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Continuous Dissolution Rate Determination as a Function of the pH of the Medium

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Abstract □ A method was developed for varying the pH of the medium during dissolution rate studies of timed-release tablets with the aid of compressed, totally soluble, alkaline powder mixtures. Commercial as well as experimental timed-release capsules or tablets were used as models, and dissolution rates were determined at pH 1.1, 2.4, and 7.4. The system can be applied to other pH values or other variations of the dissolution medium (*e.g.*, ionic strength) to: (*a*) correlate *in vitro* release rates with bioavailability data, (*b*) discriminate between alternative formulations during dosage form development, or (*c*) serve as a selective control procedure for a series of sustained-release dosage forms.

Keyphrases □ Dissolution rate—timed-release tablets, continuous determination as a function of pH □ Dosage forms—timed-release tablets, dissolution rate, continuous determination as a function of pH □ Tromethamine—buffer system for dissolution rate studies of timed-release tablets as a function of pH

Among the many parameters that influence the *in vitro* release rates of pharmaceuticals from timed-release dosage forms[1], the pH of the medium is particularly important, because these forms are designed to release their active ingredient(s) for a prolonged period (usually 8–12 hr). During this time interval, solid oral pharmaceutical formulations (such as tablets or capsules) pass from the stomach to the duodenum and on through the lower intestinal tract. Throughout this passage, they are exposed to a pH gradient ranging approximately from 1 to 8. [This range is narrower for healthy adults. The average pH of gastric fluid in men is about 1.9, while it is reported to be approximately 2.6 for women (2). The pH of the duodenal secretion for both men and women varies from 5.8 to 7.6 (3).]

This study was designed to evaluate a relatively simple method for determining the dissolution rates of timed-release solid dosage forms as a function of the pH of the medium. Model capsules and tablets were selected to meet the following requirements: (*a*) good solubility throughout the tested pH range, and (*b*) availability of a rapid assay procedure compatible with the system. The basic procedure was developed to allow for adjustment of the medium to other than the initial pH values or for the introduction of further changes (*e.g.*, ionic strength, viscosity, and composition of the medium).

[1] The term "timed release" is defined in NF XIV (1).

Alkaline phosphates and tromethamine [tris(hydroxymethyl)aminomethane] have been applied or recommended for modifying the pH of the medium (4–6), particularly for testing the dissolution rates of delayed-release formulations. In this study, completely soluble compressed tablets of these agents were formulated and used as the simplest way of achieving "in process" pH changes.

EXPERIMENTAL

Apparatus—The dissolution rate apparatus was fully described previously (6). The drug substance is extracted by a continuously flowing dissolution medium from the solid dosage forms, using a flow-through dissolution cell held at constant temperature (37°). The liquid moves through a flow-through cell, and the concentration is monitored spectrophotometrically (or by another suitable instrument). A typical set of dissolution curves is shown in Fig. 1.

Dissolution Media—Hydrochloric acid (0.1 *N*) was used as the initial dissolution fluid. The pH of this medium was changed by adding increasing amounts of tromethamine[2].

Within the scope of this study, the dissolution rates were determined at pH 1.1, 2.4, and 7.4. It was found practical to formulate 3.0-g tablets containing 1.4 g of tromethamine, 1.54 g of sucrose USP, and 0.06 g of polyethylene glycol 6000[3].

The thoroughly mixed powder was compressed directly with the aid of a manual hydraulic press[4] [compression force of 2270 kg (5000 lb)]. The excipients of the tablets were selected to form a tablet that dissolves completely in an aqueous medium and does not significantly absorb light between the wavelengths of 300 and 240 nm.

By adding four or five of these tablets, which contain 5.6 or 7.0 g of tromethamine, respectively, to the initial 500 ml of dissolution medium, the desired pH value of 2.4 or 7.4 was obtained without fluid interchange of the flowing system (Table I). (The tromethamine tablets dissolve in the medium in about 90 sec.)

Pharmaceutical Preparations—Commercially available theophylline capsules contained 259.2 mg of theophylline in coated pellets. The average total weight of the pellets in the size 0 capsules was 640.0 mg.

Phenylpropanolamine hydrochloride tablets were incorporated in an experimental timed-release matrix. [Phenylpropanolamine tablets or capsules were the subjects of several recent studies (6–8) due to their increasing therapeutic importance].

Dissolution Rate Testing Procedure—A predetermined volume of 0.1 *N* hydrochloric acid was added to each of six 1-liter beakers. For the theophylline capsules, 1000 ml was used; for the phenylpropanolamine hydrochloride tablets, 500 ml was used. The beakers were

[2] Ultrapure grade, 99.9%; Aldrich Chemical Co., Milwaukee, Wis.
[3] Sentry, Union Carbide Corp., New York, N.Y.
[4] Carver laboratory press model C, Fred S. Carver, Inc., Monomonee Falls, WI 53051

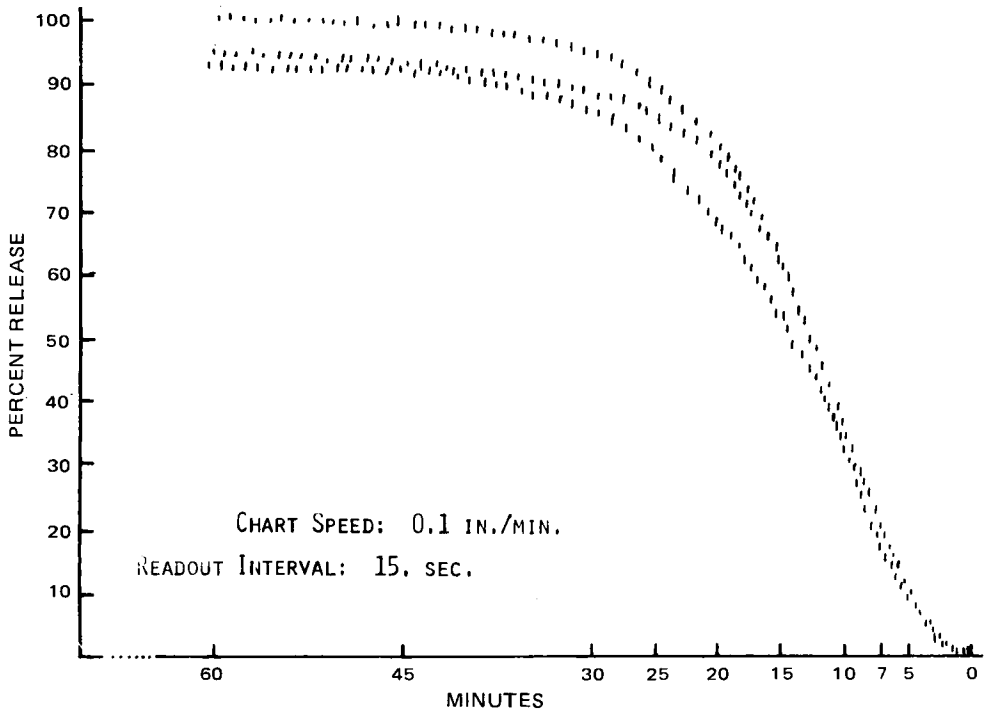

Figure 1—*Typical dissolution rate curves (three individual tablets).*

immersed in the larger constant-temperature water bath of the continuous release rate apparatus.

One dosage unit was inserted into each of five dissolution cells. The medium flowing out from the sixth beaker provided continuous baseline reference throughout the testing. The concentration of the solute in the dissolution medium was determined spectrophotometrically from the absorbance values at the approximate wavelength of the maximum absorbance (theophylline, 268 nm; phenylpropanolamine hydrochloride, 257 nm).

After 1 hr, the required number of tromethamine tablets was added to each beaker to shift the pH to 2.4. Similarly, after the 2nd hr, the pH was readjusted to 7.4 with additional tromethamine tablets.

RESULTS AND DISCUSSION

Dissolution curves obtained on the tested formulations are shown in Figs. 2 and 3.

The dissolution rate of solid particles in fluid media is described by the Noyes–Whitney equation (9):

$$\frac{da}{dt} = KS(C_s - C) \qquad \text{(Eq. 1)}$$

where da/dt, the rate of dissolution, is the amount of drug substance dissolved in a time unit; $(C_s - C)$ is the concentration gradient (the difference between the concentration of the saturated solution and the actual concentration in the medium at a given time); S is the surface area of the undissolved solute; and K is a constant, combining a large number of factors that may influence the dissolution rate.

Table I—pH Dependence of the Medium[a]

Amount of Tromethamine Added, g	pH[b]
0	1.1
5.6	2.4
6.0	2.7
6.4	7.1
7.0	7.4

[a] Initial dissolution medium was 500 ml of 0.1 *N* hydrochloric acid. [b] Variations equal ±0.1 pH unit. A Metrohm pH meter, model E300 (Metrohm Ltd., Herisau, Switzerland) was used.

These additional factors include the characteristics of the dissolution medium (*e.g.*, pH, ionic strength, and surface tension), the rates of stirring and/or the flow rates of the medium, physicochemical properties of the solute (*e.g.*, particle size), composition of the pharmaceutical formulation (*e.g.*, types and amounts of excipients), compression force, geometry of the tablets, types of capsules, and many other factors, which have been intensively studied (6–8, 10–17).

A series of papers dealt with the particular kinetics of the drug release from wax matrixes (5, 18–20) and from polymeric films (21).

For a given pharmaceutical formulation, if the other listed factors are reasonably constant, the pH of the medium may be the rate-determining factor. Therefore, Eq. 1 may be simplified as follows:

$$\frac{da}{dt} = K_1[f(\text{pH})](C_s - C) \qquad \text{(Eq. 2)}$$

where $f(\text{pH})$ represents a variable dependent upon the pH of the dissolution medium, and K_1 represents all other (constant) factors. Furthermore, under normal dissolution rate conditions, C_s is much larger than C and, for practical purposes, the concentration gradient can be substituted by C_s. As shown in Table II, the concentration gradient variation for the phenylpropanolamine hydrochloride tablets is negligible ($\Delta = 0.035\%$); it is very small (<2.5%) even for the theophylline capsules.

Manufacturers of timed-release dosage forms have recognized the possibility of utilizing the pH variations of the GI system, and pH dependence is often the basis of a timed-release delivery system.

The objective of this study was not to simulate physiological conditions but only to test *in vitro* dissolution rates in a simplified continuous system, as a means for establishing product uniformity and obtaining important information regarding the pH dependence of the dissolution rate from various pharmaceutical formulations. The initial pH 1.1 was selected as a value near the pH of simulated gastric fluid USP; pH 2.4 and 7.4 were also included since they are characteristic values in the actual range of gastric and duodenal secretions in humans (2, 3). The dosage units were exposed first for 1-hr periods at pH 1.1 and then at 2.4; thereafter, the operation of the dissolution apparatus was continued at pH 7.4 for 3–4 hr. If desired, the procedure may be varied by using different or additional pH values of the medium for shorter or longer times.

There are several methods of changing the pH of the medium. An efficient, but very tedious, way is described in NF XIV (1). It uses five individual rotating bottles and requires, after each scheduled period (ranging from 60 to 120 min): (*a*) the decanting of the medium (retaining "as much residue as is conveniently possible"), (*b*) the

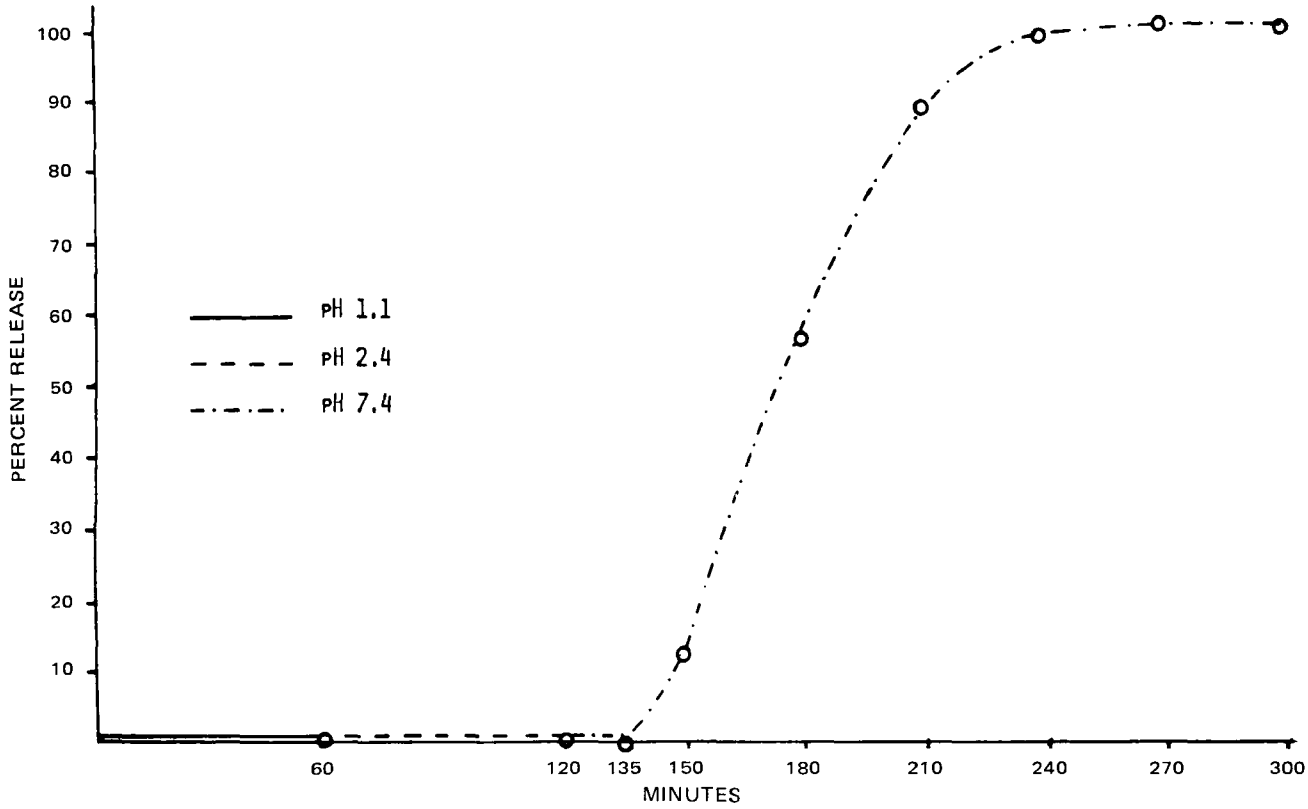

Figure 2—*Dissolution curve of theophylline capsules, 259.2 mg. Data points represent the average of four capsules. Linear regression analysis (from* t_{135} *to* t_{210}*) showed a rate of 0.98%/min* (r = 0.97).

quantitative returning of the inadvertently removed residual particles by washing them from the screens into the test bottles, (*c*) the gradual replacement of the dissolution medium of increasing pH values (from pH 1.2 to 7.5), and (*d*) the assaying of the residues. Besides the time-consuming and inconvenient operation, an additional disadvantage of this official method is that the number of residues (and, therefore, the number of parallel data) decreases at each subsequent step); at pH 7.5, only a single value can be obtained.

Other methods require the withdrawal of an assay sample at certain times and the replacement of the withdrawn volume with the same or with a different medium. These techniques involve not only a correction for the withdrawn amount (which can be simply calcu-

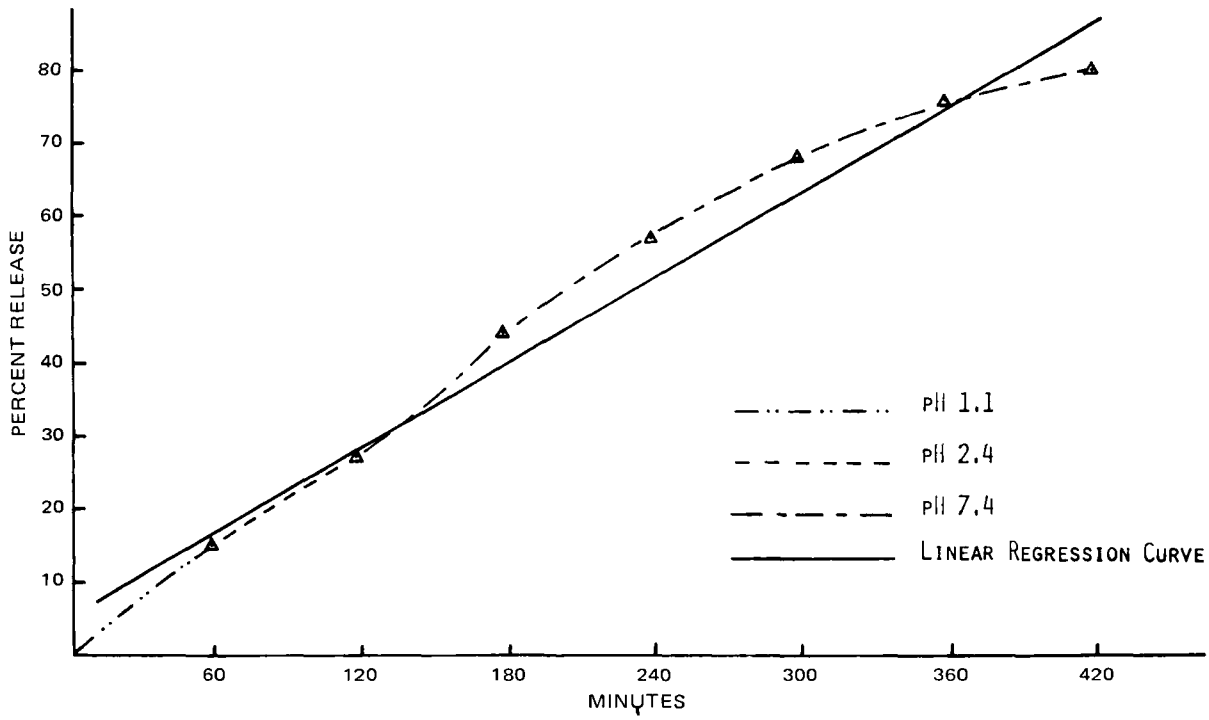

Figure 3—*Dissolution curve of experimental phenylpropanolamine hydrochloride tablets, 75 mg. Data points represent the average of five tablets. Linear regression analysis showed a rate of 0.19%/min* (r = 0.99).

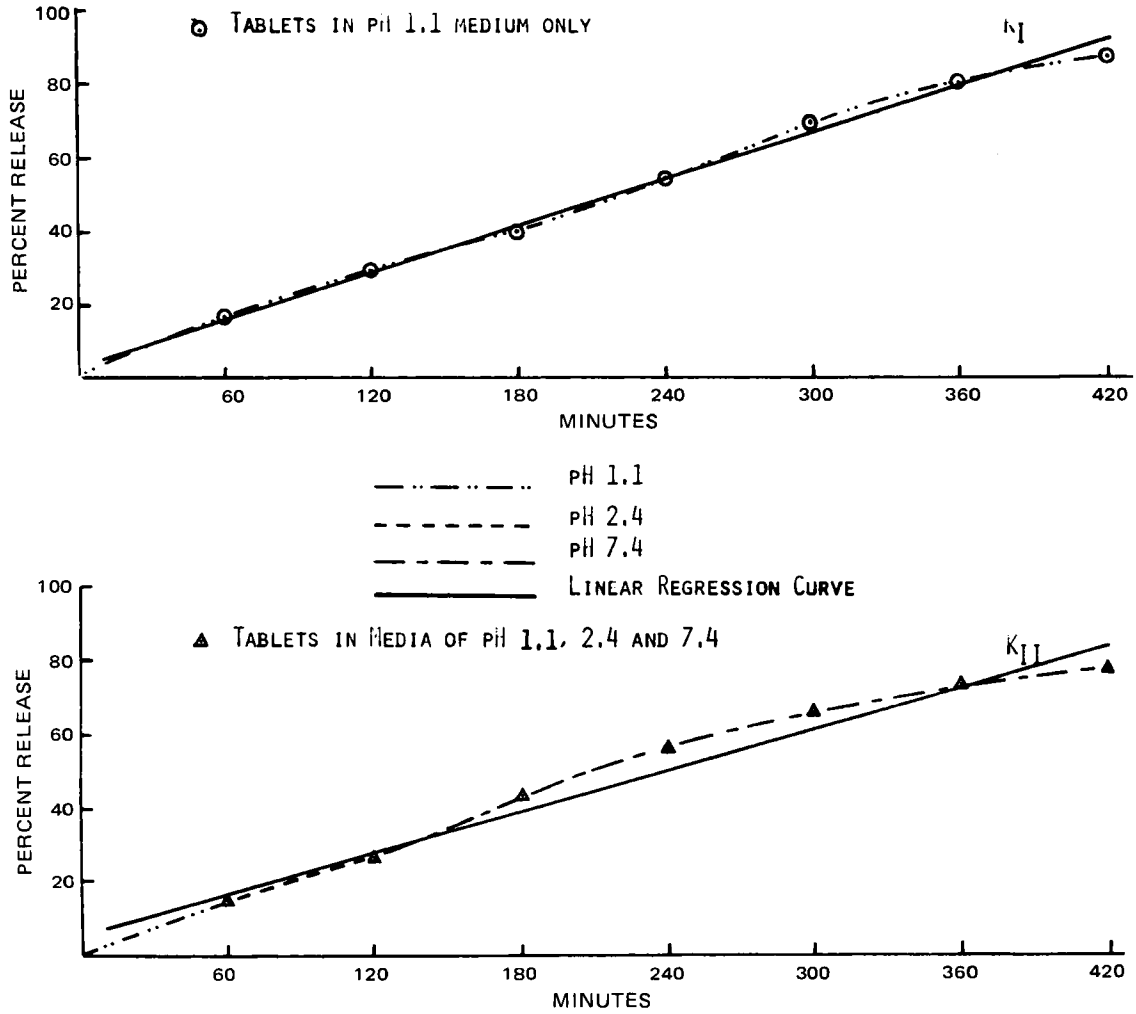

Figure 4—*Phenylpropanolamine hydrochloride tablets (comparative dissolution curves).*

lated), but also an actual change of the solute–solvent ratio. If the assay procedure or other factors necessitate comparatively larger volumes of withdrawals, the results are grossly distorted since, with the repeated replacements, less and less solute is extracted with more and more solvent. To overcome this problem, tromethamine tablets were selected. They not only change the pH of the medium without significantly changing its volume but also stabilize the pH since the hydrochloric acid–tromethamine combination is a standard buffering system (4).

Table II—Concentration Gradients in Dissolution Systems[a] at 37°

pH	Concentration Gradient ($G = C_s - C$)	Theophylline Capsules[b]	Phenylpropanolamine Hydrochloride Tablets[c]
1.1	G_{max}	1.207	42.780
	G_{min}	1.181	42.765
	$\Delta\%$	2.154	0.035
2.4	G_{max}	1.053	42.560
	G_{min}	1.027	42.545
	$\Delta\%$	2.469	0.035
7.4	G_{max}	1.120	43.110
	G_{min}	1.094	43.095
	$\Delta\%$	2.331	0.035

[a] G = concentration gradient ($C_s - C$); G_{max} = ($C_s - C$) at t_0; G_{min} = ($C_s - C$) at t_∞; C_s = saturation concentration (g/100 ml); C = concentration of the solute in the total dissolution medium (g/100 ml); and $\Delta\% = [(G_{max} - G_{min})/G_{max}] \times 100$. [b] Unit dosage = 259.2 mg; volume of the medium = 1000 ml. [c] Unit dosage = 75.0 mg; volume of the medium = 500 ml.

Another alkalizing agent, trisodium phosphate, was also considered in preliminary experiments. Due to its high water of crystallization content (56.9%), it was quite difficult to prepare the required large tablets. Additionally, there was a significant volume increase of the medium compared to the volume required for tromethamine tablets, which was only 0.7% at pH 2.4 and less than 0.75% at pH 7.4.

The dissolution curve of the commercial theophylline capsules indicated a 120-min lag time during acidic medium exposure, which corresponds to the claim that the pellets are enteric coated. After adjustment to pH 7.4, a typical S-shaped curve was obtained. After an induction period of 15 min (during which the enteric coating dissolved), the release began and continued at an almost constant rate of 0.98%/min. The release was practically complete in 2 hr after commencement of nonacidic exposure (Fig. 2).

The dissolution curve of the phenylpropanolamine hydrochloride tablets was quite different, because this formulation was designed to be pH independent in terms of release rate. Release began immediately and continued throughout the 7 hr of testing at about the same rate (0.19%/min) regardless of the pH of the medium. This result represents almost a zero-order release rate, independent of the pH of the medium within the applied limits (Fig. 3).

Figure 4 confirms this observation. In another test series, the medium was kept at pH 1.1 for 7 hr. The two curves (representing constant and variable pH conditions) show only a small rate difference (K_I = 0.21%/min; K_{II} = 0.19%/min).

CONCLUSIONS

With a simple procedural modification of a continuous dissolution rate system (namely, by adding to an initial dissolution medium of pH 1.1, at predetermined intervals, the appropriate amount of tromethamine in completely soluble tablets), the pH of the medium can

be increased and buffered as desired without any significant change in the volume of the medium. The method may serve as a highly automated alternative to the rotating-bottle method described in the official compendia. It offers the following advantages:

1. The operation of the system is very simple, and the system permits variation of conditions as required by the assay method.

2. While the system is basically designed to test the pH dependence of the dissolution rate of solid dosage forms, it may be easily adapted for determining the influence of other factors. Its flexibility may be particularly useful if *in vitro* simulation of *in vivo* bioavailability data is desired.

REFERENCES

(1) "The National Formulary," 14th ed., Mack Publishing Co., Easton, Pa., 1975, pp. 985, 986.

(2) G. Dotevall, *Acta Med. Scand.*, **170**, 59(1951).

(3) P. L. Altman, in "Blood and Other Body Fluids," D. S. Dittmer, Ed., Biological Handbooks, Federation of American Societies for Experimental Biology, Washington, D.C., 1961, pp. 416, 417.

(4) L. C. Schroeter and J. G. Wagner, *J. Pharm. Sci.*, **51**, 957(1962).

(5) F. Sjuib, A. P. Simonelli, and W. I. Higuchi, *ibid.*, **61**, 1381(1972).

(6) C. Cakiryildiz, P. J. Mehta, W. Rahmen, and D. Schoenleber, *ibid.*, **64**, 1692(1975).

(7) F. W. Goodhart, R. H. McCoy, and F. C. Ninger, *ibid.*, **62**, 304(1973).

(8) *Ibid.*, **63**, 1748(1974).

(9) H. Noyes and W. Whitney, *Z. Phys. Chem.*, **23**, 689(1897).

(10) J. G. Wagner, "Biopharmaceutics and Relevant Pharmacokinetics," 1st ed., Drug Intelligence Publications, Hamilton, Ill., 1971, pp. 115–120.

(11) J. E. Tingstad and S. Riegelman, *J. Pharm. Sci.*, **59**, 692(1970).

(12) R. E. Shepherd, J. C. Brice, and L. H. Luzzi, *ibid.*, **61**, 1152(1972).

(13) J. Cobby, M. Mayersohn, and G. C. Walker, *ibid.*, **63**, 725(1974).

(14) *Ibid.*, **63**, 732(1974).

(15) H. G. Kwee and G. A. Ulex, *Pharm. Ind.*, **36**, 576(1974).

(16) A. A. Ismail, M. W. Goudah, and M. M. Motawi, *ibid.*, **36**, 735(1974).

(17) J. B. Johnson, P. G. Kennedy, and S. H. Rubin, *J. Pharm. Sci.*, **63**, 1931(1974).

(18) J. B. Schwartz, A. P. Simonelli, and W. I. Higuchi, *ibid.*, **57**, 274(1968).

(19) *Ibid.*, **57**, 278(1968).

(20) F. Sjuib, A. P. Simonelli, and W. I. Higuchi, *J. Pharm. Sci.*, **61**, 1374(1972).

(21) M. Doubrow and M. Friedman, *ibid.*, **64**, 76(1975).

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Isolation of 10,11-Epoxide of Protriptyline in Rat Urine after Protriptyline Administration

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Abstract □ The 10,11-epoxide, 10-hydroxy, and 10,11-dihydrodiol metabolites of protriptyline were identified in rat urine collected after the administration of 40 mg/kg ip of protriptyline. Mass spectrometric characterization confirmed the structure of these metabolites.

Keyphrases □ Protriptyline—three metabolites isolated from rat urine, characterized by TLC, GLC, and GLC–mass spectrometry □ Metabolites—protriptyline, isolated from rat urine, characterized by TLC, GLC, and GLC–mass spectrometry □ Antidepressant agents—protriptyline, three metabolites isolated from rat urine, characterized by TLC, GLC, and GLC–mass spectrometry

Epoxides are intermediates in the biological conversion of the aromatic ring system into the corresponding dihydrodiols (1). However, the instability of these metabolites has made their isolation and direct identification in biological fluids difficult.

The isolation from rat urine of the 10,11-epoxide of carbamazepine (2) after administration of carbamazepine, a drug with tricyclic structure, prompted a systematic investigation on the metabolism of other drugs with the same tricyclic structure. During this study, 10,11-epoxides and *N*-desmethyl metabolites of cyproheptadine (3) and cyclobenzaprine (4) were identified.

This report summarizes findings on the metabolism of protriptyline (*N*-methyl-5*H*-dibenzo[*a*,*d*]cycloheptene-5-propylamine) (I), a drug possessing antidepressant activity (5). The 10,11-epoxide, 10,11-dihydrodiol, and 10-hydroxy metabolites of protriptyline were isolated and characterized by means of TLC, GLC, and GLC–mass spectrometry in rat urine after protriptyline administration.

EXPERIMENTAL

Biological Samples—Two male Sprague–Dawley rats, 200 g, were injected with 40 mg/kg ip of protriptyline hydrochloride[1] dissolved in saline. Following drug administration, urine samples were collected at various times over 48 hr and kept frozen until analyses were performed.

After addition of drug to untreated rat urine and incubation at 37° for 48 hr, no degradation of the product occurred.

Treatment of Biological Samples—Urine samples (20 ml)

[1] Donated by Merck Sharp and Dohme, Rahway, N.J.