with Higuchi's theoretical analysis (17) of the rate of release of solid drugs dispersed in solid matrices. In the case of the leaching of a drug from a planar system having a granular matrix which contains a

TABLE I.—AVERAGE[a] DISSOLUTION RATES OF STANDARD FERRIC CHLORIDE TABLETS AS DETERMINED BY THE NUCLEAR AND COMPARATIVE METHODS

Method	Elapsed Time, min.	% Retained	S.D.
Nuclear release apparatus	30	90.8	2.5
	60	87.2	2.2
	180	78.0	3.4
	300	71.5	4.8
	420	67.4	4.0
Rotating bottle	30	86.4	1.8
	60	81.7	1.9
	180	69.9	5.9
	300	60.5	8.8
	420	53.3	10.4
U.S.P. disintegration apparatus	30	94.0	0.5
	60	92.2	2.7
	180	87.3	4.3
	300	84.5	5.5
	420	80.6	7.2

[a] Each data point is the average dissolution value of 14 tablets.

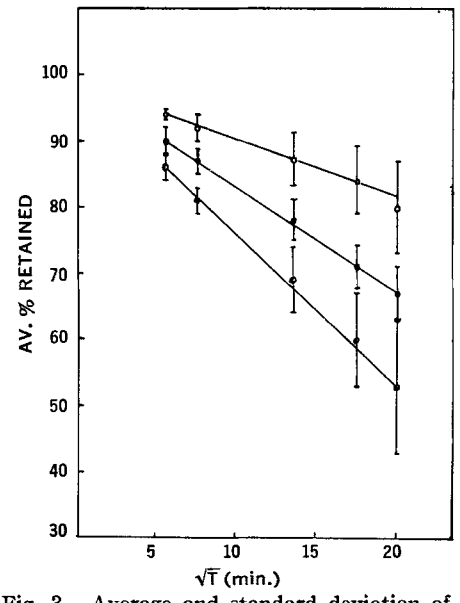

Fig. 3.—Average and standard deviation of per cent retention of ferric chloride by standard tablets. Key: ○, U.S.P. disintegration method; ●, nuclear method; ◐, rotating-bottle method.

TABLE II.—RELEASE RATE CONSTANTS OBTAINED BY THE THREE DISSOLUTION METHODS

Method	Release Rate Constant, %/min.$^{0.5}$
Nuclear	1.63
Rotating bottle	2.34
U.S.P. disintegration	0.97

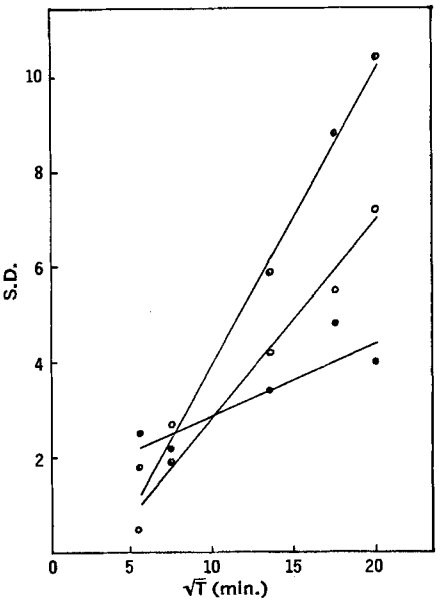

Fig. 4.—Standard deviation of the per cent retention of ferric chloride by standard tablets. Key: O, U.S.P. disintegration method; ●, nuclear method; ◐, rotating-bottle method.

high concentration of a drug, Higuchi derived the formula:

$$Q = A\sqrt{DK/\tau(2 - KC_s)\,C_s}\sqrt{t} \quad \text{(Eq. 1)}$$

where Q equals the amount of drug released after time t per unit exposed area, D equals the diffusivity of the drug in the permeating fluid, τ equals the tortuosity factor of the capillary system within the matrix, A equals the total amount of drug present in the matrix per unit volume, C_s equals the solubility of the drug in the permeating fluid, and K equals the specific volume of the drug.

In the system under study we have what amounts to a biplanar solid composed of a granular drug–matrix containing 40% drug. The shape and size of the tablet matrix studied remained virtually unchanged during the dissolution process, indicating that leaching was the prime mechanism of release. The conditions employed in this study were therefore in accord with those laid down by Higuchi in deriving the theoretical equation (Eq. 1). Since the tablets used were standardized with regard to weight, drug content, degree of compression, and surface area, Eq. 1 reduces to:

$$Q = k'\sqrt{t} \quad \text{(Eq. 2)}$$

where k' is a constant taking account of all the factors held constant in Eq. 1.

It would appear that this is the first report of actual data presented in support of the theoretical considerations made by Higuchi.

Comparison of Dissolution Methods

Release Rates.—It is apparent from the slopes in Fig. 3 that the rates of release of ferric chloride from the dosage form differ markedly according to the dissolution method employed (see Table II). This effect seems to be associated with the degree of agitation produced in each of the three types of apparatus. In the rotating bottle, the tablets were washed over and over by the 60 ml. of solvent moving back and forth in the bottle. Since the bottle was revolved at 42 r.p.m., a vigorous mixing of the solvent around the tablet was achieved. There was in addition a small amount of attrition due to the tablet's rubbing against the wall of the bottle. The release rate in the nuclear apparatus was not so rapid as in the rotating-bottle apparatus, due presumably to the reduced tablet movement and solvent flow. Of the three *in vitro* methods studied, the slowest release rate was obtained with the U.S.P. apparatus. For the type of model solid dosage form studied, this is apparently a reflection of the slower rate of presentation of new solvent to the dosage form using the U.S.P. apparatus with plastic disk in comparison to the other two *in vitro* methods.

The effect of the various degrees of agitation of the three methods was readily apparent from the external appearance of the tablets. When first placed in the solvent, all tablets were uniformly brown in color and smooth. However, after dissolution testing for variable periods, those tablets used in the rotating bottle and nuclear apparatus possessed a smooth uniformly bleached color indicative of solute leaching from the tablets. This was confirmed by vertically sectioning the various tablets which showed the presence of a uniformly decreasing brown-colored central core of soluble ferric chloride and magnesium stearate matrix. The tablets taken from the U.S.P. apparatus showed brown patches on the center of their top and bottom planes surrounded by an evenly bleached area, evidence that the tablet surface was subjected to unequal exposure to the solvent by the presence of the plastic disk. As a consequence, the rate of release was the lowest observed by the three methods since the surface area in contact with solvent was less than the total surface area of the tablet.

Precision.—Figure 4 shows the relationship between the standard deviations and time $t^{1/2}$ given in Table I. It is obvious that, of the three methods, the nuclear apparatus was the most precise and reproducible, in that the rate of increase in standard deviation per unit time is significantly lower than with the rotating-bottle or the U.S.P. methods. This is presumably a reflection of the techniques associated with the three methods. Thus, in the nuclear method which is completely automated, there are no errors involved in slight variations in sampling time, since these are taken directly from the trace drawn by the recorder. In addition, the tablet is undergoing dissolution under standard conditions throughout the entire period of evaluation. Furthermore, no samples are removed which eliminates the possibility of sampling errors. In the U.S.P. method, the samples are taken while the apparatus is in motion; however, the possibility of

small sampling and time errors exists. The rotating-bottle method is quite obviously the least precise. This is not surprising, since the apparatus has to be switched off while the bottles are removed and the samples are taken.

SUMMARY AND CONCLUSIONS

A new nuclear *in vitro* dissolution-rate measuring apparatus has been developed and preliminarily evaluated for use in measuring the dissolution release of labeled materials from solid dosage forms as a precise means of studying the effects of modifying the different variables of dosage form on the *in vitro* dissolution of model systems. The new apparatus employed Geiger–Müller detection of a γ emitter and continuous recording of the events. ^{59}Fe was the tracer incorporated in a standard tablet formulation containing ferric chloride, calcium sulfate, and magnesium stearate. The dissolution-rate constant measured in the new nuclear apparatus was compared with the dissolution-rate constants measured in the U.S.P. disintegration apparatus and the rotating bottle. The tablets did not disintegrate, but released the ferric chloride solute by a leaching mechanism according to the model, $Q = k't^{1/2}$.

The conclusions that can be drawn are as follows.

1. The nuclear method of dissolution-rate measurement is the most convenient of the three methods because (*a*) the presence of an operator was not required after the tablet was introduced; (*b*) a permanent record of the dissolution of the ^{59}Fe, representative of the ferric chloride dissolution, was made; (*c*) a chemical analysis with its inherent difficulties in sampling, measuring, etc., was not needed.

2. The nuclear method was more precise. The variation-rate constants of the U.S.P. and the rotating-bottle methods were 2.7 and 4.1 times greater than the variation-rate constant of the nuclear method.

3. Of the three *in vitro* methods studied, the U.S.P. method was the most conservative in dissolution-rate measurement (rate constant = 0.856). The nuclear method was less conservative (rate constant = 1.56), and the rotating bottle was the least conservative (rate constant = 2.17). This is related to the degree of agitation and solvent exposure of the tablet in the apparatus.

4. The data conform to the general equation $Q = k't^{1/2}$, previously deduced on theoretical grounds by Higuchi.

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Fluorometric Method for Determination of Cholesterol in Microliter Quantities of Blood

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A fluorometric method for the determination of total cholesterol is described which is based on the Tschugaeff reaction. The procedure is applicable to the analysis of as little as 2 μl. of blood, which may be obtained, for example, from the finger. In order to determine the selectivity of this reaction, a number of related steroids have been studied.

THERE HAS been a need for a rapid method for the determination of cholesterol on the micro level using macro techniques. Albers and Lowry (1) have described a fluorometric method for as little as 0.1 mcg. of cholesterol in animal tissue utilizing the Liebermann-Burchard reaction; however, the final volume to be read is less than 0.25 ml. McDougal and Farmer (2), modifying the procedures of Albers and Lowry, have developed a fluorometric procedure for the determination of total cholesterol in blood from the tip of a rat's tail. Again, however, the method of extraction and development of fluorescence involve the use of micro techniques.

The Tschugaeff reaction (3) has been shown to be more sensitive than the Liebermann-Burchard reaction and has been applied to the colorimetric determination of total cholesterol in blood by several investigators (4–6). A fluorometric method has now been developed based on the Tschugaeff reaction that is many times more sensitive than the previously described procedures and that utilizes macro techniques on a micro sample.

Received July 29, 1965, from the Research Department, Ciba Pharmaceutical Co., Summit, N. J.
Accepted for publication October 7, 1965.