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Automated *In Vitro* Dissolution-Rate Technique for Acidic and Basic Drugs

ASHOK C. SHAH

Abstract □ A continuous, automated, potentiometric titration technique for studying *in vitro* dissolution rates of acidic and basic type drugs from their compressed tablet formulations is described. The application of this technique was demonstrated by evaluating dissolution rates of sulfa- and sulfonylurea tablets, representing acidic drugs, and an antibiotic drug tablet formulation, representing a basic drug. The results obtained by the titration assay were confirmed by other analytical methods. Tablet excipients (lactose, sucrose, starch, magnesium stearate, polyvinylpyrrolidone, calcium phosphate, fumed silica, talc, and microcrystalline cellulose) present in these formulations did not interfere with the titration assay. The titration technique was utilized also in measuring release rates from drug pellets having constant surface area.

Keyphrases □ Tablets, *in vitro* dissolution rates—determination, automated potentiometric titration method □ Potentiometric titration, automated—determination, *in vitro* tablet dissolution rates

An automated method for determining *in vitro* dissolution rates of solid dosage forms was developed by Schroeter and his coworkers (1, 2). The essential features of this method were the circulation of the dissolution fluid through a spectrophotometer flowcell and the automatic recording of the change in absorbance as a function of time. Dissolution-rate determinations using this principle were reported in several studies (3–6), and this technique is still probably widely employed throughout the pharmaceutical field. The automated spectrophotometric method, however, cannot be applied to many solid dosage forms for a number of reasons, including the lack of characteristic absorbance by the drug molecule, interference by other tablet ingredients in the spectral assay, and the use of dissolution fluid containing pepsin and pancreatin enzymes. In such instances, these studies have to be conducted by withdrawal of dissolution fluid samples at several time intervals and their subsequent individual sample analysis to determine the drug concentration.

The present study was initiated to develop a convenient *in vitro* dissolution-rate procedure for the enteric coated tablet formulations of a weak basic drug. The spectrophotometric method proved to be unapplicable in this case because of the reasons mentioned earlier. Hence, a continuous titration technique was developed for the automated dissolution-rate evaluation of this product. According to this technique, the drug, as it dissolves in solution, is rapidly titrated by the addition of the required amount of titrant liquid to maintain a set constant pH of the dissolution media. This is monitored by a pH-stat titrator instrument. From the amount of titrant added as a function of time, which is recorded on a chart, the dissolution rate of the drug is estimated. A similar technique was previously employed in evaluating dissolution and reaction rates of antacid compounds (7, 8). In the present report, the general application of the titration technique as an automated dissolution method for the tablet formulation of acidic or basic drugs is evaluated. Also, the use of this technique in studying dissolution rates from a constant-surface pellet of a drug was investigated.

EXPERIMENTAL

Test Samples and Reagents—Two different 250-mg. compressed tablet formulations of an antibiotic drug and their corresponding enteric coated tablets, 500-mg. compressed tablets of a sulfa drug, and 500-mg. compressed tablets of sulfonylurea were used as samples. The excipients present in these formulations were lactose, starch, sucrose, polyvinylpyrrolidone, calcium phosphate, fumed silica, talc, magnesium stearate, and microcrystalline cellulose. The quantity of excipients present in these formulations constituted approximately 10–50% of the total tablet weight.

Hydrochloric acid and sodium hydroxide titrant solutions of known normality were prepared in carbonate-free distilled water. Pancreatin NF, polysorbate 80 USP, and other chemicals (all analytical grade) were used.

Instrumentation—The instrumental setup shown in Fig. 1 was employed in most of the experiments. It consisted of a pH-stat automatic recording titrator[1] (A), titration buret (B), dissolution test apparatus (C), a pulsating-type pump[2] (D), and a Beckman model DBG recording spectrophotometer (E). With this setup, the dissolution rates could be determined simultaneously by the automated titration technique as well as by the automated spectrophotometric analysis. Both these techniques were employed in the

[1] Radiometer, Copenhagan Type TTTI titrator and Type SBR2 Titrigraph.
[2] New Brunswick Corp. model PA-60 pump.

Figure 1—*Instrumental setup for the automated determination of dissolution rates. Key: A, pH-stat titrator; B, titration buret; C, dissolution test apparatus; D, peristaltic pump; and E, spectrophotometer.*

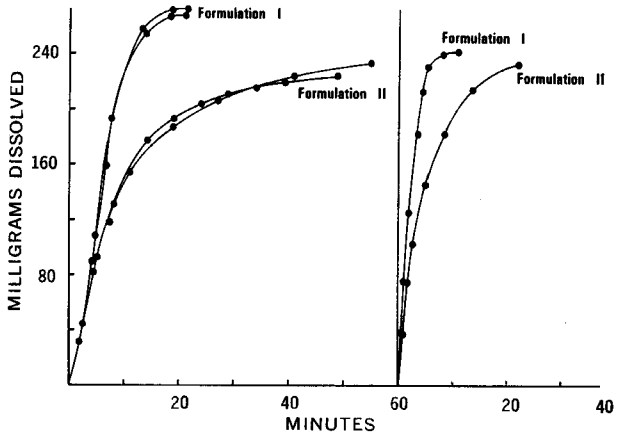

Figure 3—*Dissolution rates of the compressed tablet formulations of an antibiotic drug determined by the automated titration technique using the rotating basket apparatus (left) and the USP disintegration apparatus (right).*

evaluation of sulfonylurea tablets; for all other dissolution experiments, only parts A, B, and C were used.

A schematic diagram of the dissolution test apparatus used in the present study is shown in Fig. 2. It consisted of a thermostated beaker (A) with a stainless steel rotating basket assembly (C), held in the center about 2 cm. from the bottom of the beaker and attached to a constant-speed stirrer motor (E). The rotating basket design was essentially similar to that employed in the NF method I dissolution apparatus (9) but was further modified by placing two 1-cm. long stainless steel wire loop impeller blades (D) on two opposite sides of the basket. By this modification, it was possible to prevent formation of any stagnant layers of particles due to particles settling at the bottom of the beaker. Agitation of the dissolution liquid at a constant speed was achieved by rotation of the basket assembly at a predetermined revolutions per minute. A calomel and a glass electrode (F) were immersed into the liquid, and their terminals were connected to the pH-stat titrator unit. A fine tip Teflon tube (D) immersed into the liquid served as a buret through which titrant solution was added.

In one dissolution experiment, a USP disintegration apparatus was employed instead of the apparatus shown in Fig. 2. The electrodes and the Teflon tip buret were held in two separate cylindrical compartments of the basket.

Dissolution Procedure—The glass and calomel electrodes were calibrated with the standard buffer solution, and the pH-stat instrument was set to a desired end-point titration pH corresponding to a value at which the dissolution experiment was to be performed.

The apparatus shown in Fig. 2 was employed for the evaluation of all tablet samples. A tablet sample was placed in the basket (C); then the basket was immersed into a measured volume of unbuffered dissolution fluid equilibrated at 37° in the thermostated beaker (A). The liquid was stirred by rotating the basket at a predetermined revolutions per minute. The rate of dissolution was followed by titration of the drug as it dissolved by means of automatic intermitent additions of small volumes of titrant liquid through the Teflon tip buret (D) into the dissolution media. The dissolution rates were estimated from the rate of consumption of titrant liquid, the pH of the media, the pKa of the drug, and the normality of the titrant liquid.

Experimental Conditions—The dissolution rates of antibiotic tablets were determined in 170 ml. of simulated intestinal fluid at pH 7.5 with 100-r.p.m. stirring. The composition of simulated intestinal fluid USP was modified by replacing sodium phosphate with an equimolar quantity of sodium succinate in order to eliminate any buffer effect at pH 7.5 due to phosphate species but still maintain electrolyte concentration with the succinate ions. Dissolution studies for sulfa tablets and the powder sulfa drug were performed in 300 ml. of water at pH 7.5 with 300-r.p.m. stirring. For sulfonylurea tablets, conditions employed were 750 ml. of water at pH 7.5 with 300-r.p.m. stirring. In addition, compressed tablet samples of the antibiotic drug were evaluated using the USP disintegration apparatus. In this case, experiments were performed by following dissolution rates of three tablets in 750 ml. of simulated intestinal fluid at pH 7.5 and 37°. Since the dissolved drug existed

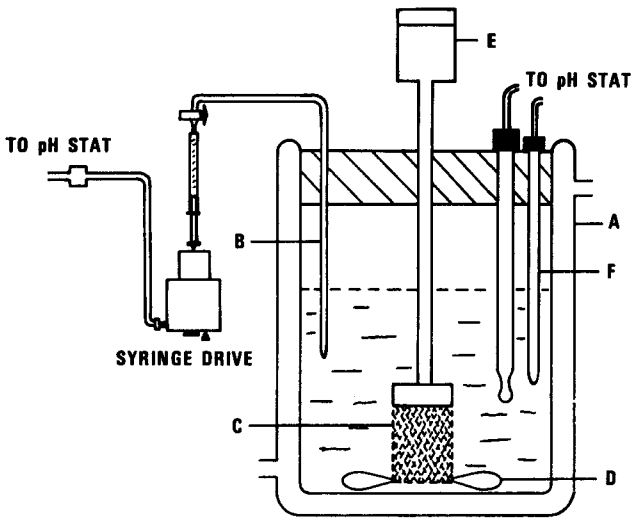

Figure 2—*Dissolution test apparatus. Key: A, thermostated beaker; B, titration buret; C, rotating basket assembly; D, wire loop impeller blades; E, constant-speed stirrer motor; and F, electrodes.*

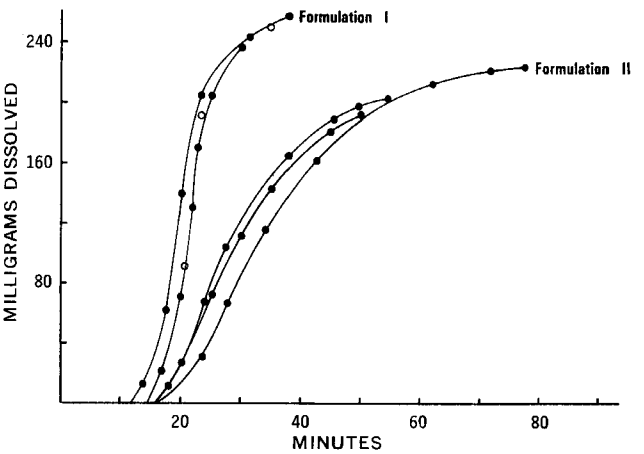

Figure 4—*Dissolution rates of the enteric coated antibiotic tablet formulations determined by the automated titration technique. The open circles represent the microbiological assay results.*

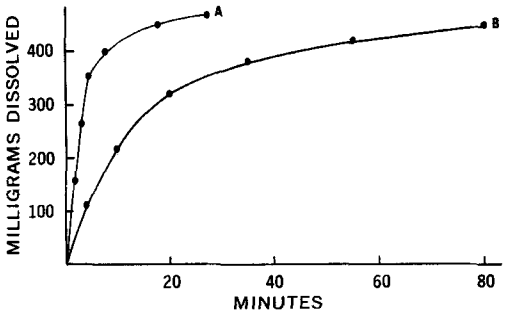

Figure 5—*Dissolution-rate profile of a sulfa drug determined by the automated titration technique. Key: A, powder drug, 500 mg.; and B, compressed tablet, 500 mg.*

in solution mostly in the ionized state, the solubility was not a problem in these experiments.

Dissolution rates from constant-surface pellets were determined in 170 ml. of water and also in 170 ml. of aqueous 0.5% polysorbate 80 solution at pH 7.5, 37°, with 300-r.p.m. stirring. The apparatus shown in Fig. 2 was used for the constant-surface pellet studies, except that the rotating basket assembly (A) was replaced with a stirrer having three 1.27-cm. (0.5-in.) impeller blades. The pellet samples were prepared by compressing a weighed amount of powder drug at 400 lb. force on a hydraulic press in a 1.27-cm. (0.5-in.) diameter stainless steel die. The pellet, held inside the die, was suspended in the apparatus at a particular height so that one flat surface of the pellet was exposed to the dissolution media.

RESULTS AND DISCUSSION

Dissolution rates determined by the continuous titration technique for the two different 250-mg. compressed tablet formulations of the weak basic antibiotic drug are represented in Fig. 3. The curves shown in the left portion of Fig. 3 were obtained when these tablets were tested in the rotating basket-type apparatus, while the curves shown in the right portion of Fig. 3 were obtained with the USP disintegration apparatus. It is evident from these results that, in both experiments, Formulation I showed a relatively rapid rate of dissolution as compared to Formulation II. It is important to notice that the total amount of drug dissolved, calculated on the basis of the amount of titrant acid consumed, was found to be more than 96% of the theoretical amount of drug present in these tablets. This finding suggests that the excipient present in the tablets did not significantly interfere with the titration assay of this drug.

The dissolution-rate results for the two enteric coated tablet formulations of the antibiotic are shown in Fig. 4. In this case, the initial lag time observed in the dissolution curve was due to the time required for the dissolution of the enteric coating. An acidic ingredient present in the enteric coating during initial dissolution time caused a slight decrease in the pH of the media. Therefore, during the dissolution of the coat, the pH had to be adjusted by the manual addition of a few drops of dilute sodium hydroxide solution. This adjustment, however, did not influence the tablet dissolution-rate results. The curves shown in Fig. 4 for each enteric coated tablet formulation were obtained upon repeated runs with the fresh samples. The titration assay results were further confirmed by the microbiological assay of the dissolution fluid samples collected at specific time intervals during a dissolution experiment with the enteric coated tablet. These results, shown as open circles in Fig. 4, are in good agreement with the titration assay results.

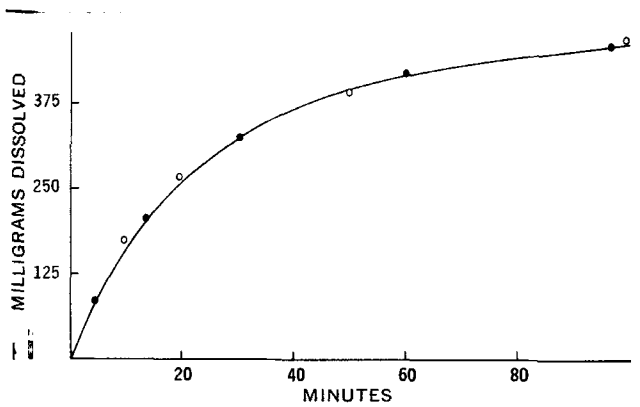

Figure 6—*Dissolution-rate profile of the sulfonylurea tablets determined by automated titration technique (smooth line with closed circles) and by automated spectrophotometric analysis (open circles).*

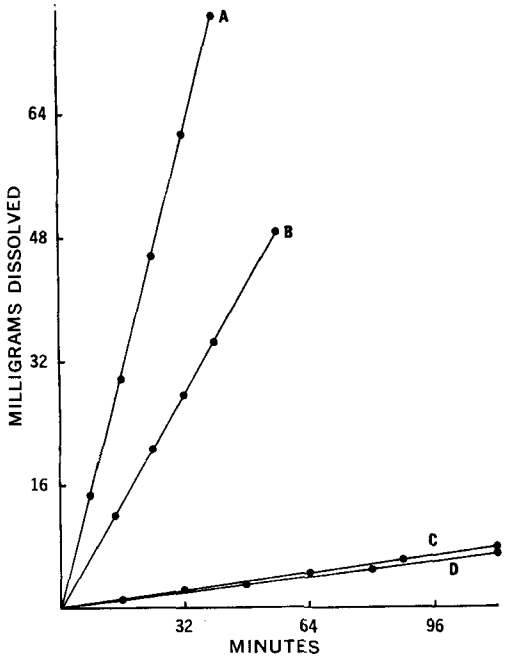

Figure 7—*Constant-surface pellet dissolution rates of two polymorphic forms of a drug determined by automated titration method. Key: A, Form I in 0.5% polysorbate 80; B, Form I in water; C, Form II in 0.5% polysorbate 80; and D, Form II in water.*

The dissolution-rate profiles for the 500-mg. weak acidic sulfa drug tablet and for 500 mg. of pure sulfa drug powder, obtained by the automated titration technique, are shown in Fig. 5. As evident from these results, the powder drug dissolved at a faster rate than the tablet. In both cases, however, the total amount of titrant consumed upon complete dissolution was equivalent to the theoretical amount needed to neutralize more than 95% of the drug, indicating thereby that the titration technique can be satisfactorily applied for the dissolution studies of this tablet.

The automated titration technique with the simultaneous automated spectrophotometric analysis technique was employed in the dissolution-rate determination of a sulfonylurea tablet. The results of this study, shown in Fig. 6, also suggest that either technique is suitable for the dissolution-rate determination of this tablet.

The drug-release rates from constant-surface pellets of acidic or basic drugs can be conveniently determined by the automated titration technique. Constant-surface pellet dissolution-rate measurements were previously studied to investigate the dissolution characteristics of crystalline states of a drug and certain other factors, like wettability and pellet porosity, that would influence the drug release rates (10, 11). The application of automated titration techniques in these types of studies is illustrated by evaluating dissolution characteristics of the two polymorphic forms of a basic drug. The results obtained for the two crystal forms of the drug in water and in 0.5% polysorbate 80 medium are shown in Fig. 7. A linear relationship between the amount dissolved as a function of time was observed in all experiments. Polymorphic Form II dissolved at a much slower rate in comparison with Form I, and both of these drug forms showed enhanced rates in the surfactant solution. The detail study of this system will be the subject of a separate communication.

The examples presented in this study suggested the applicability of the titration method in studying *in vitro* dissolution of acidic or basic drugs. However, as in the case of any other analyt-

ical technique employed for dissolution studies, one must verify that the excipients present in the formulations indeed do not interfere with the titration assay. The titration method cannot be employed in studying dissolution of capsule formulations, since the acidic nature of gelatin capsules causes overlap with the titration assay of the drug. In the automated spectrophotometric method, the dissolution fluid has to be filtered and then externally circulated through a photometric cell. Therefore, clogging of the filter screen and the lag time involved between the actual dissolution of the drug and its spectrophotometric measurement are the obvious problems encountered with this method. In the titration technique, however, these problems are nonexistent because the dissolution fluid does not have to be filtered or externally circulated.

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NOTES

Metabolites of Naloxone in Human Urine

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Abstract □ Naloxone, 7,8-dihydro-14-hydroxynormorphinone, and *N*-allyl-7,8-dihydro-14-hydroxynormorphine have been identified in a human urine specimen after naloxone administration. The metabolites were isolated, after hydrolysis with glusulase, by column chromatography and identified by TLC and spectrofluorimetry. These findings indicate that both *N*-dealkylation and the reduction of the 6-keto group of naloxone as well as glucuronide formation occur in man. Evidence is also presented indicating that *N*-allyl-7,8-dihydro-14-hydroxynormorphine formation occurs in rabbits as well as in man and the chicken.

Keyphrases □ Naloxone, metabolites—determination in human urine □ 7,8-Dihydro-14-hydroxynormorphinone, naloxone, and *N*-allyl-7,8-dihydro-14-hydroxynormorphine—isolated and identified as metabolites of naloxone in human urine □ Spectrofluorimetry—identification, naloxone metabolites □ TLC—identification, naloxone metabolites

Naloxone (*N*-allyl-7,8-dihydro-14-hydroxynormorphinone) is a potent narcotic antagonist in laboratory animals (1, 2) and in man (3). Fujimoto's (4) isolation of the *N*-allyl-7,8-dihydro-14-hydroxynormorphine-3-glucuronide metabolite of naloxone in chicken urine aroused speculation concerning its occurrence as a human metabolite. In rabbit (4) and in man (5), naloxone glucuronide has been reported as the sole metabolite of naloxone.

In the present study, an isolation procedure developed by Fujimoto and his coworkers (4–6) was used to separate the metabolites of naloxone. These were identified as the glucuronide of naloxone, 7,8-dihydro-14-hydroxynormorphinone (EN-3169), and *N*-allyl-7,8-dihydro-14-hydroxynormorphine (EN-2265).

EXPERIMENTAL

Approximately 2 l. of urine from a pooled 2-week urine collection was obtained from a patient. The urine was preserved under toluene and refrigerated. The patient was participating in a study of naloxone in the treatment of opiate dependence and was receiving between 1.0 and 1.8 g. of naloxone in single, daily oral doses during the 2-week collection period.

Column Chromatography—Metabolites were isolated from urine on a resin column[1] (5). Prior to column chromatography, 10 ml. of urine was adjusted to pH 5.3 with acetic acid and incubated overnight at 37° with 0.2 ml. of glusulase[2], an extract of *Helix pomatia* intestine which contains glucuronidase and sulfatase activity. The glusulase-treated urine was applied to a 2.5 × 10-cm. column of the resin. This process was followed by 50 ml. of distilled water and then 300 ml. of absolute methanol. The urine and water effluents were discarded. After discarding the first 10 ml. of methanol effluent, 250 ml. was collected. The methanol eluate was evaporated to dryness on a rotary evaporator[3] and redissolved in 0.2 ml. of methanol.

TLC—Five microliters of the concentrated methanol eluates was applied to a precoated silica gel TLC plate[4]. Controls and standards treated in a manner identical with that already described were also applied to the plate. Plates were developed in two solvent systems: A, chloroform–methanol–acetic acid (100:60:2 v/v); and B, chloroform–dioxane–ethyl acetate–concentrated ammonia (25:60:10:2.5 v/v). The solvents were used singly for developing

[1] Amberlite XAD-2.
[2] Endo Laboratories, Garden City, N. Y.
[3] Buchler.
[4] E. Merck ag. E. M. Reagents Division, Brinkmann Instruments.