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In Vitro Resorption Characteristics Of Selected Diuretic Compounds By Thedesaga Resomat

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IN VITRO RESORPTION CHARACTERISTICS OF SELECTED DIURETIC COMPOUNDS BY THE DESAGA RESOMAT

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IN VITRO DRUG ABSORPTION PROFILES UTILIZING THE DESAGA RESOMAT

Abstract of Dissertation

An in vitro method was tested whereby the processes of dissolution and distribution of active ingredient into a lipoid solvent were combined in one model and evaluated.

The objectives of this research project were: (1) to evaluate the efficacy of the in vitro model known as the Desaga Resomat, in the testing of gastro-intestinal absorption; (2) to apply the procedure and instrument to a group of therapeutically important products in determinig their absorption characteristics; (3) to graphically illustrate the absorption profile of a specific drug as per cent absorbed vs. time, simulating in vivo absorption; (4) to compare the profile data with experimentally determined dissolution studies; (5) to relate, biopharmaceutically the application of the profile data to absorption characteristics.

A literature survey was perfomed including the history of dissolution rate methodology and its influence on the bioavailability of medication from the dosage form.

A discussion of the experimental procedure was presented including the graphical illustration of the resorption profiles for the three commonly known drugs used in the verification of the apparatus.

The method was applied to a group of therapeutically important products, specifically, thiazide diuretics as well as non-thiazide diuretics such as Spironolactone, Acetazolamide, Ethacrynic Acid, Chlorthalidone, and Furosemide.

Dissolution properties for each drug were determined using the Desaga Resomat as well as the 'Beaker Method' of Levy and Hayes. The method used in this work involved the use of a glass model apparatus which showed the basic physical and chemical principles of the absorption process, namely, the solubility of a drug in the aqueous phase and its distribution into a lipoid phase. The dosage form was placed into an aqueous buffered solution. The pH of the buffered solution was adjusted so as to correspond to the pH of the gastro-enteral system. As a result of pressure changes corresponding to peristalsis the dissolved drug passed through a sinter and was then subjected to distribution with a suitable lipoid solvent. The absorption profiles were determined by measuring, spectrophotometrically, the concentration of the drug in the lipoid phase. Graphical displays were constructed representing the solubility and distribution of the drug at specific pH-time-intervals.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

Figure | Page

INTRODUCTION

The dissolution rate of a drug has a marked effect upon its absorption from a solid dosage form. This fact has led to an increasing interest in developing *in vitro* dissolution rate tests that can be compared with *in vivo* absorption rate studies for possible use in quality control or for establishing official standards.

Many investigators are concerned with the mechanisms and requirements for the absorption of new drugs. Pharmacologists are using the many means at their disposal in attempting to regulate the amount and rate of gastro-enteral absorption. Analysts are confronted with the problem of checking the availability of drugs in such preparations with acceptable accuracy. It is true that in the final analysis, determinations of blood levels and elimination studies on animals or humans provide the most valid answers to many of the questions on absorption. However, these studies are associated with a number of disadvantages: *in vivo* tests are very time consuming and are, therefore, expensive; because of individual differences, they often have very poor reproducibility which necessitates statements of universal validity only from a relatively large number of experimental subjects; finally, the results of a blood level analysis are always of a complex nature and are unpredictable because they are affected by absorption mechanisms, protein binding, metabolism,

and elimination (1).

Because of these disadvantages, efforts have been made for several years to learn and master the factors influencing drug absorption. Physical-chemical determinations such as dissolution rate and partitioning studies could provide a more specific picture than *in vivo* experiments.

The over-all absorption process for solid drugs consists of the dissolution step followed by drug partitioning into an essentially lipid barrier. This process of absorption is generally studied *in vitro* as two separate processes, each of which might be rate limiting.

It has been shown in recent years that the rate of disintegration of a tablet does not provide useful information on the absorption of the drug (1). Currently, *in vitro* tests do not consider this absorption factor. Consequently, an *in vitro* test apparatus was developed in which absorption model experiments on the basis of dissolution and lipid distribution can be determined.

A glass model apparatus has been developed by Dibbern (1) which simulates the human digestive tract, whereby the processes of dissolution and distribution coefficients of drugs are represented and evaluated, combined in one model. The glass model apparatus has been called the Desaga Resomat.[a]

The Desaga Resomat makes it possible to determine the amount of drug, which has been dissolved in simulated

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a - Available from Brinkmann Instruments, Co., Westbury, N. Y.

digestive juices and that has been transferred into a lipoid phase as a result of distribution. The absorption results are obtained from two factors which control in vivo absorption, namely, aqueous solubility and distribution characteristics. These distribution characteristics are currently referred to as the partition coefficients of the drug.

The drug, either as a pure substance or in an oral dosage form, is subjected to a buffered solution corresponding to digestive juice. This solution is contained in the inner cylinder of the Resomat. Alternating pressure changes within the model produced an effect corresponding to peristalsis. As a result, the dissolved drug passes through an asbestos filter where it is subjected to distribution with a suitable lipoid solvent contained in the outer vessel.

The drug concentration of the lipoid phase can be determined at definite time intervals or continuously by using the peristaltic pump, a component part of the apparatus. Thus, by using this principle of distribution, the course of gastro-enteral absorption can be followed by changing the pH of the aqueous phase stepwise to correspond with that of physiological pH. The distribution process would operate continuously throughout these pH changes. A characteristic absorption profile for the drug under test would then be plotted. Another application of the Desage Resomat is the determination of the simple solubility rate of a pure drug or its rate of release from the oral dosage form without consideration of the distribution behavior.

Consequently, this unique apparatus could be used to study any therapeutic group of drugs for evaluating their solubilities and distribution characteristics. The derived data should be of definite value in predicting *in vivo* absorption characteristics.

SURVEY OF THE LITERATURE

In 1966, Dibbern (1, 2) reported that the factors lying between the oral administration and its measurable pharmacological or clinical effects are very complicated. In the past, it was considered satisfactory to take the drug action as the summation of all these factors. However, during the last few years, attempts have been made to differentiate and study individually the sequence of events involved in drug absorption. These events include gastroenteral resorption, drug transport, protein binding, plasma levels, metabolism, excretion, and receptor site mechanisms.

The increasing knowledge of the individual mechanisms can be taken into consideration in the synthesis of new compounds and the formulation of therapeutically effective dosage forms. The separate studies of the individual processes could be rendered possible with the help of test models. Therefore, these models would differ from the complexities of <u>in vivo</u> evaluations by expressing basic principles in a manner easier to understand and visualize.

Shore and his co-workers (3, 4) showed the importance of the distribution between an aqueous and lipoid phase in the determination of the absorption of organic drugs. They demonstrate that compounds with basic or acidic functions are absorbed only in their nonionized state, lipoid soluble form. This led to the conclusion, that the higher the lipoid

solubility, the greater the gastro-enteral absorption.

The dissolution of the drug in the digestive juices can be assumed to represent the first condition for gastro-enteral resorption. A sufficiently high distribution coefficient would represent the second condition. Depending on the ionic characteristics of the drug, one or the other of the aforementioned conditions can be the limiting factor for the resorption quota. Resorption resulting from aqueous dissolution and then lipoid distribution represents a harmonious physiological process. The rate at which the drug can penetrate the lipoid barrier of the mucosa is based upon the degree of partitioning into the lipoid solvent which in turn is controlled by the aqueous characteristics of the drug and the pH of the solution. This pH variable controls the degree of nonionized state of the drug, which is the form necessary for the partitioning process. Accordingly, as the pH dependent nonionized form of the drug dissolved in the lipoid phase, more becomes available from the aqueous dissolution process with subsequent ionic conversion.

There have been several reports on the mechanics for the determination of dissolution rates, but they do not answer satisfactorily all of the questions about "resorption" in the form they are described. It was for this reason that both "resorption" conditions, i.e., water solubility and distribution coefficient, be represented and combined into the model.

As indicated formerly (1), there was adequate evidence

to conclude that the rate at which a drug dissolves from its intact or fragmented dosage forms in the human gastrointestinal tract often controlled the rate at which the drug appears in the blood. It was also shown that in many cases *in vitro* dissolution results could be used to explain observed differences in *in vivo* determinations. It should be noted, however, that the rates of the processes of disintegration and dissolution were both dependent upon the composition and method of formulation of the dosage form. Therefore, controlled pharmaceutical procedures could be used to alter the rate of release from the dosage form.

Rate of Dissolution - Historical Highlights

In 1896, Noyes and Whitney (6) published a statement of their law which concerns the rate at which solids dissolve in their own solutions. The law resulted from experiments in which they measured the amount of a substance dissolved at different time intervals when constant surface cylindrical sticks of the substance were rotated in water. They explained the dissolution process on the assumption that a very thin layer of saturated solution was formed at the surface of the solid. The rate at which the solid dissolved was governed by the rate of diffusion from this saturated layer into the main body of the solution.

Nernst and Brunner (7) advanced the Noyes-Whitney Law to include all kinds of heterogeneous reactions. They used Fick's Law of Diffusion to establish a relationship between the proportionality constant involved and the diffusion

coefficient of the solute. In this way, they were able to estimate the thickness of the diffusion layer at the surface of the solid.

In 1931, Hixson and Crowell (8) introduced their "cube root" law based upon the rate of solution of a solid in a liquid as a function of agitation. The "cube root" law was used to analyze the factors involved in the process of agitation.

Cofman (9) was the first investigator to determine the rate of dissolution of a compressed tablet by designing an apparatus known as the "solvometer." It was based on the increase of buoyancy of the apparatus in water as the tablet went into solution.

Elliott (10) illustrated graphs showing the amount of drug dissolved versus time, using the apparatus developed by Cofman. He showed the influence of the temperature of the dissolution medium and the surface area of the dosage form upon the rate of dissolution.

Oser (11), in 1945, employed urinary excretion data in proposing the concept of physiological availability of the vitamins from pharmaceutical dosage forms. Results showed that a direct relationship existed in normal subjects between the urinary excretion of water-soluble vitamins and the amount ingested.

Nelson (12) explained the differences in blood levels of theophylline salts and the prolongation of these levels of theophylline salts after oral administration by showing

the differences in the *in vitro* rates of dissolution of these salts.

Higuchi (13), in 1958, studied the influence of bases and buffers on the dissolution rates of acidic solids. He used the dissolution constants of the acids and bases involved in the study to derive a simple equation to mathematically express the dissolution rate phenomenon.

Royal (14) compared the *in vitro* rate of release of various formulations of dextroamphetamine sulfate sustained release capsules by using a modified U.S.P. tablet disintegration apparatus. He found that the *in vitro* rate of release varied with each capsule formulation. He also noted that the rate of release varied with changes in agitation making correlation of results with other methods difficult.

Wiegand (15) introduced a mathematical expression which described *in vitro* release curves of several sustained release dosage forms. His equation was useful in correlating release of active ingredient from the dosage form with physiological availability.

At the same time, Wagner (16) derived an equation which allowed the calculation of the instantaneous rate of release of active ingredient at any given time during the *in vitro* test.

Nelson (17) reviewed and discussed the theoretical basis for application of urinary excretion data to evaluation of drug absorption. He applied the concept of excretion data to show how *in vivo* solution rate, limits the absorption of

aspirin and benzyl penicillin after oral administration.

Nash and Marcus (18), in 1960, described another apparatus and method for the *in vitro* evaluation of sustained release products. The advantage of simplicity, convenience, and versatility were illustrated, as well as the variables which affected the *in vitro* test procedure.

In the same year, Campbell (19) evaluated sustained action release rates of various dosage forms. He pointed out that sustained release properties apparently were not being evaluated properly by the methods presently in use, since physiological availability could not be predicted accurately from *in vitro* and *in vivo* tests in use. It was suggested that all claims for sustained release be based on adequately controlled *in vivo* tests; quantitatively related to dose, until more information regarding *in vitro* tests becomes available.

In 1960, Levy and Hayes (20) described a dissolution assembly referred to as the "beaker" method which provided information on the physico-chemical aspects on the buffered acetylsalicylic acid controversy.

Also in 1960, Wagner and co-workers (21) presented work which involved the comparison of plasma levels of prednisolone in man after oral administration of two types of granules containing the active ingredient. The dissolution of one type was dependent on the pH while the other was not. At the same time, they attempted to correlate these results with *in vitro* data experimentation. Comparing the *in vitro*

results between the two types of granules, the rates of release differed. However, when comparing the *in vivo* results derived from both types of granules, they were not able to demonstrate significant differences in plasma levels.

Levy (22) in another study determined the absorption rates of several types of commercial aspirin tablets by a urinary excretion method. His results showed that the *in vivo* absorption rate was proportional to the *in vitro* dissolution rate. He proposed at this time that the U.S.P. tablet disintegration test be replaced by a dissolution test.

In 1961, Wagner (23) established biopharmaceutics as an important parameter in testing pharmaceutical formulations, stressing the significance of dissolution rate studies.

Hamlin and co-workers (24) determined the *in vitro* dissolution rates of two different polymorphic forms of methylprednisolone. They showed that increased agitation of the dissolution apparatus prevented them from distinguishing any difference in the dissolution rates of the two polymorphs. They emphasized the need for controlled intensities of agitation during *in vitro* dissolution tests.

Schroeter and co-workers (25) reported that a quantitative relationship existed between rate of dissolution and disintegration time in certain tablet formulations. They correlated physiological availability of p-aminosalicylate with not only dissolution rate but also with disintegration time. Also their results questioned the validity of the plastic disks used in the official tablet disintegration test.

Schroeter and Wagner (26), in 1962, described the first automated dissolution rate apparatus. They employed the use of a timer-controlled sampling system which automatically removed filtered samples from the dissolution rate apparatus and recorded the absorption of the sample as a function of time. The method was later modified by Schroeter and Hamlin (27) by using a one mm. flow cell which prevented collection of insoluble tablet additives in the cell. Further modification of the system prevented diminution of flow rate due to a clogged filter. This was accomplished by shunting the circulation stream away from the filter except during actual recording of the dissolution process.

Niebergall and Goyan (28) developed another automatic recording apparatus to follow the process of dissolution. Results were recorded as percent transmittance versus time.

Higuchi and Hiestand (29) derived an equation to describe the dissolution rate of a particle as a function of time in their diffusion controlled dissolution process. They found that variation in particle size did affect the dissolution rate. Later, Higuchi and co-workers (30) applied the equation to the dissolution rate of micronized methylprednisolone in aqueous solutions and a correlation was obtained between experimental results and theory.

Levy and Tanski (31) described a rotating disk method for the determination of dissolution rates. This method allowed for constant controlled speeds over extended periods as well as good shaft concentricity.

Stelmach and co-workers (32) studied the mechanism of release of a drug from its dosage form *in vitro*. They stated that this information was necessary to interpret absorption, distribution, and excretion data to predict physiological availability *in vivo*.

Paikoff and Drumm (33) described a simple device which is useful for dissolution rate determinations of capsule formulations. The method allowed a visual inspection of the capsule dissolution. They used the procedure in the usual chemical dissolution rate determination for predicting possible problems of drug availability from capsules.

Levy and co-workers (34) described a single *in vitro* dissolution rate test for a drug which correlated quantitatively with the gastro-intestinal absorption rate in man. They expected the *in vitro* conditions that yielded such correlations to be relatively similar to dissolution conditions found *in vivo*. They suggested that additional variables that affect availability in man be included in future *in vitro* tests to correlate in a more significant manner those *in vitro* results with *in vivo* actuality. They indicated that this could lead to a relatively generalized test procedure, suitable for product development and control purposes which could be included in official compendia as a test for physiologic availability.

Higuchi and co-workers (35, 36, 37, 38) studied the importance of a number of factors controlling the rates of drug release from a variety of plastic and wax matrices. The

factors studied included the choice of plastic, weight of drug incorporated in the matrix, solubility of the drug used, matrix additives, and the role of solvent permeation of the matrix. They developed and applied theoretical models to illustrate their results.

Knoechel and co-workers (39) studied the many variables in the tableting process using an instrumented rotary tablet machine. He also reported data on the relationship between rate of dissolution of the active ingredients with the compressional force on the tablet.

The importance and usefulness of a polymorphic form of a drug to increase rate of dissolution can be measured by the work done by Higuchi and co-workers (40). They found that a correlation existed between the two polymorphic forms of sulfathiazole and methylprednisolone. They established that the meta-unstable polymorph had a greater rate of dissolution, which decreased as it reverted to the more stable state.

Niebergall and co-workers (41) were the first to investigate the simultaneous determination of the dissolution and partitioning rate of drugs. The kinetics of their system was described and a number of methods was given for evaluating the rate constant, which conformed to other methods reported. The apparatus, however, did not allow for continuous rate determinations, or other significant variables.

Finholt and Solvang (42) studied the kinetics of *in vitro* dissolution of phenacetin and phenobarbital in human

gastric juice, comparing it to hydrochloric acid dilutions containing various amounts of the surfactant, polysorbate 80. Increased rate of dissolution in the presence of the surfactant was shown to be due to a decrease in the interfacial tension between the drug particles and the dissolution medium. The solubilizing properties of the surfactant were not significant.

Castello and co-workers (43) described an apparatus in which twenty dissolution tests could be conducted simultaneously. Automatic and simultaneous sampling at predetermined time intervals was provided in the design of the apparatus.

Pernarowski and co-workers (44) described an apparatus consisting of a closed dissolution container, a basket-stirrer assembly, and a variable speed pump. It was automated by connecting the pump to a flow cell in a suitable spectrophotometer.

Aguiar and co-workers (45) evaluated the importance of physical and pharmaceutical factors involved in chloramphenicol release and availability from four commercial samples of chloramphenicol. The study was carried out in three parts: de-aggregation determinations, dissolution studies, and *in vitro* gut permeation. A correlation between drug release as reflected by the de-aggregation and dissolution rate and chloramphenicol plasma levels in humans was demonstrated.

Barzilay and Hersey (46) described an automated dialysis method for measuring the dissolution profiles of tablet dosage forms. The suitability of the method for the evaluation of these profiles has been demonstrated by an examination

of tablets of sulfathiazole prepared under different conditions of pressure and excipient content.

Tingstad and Riegelman (47) described a continuous flow apparatus for determination of rates of dissolution. This test produces a plot of instantaneous rate of dissolution versus time rather than the usual cumulative plot of percent dissolved versus time.

Blythe (48) discussed a systematic approach to bioavailability testing. He pointed out that in future years a great deal of effort will have to be expended to obtain the human and the *in vitro* data necessary to solve the generic equivalency problem.

Langenbucher (49) described a new method for the assessment of the dissolution behavior of solid dosage forms. The method, which is based upon the mass transfer between solid and liquid phase in an exchange column, is shown to avoid some disadvantages of the commonly used beaker methods employing fixed liquid volumes. Because of its reproducibility, the method seems useful for a meaningful study of dissolution kinetics.

Fites and co-workers (50) studied the drug-permeability properties of several water insoluble films with respect to their potential application for the control of drug release from solid pharmaceutical dosage forms. Films composed of polymethylvinyl ether-maleic anhydride co-polymer, cross-linked with polysorbate 20 appeared promising for the application of film controlled drug-release applications. The

permeability of the films can be controlled by the appropriate adjustment of their polysorbate 20 content, molecular weight of the polymer, and humidity pretreatment.

Over the years, many papers have been introduced discussing the importance of dissolution rates and the variable factors that affect it. Wagner (51) pointed out that there appeared to be as many different types of dissolution rate apparatus and variations of established methods as there are investigators studying dissolution problems. However, as previously mentioned, an apparatus has been developed that very closely simulates the gastro-enteral tract. It allows variability in agitation and in pH changes as it follows the simulated gastro-enteral course. This model test has been shown to compare with *in vivo* tests. The graphical display of the resorption quota at predetermined pH-time-intervals, results in characteristic curves for the individual drugs tested. Because of these factors, it was felt that by the use of this *in vitro* test, it could be considered a valuable adjunct in predicting the absorption of drugs *in vivo*.

Wagner (51) also pointed out that future research on dissolution rate studies should be directed towards establishing scientifically realistic test apparatus for the purpose of predicting physiological availability from dosage forms. Accordingly, it was deemed feasible to apply the principles of the Desaga Resomat to investigate more thoroughly the dissolution study phenomenon. Thus, the objectives of this research project will be:

1) to establish the efficacy of the Desaga Resomat as an instrument for the *in vitro* testing of gastro-enteral absorption.
2) to apply this procedure and instrument to a group of therapeutically important products in determining their absorption characteristics. Specifically, this group will include the thiazide diuretics as well as non-thiazide diuretics such as acetazolamide, spironolactone, ethacrynic acid, chlorthalidone, and furosemide.
3) to graphically illustrate the absorption profile of a specific drug as percent absorbed versus time, simulating *in vivo* absorption.
4) to compare the profile data with experimentally determined dissolution studies by the "beaker method."
5) to relate biopharmaceutically, the application of the profile data to absorption characteristics.

EXPERIMENTAL

Verification of the Experimental Procedure

Since the Desaga Resomat is a relatively new research instrument, it was necessary to verify the physical characteristics of such a model. This apparatus, designed by Dibbern (1, 2) was employed throughout the course of this study.

When there is a system consisting of dissolved and undissolved substances in an aqueous medium, the dissolved portion of the substance would be available for distribution into an immiscible non polar phase. Distribution implies the partitioning of the dissolved substance from the aqueous layer across the interphase into the lipoid solvent. The extent of this partitioning will be based on the solvent characteristics of the substance in each of the respective solvents. To accomplish this, it will be necessary for the dissolved substance to diffuse from the aqueous layer into the lipoid phase.

The drugs employed to confirm the operation of the instrument were those selected by Dibbern (1, 2) in his original study. He selected 50 mg. of each of the following for his test samples: acetylsalicylic acid, phenacetin, and phenobarbital.

The apparatus, as illustrated in Figure 1 was set up in the following manner. "Faucets" (A) and (B) were closed,

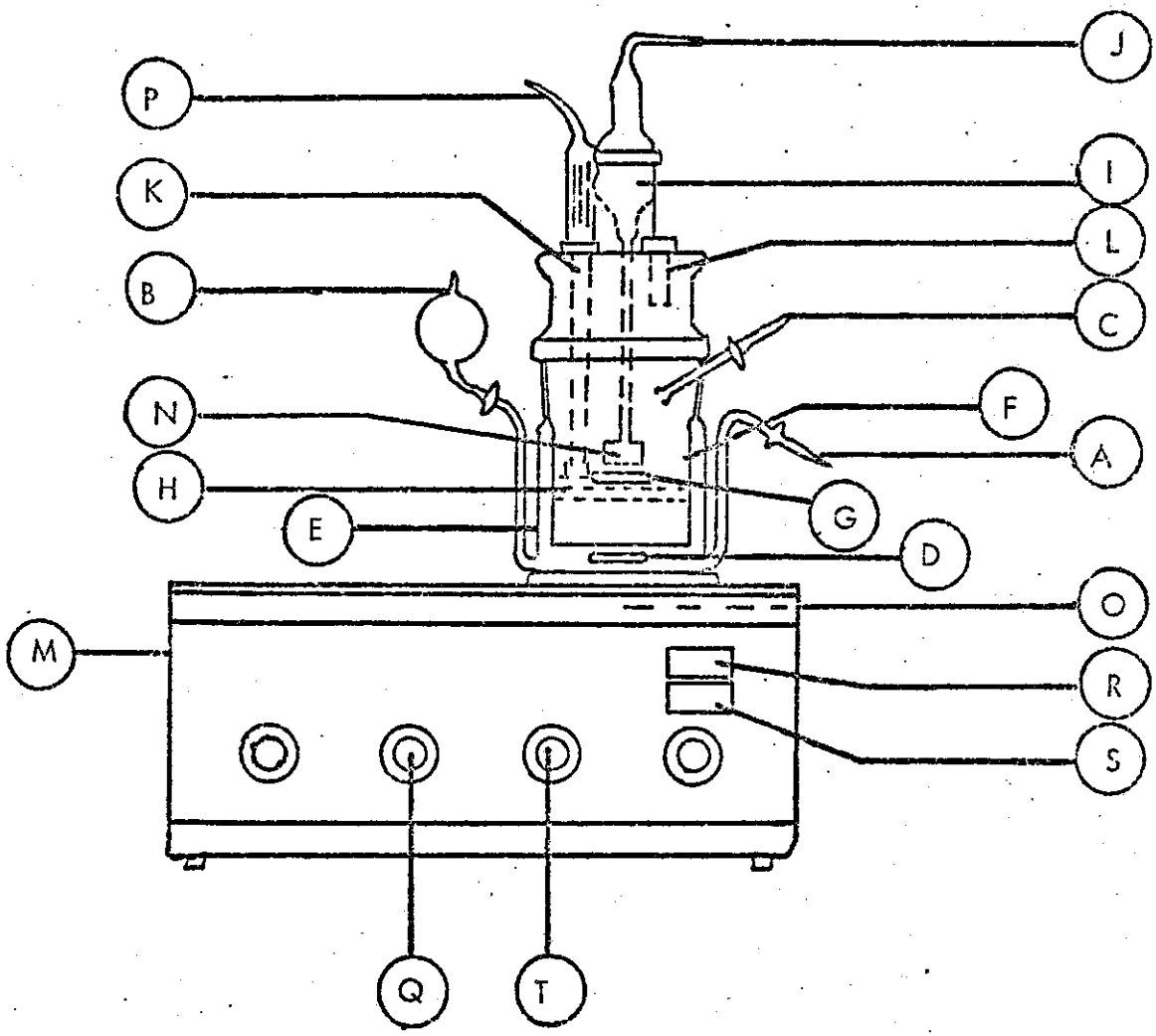

FIGURE 1

SCHEMATIC DRAWING OF THE DESAGA RESOMAT

and the pressure equalizing "faucet" (C) was opened. A magnetic stirrer (D) of 40 mm. length was inserted into the outer glass cylinder (E). Two-hundred mls. of chloroform were then introduced into this cylinder. The inner glass cylinder (F), containing a magnetic stirrer (G) of 30 mm. length, was lowered into the outer vessel so that the lower edge of the inner vessel was immersed 2 cms. into the chloroform. Precautions were taken to prevent the asbestos filter (H) from coming in contact with the chloroform layer. While holding the inner vessel in this position, 100 mls. of simulated gastric fluid were slowly poured into the inner glass cylinder through the opening (I). The simulated gastric fluid flowed dropwise through the asbestos filter onto the chloroform surface but remained within the inner cylinder. The inner glass cylinder was carefully guided onto the teflon coated standard taper joints and secured with a gentle twist. The air which remained between the filter and the aqueous phase above the chloroform was removed through the filter by sealing the tapered openings (K) and (L) and by applying a gentle vacuum at connection (J). After equalizing the pressure within the closed apparatus, the space below the filter now consisted of a two phase system of equal parts of simulated gastric fluid and chloroform.

The assembled glass apparatus was now placed upon the mechanical module (M). The 50 mg. samples of the drug under test, were placed into the tablet basket (N), which immersed the sample 1 cm. into the aqueous phase when the basket was

placed into its appropriate setting. The opening (J) was then connected to the pump (O), located in the module, with rubber tubing and the joints (K) and (L) were sealed by the introduction of a combination glass electrode[a] (P) and a ground glass stopper respectively.

The control knob (Q) located on the mechanical module was turned to the position marked "piston pump." With the pressure equalizing "faucet" open, the piston pump and the magnetic stirrer were turned on by depressing switches (R) and (S). The revolutions per minute of the magnetic stirrer were adjusted so that a vortex of about 15 mm. in diameter was formed at the two phase boundary between the water and lipoid solvent.

The control knob (Q) was turned to "single removal," to remove a chloroform sample containing the partitioning drug. This was accomplished by closing the pressure equalizing "faucet" and turning the removal pump control knob (T) in a counter-clockwise direction. This delivered a 5-ml. volume of the sample directly to a cuvette[b]. Samples were taken at sixty minute intervals and measured spectrophotometrically[c] at the appropriate wavelength. The sample was then returned

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- Semi-Micro AG/AgCl internals, available from Corning Scientific Instruments, Medfield, Ma.

- Spectrosil Cells, 10 mm. light path, available from Coleman Instruments Division, Maywood, Ill.

- Hitachi-Perkin Elmer Model 139 Spectrophotometer, available from Perkin-Elmer, Norwalk, Conn.

to the system through the "faucet" (A) by rotating the "removal pump" control knob (T) in a clockwise direction thus imparting a vacuum to the system which removed the sample from the cuvette. This dissolution and resorption distribution process was then continued by turning the control knob (Q) back to the position marked "piston pump" and by opening the pressure equalizing "faucet" (C).

The alternating positive and negative pressure imposed on the system by means of the small piston pump simulated the natural peristaltic effect which enabled the drug to pass through the filter. The aqueous layer was then in hydrostatic equilibrium with the chloroform layer.

Standard curves that would relate absorbance to drug concentration were prepared in concentrations indicated in Table 1 and illustrated in Figures 2 to 4. After one hour during which a sample had been removed, tested, and returned to the apparatus, the simulated gastric fluid at an initial pH of 1.2 was brought to the pH 4.0 by the addition of normal sodium hydroxide and acetate buffer[a]. The combination pH glass electrode was used to monitor the pH change. The dissolution and distribution process was continued for an additional hour. The pH was adjusted to 7.8 with normal sodium hydroxide and biphosphate buffer[b] and the test was continued in the above manner for an additional two hours.

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[a] - 0.2 M Potassium Acetate.

[b] - 0.2 M Potassium Biphosphate.

Drug concentration was monitored by removing samples at one hour intervals from the chloroform phase and returned to the distribution apparatus after spectrophotometric measurement. The absorbance measured was expressed as mg. percent.

Absorption profiles were illustrated for the following standard drugs: Acetylsalicylic acid, phenacetin, and phenobarbitol. The results are reported in Table II and illustrated in Figures 5 to 7.

The profile of acetylsalicylic acid (Figure 5) demonstrated a quick dissolution rate in the acidic media of the simulated gastric fluid because the acetylsalicyic acid remained in the non-ionic state and passed into the lipoid phase. At the pH of 7.8, the concentration of acetylsalicylic acid in the lipoid phase decreased. Dibbern (2) referred to the phenomenon as a type of "back resorption" occurring in the model indicating that the acetylsalicylic exists in the ionized state at this elevated pH. It should be mentioned that this would not occur in vivo because drug absorption is a dynamic process in which it would continue into the cellular fluid and finally to the blood plasma. However, the in vitro results did indicate that in vivo absorption of this drug would take place primarily in the acidic environment of the gastro-enteral tract.

The profiles for phenacetin (Figure 6) demonstrated that neither solubility nor the distribution coefficient was influenced by pH. This was expected as phenacetin is considered a neutral compound, and hence practically no ionization

TABLE I

SPECTROPHOTOMETRIC DATA OBTAINED FOR THE PREPARATION OF THE STANDARD CURVES FOR THE DRUGS EMPLOYED IN CONFIRMING THE APPARATUS PERFORMANCE

Drug Sample	Concentration[a]	Absorbance[b]
Acetylsalicylic Acid	0.01	0.14
	0.20	0.27
	0.40	0.54
	0.60	0.80
	0.80	1.10
Phenacetin	0.0125	0.16
	0.025	0.32
	0.05	0.70
	0.10	1.40
Phenobarbital	0.10	0.06
	0.20	0.17
	0.40	0.37
	0.60	0.47
	0.80	0.66

a - The standard concentration in the 5 ml. sample tested was obtained by dissolving 100 mg., accurately weighed, in 100 ml. of chloroform. Appropriate volumes were used, representing the tabulated weights.

b - Acetylsalicylic acid determinations were read spectrophotometrically at 280 mu, Phenacetin at 250 mu, and Phenobarbital at 240 mu, using a Hitachi - Perkin Elmer Model 130 Spectrophotometer.

ABSORPTION PROFILE DATA OBTAINED FOR THE DRUGS
EMPLOYED IN CONFIRMING APPARATUS PERFORMANCE

Drug Sample and Weight	Time in Hours[a]	pH of Determination[b]	Interpolated Weight for Total Volume[c]	Concentration Resorbed[d]	Resorption Pattern[e]
Acetylsalicylic acid, 50 mg.	1	1.2	15.0	30.0	+30
	2	4.0	25.0	50.0	+20
	3	7.8	23.0	45.0	
	4	7.8	12.5	25.0	-25
Phenacetin 50 mg.	1	1.2	5.5	11.0	+11
	2	4.0	10.0	20.0	+ 9
	3	7.8	18.0	36.0	
	4	7.8	24.0	49.0	+29

TABLE II (Continued)

Drug Sample and Weight	Time in Hours[a]	pH of Determination[b]	Interpolated Weight for Total Volume[c]	Concentration Resorbed[d]	Resorption Pattern[e]
Phenobarbital 50 mg.	1	1.2	4.0	7.0	+ 7
	2	4.0	26.0	52.0	+45
	3	7.8	29.0	58.0	
	4	7.8	30.0	60.0	+ 8

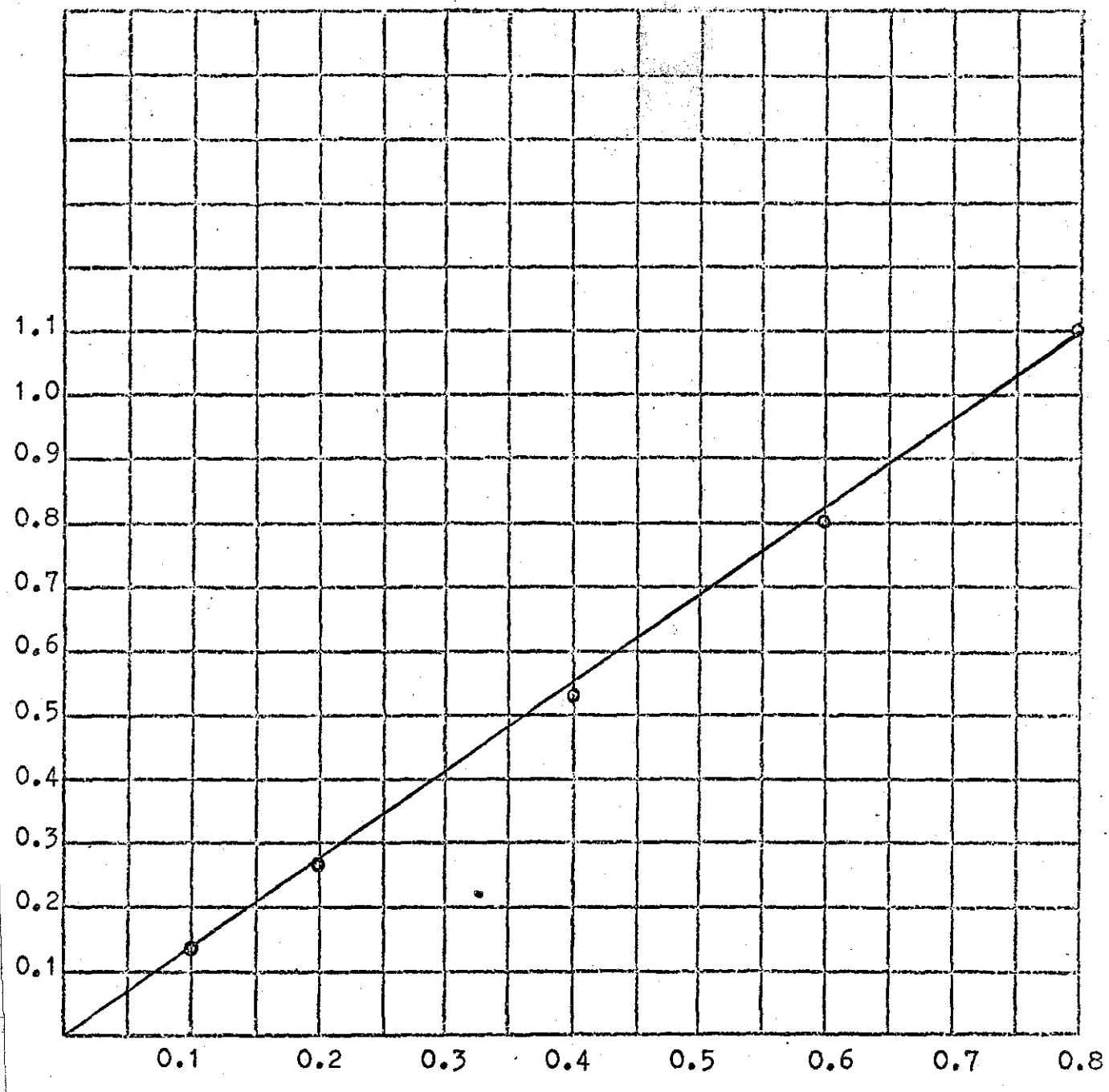
a - Represents the sampling of 5 ml. of chloroform solution at 60 minute intervals for the specific compound under test.

b - pH of 1.2 representing the pH of simulated gastric juice; pH of 4.0 - neutralized by addition of normal sodium hydroxide and buffered with 0.2 M potassium acetate; pH of 7.8 - further neutralization with normal sodium hydroxide and buffered with 0.2 M potassium biphosphate.

c - Represents the calculated weight in milligrams of the sample in 200 mls. of chloroform solution as determined by spectrophotometric analysis and interpolation from the sample under test.

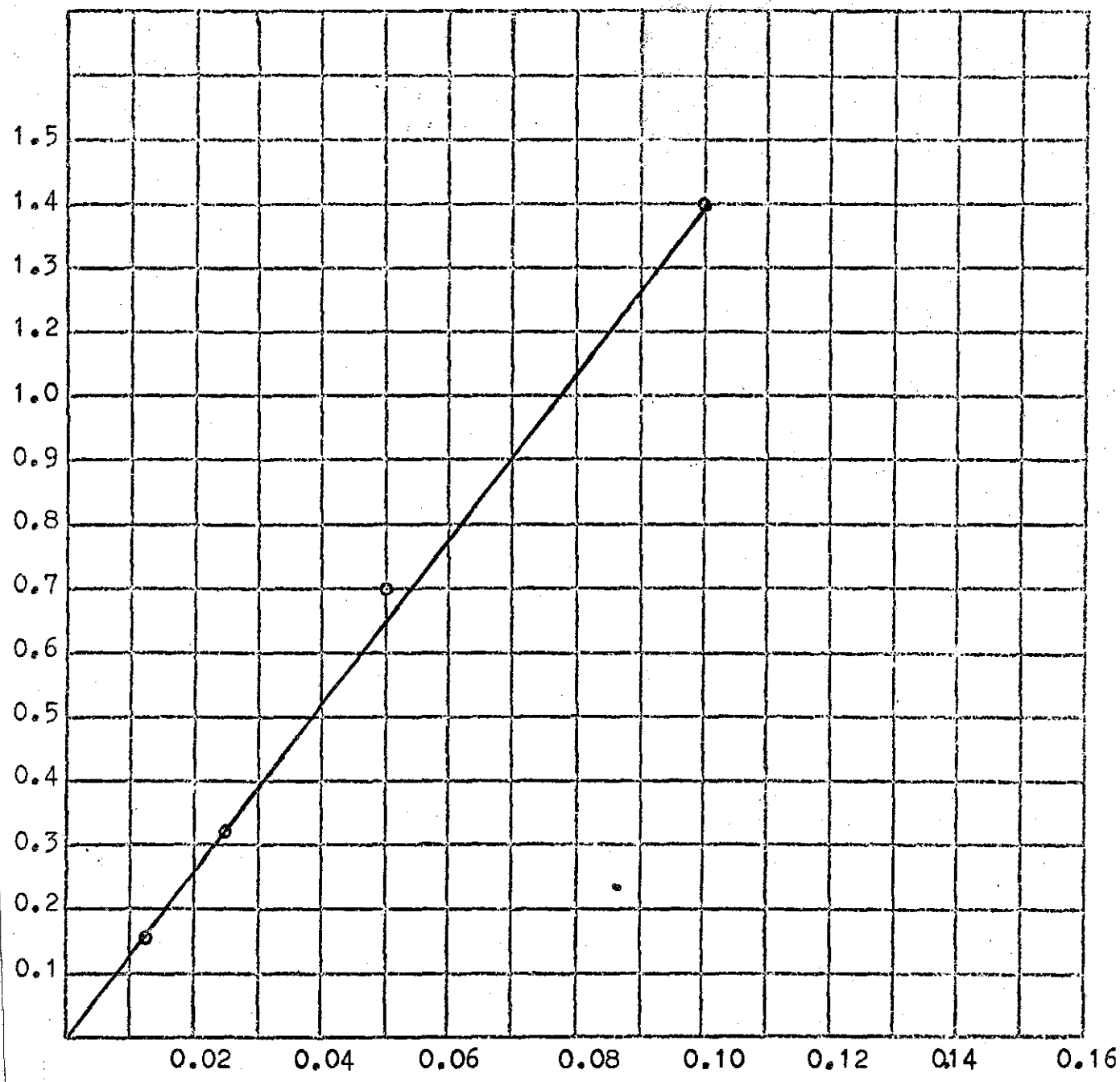
d - Represents the total percentage of the original weight of the sample that has partitioned into the chloroform layer. The values recorded represent reproducible results.

e - Represents the percentage of the original weight of the sample that had partitioned in the specific pH range under test. No percentage was reported for the third hour test. An accumulation percentage was recorded at the end of the fourth hour, since this value represents the total that had partitioned at the pH of 7.8.



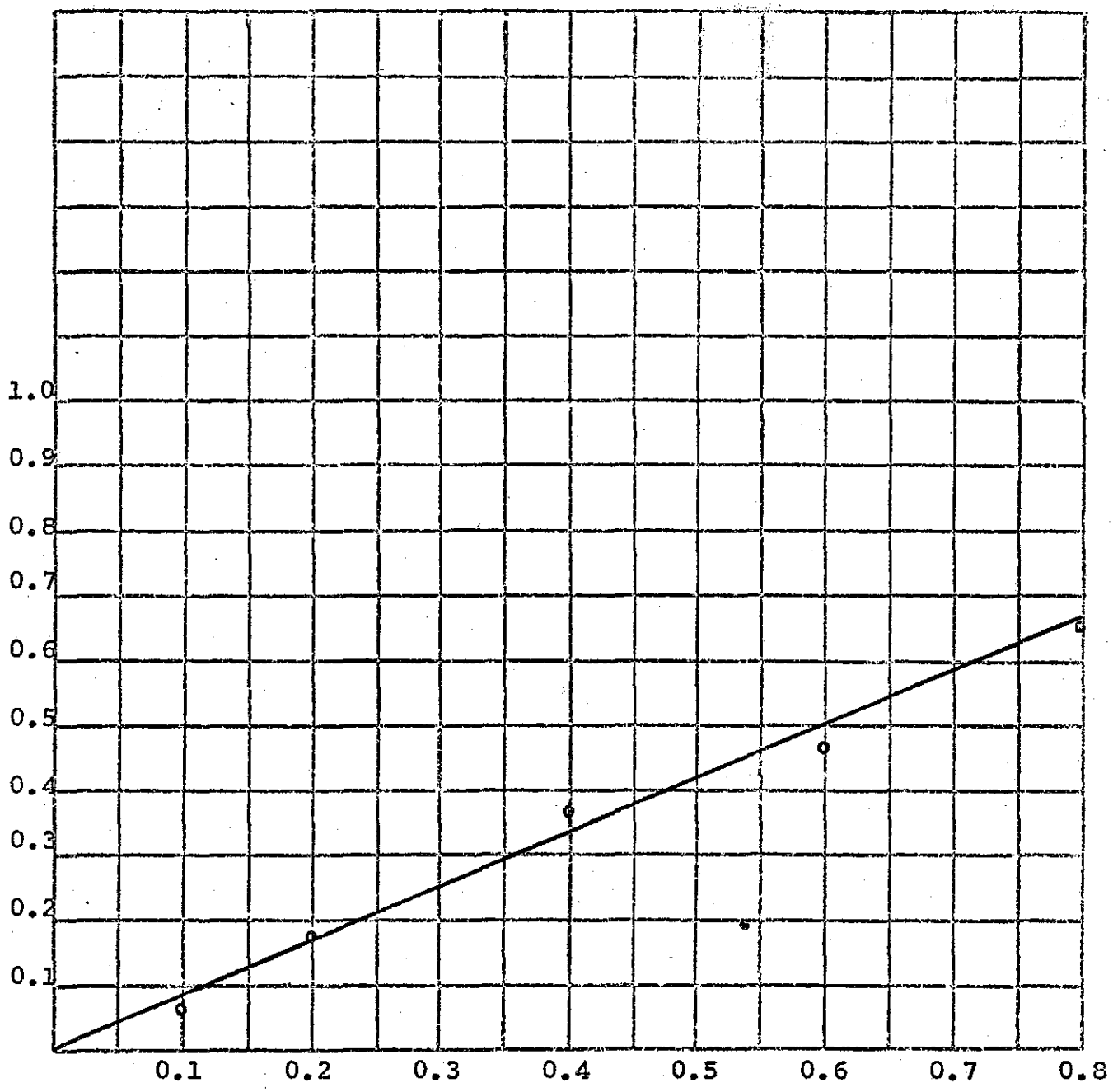
CONCENTRATION (milligrams per 5-ml.)

FIGURE 2 - STANDARD ACETYLSALICYLIC ACID CURVE



CONCENTRATION (milligrams per 5-ml.)

FIGURE 3 - STANDARD PHENACETIN CURVE



CONCENTRATION (milligrams per 5-ml.)

FIGURE 4 - STANDARD PHENOBARBITAL CURVE

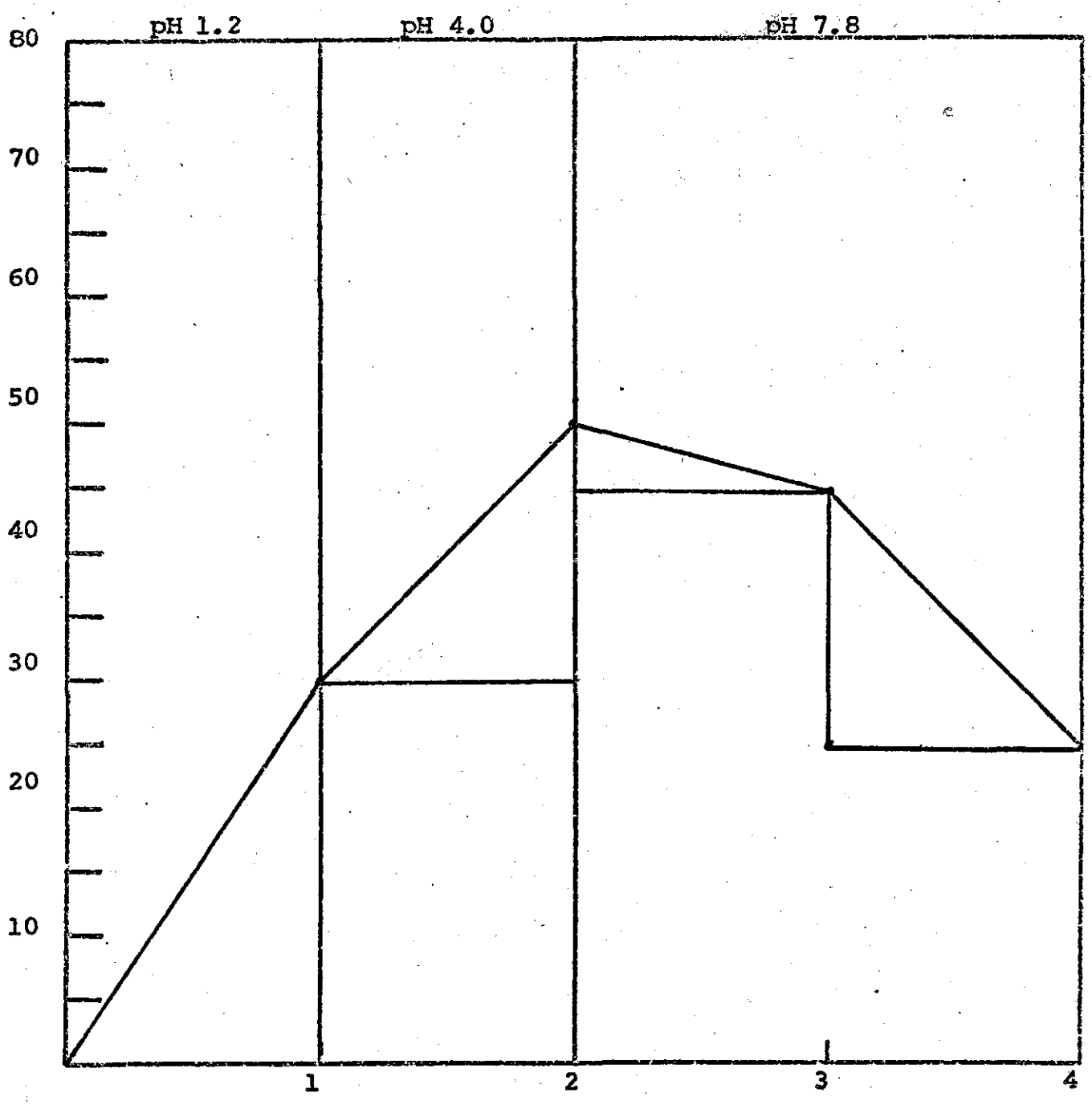


FIGURE 5 - RESORPTION PROFILE - ACETYLSALICYLIC ACID

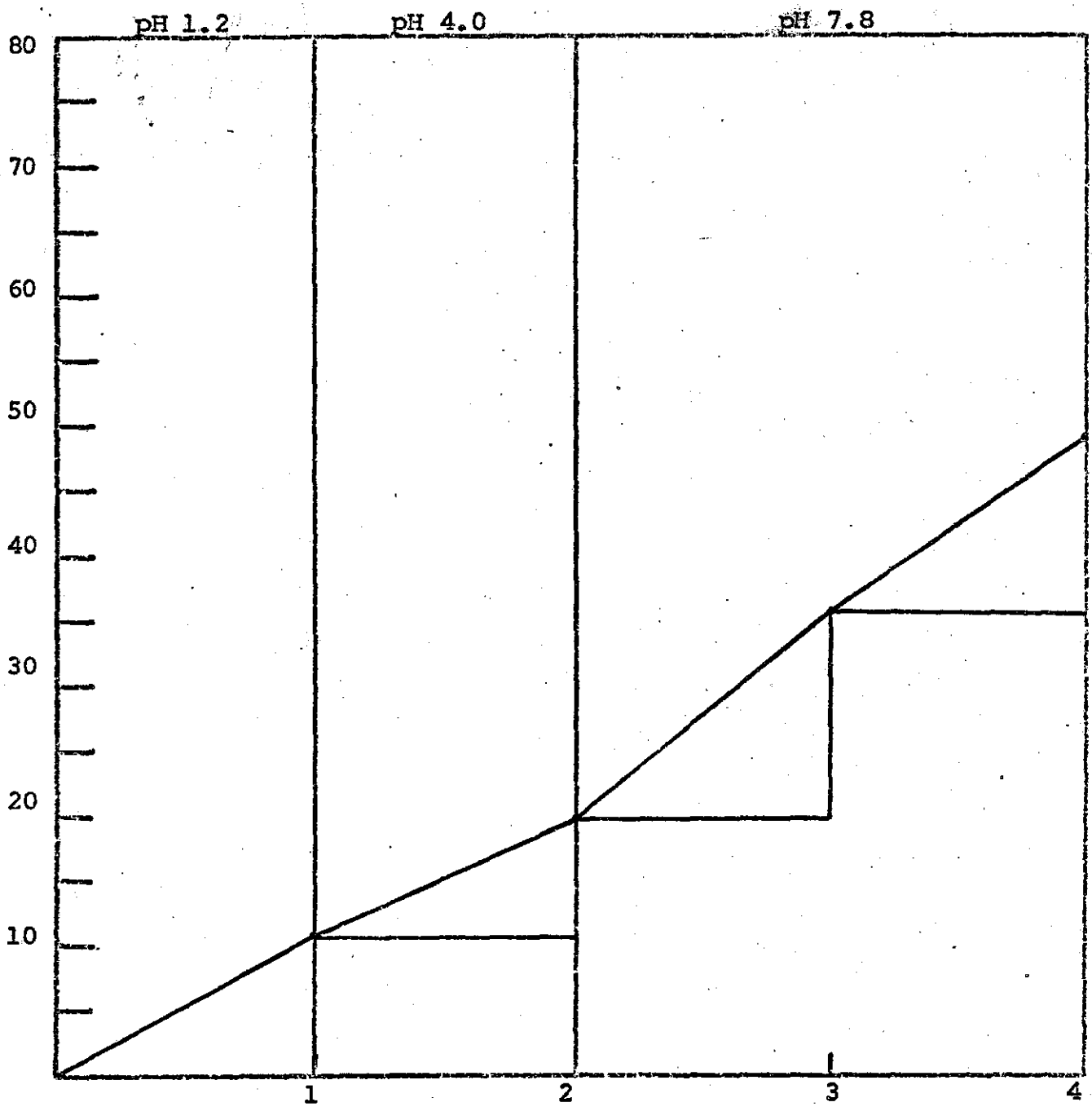


FIGURE 6 - RESORPTION PROFILE - PHENACETIN

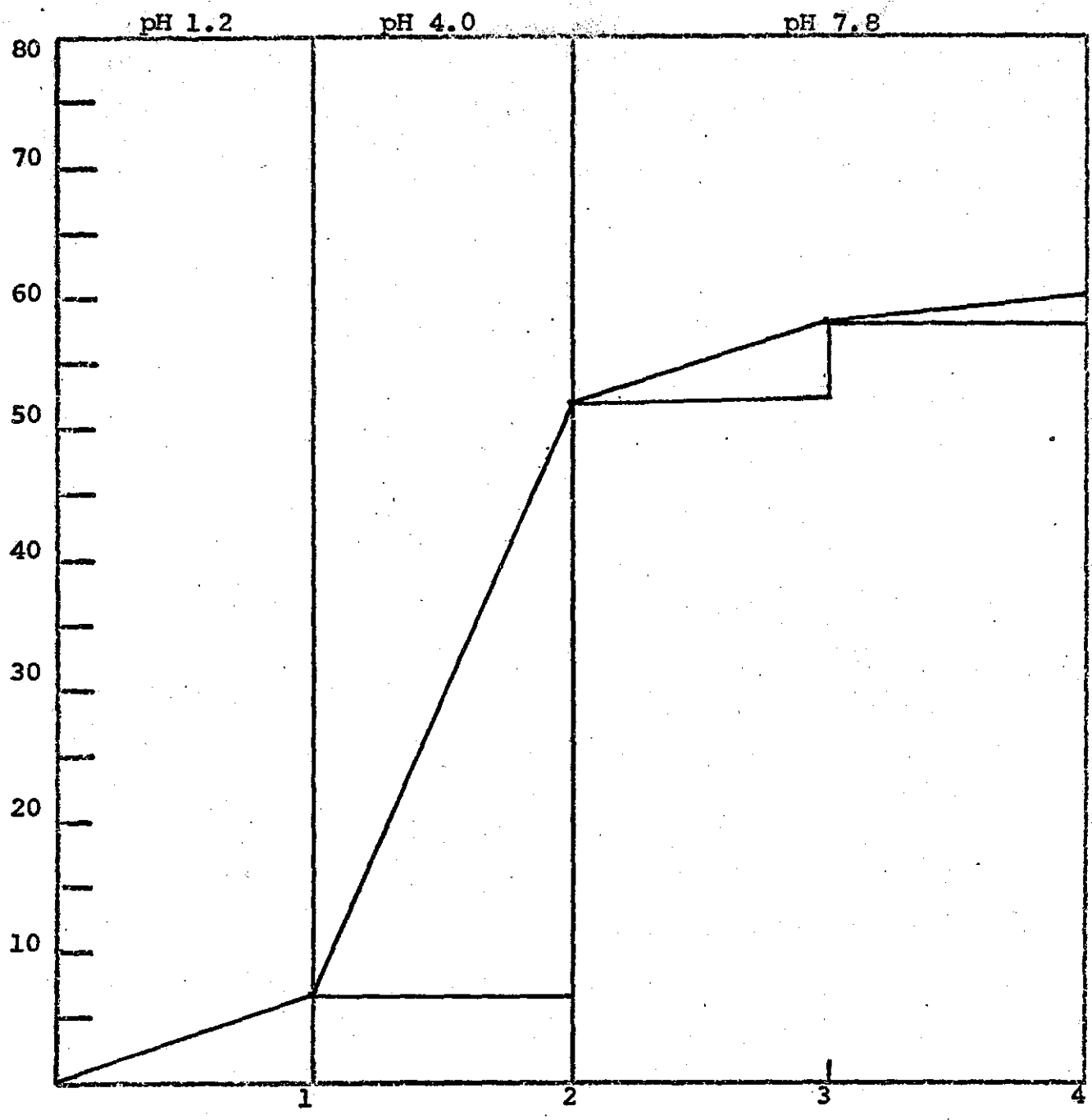


FIGURE 7 - RESORPTION PROFILE - PHENOBARBITAL

at the pH's used. This was confirmed by the fact that distribution of the phenacetin continued even though the pH of the aqueous phase was altered from acidic to an alkaline media.

The profile for phenobarbital (Figure 7), a slightly acidic compound, showed comparable distribution when compared to acetylsalicylic acid at a pH of 1.2. When the pH was increased to a 4, an increase in resorption was still noted, indicating the phenobarbital was mainly in the non-ionized state. However, when the pH was increased to 7.8, the resorption rate became weakly positive which would indicate the phenobarbital reverted to the partially ionized state.

Based upon the physical and chemical characteristics of the three drugs tested, the profiles for each of them could be forecast based upon their ionizing properties. At the same time, the profiles could be used to forecast distribution characteristics of other drugs under test based upon these principles. These preliminary results confirmed the method and results of the Desaga Resomat. Consequently, it appeared feasible to apply this *in vitro* model for comparative measurement of gastro-intestinal absorption of selected drugs.

Dissolution Rate Studies

It was apparent from the pharmaceutical literature that the term disintegration was formerly the key word in the evaluation of formulations for solid dosage forms (52). The disintegration of a tablet and the *in vivo* availability of the drug

were regarded as synonymous. The introduction of sustained release preparations precipitated the need to determine more realistic release patterns of active ingredients from dosage forms.

It was shown that disintegration tests provided no real index of availability of slightly water-soluble drugs within the body. At the same time, it was recognized that any relationship between disintegration time and biological availability was not a valid means of evaluation. However, when the biological availability was based upon dissolution rate, the significant relationship is now between dissolution and availability.

The importance of dissolution tests was best demonstrated by their inclusion in the NF XIII and the USP XVIII.

Therefore, it was decided to determine the dissolution rate of the drugs selected, in addition to the resorption studies, comparing these dissolution rates with those determined by a conventional method. This would illustrate further the versatility of this instrument.

In conventional dissolution studies, the extent of dissolution in an aqueous system is limited by the saturation concentration. This could be compared to the results obtained from a two phase system where dissolution would proceed dynamically with the solute passing from the aqueous phase into a lipoid phase.

Dissolution Rate - Beaker Method

Many models for testing dissolution rates have been

developed, however, the beaker method of Levy and Hayes (20) was chosen for this study because of its simplicity and acceptance as a significant advance in dissolution rate methodology. This method has been shown to compare favorably with in vivo biological availability. The assembly consisted of a 400-ml. Pyrex Griffin beaker which contained 250 ml. of simulated gastric, pH 1.2, fluid which was agitated by a two blade, 5-cm. diameter metal stirrer attached to an electronically controlled stirring motor[a]. The metal stirrer was immersed in the dissolution medium to a depth of 27 mm. and accurately centered by means of a guide. The stirrer was rotated at a rate of 60 r.p.m. and the test tablet was placed in the solution by dropping it along the side of the beaker. The stirring speed was sufficient to obtain a homogeneous solution for sampling purposes, but sufficiently slow, so that the fragments from the disintegrated tablet remained at the bottom of the beaker.

Five-ml. samples were taken at twenty minute intervals and the absorbance measured spectrophotometrically at the appropriate wavelengths.

Dissolution Rate - Desaga Method

The Desaga Resomat was prepared as previously described under the verification of experimental procedure, except that the outer vessel was filled with the simulated gastric fluid (pH 1.2) instead of chloroform. The tablet dosage form was

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a - Available from Eastern Industries, Hamden, Conn.

placed into the sample basket and immersed into the simulated gastric fluid. Rubber tubing was used to connect the piston pump to the glass apparatus. The system was sealed and the power control switches were turned on, regulating the piston pump and magnetic stirrers. The speed of the stirrers was adjusted to approximately 60 r.p.m.

The tablet dosage forms selected were nine commonly used diuretics consisting of: Acetazolamide[a], Chlorthalidone[b], Chlorthiazide[c], Cyclothiazide[d], Ethacrynic acid[e], Furosemide[f], Polythiazide[g], Spironolactone[h], and Trichlormethiazide[i].

Samples were removed in the same manner described for the determination of the resorption profiles for the verification of the experimental procedure. The samples were removed at twenty minute intervals over a period of two hours and the

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a - Available as Diamox 250 mg. from Lederle Laboratories, Pearl River, N. Y.

b - Available as Hygroton 100 mg. from Geigy Pharmaceuticals, Ardsley, N. Y.

c - Available as Diuril 500 mg. from Merck, Sharp and Dohme, West Point, Pa.

d - Available as Anhydron 2 mg. from Eli Lilly and Co., Indianapolis, Ind.

e - Available as Edecrin 50 mg. from Merck, Sharp and Dohme, West Point, Pa.

f - Available as Lasix 40 mg. from Hoechst Pharmaceutical Co., Somerville, N. J.

g - Available as Renese 4 mg. from Pfizer Laboratories Division, New York, N. Y.

h - Available as Aldactone 25 mg. from G. D. Searle and Co., Chicago, Ill.

i - Available as Metahydrin 4 mg. from Lakeside Laboratories, Inc., Milwaukee, Wis.

absorbance was measured spectrophotometrically. Each sample was returned through the outlet - inlet faucet by imparting a vacuum to the system.

Standard Curves

Standard curves were prepared for the diuretics in the concentrations as indicated in Table III and illustrated in Figures 8 to 16. All dilutions were prepared using simulated gastric fluid pH 1.2. These standard curves were used to relate drug concentration to absorbance.

The results of the two methods were reported in Table IV and Figures 17 to 25. The dissolution characteristics were represented graphically as percent concentration dissolved over a two hour period. In all cases, the reference points for the beaker method were represented by the small circles. The reference points for the Desaga method were represented by the triangular configurations.

Resorption Profiles

The resorption profiles were determined for the same dosage forms as evaluated under the dissolution studies. Because the solubility characteristics of each drug varied in chloroform, in some instances it was necessary to use mixtures of chloroform and ethyl acetate, or chloroform and tetrahydrofuran, as the lipoid solvent. The lipoid phase used for each drug was reported in Table V.

Standard Curves

Standard curves were prepared in concentrations of active

TABLE III

SPECTROPHOTOMETRIC DATA FOR THE PREPARATION OF THE STANDARD CURVES FOR THE DRUGS EMPLOYED IN THE DETERMINATION OF THE DISSOLUTION RATES

Drug Sample[a]	Concentration[b] (mg. per 5-ml.)	Absorbance[c]
Acetazolamide	0.010	0.14
	0.025	0.29
	0.050	0.55
	0.100	1.20
Chlorothiazide	0.010	0.06
	0.025	0.13
	0.050	0.27
	0.080	0.43
	0.100	0.53
Chlorthalidone	0.010	0.06
	0.025	0.13
	0.050	0.25
	0.100	0.49
Cyclothiazide	0.005	0.13
	0.010	0.20
	0.025	0.50
	0.050	1.00

TABLE III (Continued)

Drug Sample[a]	Concentration[b] (mg. per 5-ml.)	Absorbance[c]
Ethacrynic Acid	0.005	0.09
	0.010	0.17
	0.025	0.40
	0.050	0.80
Furosemide	0.003	0.19
	0.006	0.38
	0.013	0.75
	0.025	1.50
Polythiazide	0.013	0.17
	0.025	0.34
	0.050	0.68
	0.100	1.30
Spironolactone	0.010	0.10
	0.025	0.26
	0.050	0.52
	0.100	1.00
Trichlormethiazide	0.010	0.13
	0.025	0.33
	0.050	0.64
	0.100	1.20

TABLE III (Continued)

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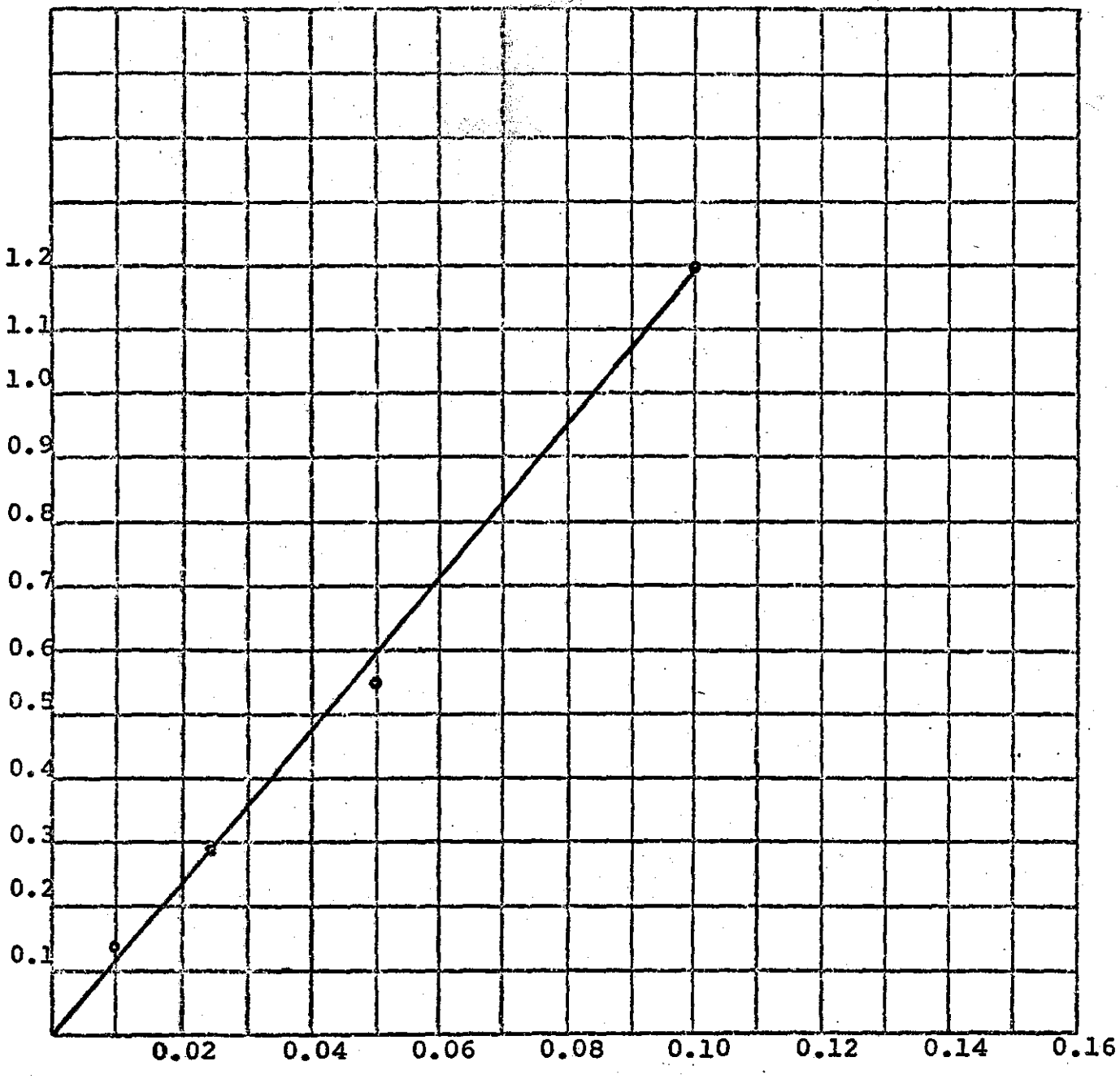
a - Samples used were the pure drug furnished by each of the respective pharmaceutical manufacturers.

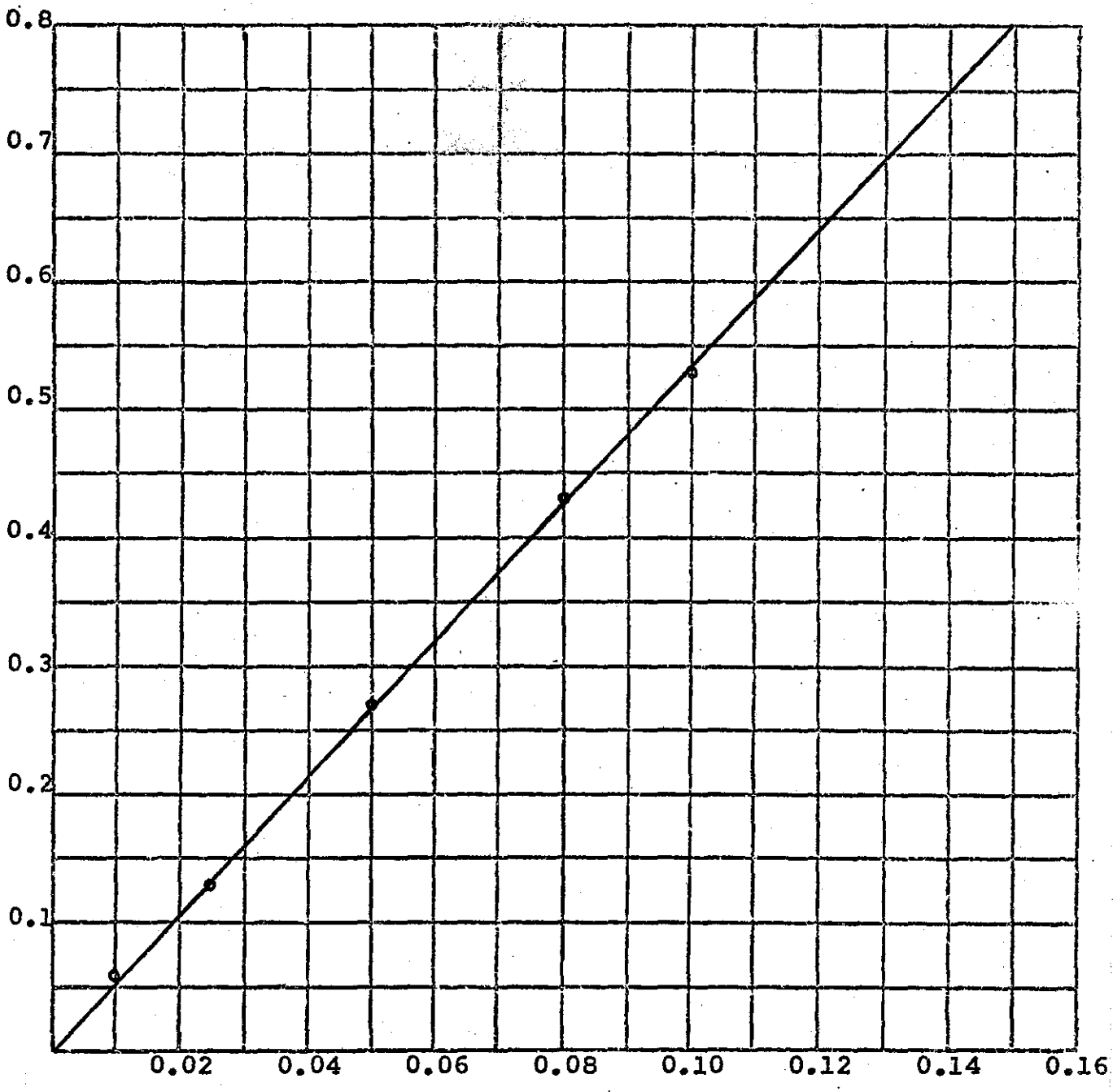
b - The standard concentration in the 5 ml. sample tested was obtained by dissolving 50 mg. in 100 ml. of gastric fluid. Appropriate aliquots, volumetrically measured, were taken representing the tabulated weight.

c - Acetazolamide determinations were read at 269 mu, Chlorothiazide at 292 mu, Chlorthalidone at 275 mu, Cyclothiazide at 271 mu, Ethacrynic Acid at 271 mu, Furosemide at 274 mu, Polythiazide at 270 mu, Spironolactone at 245 mu, and Trichlormethiazide at 267 mu.



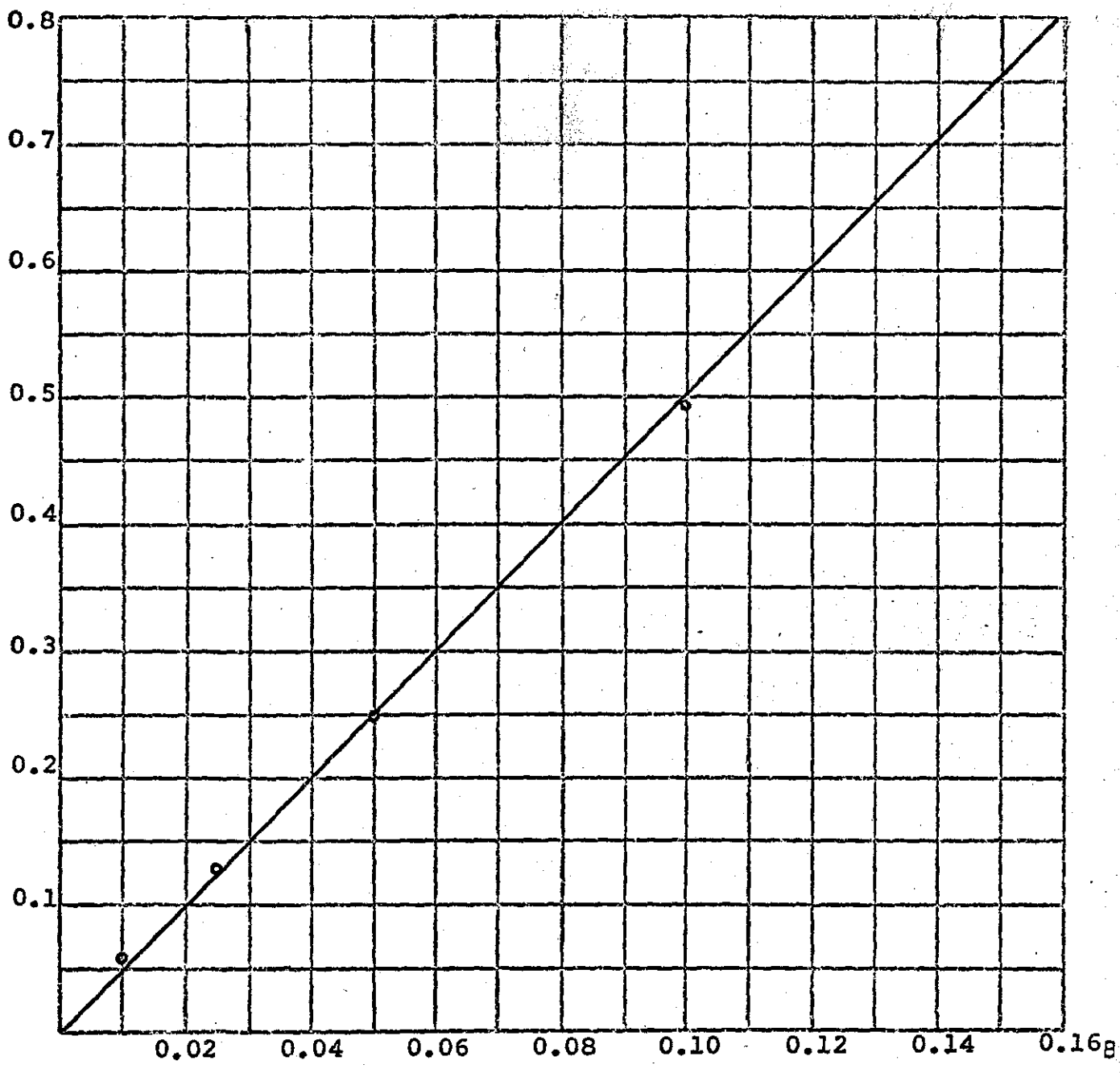
CONCENTRATION (milligrams per 5-ml.)

FIGURE 8 - STANDARD ACETAZOLAMIDE CURVE



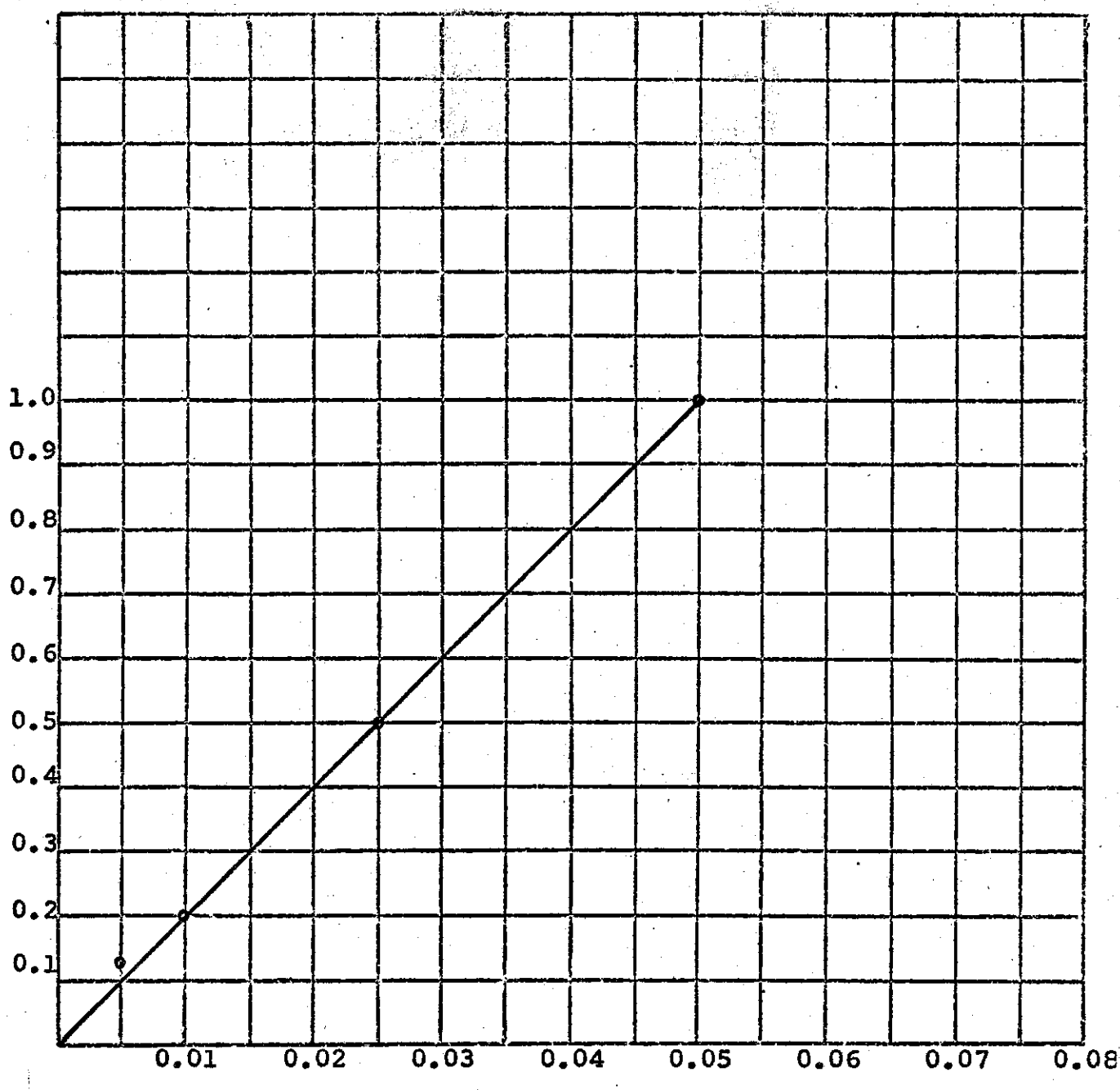
CONCENTRATION (milligrams per 5-ml.)

FIGURE 9 - STANDARD CHLOROTHIAZIDE CURVE



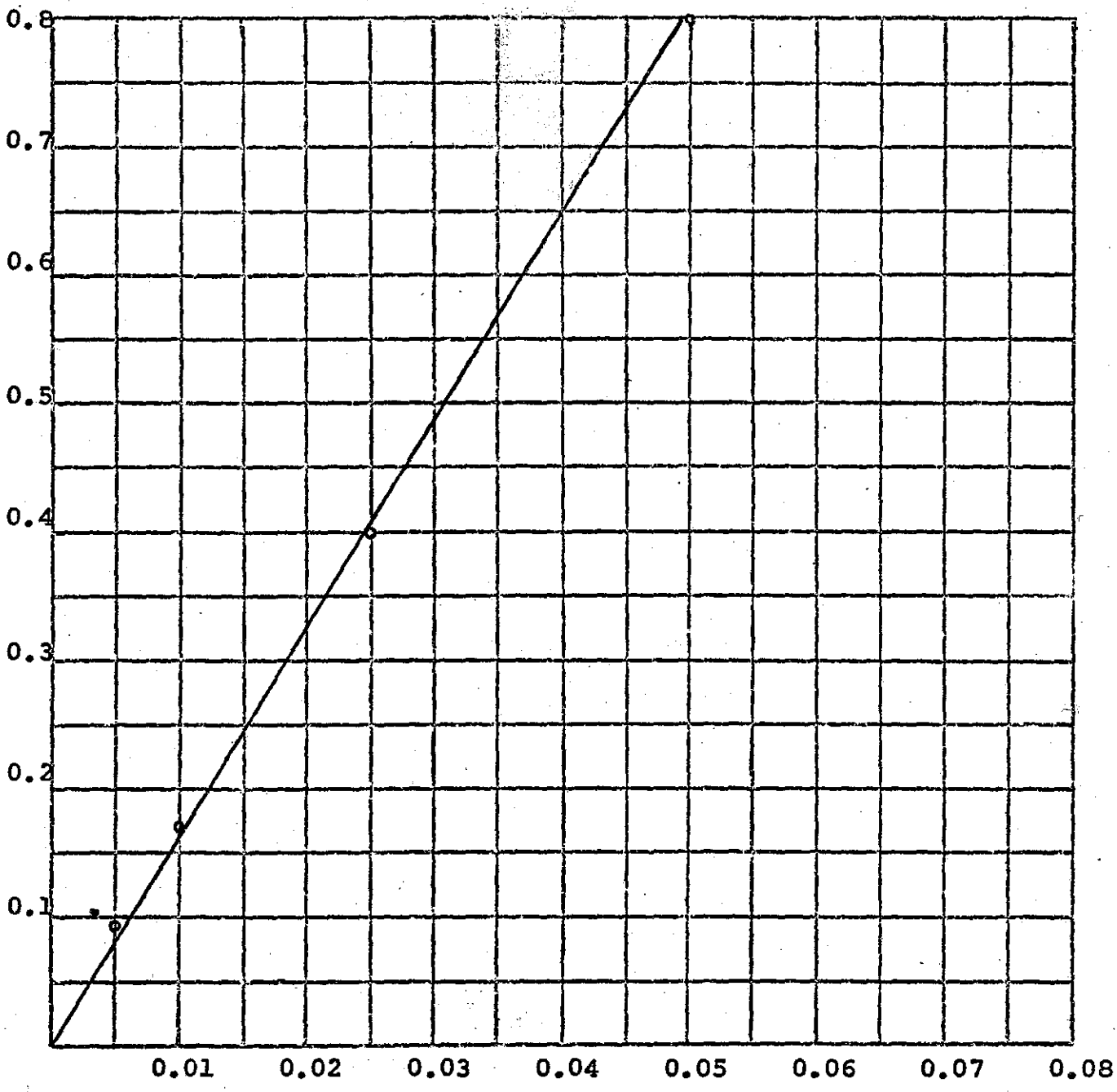
CONCENTRATION (milligrams per 5-ml.)

FIGURE 10 - STANDARD CHLORTHALIDONE CURVE



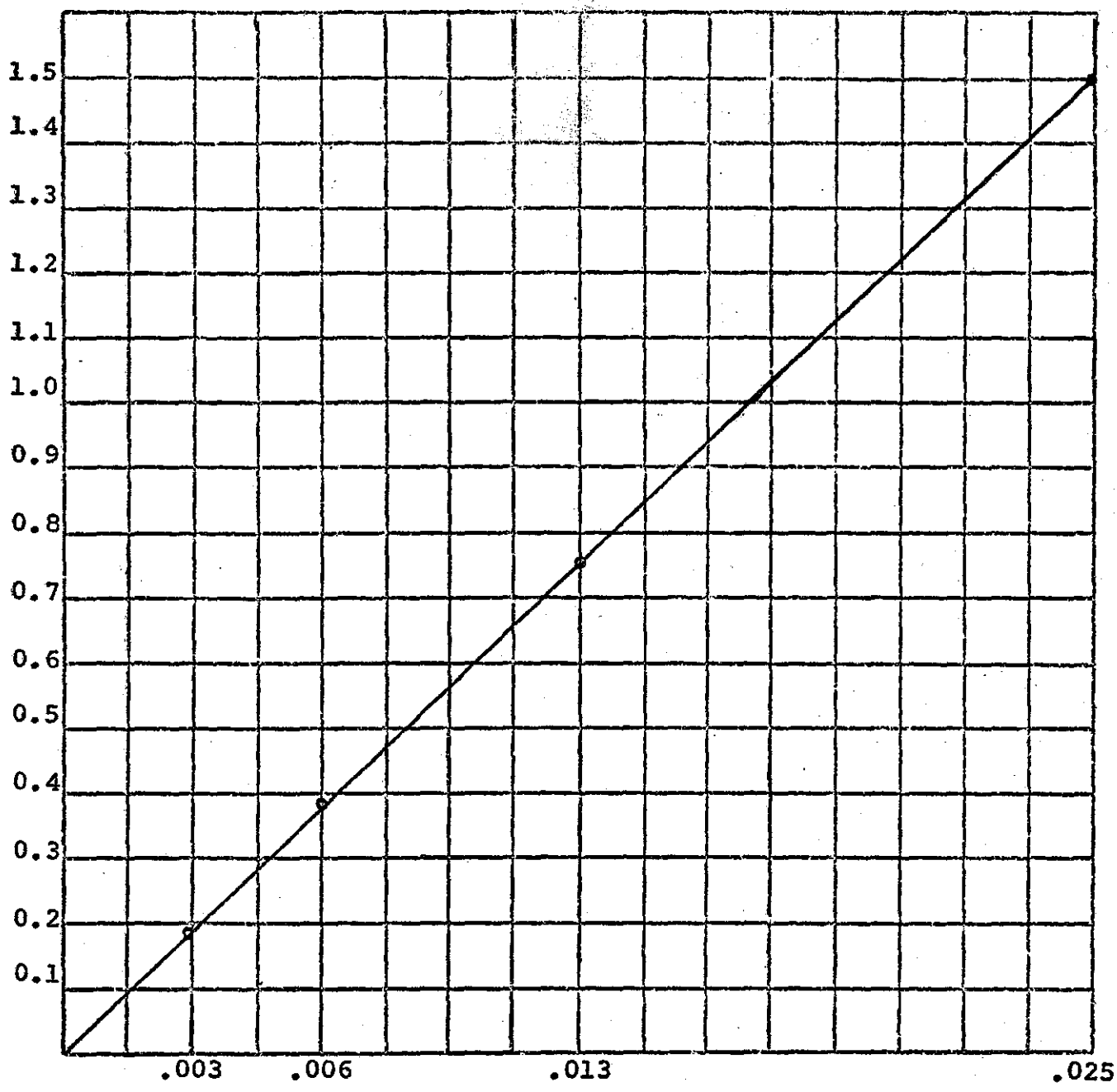
CONCENTRATION (milligrams per 5-ml.)

FIGURE 11 - STANDARD CYCLOTHIAZIDE CURVE



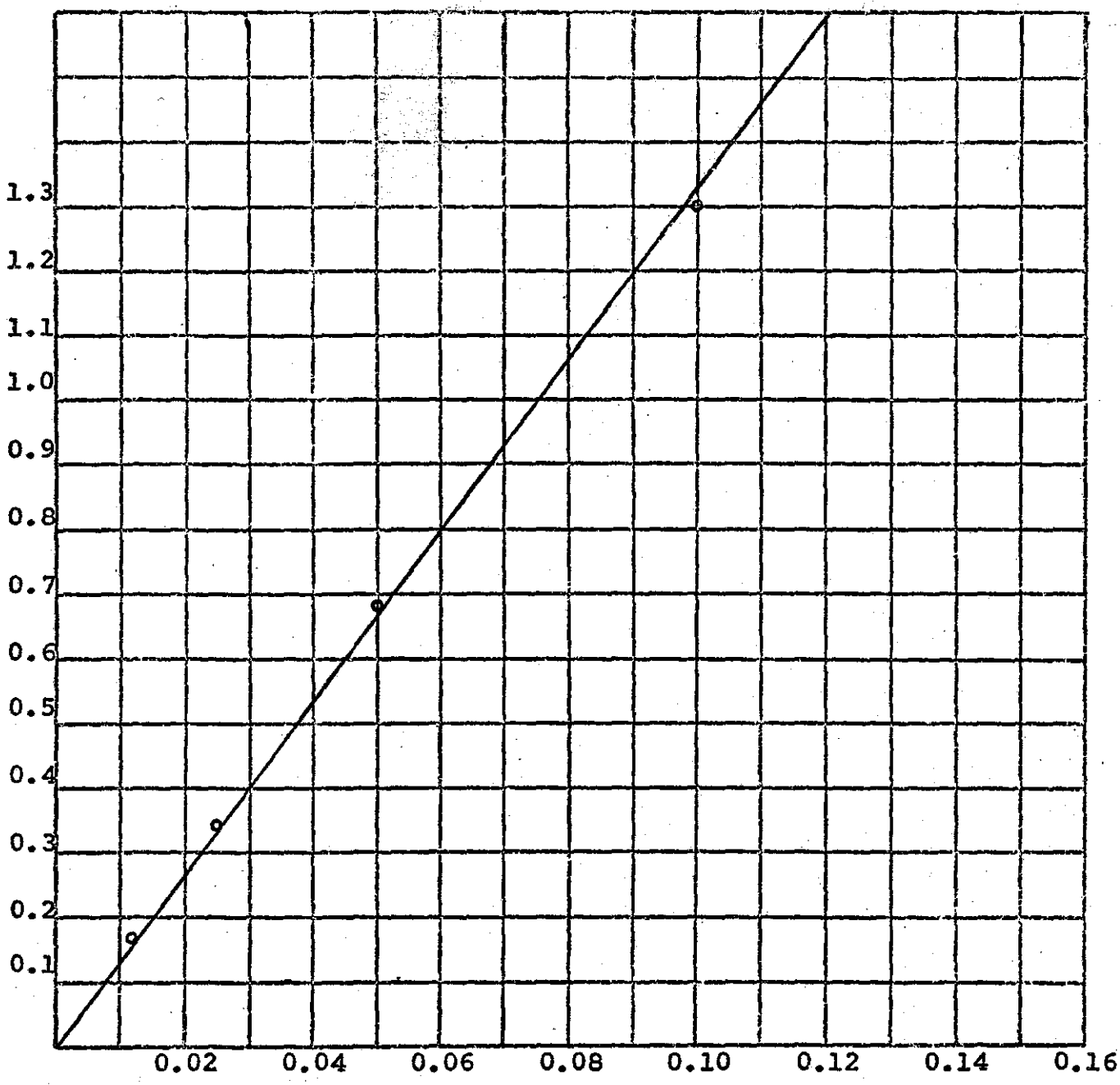
CONCENTRATION (milligrams per 5-ml.)

FIGURE 12 - STANDARD ETHACRYNIC ACID CURVE



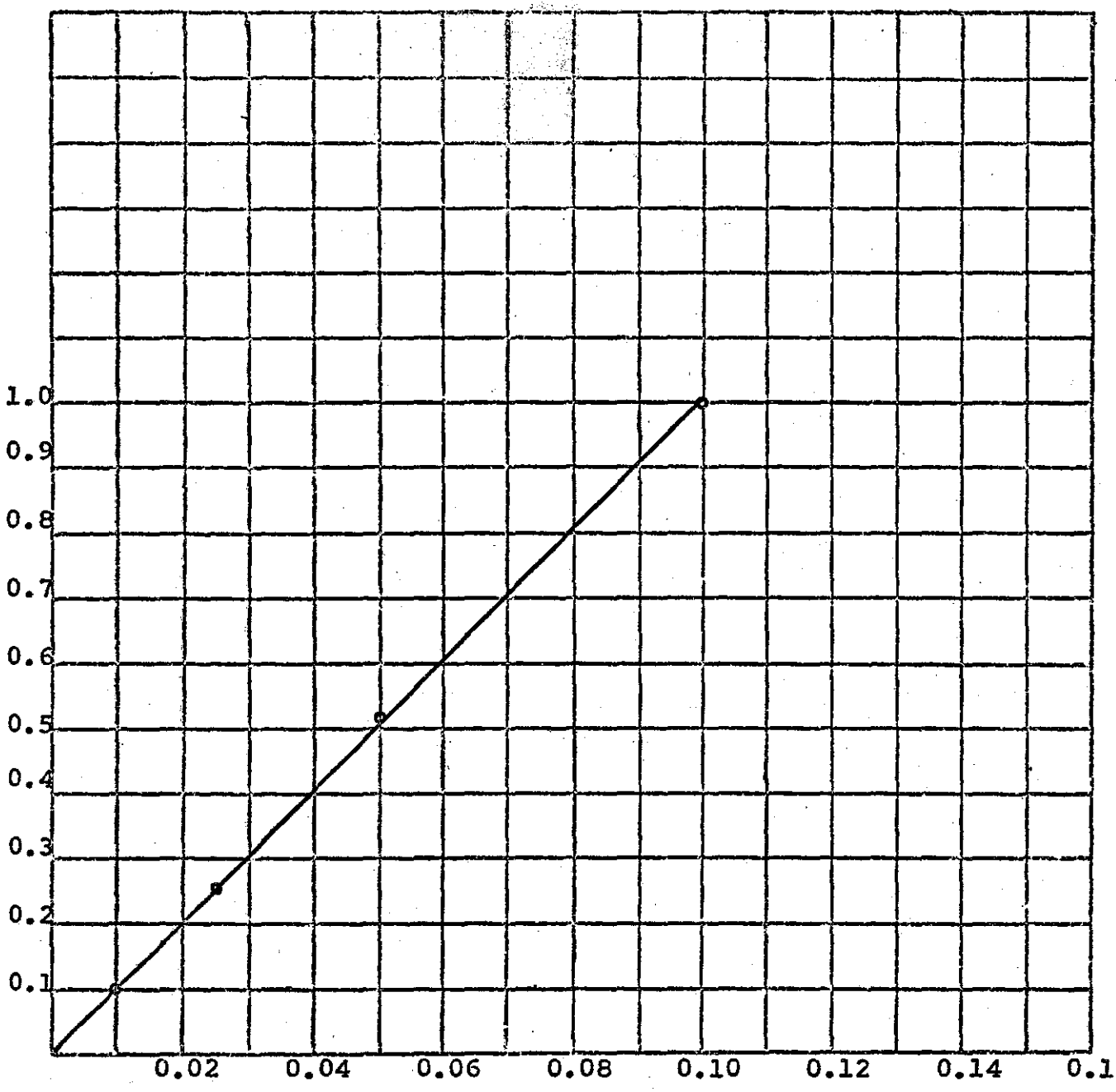
CONCENTRATION (milligrams per 5-ml.)

FIGURE 13 - STANDARD FUROSEMIDE CURVE



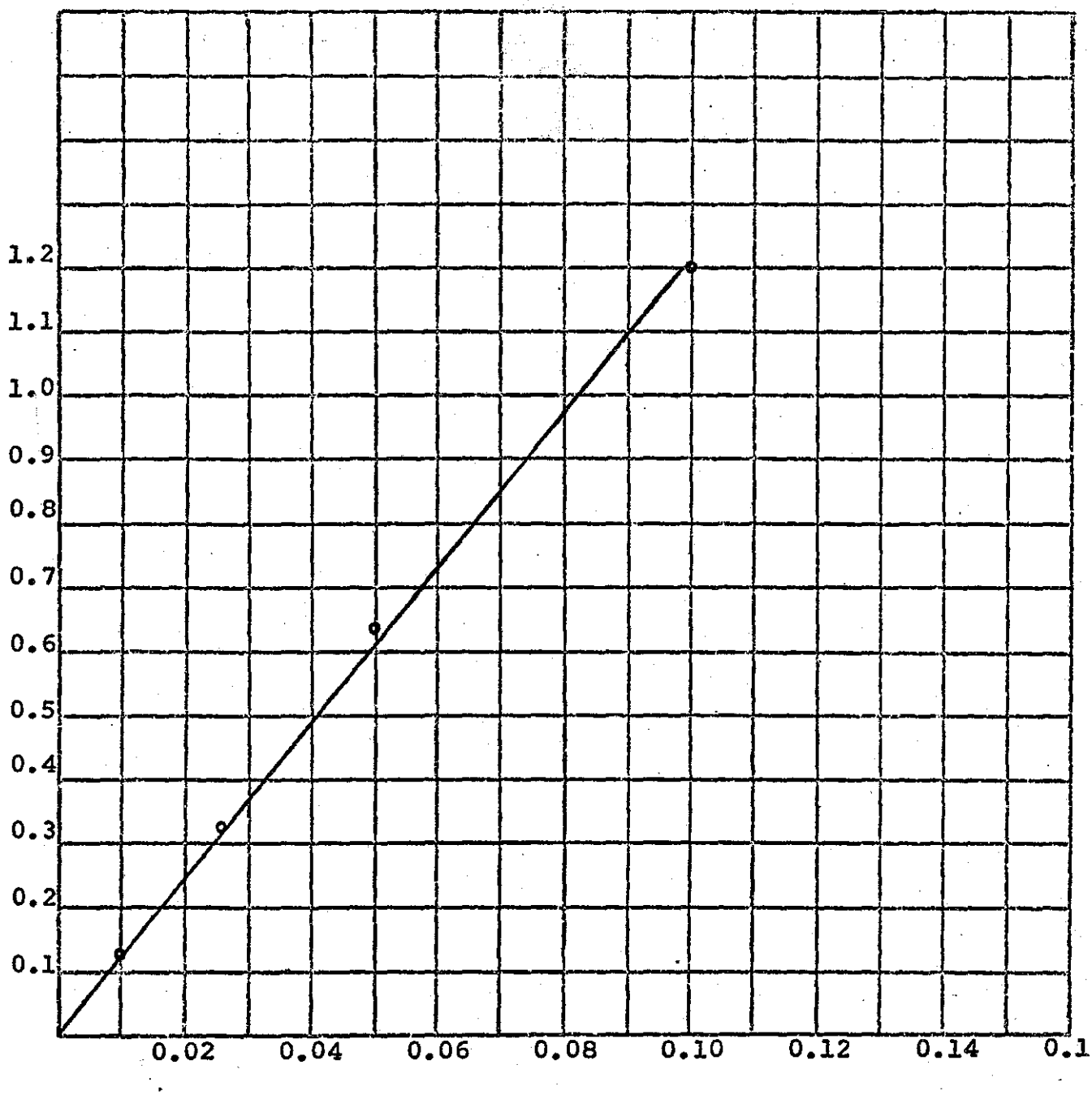
CONCENTRATION (milligrams per 5-ml.)

FIGURE 14 - STANDARD POLYTHIAZIDE CURVE



CONCENTRATION (milligrams per 5-ml.)

FIGURE 15 - STANDARD SPIRONOLACTONE CURVE



CONCENTRATION (milligrams per 5-ml.)

FIGURE 16 - STANDARD TRICHLORMETHIAZIDE CURVE

TABLE IV

DATA OBTAINED FOR THE TABLET DOSAGE FORMS EMPLOYED IN THE DISSOLUTION STUDIES

Dosage Form and Weight[a]	Time in Minutes[b]	Interpolated Weight for Total Volume-Beaker Method[c]	Interpolated Weight for Total Volume-Resomat[d]	Concentration Dissolved Beaker Method[e]	Concentration Dissolved Resomat[f]
Acetazolamide 250 mg.	20	24	9	10	4
	40	33	9	13	10
	60	40	24	16	13
	80	40	33	16	14
	100	43	48	17	19
	120	46	49	19	19
Chlorothiazide 500 mg.	20	2.5	1.5	0.5	0.3
	40	3.5	1.9	0.7	0.4
	60	4.3	3.6	0.9	0.7
	80	5.6	4.8	1.1	1.0
	100	6.4	6.0	1.3	1.2
	120	7.0	7.2	1.4	1.5

TABLE IV (Continued)

Dosage Form and Weight[a]	Time in Minutes[b]	Interpolated Weight for Total Volume-Beaker Method[c]	Interpolated Weight for Total Volume-Resomat[d]	Concentration Dissolved Beaker Method[e]	Concentration Dissolved Resomat[f]
Chlorthalidone 100 mg.	20	0.9	1.2	0.9	1.2
	40	0.9	1.5	0.9	1.5
	60	2.0	2.0	2.0	2.0
	80	2.0	2.5	2.0	2.5
	100	2.7	3.0	2.7	3.0
	120	3.2	3.6	3.2	3.6
Cyclothiazide 2 mg.	20	0.25	0.18	13.0	9.0
	40	0.30	0.24	15.0	12.0
	60	0.35	0.30	18	15.0
	80	0.40	0.36	20.0	18.0
	100	0.45	0.42	23.0	21.0
	120	0.45	0.48	23.0	24.0

TABLE IV (Continued)

Dosage Form and Weight[a]	Time in Minutes[b]	Interpolated Weight for Total Volume-Beaker Method[c]	Interpolated Weight for Total Volume-Resomat[d]	Concentration Dissolved Beaker Method[e]	Concentration Dissolved Resomat[f]
Ethacrynic Acid 50 mg.	20	0	0.12	0	0.3
	40	0	0.18	0	0.4
	60	0.05	0.18	0.1	0.4
	80	0.10	0.18	0.2	0.4
	100	0.15	0.18	0.3	0.4
	120	0.15	0.18	0.3	0.4
Furosemide 40 mg.	20	0.10	0.03	0.08	0.3
	40	0.16	0.06	0.14	0.4
	60	0.22	0.08	0.2	0.5
	80	0.25	0.11	0.3	0.6
	100	0.27	0.13	0.4	0.7
	120	0.28	0.19	0.5	0.7

TABLE IV (Continued)

Dosage Form and Weight[a]	Time in Minutes[b]	Interpolated Weight for Total Volume-Beaker Method[c]	Interpolated Weight for Total Volume-Resomat[d]	Concentration Dissolved Beaker Method[e]	Concentration Dissolved Resomat[f]
Polythiazide 4 mg.	20	0.4	0.36	10.0	9.0
	40	0.7	0.84	18.0	21.0
	60	0.8	1.14	20.0	29.0
	80	1.0	1.4	25.0	35.0
	100	1.10	1.6	28.0	39.0
	120	1.2	1.7	30.0	42.0
Spironolactone 25 mg.	20	0.1	0.3	0.3	1.2
	40	0.15	0.6	0.6	2.4
	60	0.2	1.00	0.8	4.3
	80	0.35	1.4	1.4	5.7
	100	0.4	1.7	1.6	6.9
	120	0.5	1.9	2.0	7.4

TABLE IV (Continued)

Dosage Form and Weight[a]	Time in Minutes[b]	Interpolated Weight for Total Volume-Beaker Method[c]	Interpolated Weight for Total Volume-Resomat[d]	Concentration Dissolved Beaker Method[e]	Concentration Dissolved Resomat[f]
Trichlor-methiazide 4 mg.	20	0.3	0.6	8.0	15.0
	40	0.7	1.2	18.0	30.0
	60	1.3	1.7	31.0	42.0
	80	1.4	1.9	35.0	48.0
	100	1.5	2.2	38.0	54.0
	120	1.7	2.3	43.0	57.0

a - All samples tested were tablet dosage forms. The weight indicated represent the labeled amount of active ingredient in each of the tablet dosage forms specified by each of the pharmaceutical manufacturers.

b - Represents the sampling of 5 ml. of simulated gastric juice at 20 minute intervals for the specific compound under test.

c - Represents the calculated weight in milligrams of the sample in 250 mls. of simulated gastric juice as determined by spectrophotometric analysis and interpolation from the sample under test.

d - Represents the calculated weight in milligrams of the sample in 300 mls. of simulated gastric juice as determined by spectrophotometric analysis and interpolation from the sample under test.

TABLE IV (Continued)

e - Represents the percentage of the original weight of the sample that has dissolved in the simulated gastric juice.

f - Represents the percentage of the original weight of the sample that has dissolved in the simulated gastric juice.

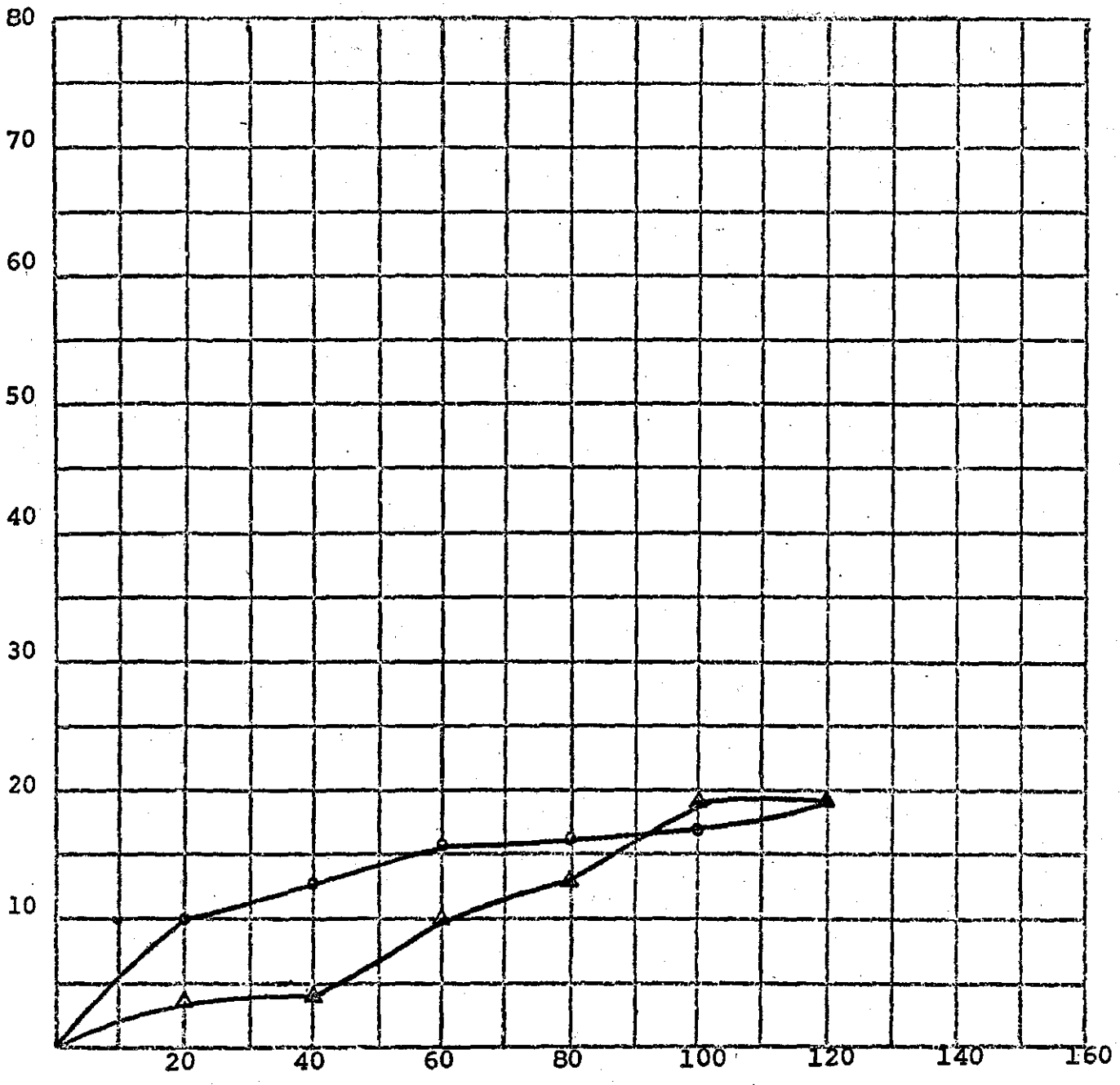


FIGURE 17 - DISSOLUTION CURVES OF ACETAZOLAMIDE

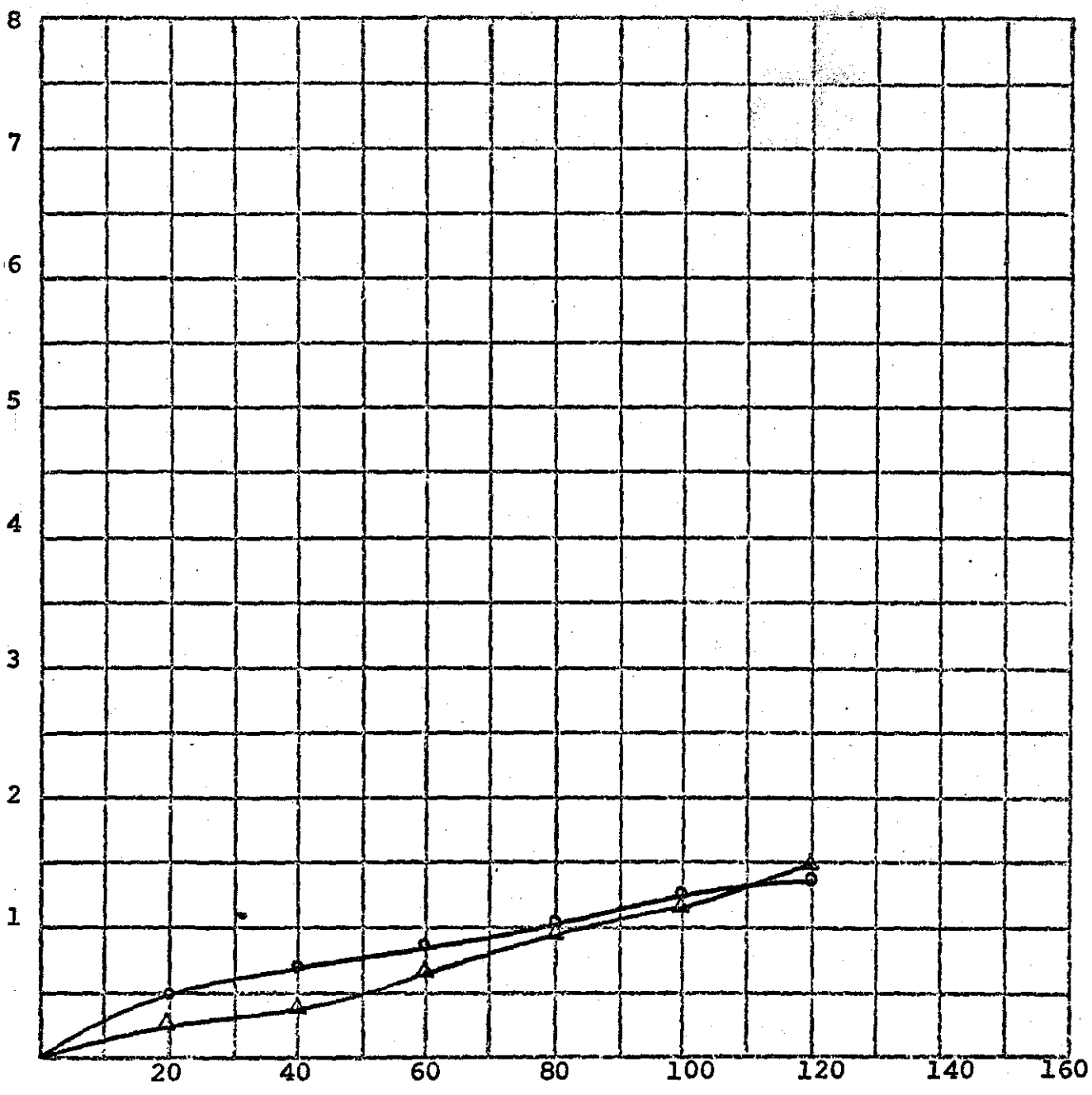


FIGURE 18 - DISSOLUTION CURVES OF CHLOROTHIAZIDE

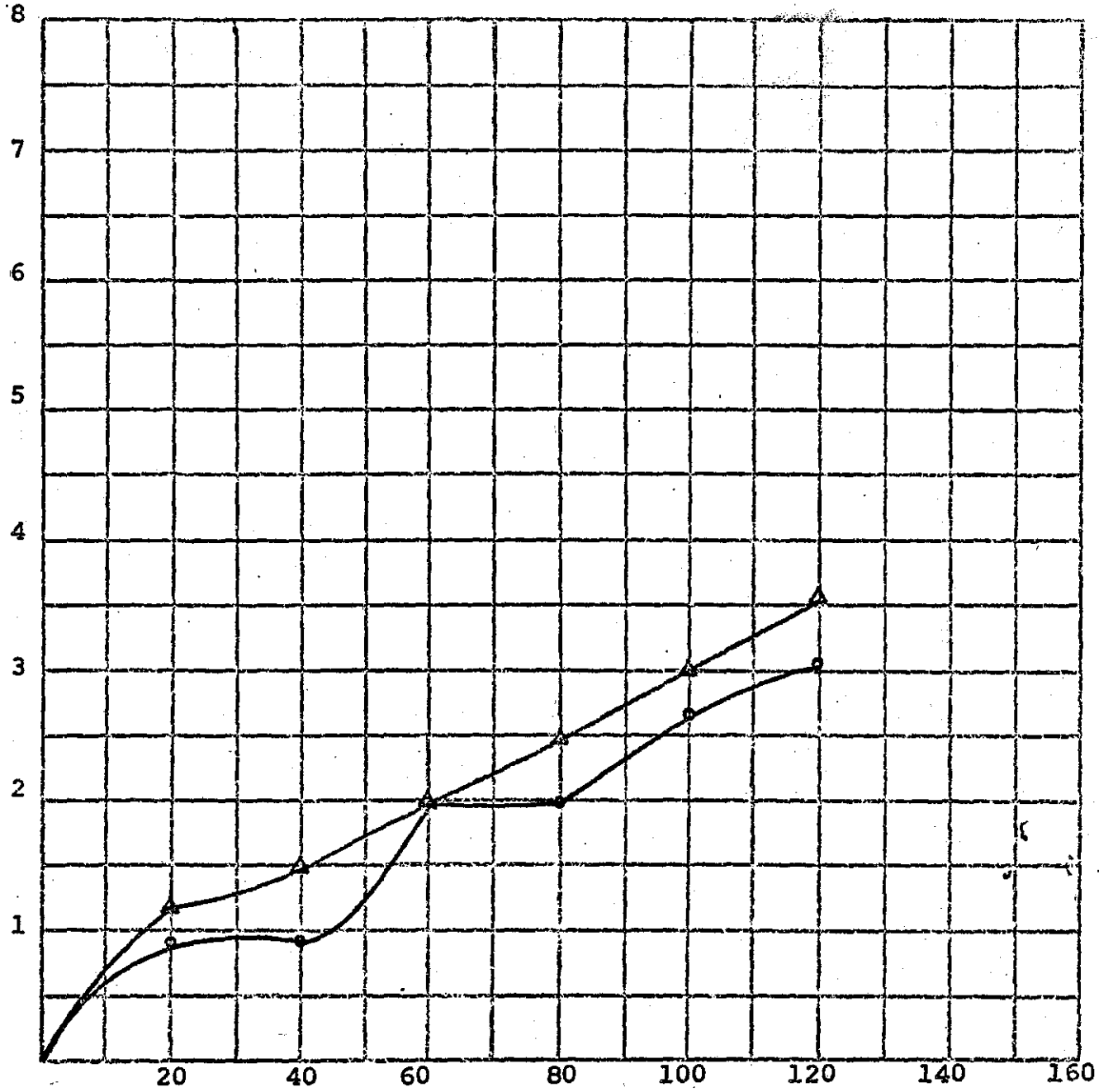


FIGURE 19 - DISSOLUTION CURVES OF CHLORTHALIDONE

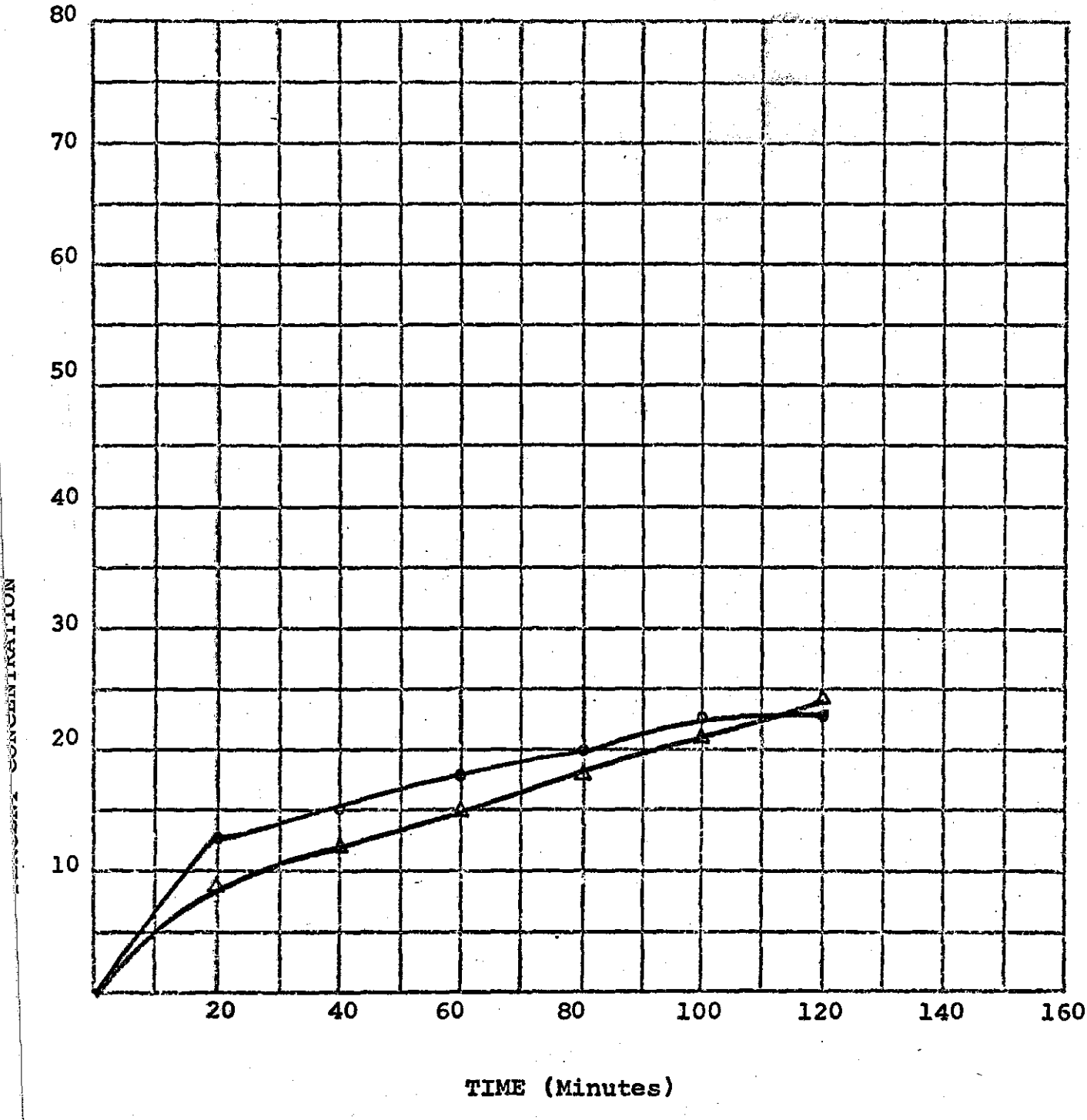


FIGURE 20 - DISSOLUTION CURVES OF CYCLOTHIAZIDE

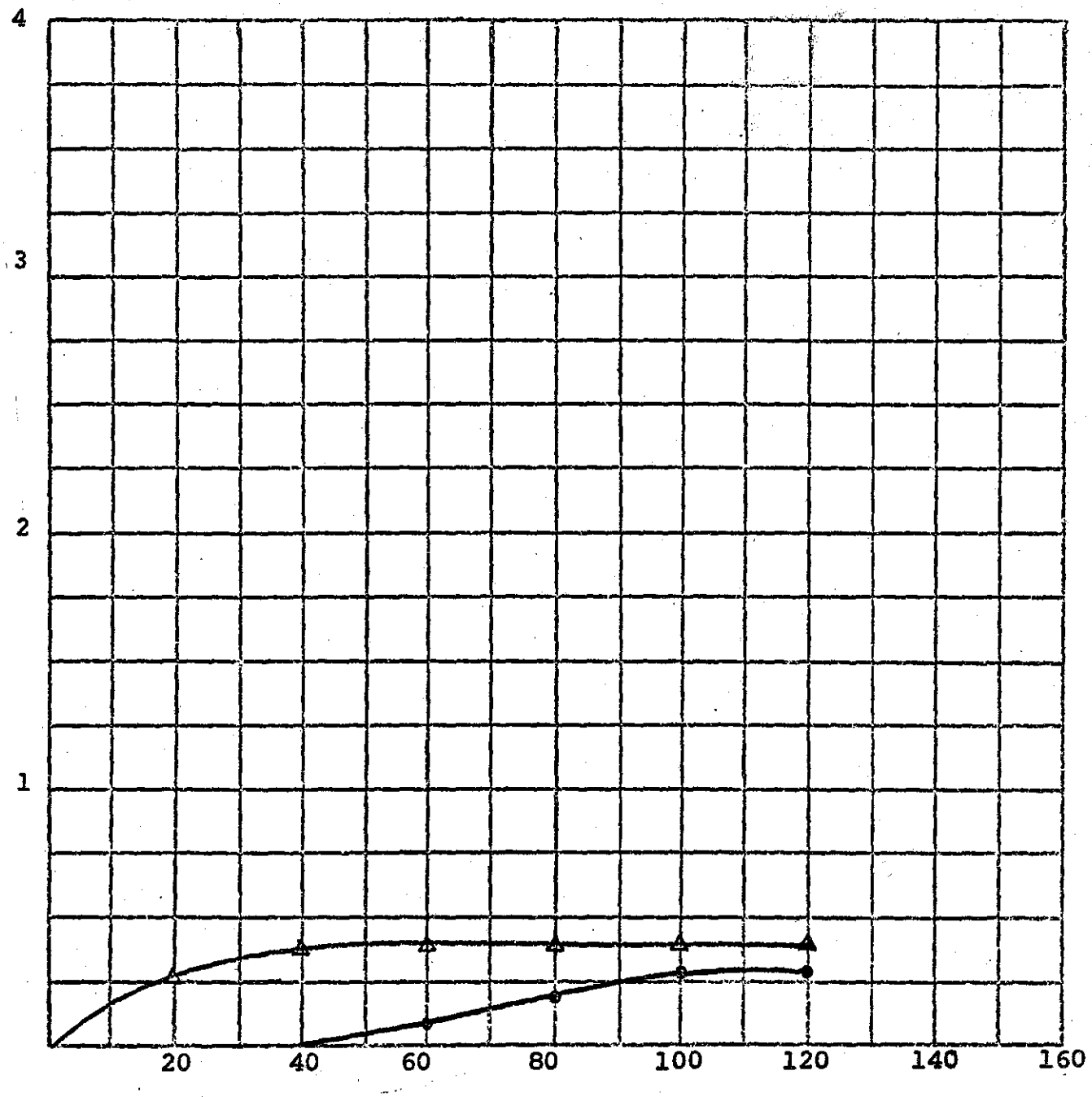


FIGURE 21 - DISSOLUTION CURVES OF ETHACRYNIC ACID

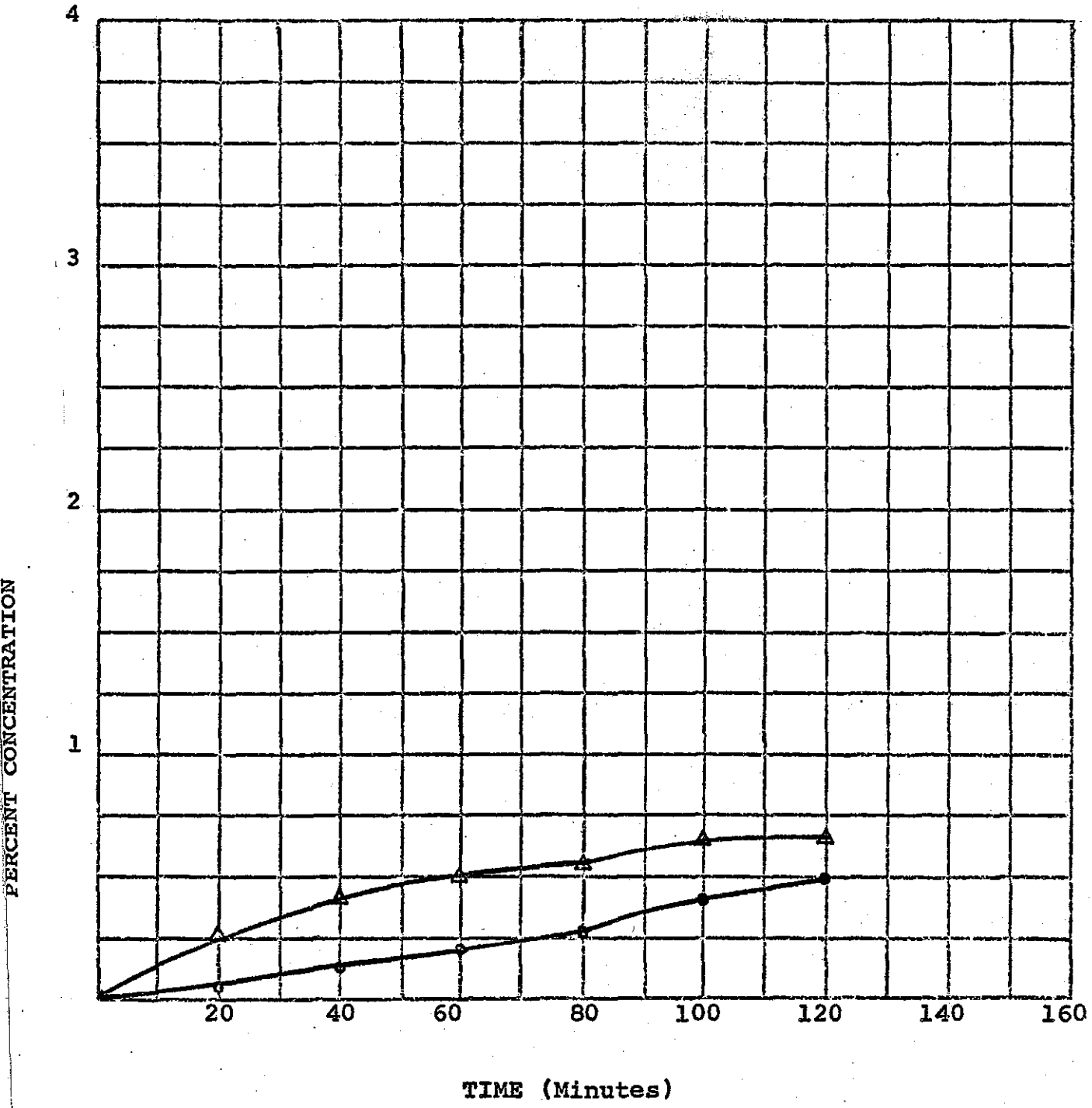


FIGURE 22 - DISSOLUTION CURVES OF FUROSEMIDE

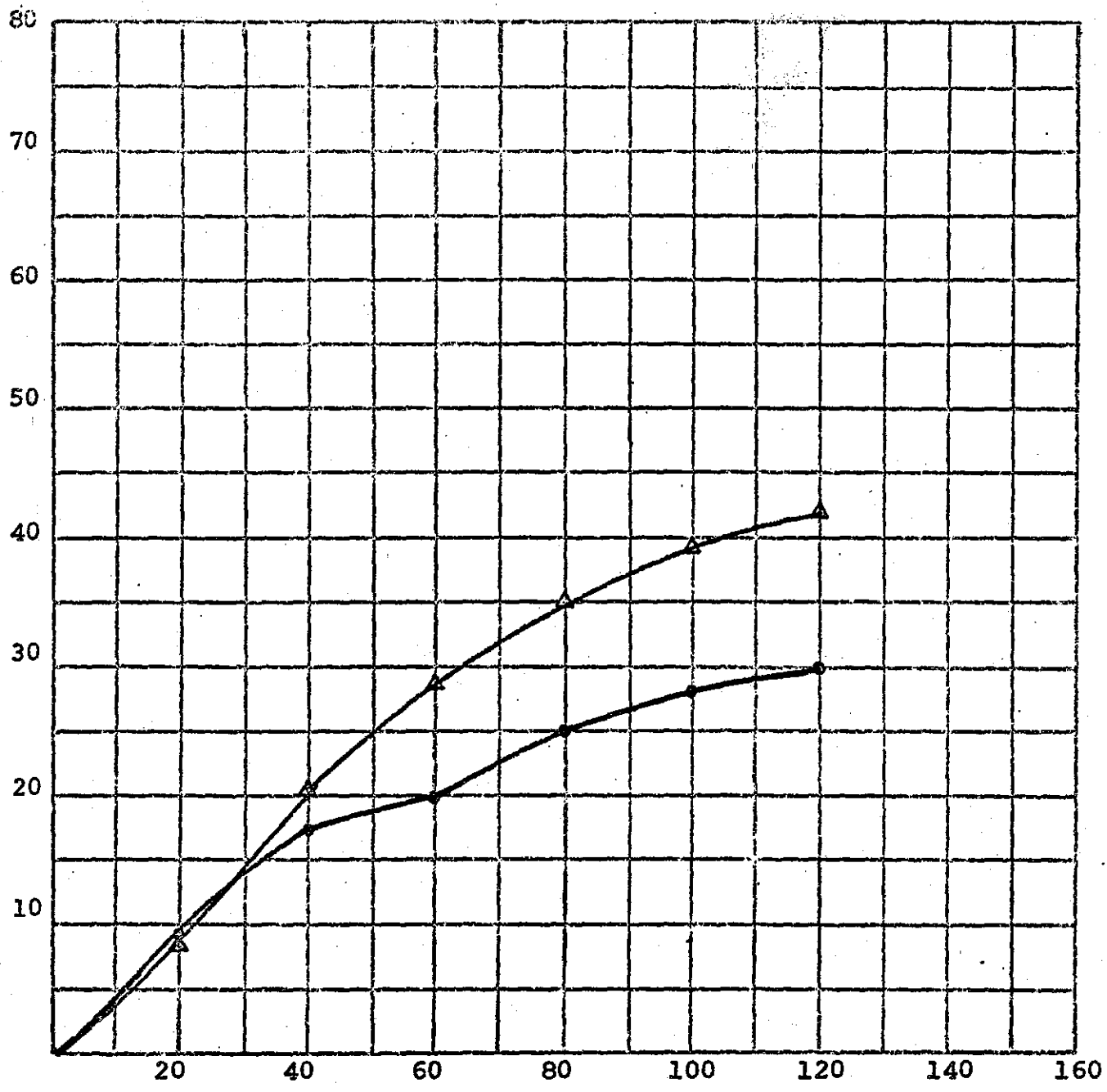


FIGURE 23 - DISSOLUTION CURVES OF POLYTHIAZIDE

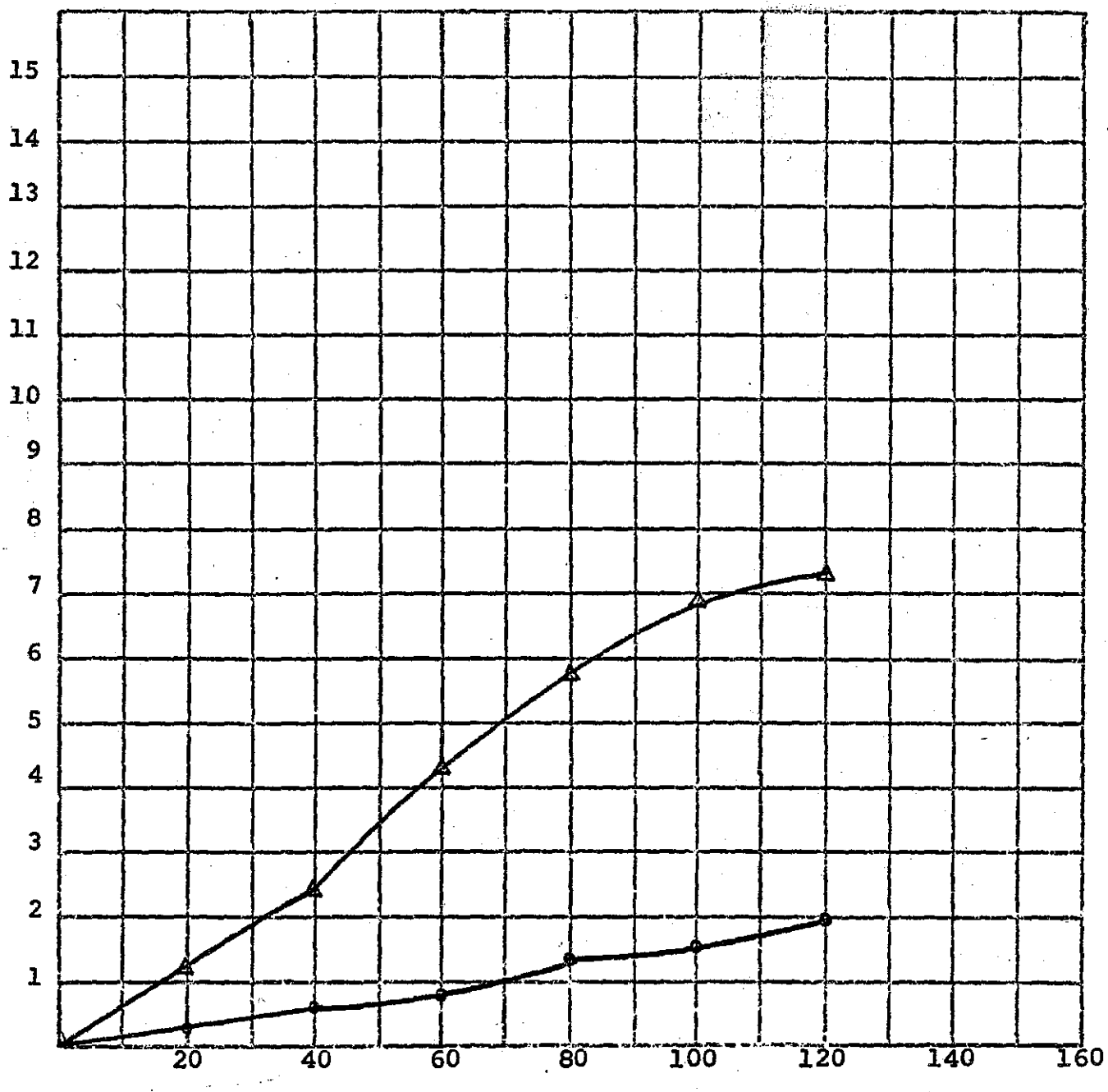


FIGURE 24 - DISSOLUTION CURVES OF SPIRONOLACTONE

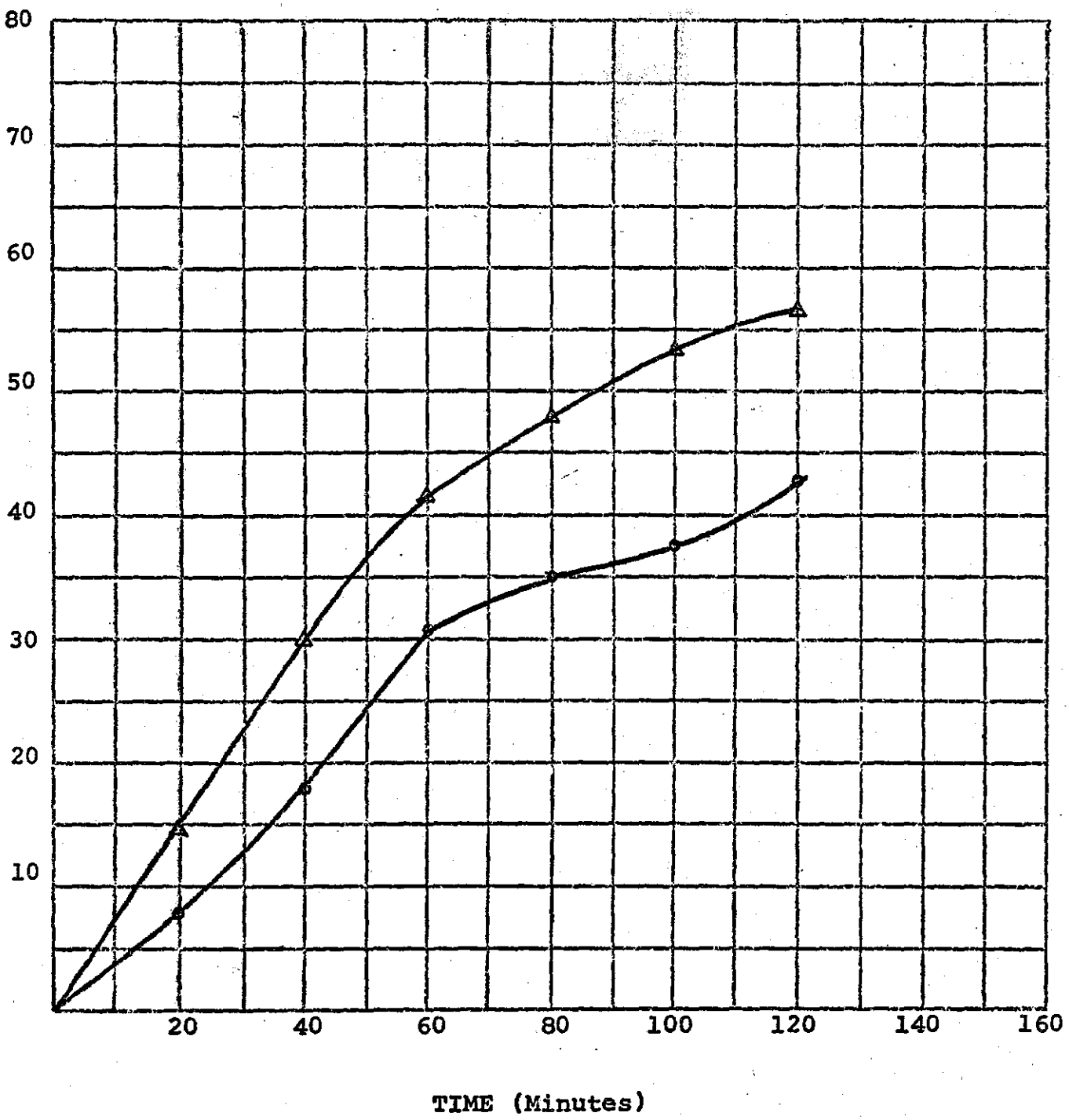


FIGURE 25 - DISSOLUTION CURVES OF TRICHLORMETHIAZIDE

TABLE V

SOLVENT SYSTEMS USED AS THE LIPOID PHASE
IN RESORPTION PROFILE DETERMINATIONS

Active Ingredient in Dosage Form	Solvent[a]	Proportions[b]
Acetazolamide	chloroform/tetrahydrofuran	1:1
Chlorthalidone	chloroform/tetrahydrofuran	1:1
Chlorothiazide	chloroform/tetrahydrofuran	1:1
Cyclothiazide	chloroform/ethyl acetate	1:1
Ethacrynic Acid	chloroform	
Furosemide	chloroform/ethyl acetate	1:1
Polythiazide	chloroform/tetrahydrofuran	1:1
Spironolactone	chloroform	
Trichlormethiazide	chloroform/ethyl acetate	1:1

a - All solvents used were reagent grade.

b - The proportions indicate the parts by volume of each solvent used in the lipoid phase. These systems used were selected on the basis of their solvent characteristics on the specific active ingredient. Where no co-solvent is indicated, the active ingredient exhibited satisfactory solubility characteristics in the chloroform.

ingredient as indicated in Table VI. Where a solvent system other than chloroform, such as chloroform - ethyl acetate or chloroform - tetrahydrofuran was used as the lipoid phase in the determination of the resorption profiles, then these solvent mixtures were used in the preparation of the dilutions required for the standard curves. Spectrophotometric determiniations were performed at the wavelength specified in Table VI.

Resorption Profile Procedure

In the design set up of the standard taper glass apparatus, the outlet-inlet faucets were closed, and the pressure equalizing "faucet" was opened. A magnetic stirrer of 40 mm. length was inserted into the outer glass cylinder. Two-hundred ml. of lipoid solvent was then introduced into this cylinder. The inner glass cylinder containing a magnetic stirrer of 30 mm. length was lowered into the outer vessel so that the lower edge of the inner vessel was immersed 2 cms. into the lipoid solvent. Precautions were taken to prevent the asbestos filter from coming in contact with the lipoid layer. While holding the inner vessel in this position, 100 mls. of simulated gastric fluid at a pH of 1.2, was slowly poured into the inner glass cylinder through the opening. The simulated gastric fluid flowed dropwise through the asbestos filter onto the lipoid surface but remained within the inner cylinder. The inner glass cylinder was carefully guided onto a teflon coated standard taper joint and secured with a gentle twist. The air which remained between the filter and the

aqueous phase above the lipoid solvent was removed through the filter by sealing the tapered openings and by applying a gentle vacuum to the system (J). After equalizing the pressure within the closed apparatus, the space just below the filter now consisted of a two phase system consisting of equal parts simulated gastric fluid and lipoid solvent.

The assembled glass apparatus was now placed upon the mechanical module. The tablet dosage form representing the diuretic under test was placed into the tablet basket which was immersed 1 cm. into the aqueous phase when the basket was placed into its appropriate setting. The glass module then was joined to the pump located in the module using rubber tubing between the appropriate connections. The system was sealed. So that the pH change in the aqueous phase could be monitored, one of the glass stoppers was replaced by the combination glass electrode.

The switch located on the mechanical module was turned to the position marked "piston pump." With the pressure equalizing "faucet" open, the piston pump and the magnetic stirrer were turned on by depressing the power switches. The revolutions per minute of the magnetic stirrer were adjusted so that a vortex of about 15 mm. in diameter was formed at the two phase boundary between the water and lipoid solvent.

The control knob was then turned to "single removal," thus removing a sample of the lipoid solvent containing the resorbed diuretic. This was accomplished by closing the pressure equalizing "faucet" and turning the removal pump control

knob in a counter-clockwise direction. Samples were taken at sixty minute intervals and measured spectrophotometrically. The sample was then returned to the system through the outlet-inlet "faucet" by rotating the "removal pump" control knob in a clock-wise direction thus imparting a vacuum to the system which removed the sample from the cuvette. This dissolution and resorption process was continued by turning the control knob back to the position marked "piston pump" and by opening the pressure equalizing "faucet."

Standard curves that would relate absorbance to drug concentration were prepared in concentrations as indicated in Table VI and illustrated in Figures 26 to 34. After one hour during which a sample had been removed, tested, and returned to the apparatus, the simulated gastric fluid at a pH of 1.2 was neutralized by the addition of normal sodium hydroxide. Then acetate buffer[a] was added to bring the pH to 4.0. The combination pH glass electrode was used to monitor this pH change. The dissolution and resorption process was continued for an additional hour. Sampling and testing was carried out as above. The pH was adjusted to 7.8 with normal sodium hydroxide and biphosphate buffer[b]. The test was continued in the above manner for an additional two hours and the sampling was repeated.

Drug concentration was determined spectrophotometrically

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a - 0.2 M Potassium Acetate.

b - 0.2 M Potassium Biphosphate.

TABLE VI

SPECTROPHOTOMETRIC DATA OBTAINED FOR THE PREPARATION OF THE STANDARD CURVES FOR THE DRUGS EMPLOYED IN THE DETERMINATION OF THE RESORPTION PROFILES

Drug Sample[a]	Concentration[b]	Absorbance[c]
Acetazolamide	0.025	0.19
	0.030	0.21
	0.050	0.39
	0.100	0.78
Chlorothiazide	0.025	0.19
	0.050	0.38
	0.080	0.61
	0.100	0.77
Chlorthalidone	0.100	0.12
	0.250	0.27
	0.400	0.43
	0.500	0.54
	0.800	0.86
Cyclothiazide	0.013	0.17
	0.025	0.28
	0.030	0.33
	0.050	0.54
	0.075	0.82

TABLE VI (Continued)

Drug Sample[a]	Concentration[b]	Absorbance[c]
Ethacrynic Acid	0.050	0.09
	0.100	0.18
	0.200	0.38
	0.250	0.45
	0.500	0.91
Furosemide	0.050	0.09
	0.100	0.17
	0.200	0.34
	0.250	0.41
	0.500	0.82
Polythiazide	0.020	0.17
	0.025	0.20
	0.030	0.25
	0.050	0.42
	0.100	0.84
Spironolactone	0.125	0.12
	0.250	0.23
	0.300	0.26
	0.500	0.43
	1.000	0.86

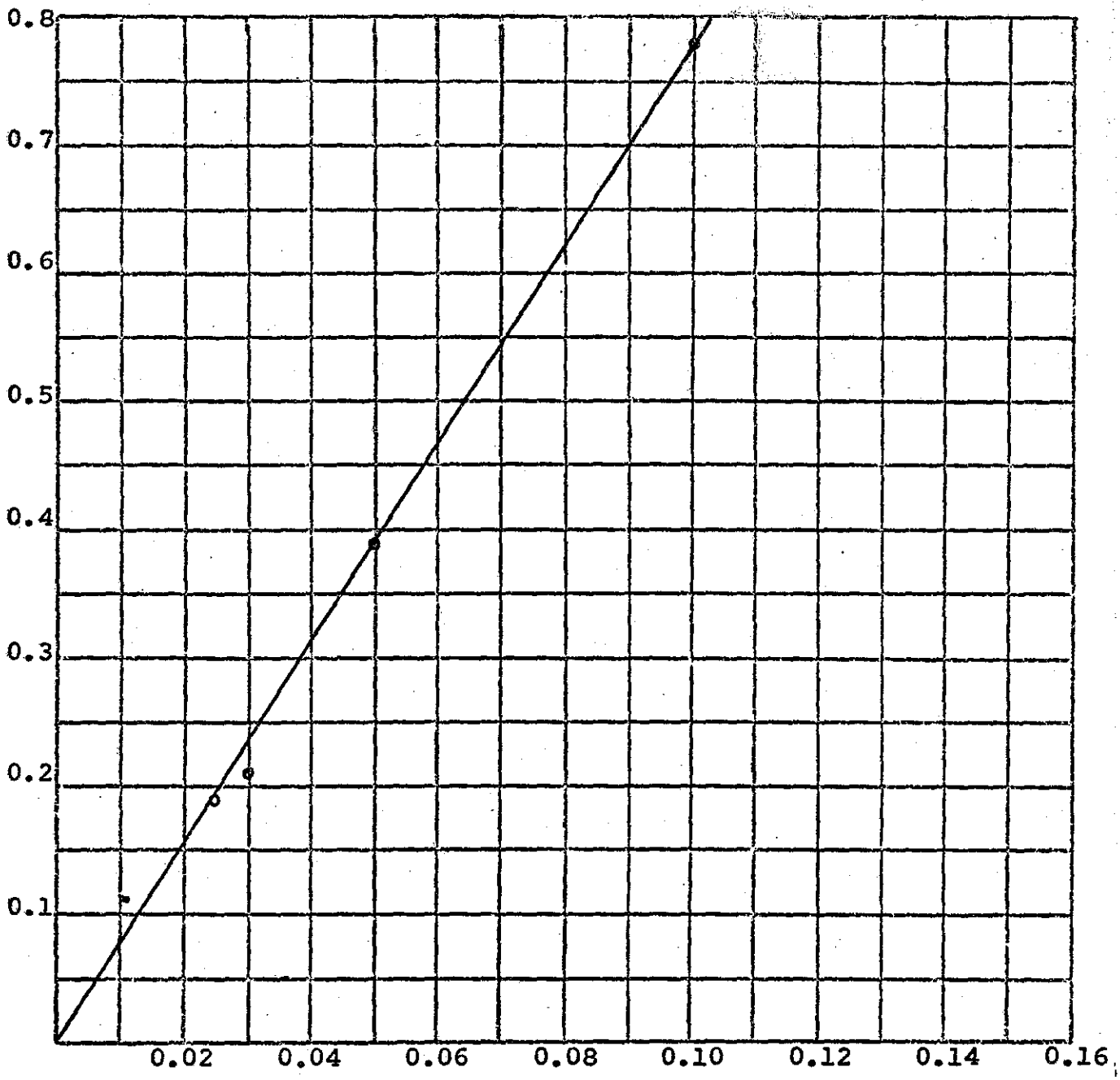
TABLE VI (Continued)

Drug Sample[a]	Concentration[b]	Absorbance[c]
Trichlormethiazide	0.013	0.11
	0.025	0.215
	0.050	0.43
	0.100	0.84

a - Samples used were the pure drug furnished by each of the respective pharmaceutical manufacturers.

b - The standard concentration in the 5 ml. sample tested was obtained by dissolving 50 mg., accurately weighed, in 100 ml. of chloroform. Appropriate volumes were used representing the tabulated weights.

c - Acetazolamide absorbancy determinations were read spectrophotometrically at 269 mu, Chlorothiazide at 292 mu, Chlorthalidone at 275 mu, Cyclothiazide at 271 mu, Ethacrynic Acid at 271 mu, Furosemide at 274 mu, Polythiazide at 270 mu, Spironolactone at 245 mu, and Trichlormethiazide at 267 mu.



CONCENTRATION (milligrams per 5-ml.)

FIGURE 26 - STANDARD ACETAZOLAMIDE CURVE

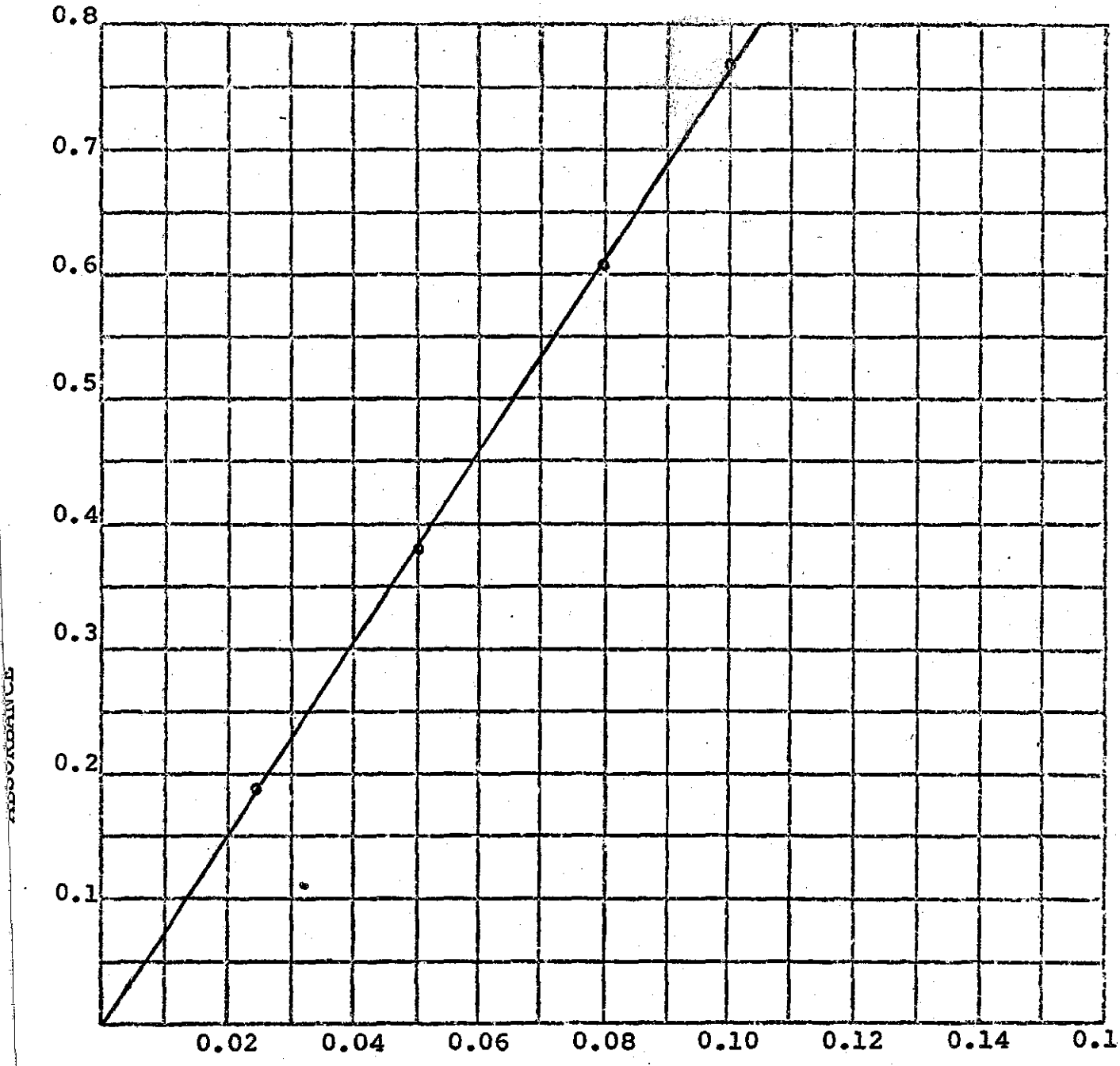
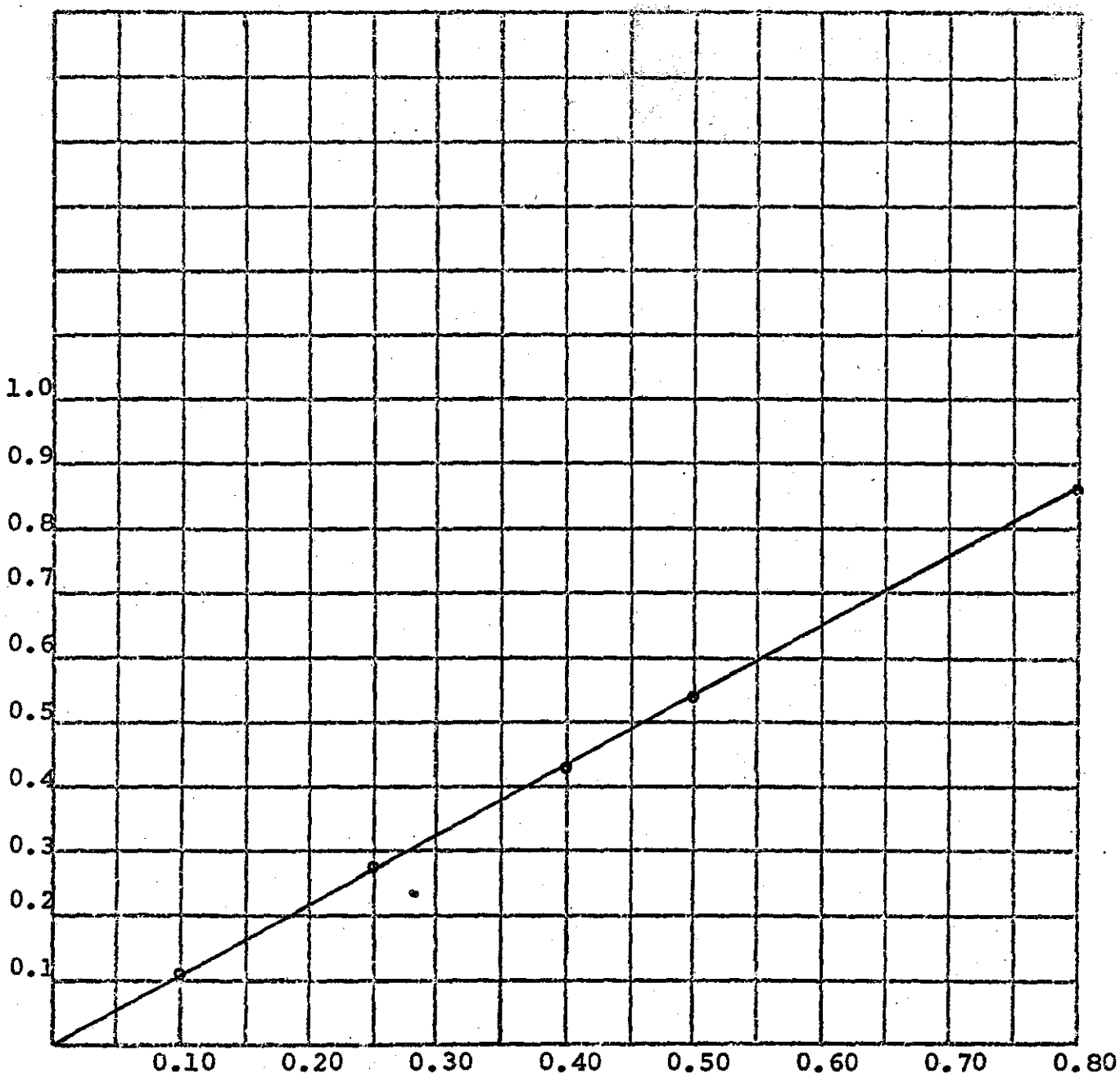
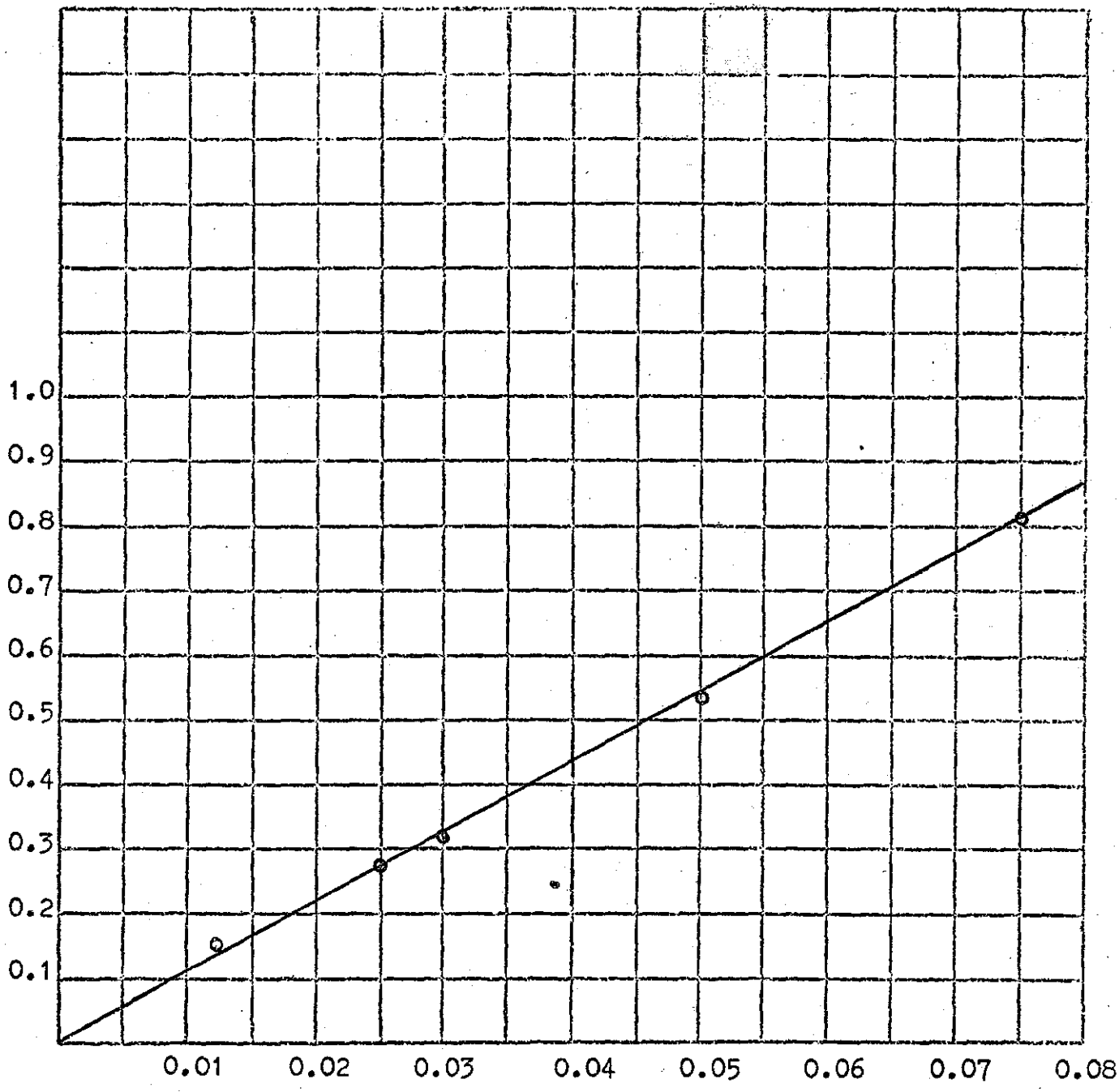


FIGURE 27 - STANDARD CHLOROTHIAZIDE CURVE



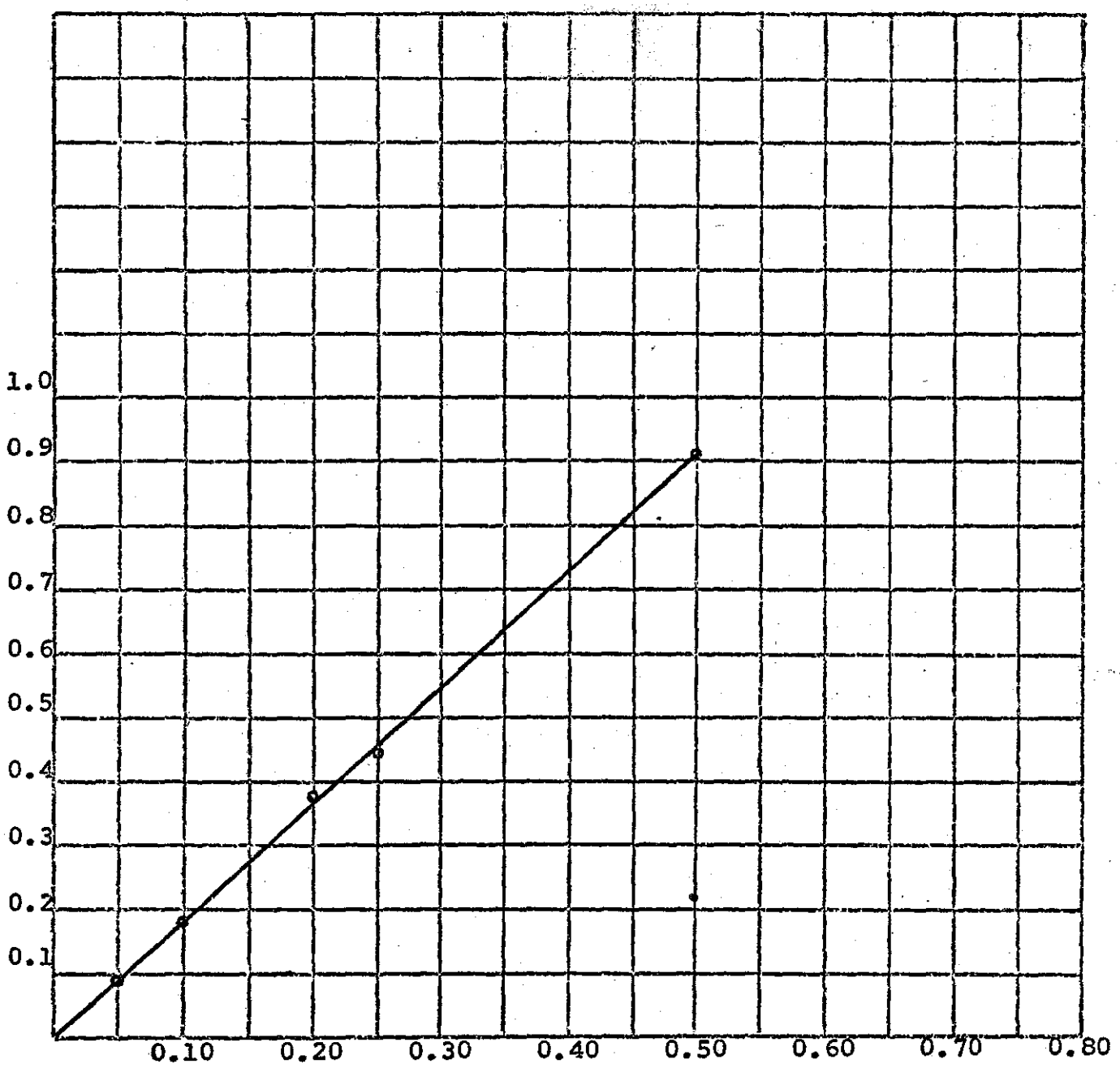
CONCENTRATION (milligrams per 5-ml.)

FIGURE 28 - STANDARD CHLORTHALIDONE CURVE



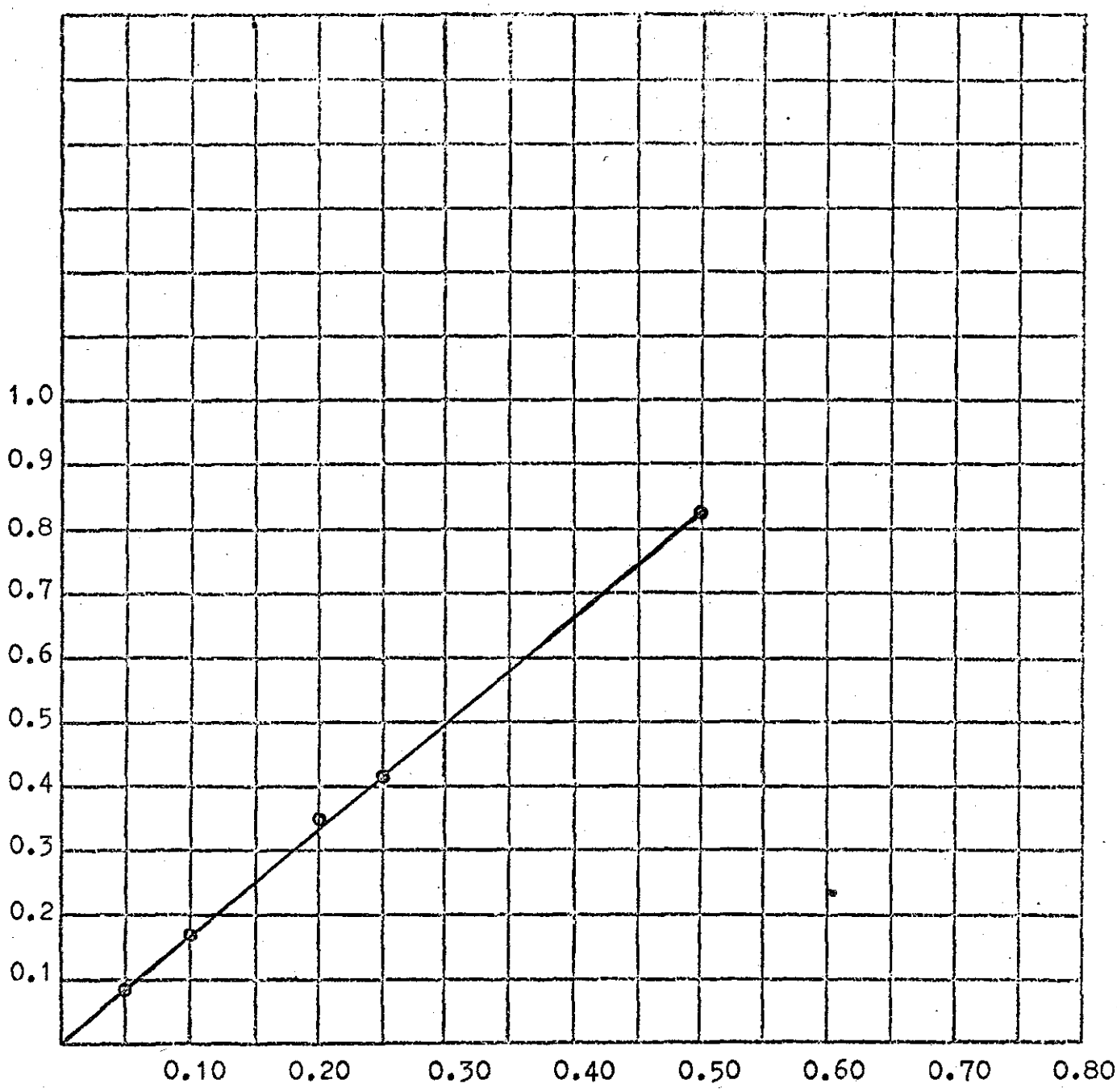
CONCENTRATION (milligrams per 5-ml.)

FIGURE 29 - STANDARD CYCLOTHIAZIDE CURVE



CONCENTRATION (milligrams per 5-ml.)

FIGURE 30 - STANDARD ETHACRYNIC ACID CURVE



CONCENTRATION (milligrams per 5-ml.)

FIGURE 31 - STANDARD FUROSEMIDE CURVE

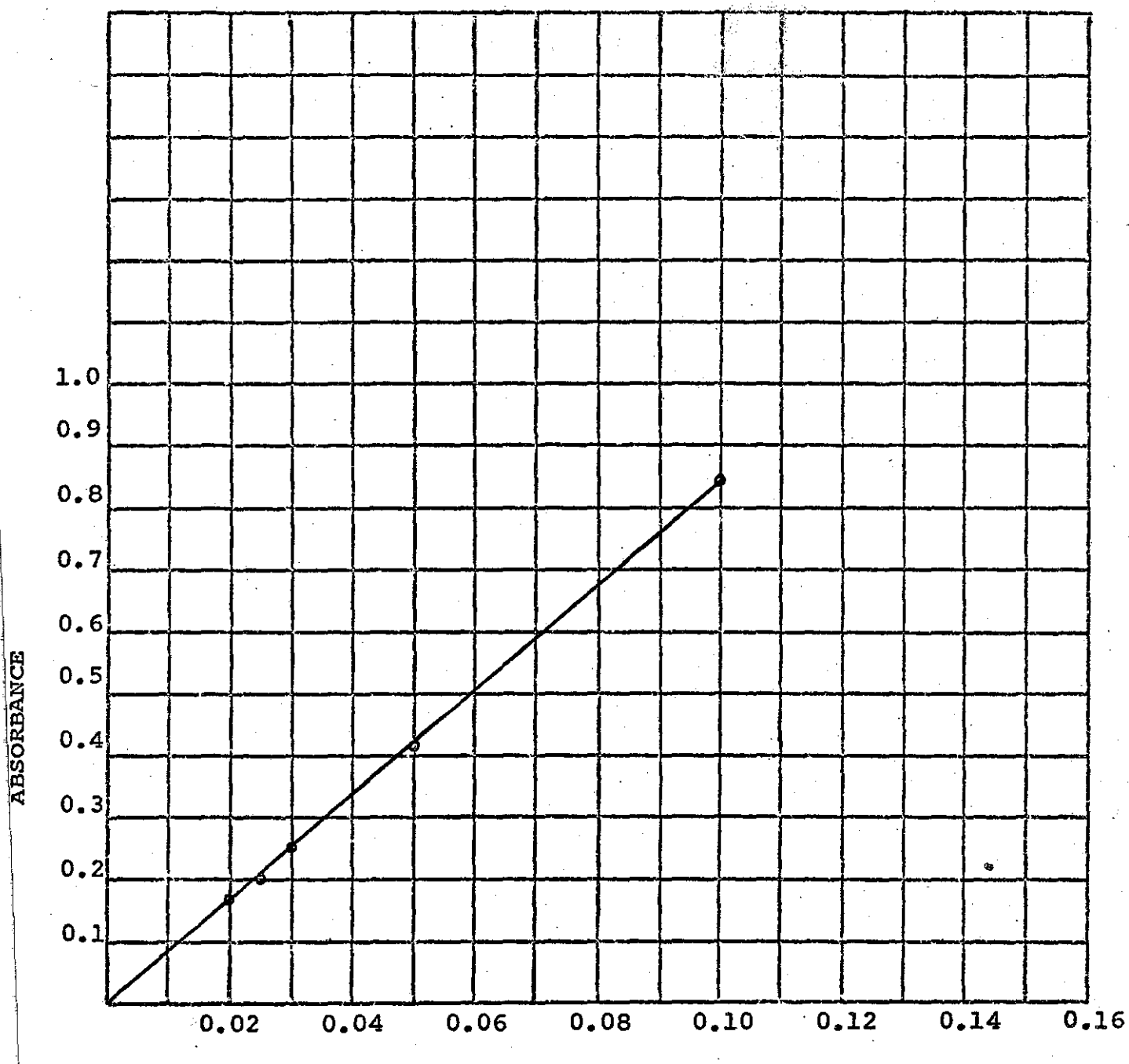
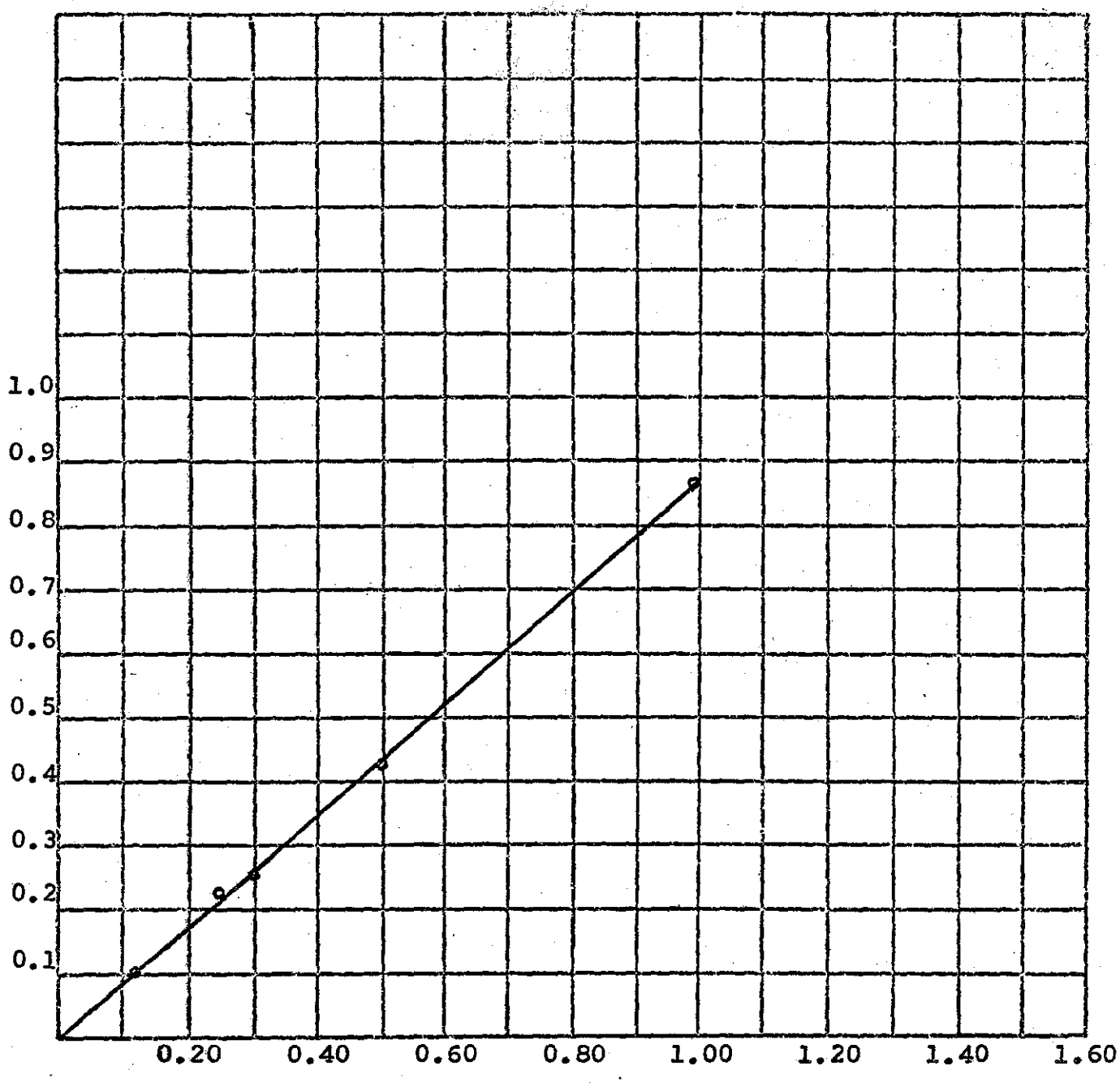
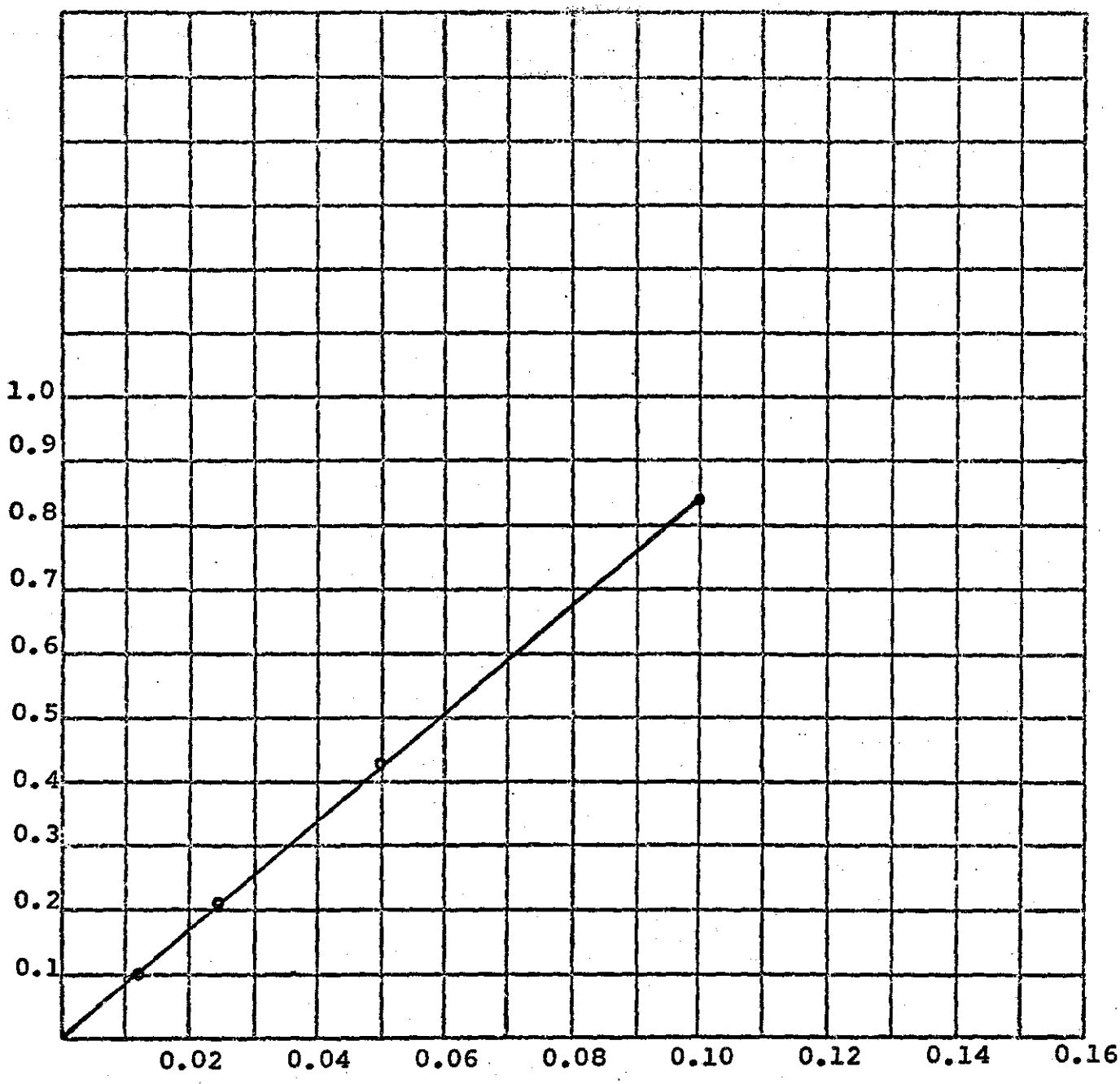


FIGURE 32 - STANDARD POLYTHIAZIDE CURVE



CONCENTRATION (milligrams per 5-ml.)

FIGURE 33 - STANDARD SPIRONOLACTONE CURVE



CONCENTRATION (milligrams per 5-ml.)

FIGURE 34 - STANDARD TRICHLORMETHIAZIDE CURVE

by removing samples from the lipoid phase at one hour intervals. The samples were returned to the distribution apparatus after each measurement.

The results were reported in Table VII and the resorption profiles are illustrated in Figures 35 to 43.

TABLE VII

RESORPTION PROFILE DATA OBTAINED FROM THE DOSAGE FORMS AND USED FOR THE CONSTRUCTION OF THEIR ABSORPTION PROFILES

Dosage Form and Weight[a]	Time in Hours[b]	pH of Determination[c]	Interpolated Weight for Total Volume[d]	Percent Resorbed[e]	Resorption Pattern[f]
Acetazolamide 250 mg.	1	1.2	12	5	+ 5
	2	4.0	29	12	+ 7
	3	7.8	42	17	
	4	7.8	65	26	+14
Chlorothiazide 500 mg.	1	1.2	0.04	0.2	+ 0.2
	2	4.0	0.3	1.5	+ 1.3
	3	7.8	0.36	1.8	
	4	7.8	0.4	2.0	+ 0.7

TABLE VII (Continued)

Dosage Form and Weight[a]	Time in Hours[b]	pH of Determination[c]	Interpolated Weight for Total Volume[d]	Percent Resorbed[e]	Resorption Pattern[f]
Chlorthalidone 100 mg.	1	1.2	8.0	8.0	+ 8
	2	4.0	16.0	16.0	+ 8
	3	7.8	25.0	25.0	
	4	7.8	35.0	35.0	+19
Cyclothiazide 2 mg.	1	1.2	0.15	8.0	+ 8
	2	4.0	0.65	33.0	+25
	3	7.8	0.95	48.0	
	4	7.8	1.25	63.0	+38

TABLE VII (Continued)

Dosage Form and Weight[a]	Time in Hours[b]	pH of Determination[c]	Interpolated Weight for Total Volume[d]	Percent Resorbed[e]	Resorption Pattern[f]
Ethacrynic Acid 50 mg.	1	1.2	4.5	9.0	+ 9
	2	4.0	3.0	6.0	- 3
	3	7.8	2.3	5.0	- 3
	4	7.8	1.4	3.0	
Furosemide 40 mg.	1	1.2	4.0	9.0	+ 9
	2	4.0	11.0	28.0	+19
	3	7.8	36.0	90.0	
	4	7.8	36.0	90.0	+62

TABLE VII (Continued)

Dosage Form and Weight[a]	Time in Hours[b]	pH of Determination[c]	Interpolated Weight for Total Volume[d]	Percent Resorbed[e]	Resorption Pattern[f]
Polythiazide 4 mg.	1	1.2	0.64	16.0	+16.0
	2	4.0	1.00	25.0	+ 9.0
	3	7.8	1.76	44.0	
	4	7.8	2.00	50.0	+25.0
Spironolactone 25 mg.	1	1.2	7.00	28.0	+28.0
	2	4.0	10.00	40.0	+12.0
	3	7.8	15.00	60.0	
	4	7.8	16.0	64.0	+24.0

TABLE VII (Continued)

Dosage Form and Weight[a]	Time in Hours[b]	pH of Determination[c]	Interpolated Weight for Total Volume[d]	Percent Resorbed[e]	Resorption Pattern[f]
Trichlormethiazide 4 mg.	1	1.2	2.0	50.0	+50.0
	2	4.0	2.7	68.0	+18.0
	3	7.8	2.85	71.0	
	4	7.8	3.0	75.0	+ 7.0

a - All samples tested were tablet dosage forms. The weight indicated represents the labeled amount of active ingredient in each of the tablet dosage forms as specified by each of the pharmaceutical manufacturers.

b - Represents the sampling of 5 ml. of lipoid solvent at 60 minute intervals for the specified dosage form under test.

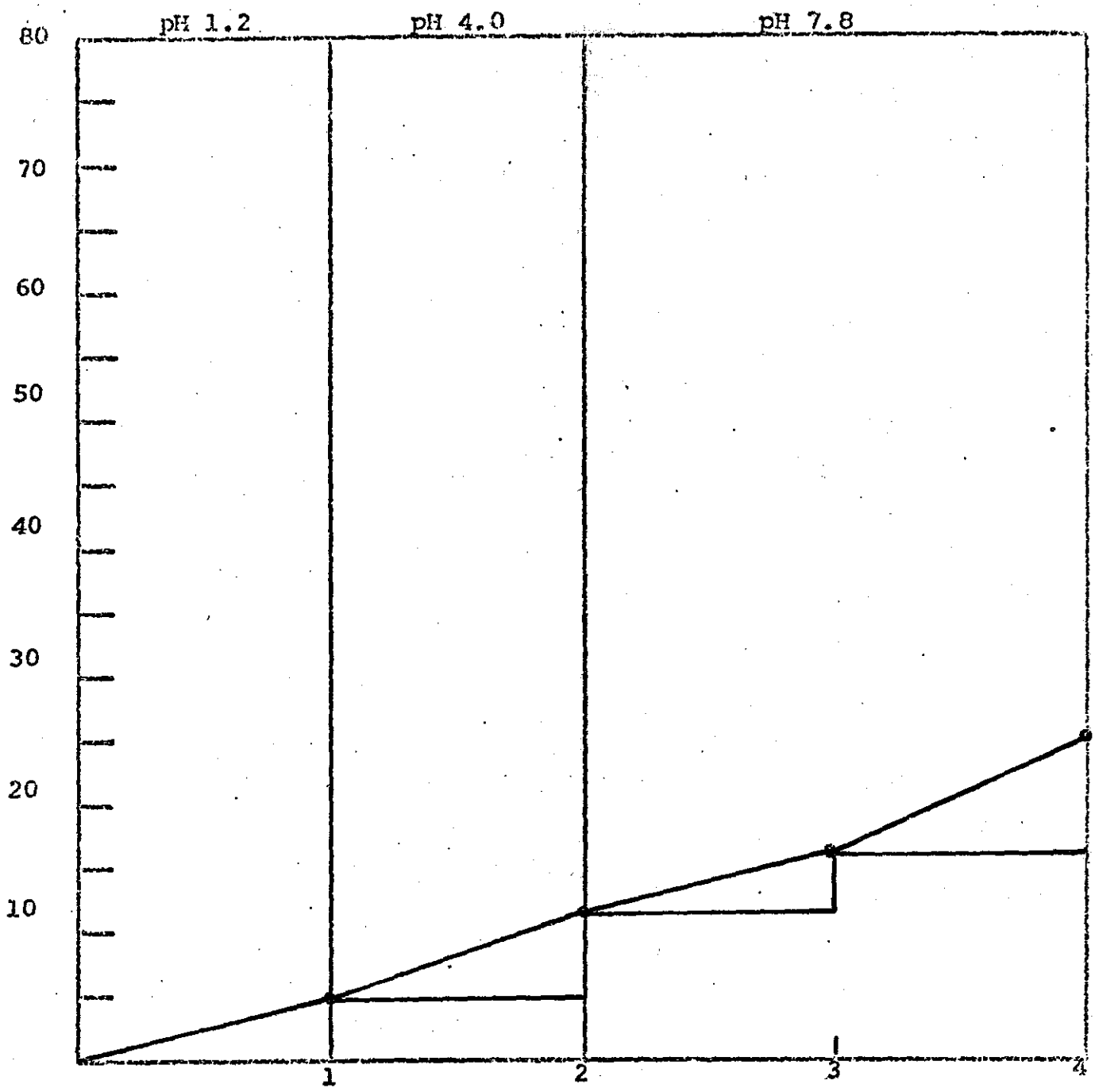
c - pH of 1.2 represents the pH of simulated gastric juice; pH of 4.0 - neutralized by addition of normal sodium hydroxide and buffered with 0.2 M potassium acetate; pH of 7.8, further neutralization with normal sodium hydroxide and buffered with 0.2 M potassium biphosphate.

d - Represents the calculated weight in milligrams of the active ingredient in 200 mls. of lipoid solvent as determined by interpolation of spectrophotometric analysis for each sample under test.

TABLE VII (Continued)

e - Represents the total percentage of the original weight of the sample that had partitioned into the lipoid phase at the various time intervals.

f - Represents the percentage of the original weight of the sample that had partitioned in the specific pH range under test. No percentage increase was reported for the third hour test. The percentage increase was recorded at the end of the fourth hour, since this value represented the total that had partitioned at the pH of 7.8.



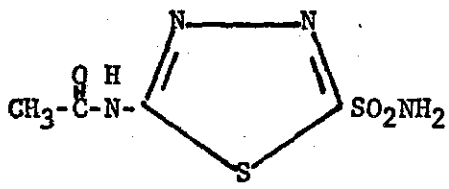
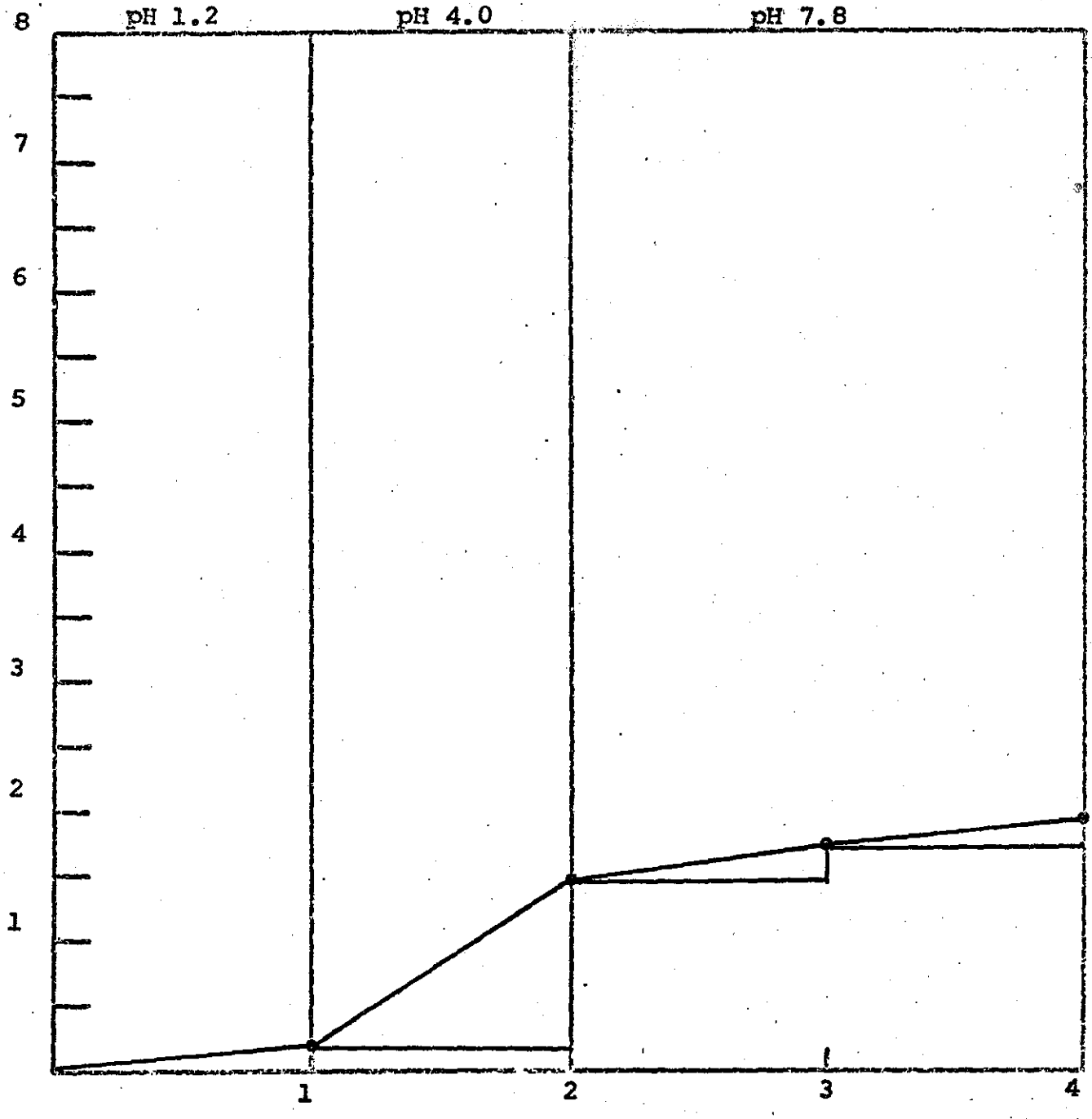


FIGURE 35 - RESORPTION PROFILE - ACETAZOLAMIDE



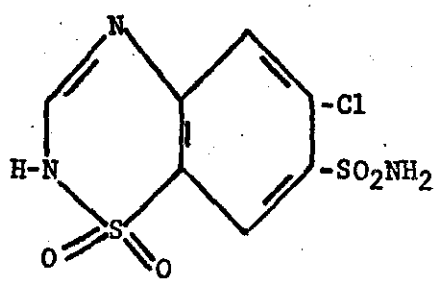
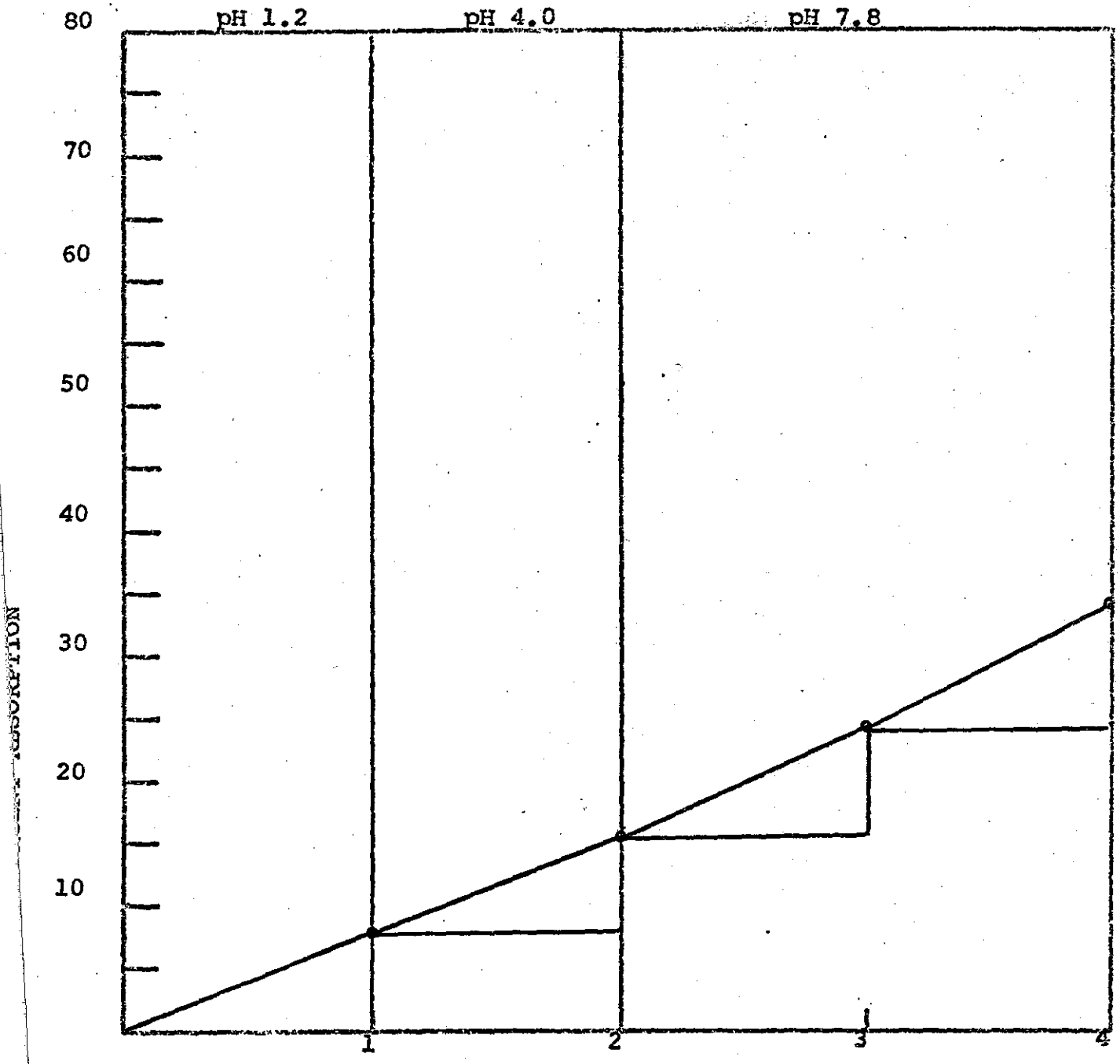


FIGURE 36 - RESORPTION PROFILE - CHLOROTHIAZIDE



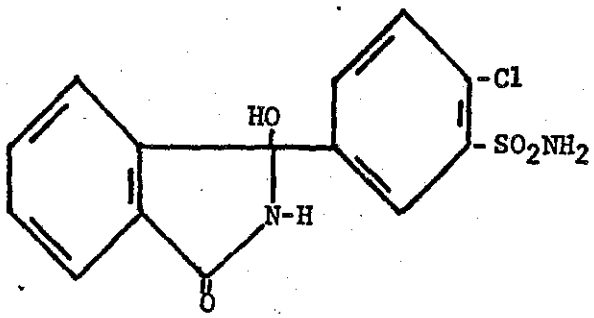
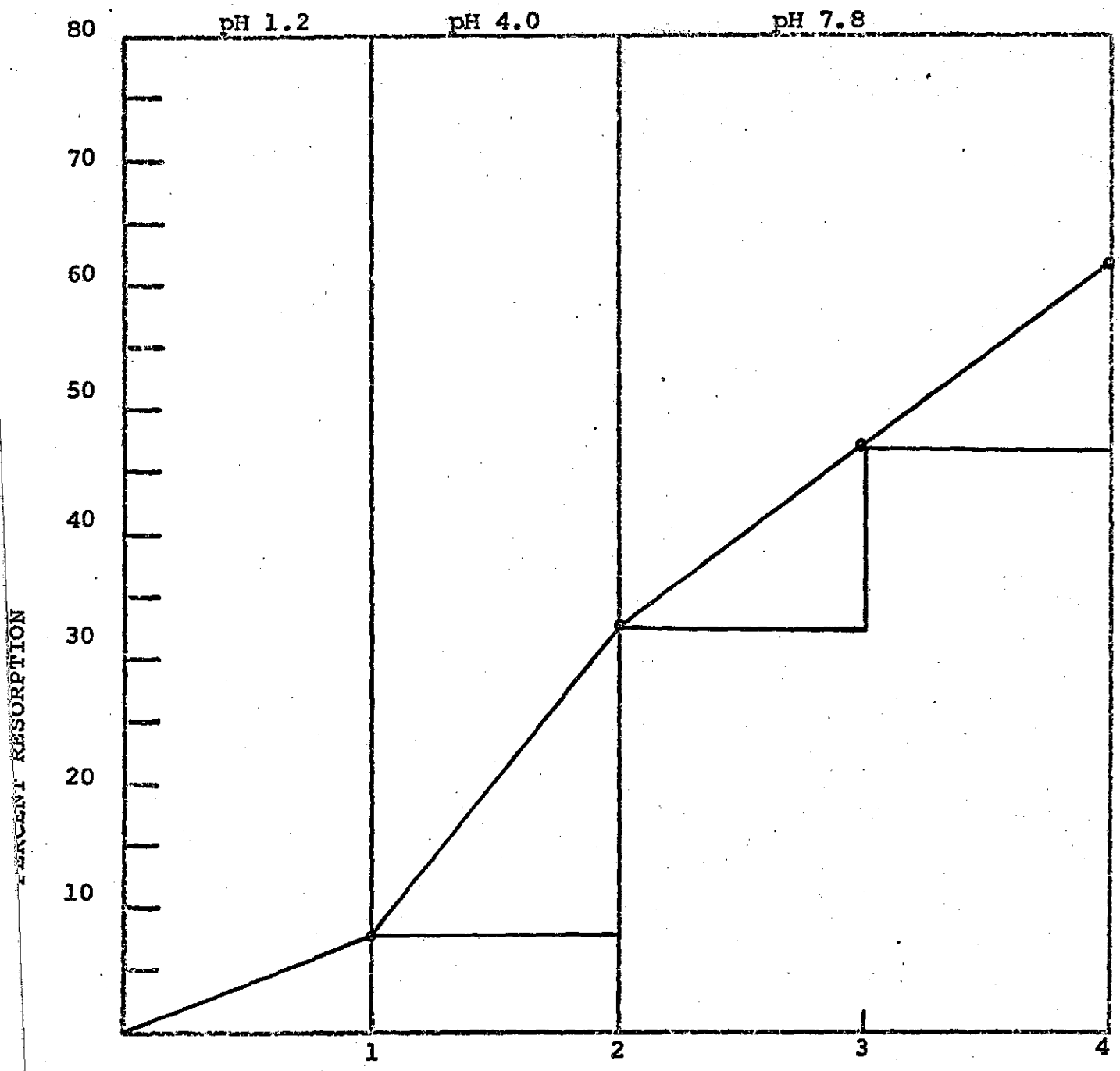


FIGURE 37 - RESORPTION PROFILE - CHLORTHALIDONE



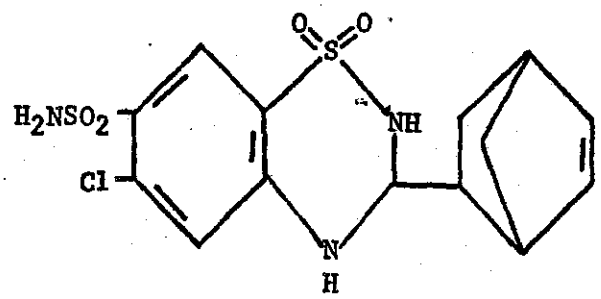
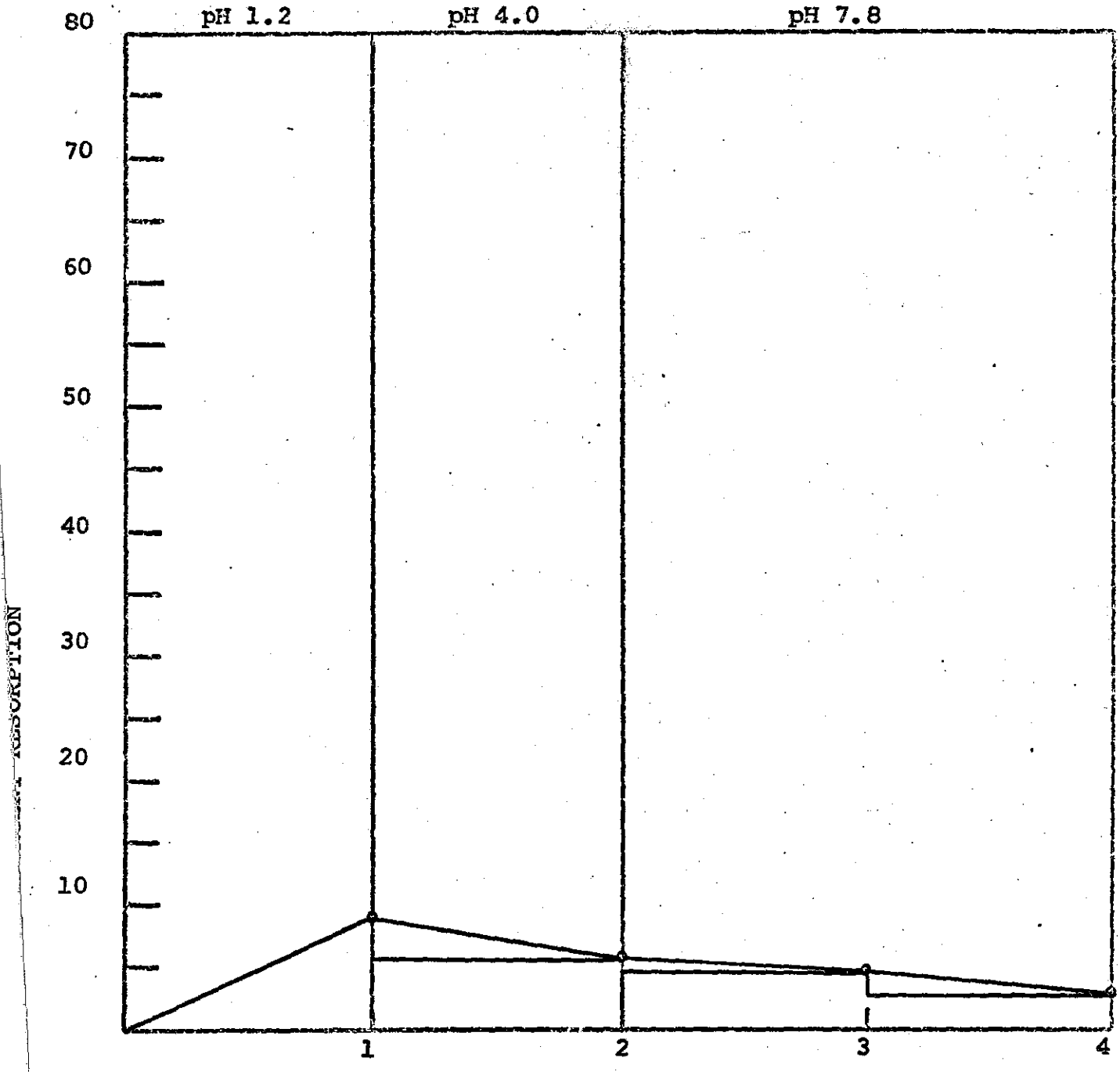


FIGURE 38 - RESORPTION PROFILE - CYCLOTHIAZIDE



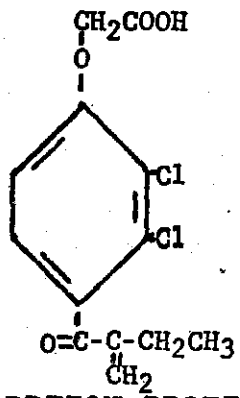
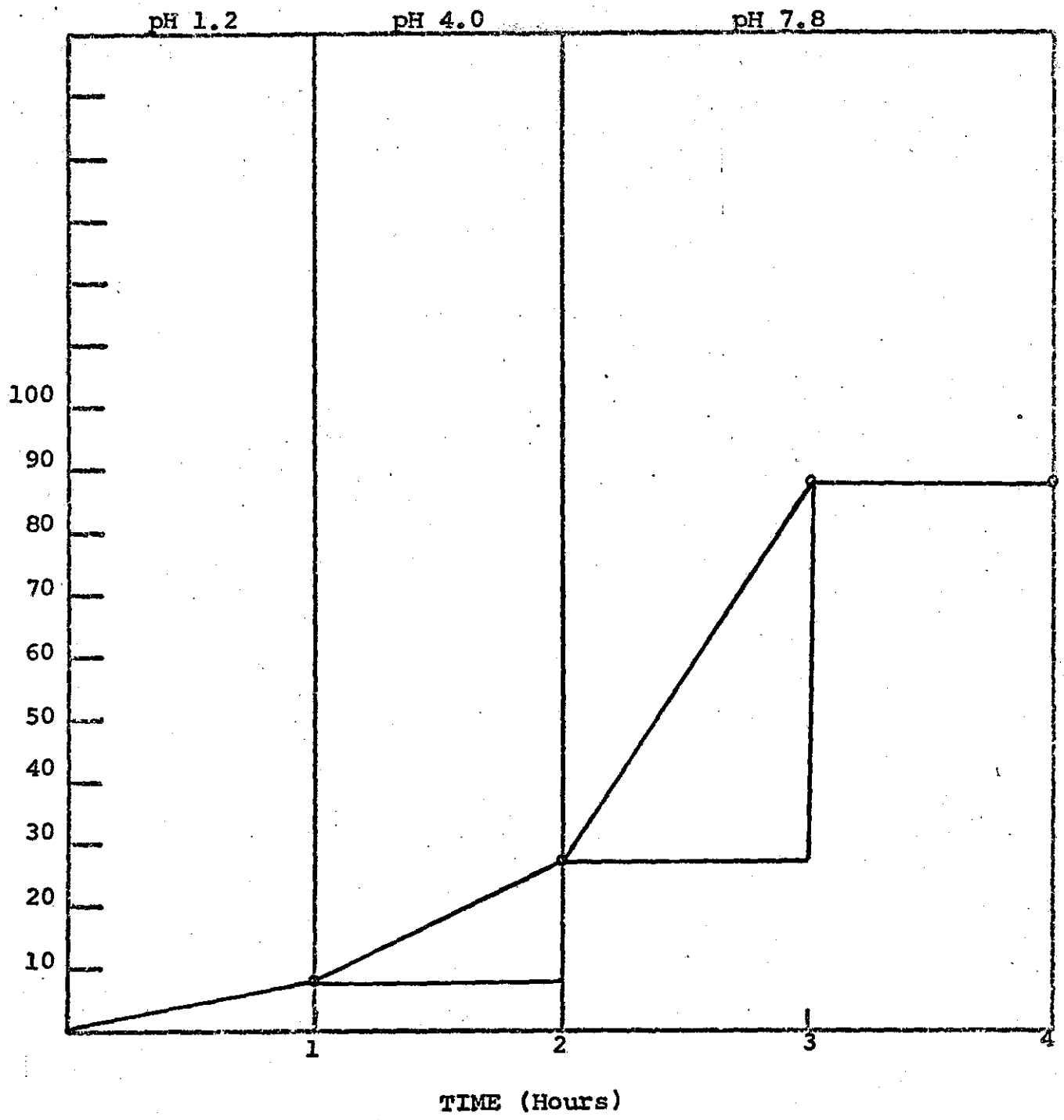


FIGURE 39 - RESORPTION PROFILE - ETHACRYNIC ACID



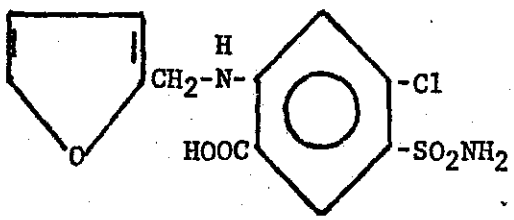
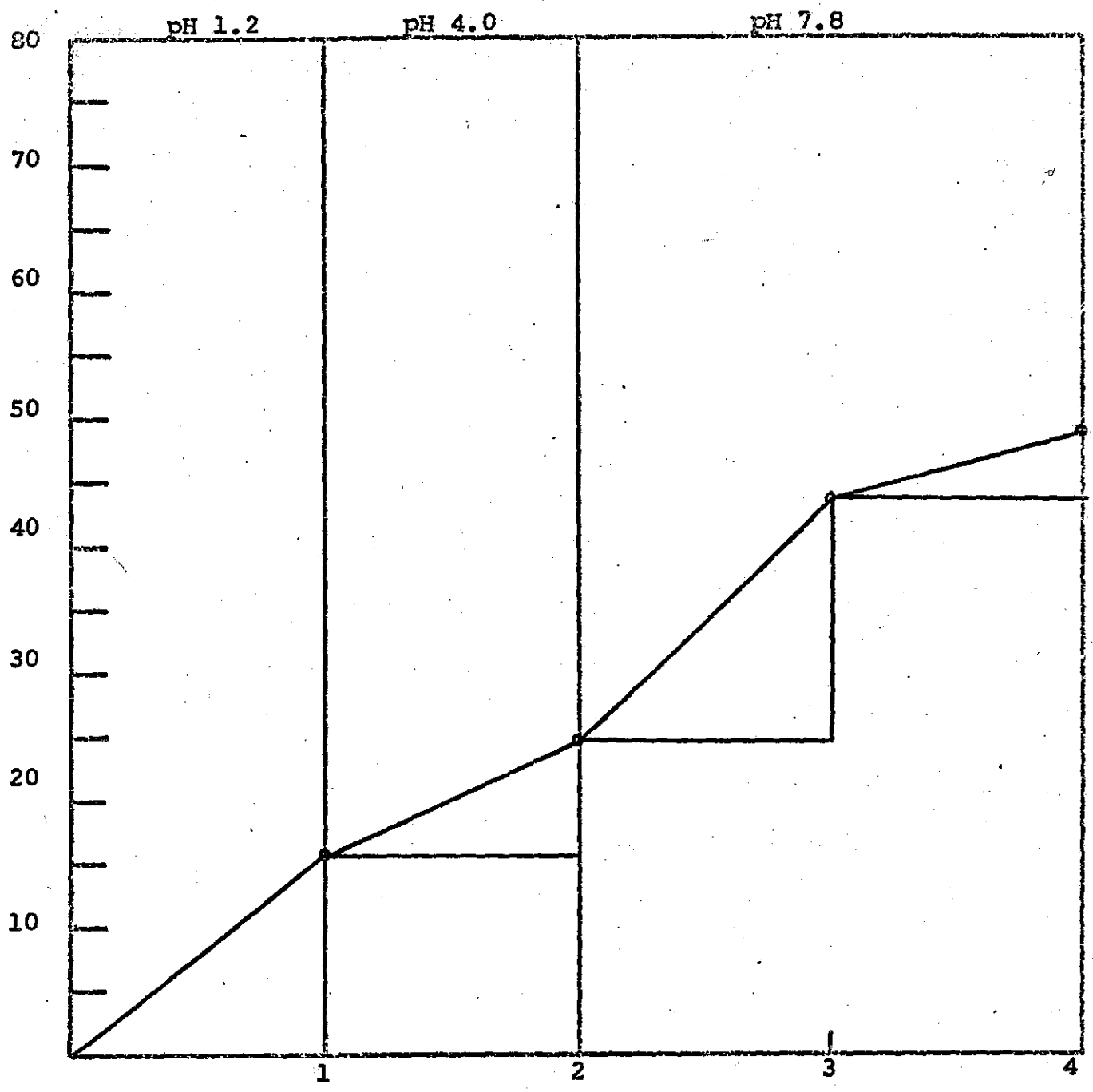


FIGURE 40 - RESORPTION PROFILE - FUROSEMIDE



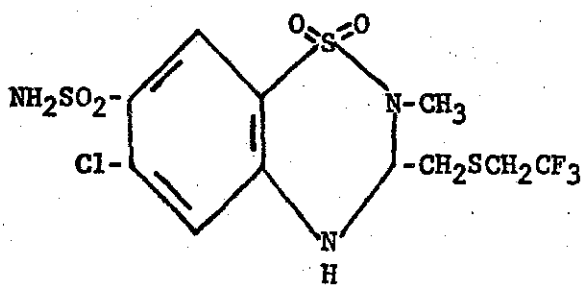
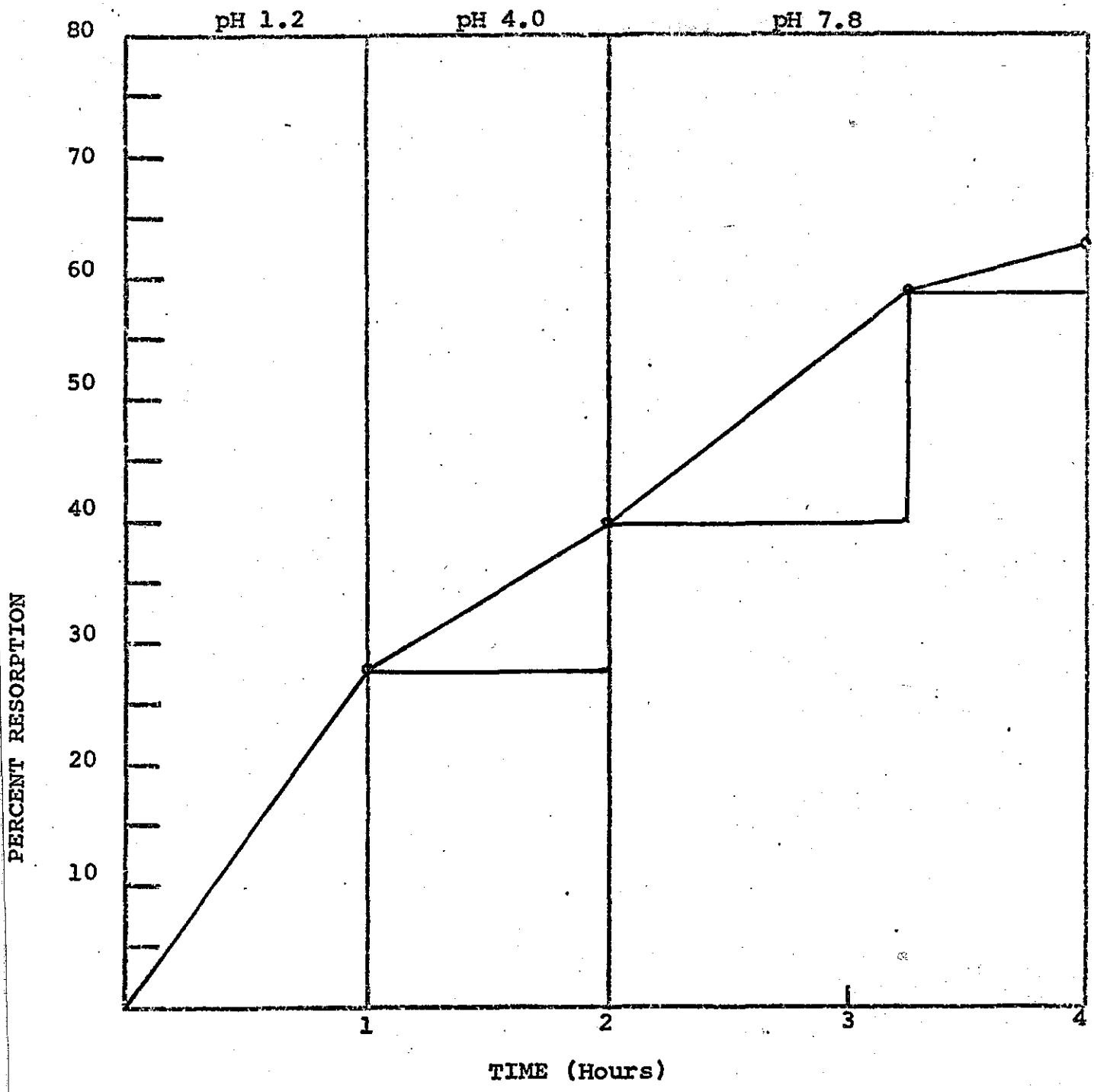


FIGURE 41 - RESORPTION PROFILE - POLYTHIAZIDE



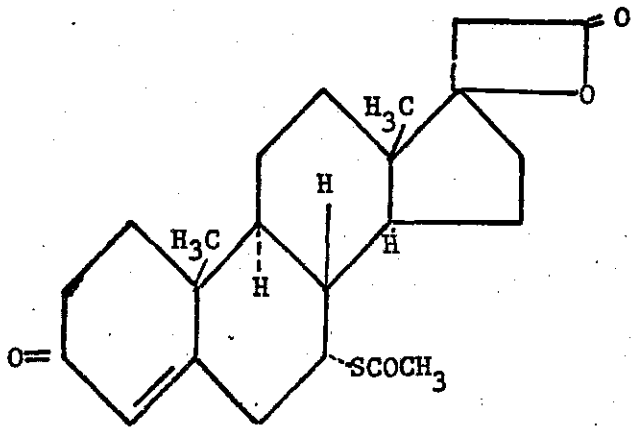
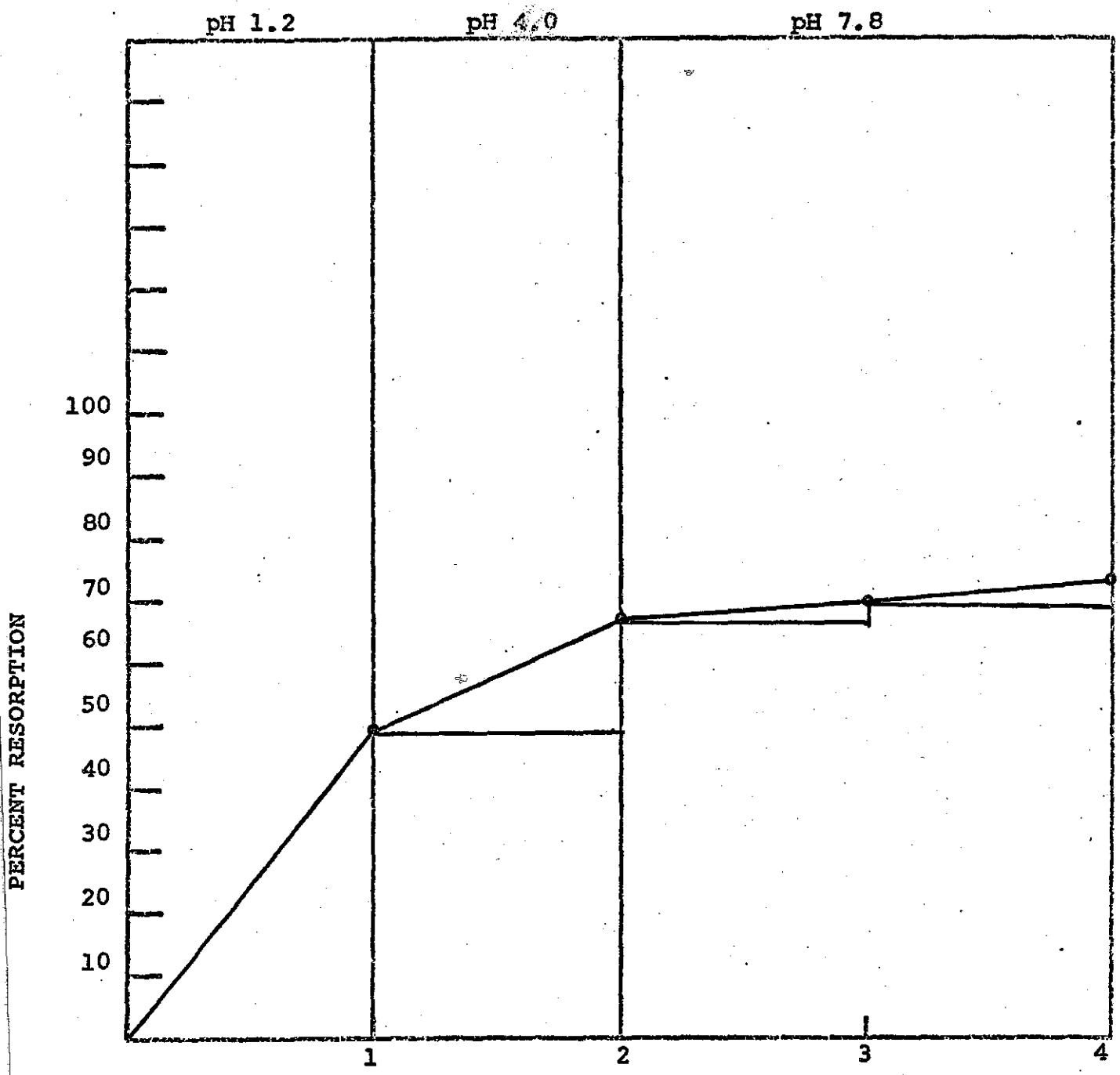


FIGURE 42 - RESORPTION PROFILE - SPIRONOLACTONE



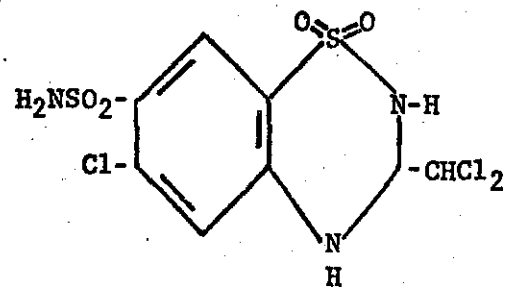


FIGURE 43 - RESORPTION PROFILE - TRICHLORMETHIAZIDE

DISCUSSION

The results obtained from experiments conducted with the Desaga Resomat provided useful information for application to other projects involving resorption or absorption of organic drugs.

A preliminary discussion of the absorption profiles used to verify operational principles has already been included in the experimental procedure. It was included at that time to support the experimental results that would establish the instrument as a valuable tool in predicting absorption characteristics of organic compounds other than those under test. However, a further discussion of these results was deemed feasible to fully evaluate the *in vitro* model and its application.

In a distribution system, a situation exists where the active ingredient was partially dissolved in its aqueous dissolution medium. Only this dissolved portion of the drug would be available for distribution or partitioning into the adjoining lipoid phase. Dibbern (1, 2) used this assumption as his first criterion or principle for the development of his test apparatus. For the dissolved portion of the drug to partition, it was also necessary for the aqueous phase of the binary system to be in an active state of "physical involvement" with the lipoid phase. The procedure was designed so that the aqueous phase compared favorably with the *in vivo* conditions

by using simulated gastric juice. Also, the non-polar solvent as the adjoining phase would simulate the lipoid barrier or membrane. The asbestos filter would prevent any fragments of the drug or its dosage form from entering this lipoid phase. At the same time, the alternating pressure on the system produces a movement comparable to peristalsis and would bring the dissolved drug to the lipoid boundary, setting up conditions for partitioning. The extent of the drug partitioning could be monitored by a suitable analytical procedure.

Generally, most organic compounds are absorbed or partitioned by passive diffusion (3). For passive diffusion to take place, certain conditions must prevail in the system. There must be a drug concentration gradient on each side of the membrane or barrier. As the concentration begins to equalize itself in each of the phases, diffusion will be reduced or will cease. Also, the diffusion rate will be based on the partition coefficient characteristics of the drug. Probably, the most important factor controlling the diffusion is referred to as the "pH-partition" theory (3). This theory defines the role that pH exerts on the diffusion of the compound as it passes along the natural absorption sites from the stomach to the large intestine. Since drugs can diffuse into the lipoid phase only as the undissociated molecule, it would actually be the pH of the environment that would control dissociation and hence the ability to partition. The pH characteristics promoting the ionization of the compound would inhibit diffusion, since the ionized state of a compound lacks

lipoid solubility properties.

Weakly acidic drugs exist primarily as the undissociated molecule at the pH of the stomach. Hence, their absorption by partitioning would be favored from this acidic environment. Conversely, weakly basic drugs would exist in the same site as the dissociated or cationic state and, therefore, would not be absorbed. The difference in absorption of two compounds of the same acidic character, would depend on their pK_a. Weakly basic drugs, which would be present in the dissociated form in the gastric contents, would revert to the undissociated state at the slightly alkaline pH of the small intestine. This would favor their absorption across the barrier at this site.

Neutral compounds would not be affected by the pH of the environment in either the stomach or the small intestine. Hence, their absorption would proceed favorably along the entire absorption area. The only controlling factors would be the solubility or partitioning characteristics of the particular drug, as well as the concentration gradient of the drug on each side of the partitioning membrane.

These principles or theories were supported by the absorption profiles for the drugs initially tested with the Desaga Resomat. Figure 5 illustrated the absorption profile for acetylsalicylic acid. Acetylsalicylic acid is a weak acid with a pK_a of 3.4. The profile indicated that partitioning had proceeded readily, even when the pH of the medium had been increased to 4.0. However, as the pH was increased to 7.8, the acetylsalicylic acid should have almost completely ionized

to the dissociated state. Hence, it would no longer partition into the lipoid solvent. Because of the extensive resorption of the acetylsalicylic acid in the acidic pH, and also the fact that the compound had ionized in the alkaline medium, the concentration gradient favored the diffusion of the ionized acetylsalicylic acid back into the aqueous phase. This phenomenon is referred to as reverse diffusion or more commonly as "back resorption." This condition would not occur in vivo, since a dynamic state would exist which would dissipate the partitioned acetylsalicylic acid as quickly as it diffused. In summary, acetylsalicylic acid would be readily absorbed from the stomach but not from the small intestine. This fact has been quantitatively illustrated in Table II.

Phenacetin is considered to be a neutral compound with no pH dependent ionizing characteristics. Therefore, partitioning mechanisms would be independent of any changes in pH. This fact was illustrated in Figure 6, the resorption profile for this compound. Because phenacetin is only slightly soluble in water, this property was the controlling factor for the low resorption quota. Specifically, the profile did indicate that resorption or partitioning was independent of the pH environment.

Phenobarbital is considered to be a weak acid, having a pK_a of 7.4. The drug would be expected to exist primarily in its undissociated form at the pH of the stomach with an anticipated high resorption value. However, phenobarbital is a relatively insoluble compound at the pH of the simulated

gastric contents. Hence, it was the low solubility properties of the phenobarbital that were responsible for the low resorption properties. This was illustrated in the resorption profile in Figure 7. When the pH of the medium is increased, the solution of phenobarbital in water also increases. This was noted in the profile when the pH was increased to 4.0 where a higher resorption value was illustrated. A further increase in pH to 7.8, promoted the ionization of the compound. This condition became the limiting factor in the resorption of the phenobarbital. This was illustrated by the weak resorption in the alkaline pH.

Dissolution Studies

Dissolution implies the solubilizing of a compound in a specific solvent. One of the factors controlling the dissolution rate of a compound is the extent to which the concentration builds up in the dissolution medium. This increase in concentration will have a retarding effect on the rate of dissolution, up to the point of saturation, where the dissolution will cease. With sparingly soluble drugs, the effect of increased concentration becomes very apparent. Therefore, many of the established *in vitro* dissolution rate methods were unable to accurately evaluate the dissolution rate of these sparingly soluble drugs. Consequently, dissolution studies were included in this project primarily, to illustrate and evaluate the solubility characteristics of the diuretics. By using the Desaga Resomat for these determinations, the versatility of this instrument would be demonstrated. To confirm

the Resomat dissolution rates, they were compared to the Beaker Method (20), a procedure that has proven satisfactory for expressing dissolution rate characteristics of compounds in oral dosage forms. Comparative dissolution characteristics of the diuretics were indicated in Table IV and their dissolution rates illustrated graphically in Figures 17 to 25.

It was noted that while the dissolution characteristics were not the same for each period of testing by either method, the over-all extent of dissolution for the majority of the diuretics compared favorably at the end of the test period. Where differences were noted, several factors should be considered. It was possible that the dissolution rate of the active ingredient was affected by the disintegration rate of the tablet. Variations in disintegration rates have been noted to occur with tablets having the same lot numbers.

A possible explanation for the slightly slower rate of dissolution with the Desaga Resomat was the fact that after the active ingredient dissolved, it had to diffuse through the asbestos pad into the medium that was actually tested. Also, the rate of agitation could account for differences in the dissolution rate. With the Beaker Method, agitation was controlled in such a manner that the tablet fragments were restricted to the bottom of the beaker. However, in the Resomat, the two magnetic stirrers and the alternating pressure imposed on the system maintained the fragments in active dispersion. Finally, the variations in test volumes for each of the methods could be a contributing factor to the differences.

Therefore, it was established that the Desaga Resomat could be used as an instrument for the determination of dissolution rates of tablet.

Resorption Profiles

A resorption profile is a graphical illustration of the partitioning of a drug from an aqueous phase into a lipoid medium at specific pH values. The Desaga Resomat is designed in such a manner that the aqueous medium will be in equilibrium with a non-polar solvent. The aqueous medium with its varying pH would simulate the physiological variation from the gastric contents to that of the small intestine. The non-polar solvent would approximate the properties of the lipoid membrane through which the drug partitions in the absorption process.

Profiles were obtained by plotting percent resorption as a function of time. Changes in pH characteristics were made at time intervals that would approximate the physiological process.

Acetazolamide Profile

Acetazolamide is described as a weak acid with a pK_a of 7.2. It possesses low water solubility properties as illustrated in Figure 17. It would be expected, based upon the "pH-partition" theory, that the distribution coefficient would be higher in the more acidic medium. However, the profile indicated a relatively low distribution at this pH. This could be explained on the basis of the low solubility characteristics of the compound. As the pH increased, it would be expected

that the distribution would decrease. Evidently, there was sufficient drug in the nonionized state to partition, thus maintaining an unaltered distribution rate. Therefore, the profile would indicate absorption of this drug taking place over the entire pH range. However, distribution is limited because of its solubility characteristics. Reported data (53) indicate that the compound was stable *in vivo*, and that the compound was absorbed along the entire gastro-intestinal tract and excreted unchanged.

Chlorothiazide Profile

Chlorothiazide is described as a weak acid with a pK_a of 6.7 (54). It exhibits low water solubility as illustrated in Figure 18. The profile, Figure 36, indicated a low drug distribution into the lipoid phase. The property of low aqueous solubility, would be a contributing factor. However, as the pH increases, chlorothiazide's water solubility also increases (55). This accounted for the increase in distribution occurring at a pH of 4.0. The increased solubility favored distribution even though dissociation may have occurred. However, as the pH increased to 7.8, ionizing characteristics of the compound would become the limiting factor. Thus accounting for reduced resorption quota. Therefore, from the analysis of the solubility characteristics and the profile for chlorothiazide, it may be postulated that chlorothiazide would be primarily absorbed as the compound passed into the duodenum.

Chlorthalidone Profile

Chlorthalidone is a weak acid with a pK_a of 7.0 (55). Figure 19, indicated that the compound showed some solubility in the aqueous medium. The solubility increased as the pH increased (55). Figure 37, the resorption profile for chlorthalidone, indicated that the partitioning properties of the compound were independent of any changes in pH. The uniformity of partitioning over the entire pH range was due to the increased solubility characteristics compensating for any inhibiting effect due to ionization. Thus this profile would forecast that the *in vivo* absorption sites would be over the entire gastro-intestinal course.

Cyclothiazide Profile

Cyclothiazide is a weak acid with a reported pK_a approximating 7 (55). Its solubility characteristics, Figure 20, at a pH of 1.2 were considerably higher than for other compounds tested. This dosage form exhibited particularly prolonged disintegration properties. The absorption profile, Figure 38, indicated a relatively low distribution at a pH of 1.2. The "pH-partition" theory would forecast good distribution characteristics. The low distribution at this pH could be due to a decreased availability of the cyclothiazide due to the poor disintegration properties of the tablet. However, as the pH was increased to 4.0, a marked increase in distribution became apparent from the profile. The increased partitioning could be explained on the basis of a high solubility of cyclothiazide in the lipoid solvent system employed in this test. These

partitioning properties would also account for the continued distribution at a pH of 7.8. The profile would, therefore, indicate a slower onset of absorption but that the absorption would continue over the entire gastro-intestinal absorption sites.

Ethacrynic Acid Profile

Ethacrynic acid is a weak acid and has a reported pK_a of 3.5 (56). It exhibited poor water solubility characteristics at a pH of 1.2 as illustrated in Figure 21. Based upon its pK_a, a high distribution could be anticipated. This postulation can be made because the compound would exist as the undissociated molecule at the pH 1.2 which would favor marked partitioning into the lipoid solvent. However, the low dissolution properties of the compound evidently restricted the distribution at this pH. The dosage form also exhibited prolonged disintegration characteristics in the test medium. This would also account for the limited availability of the ethacrynic acid for distribution. Due to the pK_a value of the compound, the ethacrynic acid would become highly ionized with an increase in pH. The concentration gradient would favor a "reverse resorption" of the ionized ethacrynic acid back into the aqueous medium. The profile indicated that the compound would be absorbed only from the stomach.

Furosemide Profile

Dibbern (1) has described Furosemide as a weak acid. It also possesses a high partition coefficient in lipoid

solvents. From the dissolution study, Figure 22, Furosemide exhibited a low water solubility. This would increase with an increase in pH (55). From the absorption profile, the low resorption rate was attributed to the low water solubility characteristics at the lower pH. However, as the pH increased, even though a reduced partitioning might be anticipated due to ionization, the reverse was noted in the profile, Figure 40. The increase in the resorption quota at the elevated pH values was probably due to the enhanced solubilizing affect of the medium. From the profile data, particularly in the pH range of 7.8, it could be assumed that almost all of the nonionized Furosemide was partitioned. The data presented would indicate that Furosemide would partition over the entire gastro-intestinal tract with the absorption increasing as the pH of the absorption sites increased.

Polythiazide Profile

Polythiazide is a weak acid. The data, as shown in Figure 23, showed that the compound was soluble in simulated gastric fluid. The resorption profile of Polythiazide, Figure 41, resembled that of a neutral compound. However, Polythiazide is readily soluble in an alkaline medium (55). The high degree of solubility appeared to compensate for any effect that ionization may have had upon resorption. A good resorption quota was shown in the strongly acidic pH. As the pH was raised, a slight decrease in the resorption quota was illustrated. The data, as shown in Figure 41, would suggest

that absorption would proceed over the entire gastro-intestinal tract. Reported data (57) supported this profile. Polythiazide was described as a potent diuretic with an onset of action occurring within two hours, and with a prolonged action.

Spironolactone Profile

Spironolactone is described as an ester. The data, as shown in Figure 24, indicated that the compound has low water solubility characteristics. From the profile data, Figure 42, the compound adhered to the "pH-partition" theory with a more pronounced distribution occurring in the more acidic medium. As the Spironolactone partitioned, however, more would become available through dissolution. Thus, distribution continued at both increased pH values. The marked reduction in this process at the end of the third hour of testing was possibly due to two factors. It was possible that the lipoid solvent had approached saturation with respect to Spironolactone in solution. Also, the Spironolactone tablet formulation appeared to release an "oily" material which tended to clog the sintered filter. This had an impeding effect on the passage of the aqueous medium through the filter thus inhibiting contact with the lipoid solvent. The profile would predict that Spironolactone would be absorbed *in vivo* over the entire pH range of the gastro-intestinal tract but would be time limited at the pH of the small intestine.

Trichlormethiazide Profile

Trichlormethiazide is a weak acid and exhibited good

solubility in simulated gastric fluid. The solubility was illustrated in Figure 25. Trichlormethiazide would be expected to show a high resorption at a pH of 1.2 because of minimal ionization and the enhanced solubility. The resorption profile, Figure 43, illustrated this phenomenon. When the pH was increased to 4.0, the resorption rate decreased slightly. This indicated that Trichlormethiazide had undergone ionization. However, the solubility of Trichlormethiazide increased as the pH was increased into an alkaline range (57). Therefore, partitioning would still occur as a result of this increased solubility. The effect of ionization on the drug was illustrated in the alkaline pH as only a slight increase in resorption was noted in this range. Increased ionization of the compound would be the contributing factor. In summary, it appeared that Trichlormethiazide would be absorbed from the entire gastro-intestinal tract, with the highest resorption quota occurring in the stomach. This profile compared with reported data (57) that Trichlormethiazide is a potent diuretic. Onset of action occurs within two hours with moderate diuresis continuing for twenty-four hours.

SUMMARY

1. The literature surveying the history of dissolution rate methodology and its influence on the bio-availability of medication from the dosage form has been reviewed.

2. Experiments to verify the Desaga Resomat as a useful instrument in predicting gastro-intestinal absorption have been conducted.

3. Resorption profiles for acetylsalicylic acid, phenacetin, and phenobarbital were used in the verification of the Desaga Resomat were graphically illustrated.

4. Standard curves were prepared relating concentration of drug in simulated gastric fluid to absorbance by spectrophotometric analysis. These curves were used to determine the amount of drug dissolved in the dissolution medium.

5. Experimental dissolution rate studies were carried out and graphically illustrated, by two methods; the Desaga Resomat and a previously reported procedure known as the Beaker Method.

6. Standard curves were prepared which related concentration of drug in the various lipoid solvents used in this study.

7. Nine diuretics, of current interest, were tested by the Desaga Resomat procedure and their resorption profiles graphically illustrated..

CONCLUSIONS

The results obtained from experiments with the Desaga Resomat provided useful information about dosage form characteristics and the availability of the active ingredient from the formulation.

The Desaga Resomat was developed primarily for absorption model experiments utilizing a binary system. The instrument, however, is not limited to just this application. Disregarding distribution into an immiscible solvent, the Desaga Resomat could be used for the determination of the simple rate of release of the active ingredient from tablet dosage form. The results of this study compared favorably with data supplied by an accepted method used in dissolution rate methodology. The advantages of the instrument used for this purpose over other methods arise from the convenience and ease in varying the conditions which alter dissolution rate characteristics.

The Desaga Resomat permitted the determination of resorption profiles which illustrated the result of aqueous solubility and the distribution of a drug into a lipoid phase.

The resorption profiles of the drugs used in establishing the efficacy of the Desaga Resomat illustrated that drug absorption is primarily dependent upon the "pH-partition" theory. These profiles supported this theory that a membrane is more permeable to the nonionized form of a drug due to its greater lipoid solubility. Therefore, partitioning through

the membrane becomes a function of the pH of the internal environment and the pK_a value of the particular drug. Consequently, a weak acid would be absorbed more extensively at a lower pH because of its minimal dissociation. Conversely, a weak base would have enhanced absorption at the pH values common to the small intestine.

The resorption profiles were determined for nine diuretic drugs. Their in vivo absorption characteristics could be predicted based upon these profiles. The interdependency of aqueous solubility, distribution coefficient, and changing pH was shown to influence the resorption of these drugs in vitro. These factors also control the absorption process in the gastro-intestinal tract (1-5).

The ideal situation, based upon the "pH-partition" theory, was shown with those drugs having a low pK_a. Dissociation of these drugs was minimal in the strongly acidic medium, favoring absorption from this site. However, the profiles illustrated the effect of other factors on absorption. Initial availability of active ingredient was controlled by the disintegration rate of the dosage form, solubility characteristics of active ingredient in aqueous medium and the degree of ionization of the drug at the specific pH value. The profiles also illustrated the influence of concentration gradients existing in the binary system. Conditions favoring approaching saturation of active ingredient in the lipoid phase would be a rate limiting factor in in vitro drug absorption.

Significant information obtained from this test model

could be applied to prediction of *in vivo* availability characteristics of tablet dosage forms. There is still some doubt associated with correlating results of *in vitro* tests with actual *in vivo* conditions (58). However, it is agreed that if a dosage form does not perform satisfactorily in an *in vitro* test, it would be doubtful that release of active ingredient from the dosage form would occur *in vivo*. It is also agreed that a well-formulated tablet that passes the rigorous *in vitro* testing for drug availability will have a much greater chance of clinical success. Thus, the Desaga Resomat presents an instrument with the potential of providing information which previously was only available from *in vivo* studies.

BIBLIOGRAPHY

1. H. W. Dibbern, Arzneimittel-Forsch., 16, 177(1966).

2. H. W. Dibbern, Ibid, 16, 1304(1966).

3. P. A. Shore, B. B. Brodie, and C. A. M. Hogben, J. Pharmacol., 119, 361(1957).

4. L. S. Schanker, P. A. Shore, B. B. Brodie, and C. A. M. Hogben, J. Pharmacol., 120, 528(1957).

5. J. G. Wagner, Drug Intelligence and Clin. Pharm., 4, 32(1970).

6. A. A. Noyes and W. R. Whitney, J. Am. Chem. Soc., 19, 930(1897).

7. W. Nernst and E. Brunner, Zeit. Phys. Chem., 47, 52(1904) c.f., J. G. Wagner, Drug Intelligence and Clin. Pharm., 4, 34(1970).

8. A. W. Hixon and J. H. Crowell, Ind. Eng. Chem., 23 923(1931): through Chem. Abstr. 25, 5074(1931).

9. V. Cofman, Chem. and Ind., 50, 1057(1931): through Chem. Abstr., 26, 878(1932).

10. G. H. Elliott, Pharm. J., 131, 514(1933).

11. B. L. Oser, D. Meinick and M. Hochberg, Ind. Chem. Anal. Ed. 17, 401(1945): through Chem. Abstr. 39, 46523(1945).

12. E. Nelson, J. Am. Pharm. Assoc., Sci. Ed., 46, 607(1957).

13. W. I. Higuchi, E. L. Parrott, D. E. Wurster, and T. Higuchi, J. Am. Pharm. Assoc., Sci. Ed., 47, 376(1958).

14. J. Royal, Drug Std., 27, 1(1959).

15. R. G. Wiegand and J. D. Taylor, Drug Std., 27, 161(1959).

16. J. G. Wagner, Drug Std., 27, 178(1959).

17. E. Nelson, J. Am. Pharm. Assoc., Sci. Ed., 49 437(1960).

18. R. A. Nash and A. D. Marcus, Drug Std., 28, 1(1960).

19. J. A. Campbell, Drug Cosmetic Ind., 87, 620(1960).

20. G. Levy and B. A. Hayes, New Engl. J. Med., 262, 1053(1960).

21. J. G. Wagner, O. S. Carpenter and E. J. Collins, J. Pharmacol. Exp. Therap., 129, 101(1960).

22. G. Levy, J. Pharm. Sci., 50, 359(1961).

23. J. G. Wagner, J. Pharm. Sci., 50, 359(1961).

24. W. E. Hamlin, E. Nelson, B. E. Ballard, and J. G. Wagner, J. Pharm. Sci., 51, 915(1962).

25. L. C. Schroeter, J. E. Tingstad, E. L. Knoechel, and J. G. Wagner, J. Pharm. Sci., 51, 915(1962).

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

27. L. C. Schroeter and W. E. Hamlin, J. Pharm. Sci., 52, 1047(1963).

28. P. J. Niebergall and J. E. Goyan, J. Pharm. Sci., 52, 29(1963).

29. W. I. Higuchi and E. N. Hiestand, J. Pharm. Sci., 52, 67(1963).

30. W. I. Higuchi, E. L. Rowe, and E. N. Hiestand, J. Pharm. Sci., 52, 162(1963).

31. G. Levy and W. Tanski, J. Pharm. Sci., 53, 656(1964).

32. H. Stelmach, J. R. Robinson, and S. P. Erikson, J. Pharm. Sci., 54, 1453(1965).

33. M. Paikoff and G. Drumm, J. Pharm. Sci., 54, 1693(1965).

34. G. Levy, J. R. Leonards and J. A. Procknall, J. Pharm. Sci., 54, 1719(1965).

35. S. J. Esai, A. P. Simonelli, and W. I. Higuchi, J. Pharm. Sci., 54, 1459(1965).

36. S. J. Desai, P. Singh, A. P. Simonelli, and W. I. Higuchi, J. Pharm. Sci., 55, 1224(1966).

37. P. Singh, S. J. Desai, A. P. Simonelli, and W. I. Higuchi, J. Pharm. Sci., 57, 217(1968).

38. J. B. Schwartz, A. P. Simonelli, and W. I. Higuchi, J. Pharm. Sci., 57, 278(1968).

39. E. L. Knoechel, C. C. Sperry, and C. J. Lintner, J. Pharm. Sci., 56, 116(1967).

40. W. I. Higuchi, P. D. Bernardo, and S. C. Mehta, J. Pharm. Sci., 56, 200(1967).

41. P. J. Niebergall, M. Y. Patel, and E. T. Sigita, J. Pharm. Sci., 56, 943(1967).

42. P. Finholt and S. Solvang, J. Pharm. Sci., 57, 1322(1968).

43. R. A. Castello, G. Jellinick, J. M. Koniecny, K. C. Kwan, and R. O. Toberman, J. Pharm. Sci., 57, 485(1968).

44. M. Pernarowski, W. Woo, and R. O. Searl, J. Pharm. Sci., 57, 1419(1968).

45. A. J. Aguilar, R. M. Wheeler, S. Fusari, and J. E. Zelmer, J. Pharm. Sci., 57, 1844(1968).

46. R. R. Barzilay and J. A. Hersey, J. Pharm. Pharmacol., 20, 2325(1968).

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

48. R. H. Blythe, c.f., Drug Intelligence and Clin. Pharm., 4, 37(1970).

49. F. Langenbucher, J. Pharm. Sci., 58, 1265(1969).

50. A. L. Fites, G. S. Banker, and V. F. Smolen, J. Pharm. Sci., 59, 610(1970).

51. J. G. Wagner, Drug Intelligence and Clin. Pharm., 4, 96(1970).

52. J. Swarbrick, "Current Concepts in the Pharmaceutical Sciences: Biopharmaceutics," Lea and Febiger, Philadelphia, Pa., 1970, p. 266.

53. Anon., "Diamox - Acetazolamide," Medical Advisory Department, Lederle Laboratories, Pearly River, N. Y., p. 2.

54. Anon., "Chlorothiazide - Diuretic and Antihypertensive Agent," Medical Publications Department, Merck, Sharp and Dohme, Division of Merck & Co., West Point, Pa., 1961, p. 6.

55. "The Merck Index," 8th Ed., Merck & Co., Inc., Rahway, N. J., 1968.

56. "Ethacrynic Acid, Product Information Summary," Merck Sharp & Dohme, Division of Merck & Co., Inc., West Point, Pa., 1966.

57. E. A. Swinyard and S. C. Harvey, in "Remington's Pharmaceutical Sciences," 14th ed., J. E. Hoover, Ed., Mack Publishing Co., Easton, Pa., 1970, pp. 936-950.

58. J. H. Wood, "In Vitro Evaluation of the Release From Drug Dosage Forms," presented before the Industrial Pharmacy Section, 113th Annual Meeting, Amer. Pharm. Assoc., at Dallas, Texas, April 25 - 29, 1966.