SOME CHEMISTRY AND PHARMACEUTICAL APPLICATIONS
OF CERTAIN POLYCARBOXYLIC ACID DERIVATIVES

by

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Under the Supervision of Professor Takeru Higuchi

Many organic acid derivatives find wide usage in pharmaceutical systems. A thorough understanding of the chemistry of these compounds is essential for proper utilization for pharmaceutical purposes. The present study has been concerned with: 1) investigation of the interaction between acid anhydrides and polycarboxylic acids in aqueous solution, such as may occur at ambient temperature and under autoclaving conditions; and 2) application of anhydro carboxy derivatives of these acids to pharmaceutical dosage forms.

Anionic forms of succinic and citric acid were found to interact reversibly and rapidly with glutaric anhydride in aqueous solution at room temperature to produce species which undergo subsequent hydrolysis. The species formed from citrate was reacted *in situ* with aniline and the resulting products isolated by column chromatography. Results of the chromatographic study and spectrophotometric observations on the reacting system suggested a mechanism based on the initial formation of a mixed anhydride which cleaved to produce an anhydride of the attacking anionic

species. Spectrophotometric investigations have shown that the rate of formation and subsequent hydrolysis of the presumed citric anhydride were dependent upon the pH and the buffer concentration. These interactions were assumed to be highly reversible through intermediate formation of a mixed anhydride.

Spectrophotometric determinations were also carried out on the above system in which acetic anhydride replaced glutaric anhydride, the reaction in this instance being presumed to be essentially irreversible. The linear anhydride apparently again reacted with citrate ions in aqueous solution to form the presumed citric anhydride species which underwent rapid hydrolysis. The rate of the initial reaction and the rate of the subsequent step depended as expected on the citrate concentration and pH. Results of chromatographic studies on products obtained by reaction with aniline at different phases of the reaction were as expected.

An investigation was also conducted on the possible application of organic acid derivatives such as anhydrides to formulations of effervescent dosage forms. Glutaric anhydride was used as a model latentiated acidifier to aid in achieving a superior degree of carbonation when used with sodium bicarbonate. The physical chemical basis of the formulation depended in this approach on essentially total dissolution of the bicarbonate salt and the

latentiated acidifier prior to homogeneous formation of any free carbonic acid. The system achieved a markedly greater degree of supersaturation with respect to carbon dioxide than was possible by the conventional method of dissolving sodium bicarbonate and a solid acid in water.

Another possible application of anhydro carboxylic acids to effervescent dosage forms was based on availability of compounds which when placed in aqueous solution hydrolyze, yielding only carbon dioxide and an organic acid. A similar approach was recently suggested (1) in a patent which appeared subsequent to initiation of this program. Three compounds were prepared for this purpose; ascorbic carbonate, anhydro-bis-O-carboxy tartaric acid, and anhydro-O-carboxy citric acid. The bis-O-carboxy anhydride of tartaric acid has been previously prepared, while anhydro-O-carboxy citric acid and ascorbic carbonate were presumed to be new compounds. The half lives of hydrolysis in aqueous solution of all three compounds were relatively short being of the order of a few seconds. Anhydro-bis-O-carboxy tartaric acid contained the greatest percentage by weight of carbon dioxide, followed by ascorbic carbonate and finally anhydro-O-carboxy citric acid. Evaluation of the compounds, from a pharmaceutical point of view, has shown them all to be potentially useful in pharmaceutical formulations.

(1) Feldman, J. R. and Foltz, R. L., United States Patent #3,218,338 Nov. 16, 1965.

APPROVED Takeru Higuchi

DATE July 6th 1966

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A thesis submitted in partial fulfillment of the requirements for the degree of

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1966

TO

My Wife

My Parents

and to

My Children

James and Nancy

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INTRODUCTION

The active roles that carboxylic acids and carboxylic acid derivatives play in pharmacy are numerous and varied. A recent publication by Higuchi *et al.*, for example, on the behavior of common buffer systems composed of carboxylic acids, reported that many carboxylic acids exist in equilibrium with their corresponding acid anhydrides in aqueous solution. Because of the sensitivity of these anhydrides to nucleophilic attack, it was postulated that their formation in the presence of drug species presented a possible pathway for drug loss from solution. Situations may exist on the other hand, where such anhydrides may serve a useful purpose. Greater insight into their chemistry and new applications to pharmaceutical systems are obviously of significant importance.

The first part of the study was designed to investigate possible interactions between acid anhydrides and carboxylic acids. It was previously observed in our laboratory that when acid anhydrides were placed in buffers of polycarboxylic acids, spectral changes occurred which suggested formation and subsequent hydrolysis of an intermediate species. The study included observations on:

1) systems containing a cyclic anhydride, such as glutaric anhydride, which would yield a reversible pathway for formation of this intermediate species, and

2) systems containing a non-cyclic anhydride, such as acetic anhydride, which would be expected to yield an irreversible pathway for formation of such species. Both systems were investigated to see the effect of pH and buffer concentration with the overall goal of gaining insight into the reaction mechanism.

The second part of the study was devoted to investigations of possible applications of these carboxylic acid derivatives in formulating effervescent type dosage forms. These products have undergone very little change from the original concept, although they suffer from many disadvantages. Classical carbonated systems are composed of an organic acid such as citric, tartaric, adipic, etc. with sodium bicarbonate.

One apparent disadvantage to this type of system is the loss of carbon dioxide to the atmosphere which occurs during dissolution of the two components. The solid organic acid for example may dissolve more rapidly than the solid sodium bicarbonate, with formation of carbonic acid occurring in the immediate vicinity of the undissolved sodium bicarbonate particles. The solid bicarbonate particles in such an instance can act as nucleation points for loss of carbon dioxide to the atmosphere. A possible means of overcoming this mode of loss through the use of a latentiated acidifier has been explored. The latentiated acidifier chosen as a model was glutaric anhydride. A non-acid species such as glutaric anhydride would produce a

lag time which may allow total dissolution of the bicarbonate particles before the acid portion becomes available, and thus, a homogeneous reaction in solution can occur.

Other apparent disadvantages to the classical effervescent system include, the saline taste that is associated with salt formation, and the rate at which carbon dioxide is evolved in this type of system. The second section of this part of the study was devoted to the possibility of overcoming the taste problem as well as the rate of evolution problem by resorting to carbonating agents. These potential carbonating agents when placed in water would hydrolyze to yield carbon dioxide and biologically acceptable hydrolysis products.

Section 1

Interaction of Di- and Tri-carboxylic Acids With Anhydrides

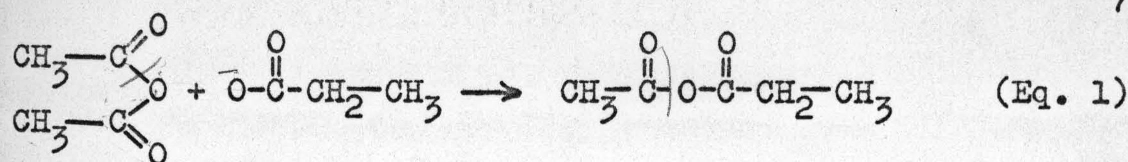
Part 1

Interaction of Di- and Tri-Carboxylic Acids with Glutaric Anhydride in Aqueous Solution

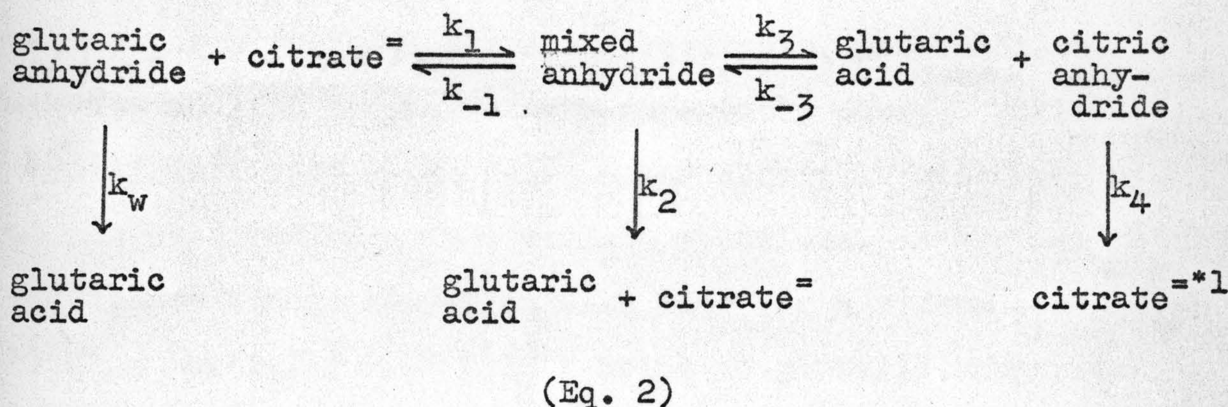
INTRODUCTION

Many biochemical systems as well as pharmaceutical formulations contain polycarboxylic acids. Citric acid, tartaric acid, malic acid, aconitic acid and succinic acid are examples of food acids which occur naturally and are also often used in pharmaceutical preparations. In an earlier report (1) it was shown that these acids exist in solution in equilibrium with their corresponding cyclic acid anhydride forms which are capable of reacting with any nucleophilic species present. The present communication is concerned with results of studies designed to determine the rate and mechanism of transference of this anhydride character in a mixture containing two of these polycarboxylic acid species. Specifically the interaction of citrate species with glutaric anhydride was investigated.

In aqueous solution, since water is a weak but an effective nucleophile, acid anhydrides undergo relatively rapid hydrolysis with half lives of the order of minutes. In the presence of other more potent electron donors which are often constituents of frequently used pharmaceutical buffers, other reactions may take place preferentially. Thus it has been suggested that acetic anhydride reacts with propionate ions with subsequent formation of a mixed anhydride (2).



Reaction -1- as written would be expected to be largely irreversible in the presence of a large excess of the attacking carboxylate species. If an analogous reaction takes place between a cyclic anhydride and a polycarboxylic acid species, on the other hand, an equilibrium system such as the following may be expected:



(Eq. 2)

Formation of citric anhydride is a probable pathway due to the fact that the carbonyl carbon in the mixed anhydride can undergo intramolecular nucleophilic attack from the neighboring carboxyl group of the citrate moiety. The mixed anhydride may also revert back to glutaric anhydride by the same mechanism, and therefore statistically it would be expected that the mixed anhydride would be present in relatively low concentration.

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*1. Formation of anhydrides from their corresponding acids is negligible at ambient condition, (estimated for succinic acid to be two parts per 10^7 at 25° C.) (1), therefore k_w, k_2 and k_4 are written irreversibly.

RESULTS AND OBSERVATIONS

Spectrophotometric Studies.--

Glutaric anhydride has a characteristic ultraviolet absorbance spectrum that can be utilized to follow its hydrolysis in aqueous solution. When citric acid is employed as a buffer at a pH where substantial quantities of the di-ionized species are present, a change in ultraviolet absorbance is observed. This interaction can be conveniently followed since the reactants, intermediate species and the final products apparently possess sufficiently different ultraviolet molar absorptivities to permit observation of the various reactions. A typical absorbance change at 248 mu with time for a system containing initially 7.05×10^{-3} moles of glutaric anhydride in 0.3 molar citrate buffer at pH = 5.0 is shown in Figure 1. There is a small increase in absorbance initially, followed by a logarithmic approach to an equilibrium value. This corresponds presumably to a $G + B \rightleftharpoons C \longrightarrow D$ type relationship where species C possesses a slightly higher molar absorbance than the reactants or the final product. The experimental observation noted above is in agreement, at least in form, to the reactions implicit in equation -2-.

It is apparent from the proposed reaction scheme that there are two possible species which could correspond to

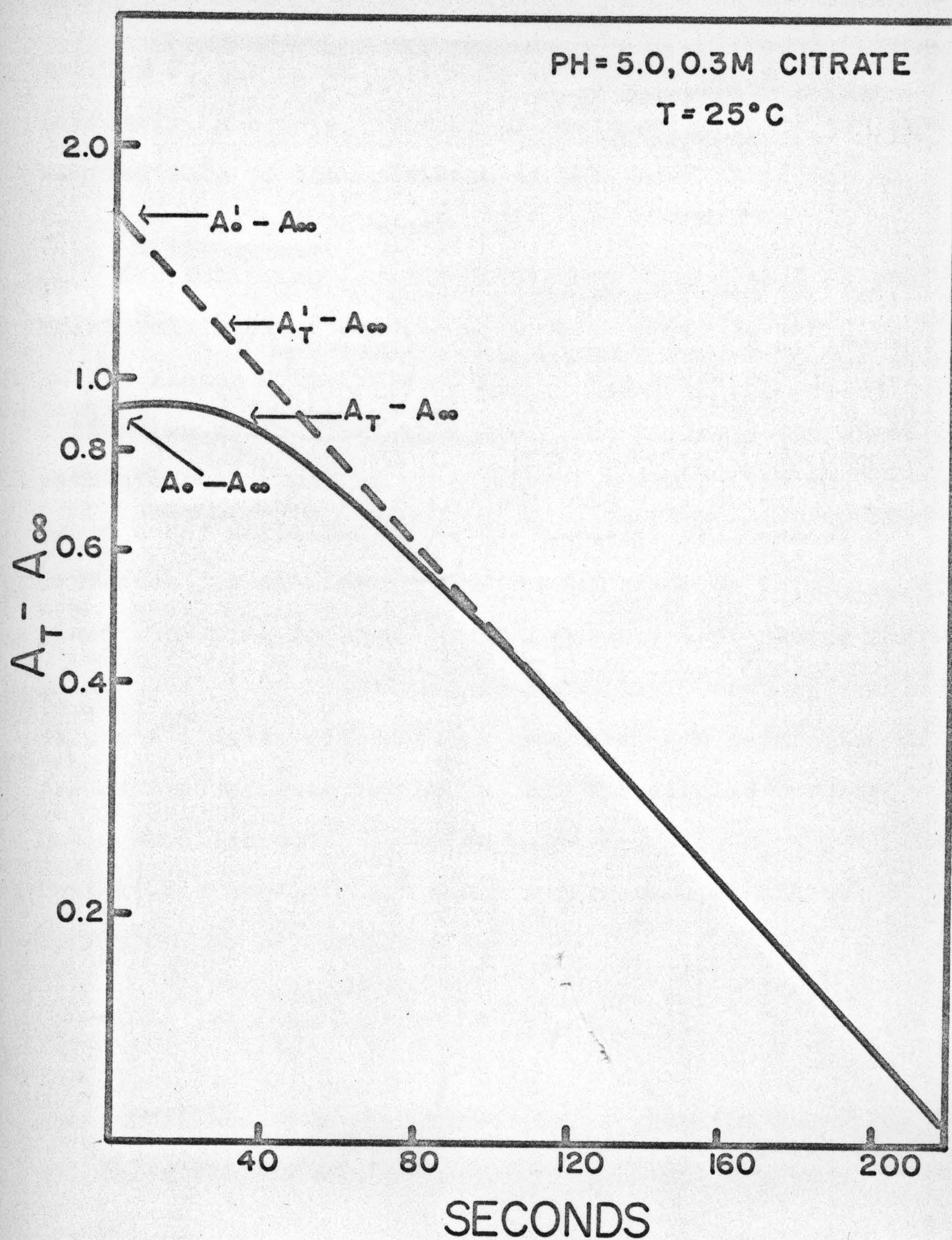

Figure 1. Semi-logarithmic plot of absorbance change at 248 mu for the system glutaric anhydride in citrate buffer.

species C, the mixed anhydride and citric anhydride. Similarly, the deterioration of the species could be attributable to decomposition of the mixed anhydride or hydrolysis of citric anhydride.

The following derivation assumed that G to C is the relatively rapid formation of citric anhydride and C to D is the slower hydrolysis of citric anhydride to the corresponding acid. Obviously there are at least two other pathways that would be kinetically equivalent to the proposed case; formation of mixed anhydride followed by a corresponding slow hydrolysis to the acids or formation of mixed anhydride followed by slow formation of citric anhydride which then hydrolyzes very rapidly. Each pathway allows a similar mathematical treatment for separation of the individual rate constants, but the species involved would be different. Assuming $k_3 \ggg k_1$, k_{-1}, k_4 and k_w and that k_{-3} is negligible,[2] the concentration of citric anhydride can be expressed as:

$$[CA] = \frac{k_1[B][GA]_0}{k_1[B]+k_w-k_4}\left[e^{-k_4 t} - e^{-(k_1[B] + k_w)t}\right] \qquad \text{(Eq. 3)}$$

where [CA] = citric anhydride, [GA] = glutaric anhydride, and [B] = citrate buffer.

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2. Pseudo-first order conditions were maintained and there was always at least a 60 fold excess of citrate buffer.

If the absorbance of a reacting system initially containing glutaric anhydride and citrate is followed at 248 mu, a plot of log $[A_T - A_\infty]$ against time (where A_T is the absorbance at any time t and A_∞ is the limiting absorbance) will yield a straight line in the terminal phase, provided $(k_1[B] + k_w) \gg k_4$. This is because under such a circumstance $(A_T - A_\infty)$ will be expected to be essentially proportional to the concentration of citric anhydride when the total anhydride concentration becomes very small. The slope of the logarithmic plot would then be equal to $-k_4$. Extrapolation of this line will give an imaginary $(A_o' - A_\infty)$ value (which is proportional to [CA]) at time zero. A semi-logarithmic plot of $[(A_T' - A_\infty) - (A_T - A_\infty)]$ against time will yield a linear relationship with a slope equal to $-(k_1[B] + k_w)$.

The rates of appearance (corresponding to $(k_1[B] + k_w)$) of the strongly absorbing intermediate species (citric anhydride?) were determined from plots of $(A_T' - A_T)$ for systems at several pH values and different buffer concentrations. Results are shown in Figure 2. The data suggests that the initial reaction between the anhydride and citrate probably involves the di- and/or tri-ionized form of citric acid.

The apparent rates of loss of the active species (k_4) determined as outlined above are shown in Figure 3 for several values of pH and various buffer concentrations. The pH range studied was limited to that where citrate behaved

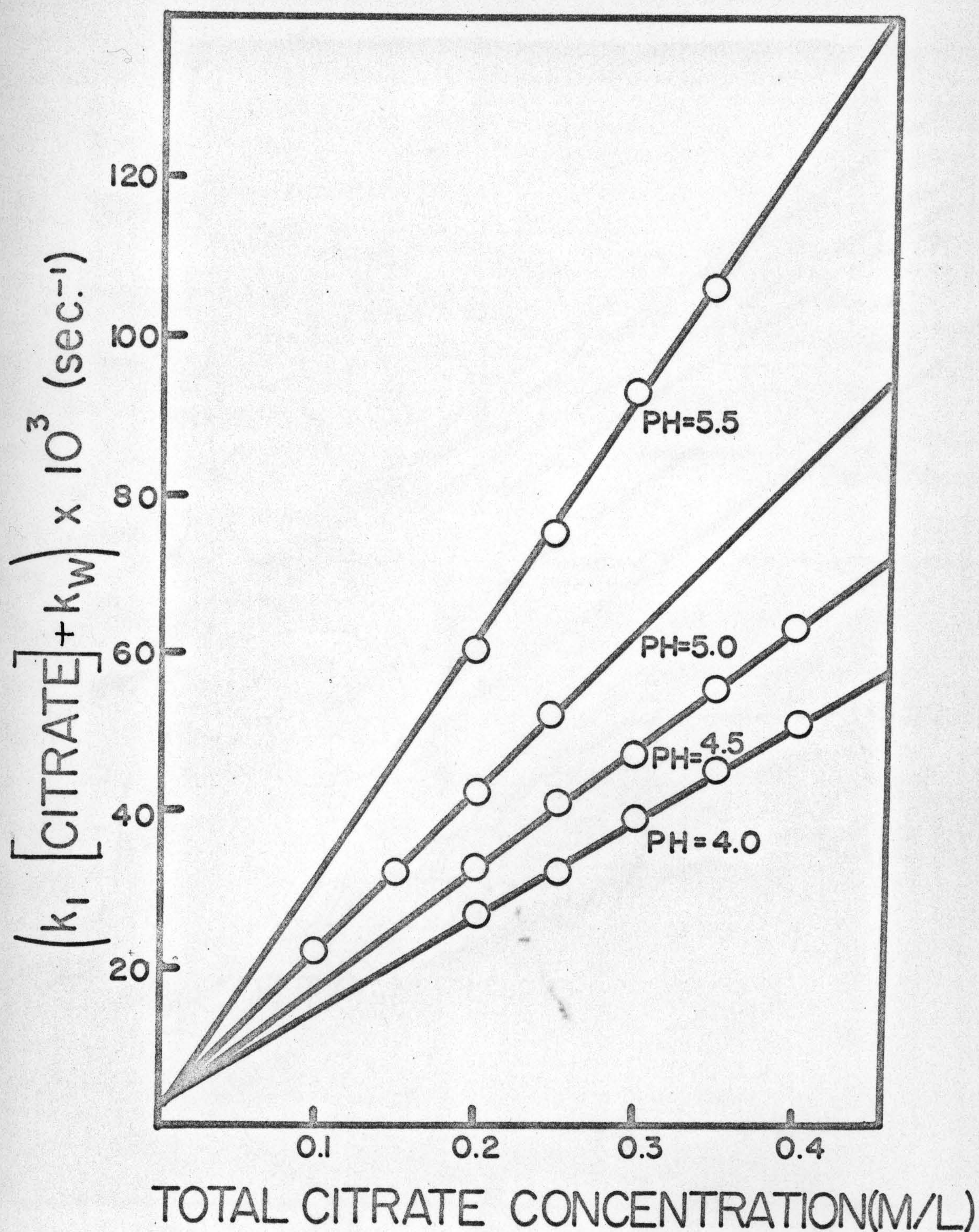

Figure 2. Rate of formation of the species formed from the glutaric-citric system at various pH values and different buffer concentrations.

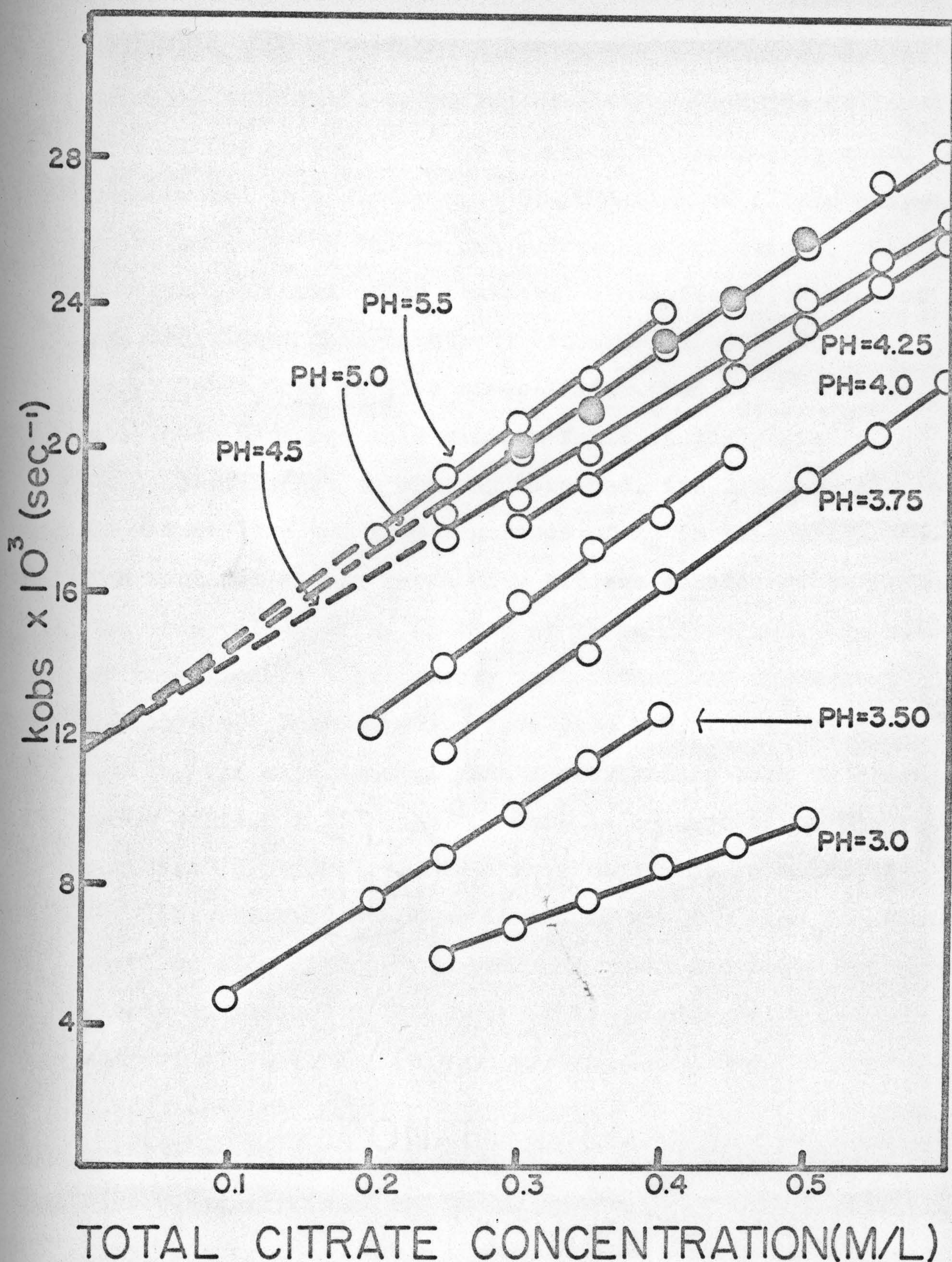

Figure 3. Observed rate of hydrolysis of the species formed from the interaction of glutaric anhydride in citrate buffer at various pH values and different buffer concentration: ● = 1 M KCl, O = H_2O.

effectively as a buffer. Based on the proposed mechanism of relatively rapid formation of citric anhydride followed by a slower hydrolysis, the apparent pH dependency could be ascribed to a slower rate of formation of citric anhydride at lower pH values and buffer concentrations. The mathematical derivation presented above for separation of the individual rate constants requires that $(k_1[B] + k_w)$ be at least four to five times larger than k_4. Since in the lower pH range this minimum ratio in the reaction velocities is not apparently obtained, the limiting slopes do not reflect the true magnitude of k_4 in this range. The terminal slopes above pH = 4.5 are considered to reflect the true values of k_4 in that at these pH values, the rate of formation is considerably faster than the subsequent hydrolysis. In Figure 3, above pH = 4.5, the lines connecting the experimental points extrapolate back to a common value of 11.5×10^{-3} sec^{-1}. This is assumed to be the rate constant for hydrolysis of citric anhydride in water.

The observed decrease in absorbance may also be attributed to slow disappearance of the mixed anhydride (to form citric anhydride) followed by rapid hydrolysis of citric anhydride. A mechanism such as this would require that the glutaric-citric mixed anhydride would be a relatively stable species which would not be expected based on the structure. Direct hydrolysis of the mixed anhydride is also a possibility, but again a stable mixed anhydride would be required. Further work is required to definitely establish which

mechanism is correct, but intuitively the scheme involving rapid formation of citric anhydride followed by a relatively slow hydrolysis would seem the more likely.

Chromatographic Studies.--Although results of rate studies were strongly indicative of formation of citric anhydride species from mixtures of glutaric anhydride and citrate buffers, the data were not considered definite by themselves. Attempts were made to substantiate these findings by direct chemical examination of the reacting systems. The procedure adopted was isolation and quantification of the reaction products resulting from addition of aniline to the anhydride mixture, all anhydride species present presumably reacting extremely rapidly with the strong nucleophile aniline.

Separation of reaction products following addition of aniline to the reacting systems are evident in the chromatogram shown in Figure 4. The particular run contained initially 0.3 molar pH = 5 citrate buffer and 3.95×10^{-5} moles of glutaric anhydride with a trace of dioxane. The reaction was quenched with aniline after 30 seconds and separated by column partition chromatography and analyzed by ultraviolet spectrophotometry as described earlier (3). The double peak ascribed to citric monoanilide probably corresponds to the isomeric forms as previously suggested (3). Elemental analysis, equivalent weight, ultraviolet spectrum and infrared spectrum of the compound corresponding to peak 1 and 2 were determined and found to be

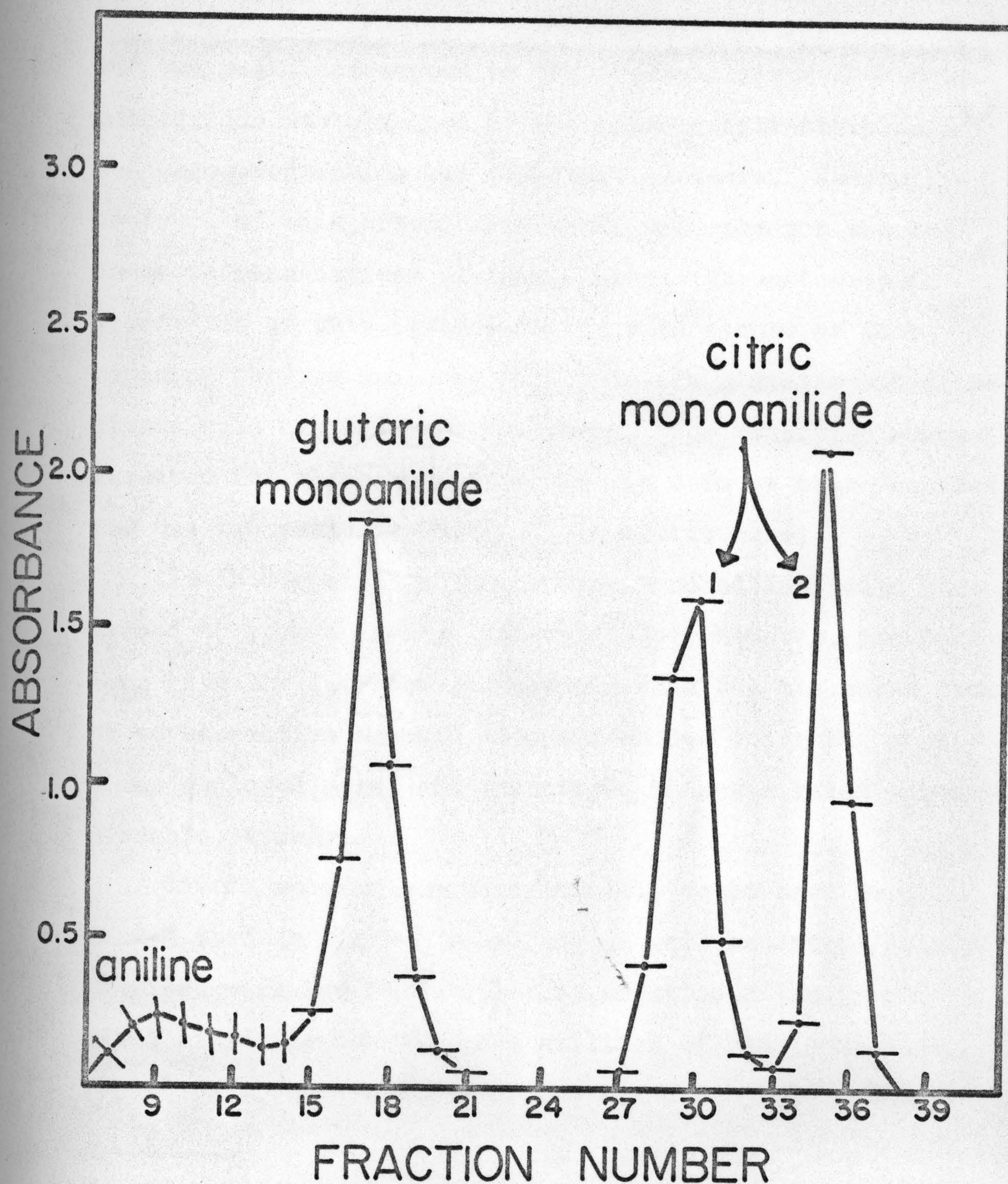

Figure 4. Typical chromatogram for the anilides formed by addition of aniline to the reaction mixture. The reaction was quenched with aniline after 30 seconds. The eluate was extracted with 0.1 N NaOH and the alkaline solution analyzed spectrophotometrically at 241 mu.

compatible with that of citric monoanilide.

Supportive evidence to corroborate the postulate that the two peaks correspond to the isomeric forms of citric monoanilide was obtained by hydrolyzing citranilic acid[3] and chromatographing the resultant products. Random hydrolysis of this citric acid imide will produce the two isomeric monoanilides of citric acid. Chromatographic separation of this mixture in the same manner as that employed for the anilides formed in the glutaric anhydride-citric acid mixture gave two peaks. The retention volume required for these two peaks was the same as that required for the two peaks in the glutaric-citric case.

It is conceivable that citric monoanilide could have formed through a lactone intermediate. However, previous work with the lactones of tartaric acid (4) has shown them to be unreactive towards aromatic amines under the conditions employed here, and therefore, this was ruled out as a possible pathway.

Citric monoanilide theoretically could have been formed through a mixed anhydride as well as citric anhydride. Previous work has shown (5) that addition of aniline to a mixed anhydride usually gave anilides of both compounds. Depending upon the nature of the mixed anhydride, the yield

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3. Citranilic acid was prepared according to Higuchi, *et al.* (3) m.p. 187-188° (literature values vary from 185-189°). Molecular weight determined by direct titration with sodium hydroxide was found to be 253 (calculated 249).

of one of the anilides may be favored over the other. When aniline was added to the reaction mixture where the pH of citrate buffer was 2.0, citric monoanilide could not be isolated.[4] At a pH = 2.0, using 3.08 and 4.74 as the respective first and second ionization constants of citric acid (6), the monoionized and free acid would be present almost exclusively with very little of the di-ionized species present. If the monoionized citrate species can attack glutaric anhydride, and since citric monoanilide was not isolated, it is reasonable to assume that either the mixed anhydride is present in a very low concentration or that glutaric monoanilide is exclusively favored in this system.

An additional system that was tried was glutaric anhydride in a pH = 5.5 acetate buffer. Addition of aniline to this reaction mixture did not produce any acetanilide. This would indicate that this system, like the previous one, hydrolyzes *via* an unstable, mixed anhydride or formation of glutaric monoanilide is exclusively favored. An alternative possibility is that acetate ion serves as a general base in this system.

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4. At pH = 2.0, the fraction of aniline in the protonated form is quite high and therefore a large excess was employed. Isolation of glutaric monoanilide from the reaction mixture indicated that sufficient unprotonated aniline was present to react with anhydrides in the system.

To demonstrate the generality of the reaction, phthalic anhydride was used in place of glutaric anhydride. Phthalic anhydride hydrolysis was followed at 315 mu in citrate buffer. Unlike the glutaric case, there was no apparent anomaly observed in the ultraviolet spectrum indicating that if a new species was being formed in solution, it had less absorbance at this wavelength than the reactants. The rate of hydrolysis, of phthalic anhydride as a function of pH and buffer concentration, is shown in Figure 5. Apparently this system, like glutaric anhydride, is sensitive to the di- and/or tri-ionized species of citric acid. Addition of aniline to the reaction mixture in the same manner as before gave substantial quantities of citric monoanilide. This indicates that phthalic anhydride like glutaric anhydride is capable of reacting with citrate to form a species in solution which has anhydride properties.

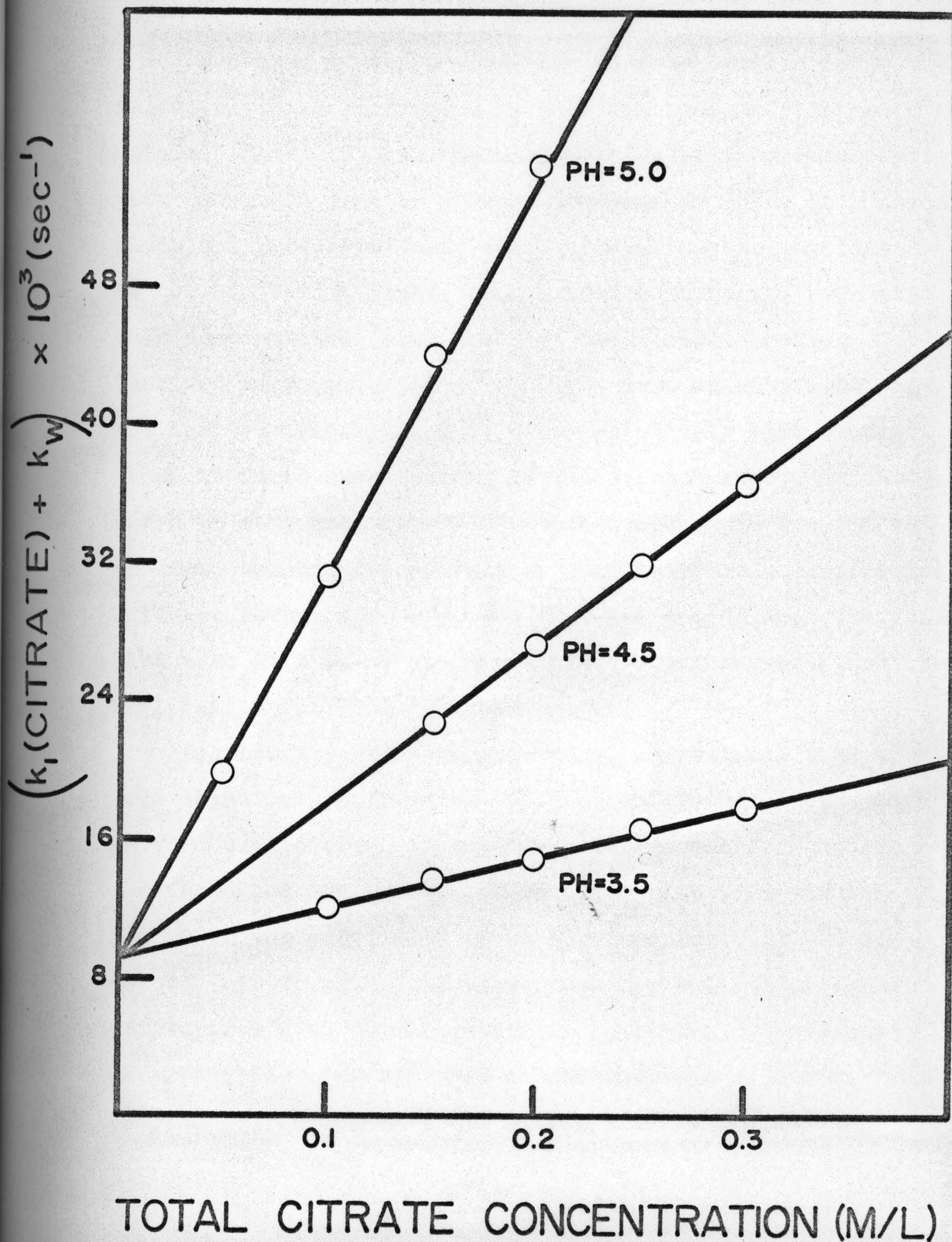

Figure 5. Disappearance of phthalic anhydride at 302 mu as a function of pH and buffer concentration.

DISCUSSION

Reactions of anhydrides with various species under relatively mild conditions to form new compounds is not unique. Flynn (7) has shown that phthalic anhydride reacts with phosphate ions in aqueous solution at 25°C. to produce phthaloyl phosphate which can be stabilized at alkaline pH's. Similarly, acetic and propionic phosphate have also been demonstrated (8). However, the formation of an "Active" carboxylic compound formed from an anhydride and a diionized carboxylic acid under relatively mild conditions is first demonstrated in the present study. Although there is some question as to which compound is the active species, the mixed anhydride or a new anhydride, supportive evidence favors the formation of a new anhydride. The relative ease with which these reactions occur suggests possible implications in biological pathways.

Another important consideration is possible drug loss from solution. A previous publication (3) has shown that polycarboxylic acids in aqueous solution can form active species which are capable of reacting with aromatic amines, and which was postulated to be an anhydride. It was suggested that formation of anhydrides presented a possible pathway for drug deterioration in solution. Very often dicarboxylic acids are used in combinations as buffer components; in light of the findings of this study an

exchange of anhydride character also presents a possible pathway for drug loss from solution. Equally important is the possibility that a myriad of products could result from an anhydride exchange and subsequent reaction with nucleophiles.

EXPERIMENTAL

Equipment and Reagents.--A Cary model 11 M.S. recording spectrophotometer was utilized for the spectrophotometric determinations. All pH measurements were made with a Beckman Zeromatic pH meter with an expanded scale.

Aniline was purified by repeated distillation and was stored under nitrogen prior to use. Dioxane was purified according to Vogel (9). Glutaric anhydride was recrystallized from ether until a m.p. of 56-57°C was obtained. All other chemicals were of analytical or reagent grade.

Procedure for Kinetic Runs on Citric Acid-Glutaric Anhydride Reactions.--Citrate buffers of appropriate concentration and pH were prepared. One hundred microliters of a 0.450 molar glutaric anhydride in dioxane solution were mixed with 6 ml of citrate buffer in a 2 cm photometer cell. The reaction was followed spectrophotometrically at 248 mu. All reaction solutions were equilibrated at 25 $\pm$ 0.1°C prior to use. The concentration of dioxane used in all cases was determined to have a negligible effect on the rate constants.

Procedure for Kinetic Runs on Citric Acid-Phthalic Anhydride Reactions.--Citrate buffers of appropriate concentration and pH were prepared. Fifty microliters of a

0.0338 molar phthalic anhydride in dioxane solution were mixed with 6 ml of citrate buffer in a 2 cm photometer cell. The reaction was followed spectrophotometrically at 302 mu. All reaction solutions were equilibrated at 25 ± 0.1°C prior to use.

Chromatographic Separation of the Reaction Mixture.--The chromatographic columns were prepared as outlined in a previous communication (3). The reaction mixture was prepared by adding 15 ml of citrate buffer to 100 microliters of a 1.58 molar glutaric anhydride in dioxane solution. The reaction was allowed to continue the requisite period of time and was quenched with 4 ml of a 0.33 molar aqueous aniline solution. The pH was adjusted to 3.13 with HCl and a 5 ml sample was placed on the column with 5 gms of silicic acid. The eluting solutions consisted of 100 ml each of chloroform, 1.5% butanol in chloroform, 10% butanol in chloroform and 30% butanol in chloroform; each solution was saturated with the internal phase prior to use. The eluate was collected in 10 ml fractions; the collecting vessel was rinsed with 10 mls of chloroform and added to the 10 ml fraction. Each sample was extracted with 20 mls of 0.1 N NaOH.

Preparative Chromatography of the Reaction Mixture.--A chromatographic column with the following dimensions was employed: length = 60 cm, I.D. = 3.40 cm. Three hundred gms of silicic acid was utilized as the support and 300 mls of pH 3.13 phosphate buffer was employed as the internal

phase. The eluting solutions consisted of 500 mls each of chloroform, 1.5% butanol in chloroform, 5% butanol in chloroform, 15% butanol in chloroform and 35% butanol in chloroform; each solution was saturated with the internal phase prior to use. The reaction mixture was prepared by mixing 100 mls of 0.3 molar pH 5 citrate buffer with 3.2 mls of 1.58 molar glutaric anhydride in dioxane solution. The reaction mixture was quenched at the end of 30 seconds with 16 mls of a 0.33 molar aqueous aniline solution. The pH of the resulting solution was adjusted to pH 3.13 with HCl and 30 mls of this solution was placed on the column with 30 gms of silicic acid. The eluate was collected in 50 ml fractions. The solvent was removed from each sample under reduced pressure and a mixture of chloroform-petroleum ether added to precipitate the compounds.

Characterization of Reaction Compounds.--Glutaric monoanilide: was recrystallized from methanol-water m.p. 129-130° C. Equivalent weight found by direct titration against standard NaOH was 102.5 (calculated 103.5). Known glutaric monoanilide was prepared by adding glutaric anhydride dissolved in dioxane to an aqueous solution of aniline. The sparingly soluble anilide precipitated and when recrystallized gave a m.p. 129-130° C. Both known and unknown compounds gave identical infrared and ultraviolet absorption spectra.

Citric Monoanilide-1: was recrystallized from chloroform, m.p. 136-137° C. Elemental analysis gave C = 53.90, H = 4.94, N = 5.3 required C = 53.94, H = 4.87, N = 5.23. Equivalent weight determined by direct titration against sodium hydroxide solution was found to be 134.5 (calculated 134). Infrared and ultraviolet absorption spectra was characteristic of an anilide. N.M.R. spectrum strongly suggested this to be the symmetrical isomer; 2-hydroxy, 2-N phenyl carbamide-1,3 propane dicarboxylic acid.

Citric Monoanilide-2: was recrystallized from chloroform, m.p. 127-128° C. Equivalent weight determined by direct titration against sodium hydroxide solution was 135 (calculated 134). Elemental analysis gave C = 53.87, H = 4.85, N = 5.20, required C = 53.94, H = 4.87, N = 5.23. Infrared and ultraviolet absorption spectra was characteristic of an anilide. N.M.R. spectrum suggested this to be the unsymmetrical isomer; 2-hydroxy, 1-N phenyl carbamide, 2,3 propane dicarboxylic acid.

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Part 2

Interaction of Acetic Anhydride with Di- and Tri-Carboxylic Acids in Aqueous Solution

INTRODUCTION

In an earlier report (1), experimental evidence was presented which suggested that glutaric and presumably other cyclic anhydrides formed an equilibrium system in the presence of a large excess of citrate buffer in which the total anhydride concentration was distributed among glutaric anhydride, citric anhydride and perhaps glutaric-citric anhydride. An attempt has been made in the present study to obtain a clearer picture of the reacting system by employing acetic anhydride to furnish the initial anhydride concentration; the non-cyclic anhydride not being expected to participate effectively in any reversible process.

If the forward reaction pathway remains essentially the same as in the cyclic anhydride case, a mixed acid anhydride species would be formed by interaction of acetic anhydride and citrate ion. The loss of the acetate in this step would, however, make formation of the mixed anhydride irreversible. The net effect of this would be to drive the reaction to the right. The proposed reaction scheme can be written:[1]

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1. The reaction steps k_w, k_2 and k_4 are considered irreversible, since under the conditions employed here (25° C., aqueous solution) the reverse reaction would be negligible (2).

$$\begin{array}{ccccc} \text{acetic anhydride} + \text{citrate} & \xrightarrow{k_1} & \text{mixed anhydride} & \underset{k_{-3}}{\overset{k_3}{\rightleftharpoons}} & \text{citric anhydride} + \text{acetic acid} \\ \downarrow k_w & & \downarrow k_2 & & \downarrow k_4 \\ \text{acetic acid} & & \text{acetic acid} + \text{citrate} & & \text{citrate} \end{array} \qquad \text{(Eq. 1)}$$

In the present investigation the differences in the characteristics of this irreversible system from those of the previously studied reversible system have been explored.

RESULTS AND OBSERVATIONS

Spectrophotometric Studies.--Although acetic anhydride hydrolyzes rather rapidly in cold water ($t_{1/2}$ = 5 minutes at 25° C), it appears to be capable of reacting with nucleophilic species such as citrate anions. When the hydrolysis is followed spectrophotometrically at 248 mu in citrate buffer, at pH values where there is an appreciable quantity of diionized citrate, a species appears to be formed which has an ultraviolet absorptivity greater than the original anhydride. The absorbance-time profile observed for a system initially containing 1.08×10^{-3} M acetic anhydride in 0.3 M citrate buffer, pH = 5 and 25° C. is shown in Figure 1. The shape of the plot suggests that the reaction involves relatively rapid formation and subsequent hydrolysis of some intermediate species.

As shown previously (1), the concentration of citric anhydride can be expressed[2] as:

$$[CA] = \frac{k_1[B][AA]_o}{(k_1[B] + k_w - k_4)} \left[e^{-k_4 t} - e^{-(k_1[B] + k_w)t} \right]$$

If $(k_1[B] + k_w) \gg k_4$, a semi-logarithmic plot of $(A_T - A_\infty)$ against time (where A_T is the absorbance at any time t and A_∞ is the limiting absorbance) will have a terminal slope

2. The assumptions involved in this derivation are the same as previously reported (1). The reaction step k_1 is considered irreversible since at least a sixty fold excess of buffer was employed at all times.

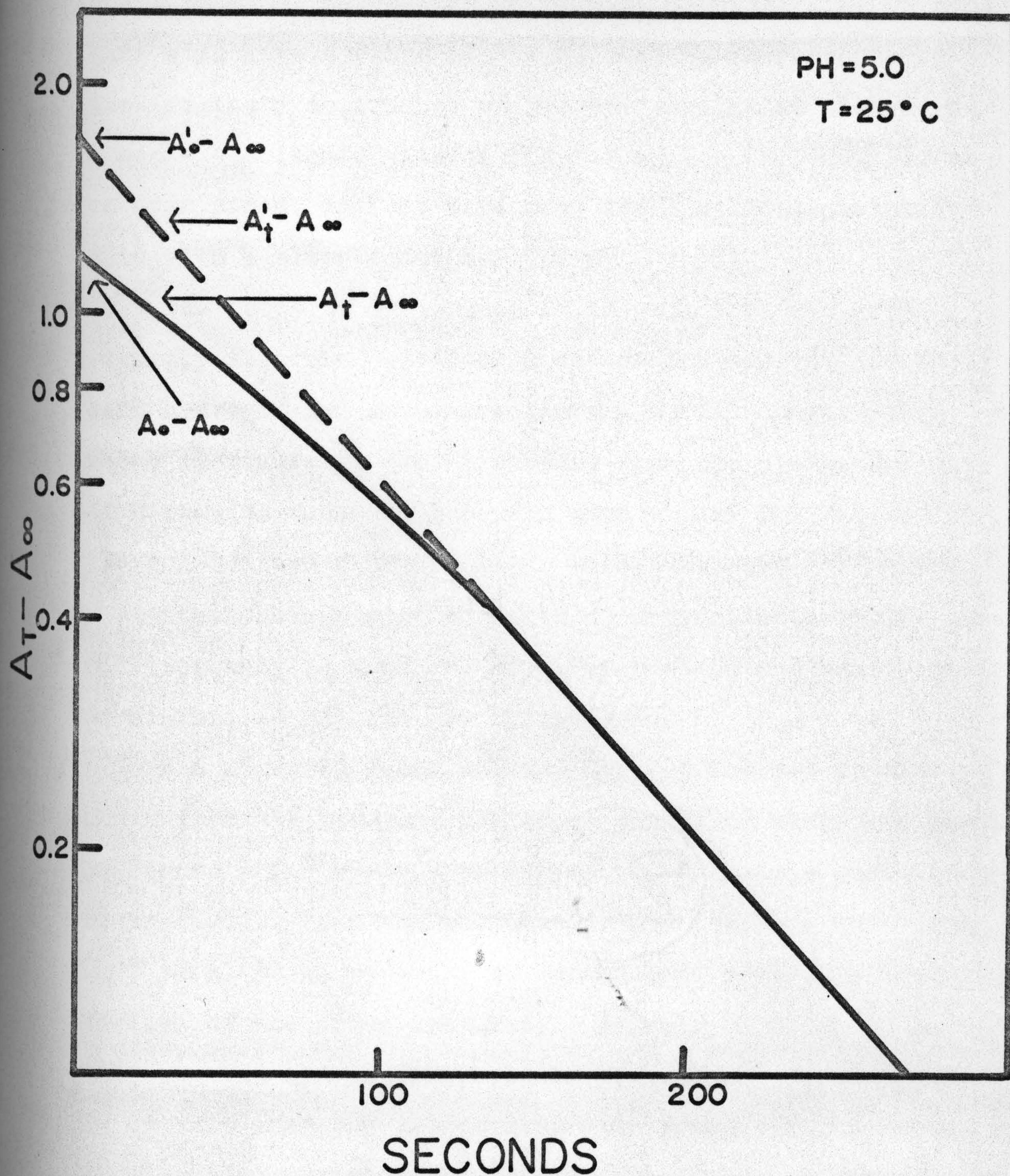

Figure 1. Semi-logarithmic plot of absorbance change at 248 mu for the system acetic anhydride in citrate buffer.

equal to $-k_4$. Extrapolation of this terminal line to zero time will give a value $(A_o' - A_\infty)$ which corresponds to the imaginary absorbance of the new species at time zero. A semi-logarithmic plot of $[(A_T' - A_\infty) - (A_T - A_\infty)]$, at various times, against time will yield a linear relationship with a slope equal to $-(k_1[B] + k_w)$.

The rates of appearance $(k_1[B] + k_w)$ of the more strongly absorbing species in the system are influenced by both hydrogen ion concentration and buffer concentration as shown in Figure 2. As is evident from the plots, the rate of formation appears to depend upon either the di- and/or the tri-ionized form of citric acid. It should be noted here that the apparent transfer of anhydride character was observed only at a pH = 3 or greater where the concentration of di-ionized citrate was appreciable.

The observed rates of loss (k_4) of the new species, found from the terminal slopes as described above are shown in Figure 3 for various pH values and different buffer concentrations. From the proposed reaction scheme (equation -1-) if $k_4 \gg k_3$ we would be essentially observing the rate of loss of the mixed anhydride. Similarly, if $k_3 \gg k_4$, the observed hydrolysis would be that of citric anhydride. It is felt that the latter situation probably exists and that acetic anhydride reacts with the citrate buffer to produce citric anhydride which subsequently undergoes hydrolysis.

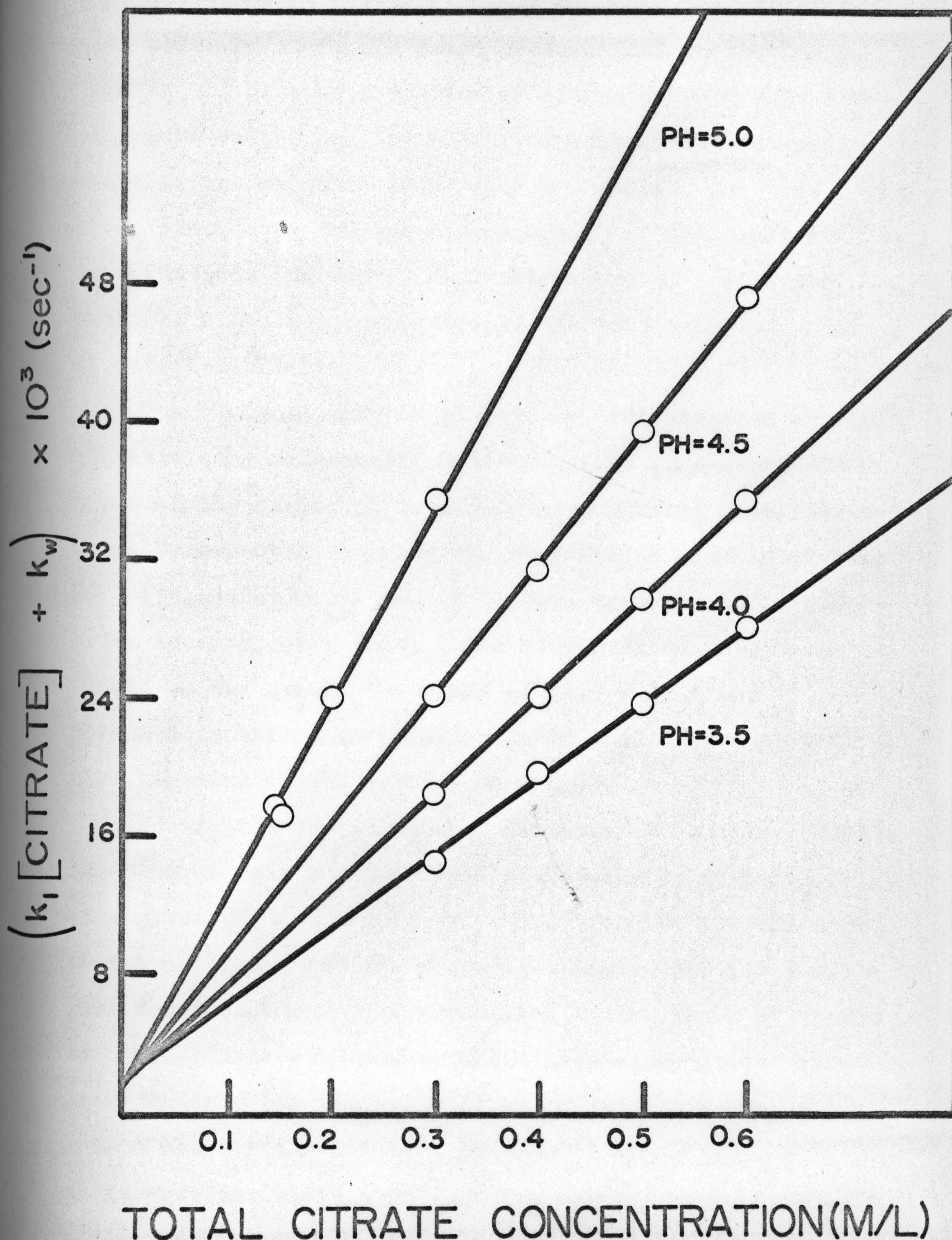

Figure 2. Rate of formation of the species formed from the acetic anhydride-citrate system at various pH values and different buffer concentrations.

The apparent dependency upon hydrogen ion concentration observed in Figure 3 can be explained on the basis that if the rate of formation of citric anhydride is slow at low pH values and low buffer concentration, extrapolations of the terminal slope does not reflect the true hydrolytic rates for the disappearance of the species. It is suggested, therefore, that only above pH = 6 is the terminal slope representative of the true hydrolytic rate of citric anhydride.

The pH dependency is similar to that observed in the glutaric anhydride-citric acid case (1). The extrapolations of the higher pH values to zero buffer concentration would be expected to yield the uncatalyzed hydrolytic rate for citric anhydride. Since in both systems citric anhydride is apparently formed, the extrapolated rate value should be the same. The value of 11.5×10^{-3} sec^{-1} obtained in this investigation is in good agreement with that reported in the earlier work (1).

Apparently the pH values, necessary to obtain a limiting maximum rate constant upon extrapolation to zero buffer at a given pH, are higher (pH = 6.0) for the present case than those found for the glutaric-citrate case (pH = 4.5). This dependence may be rationalized on the basis of steric effects. Since both glutaric and acetic anhydrides have approximately the same rate of hydrolysis in water, we cannot ascribe the observed phenomenon singly to the different sensitivities of the two species to nucleophilic attack.

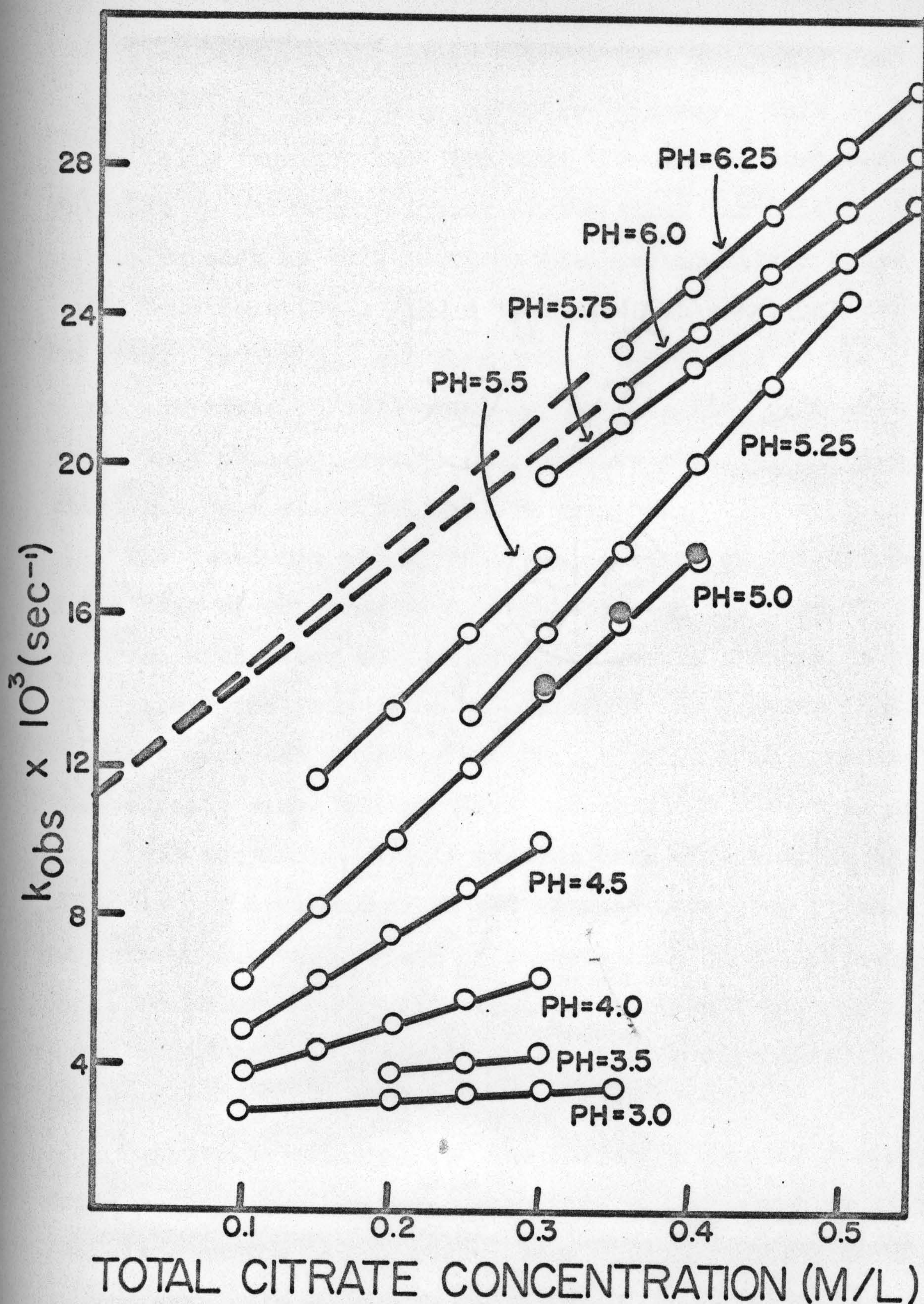

Figure 3. Observed rate of hydrolysis of the species formed from the interaction of acetic anhydride in citrate buffer at various pH values and different buffer concentrations: ● = 1 M KCl, O = H_2O.

As previously mentioned (1) the data presented can also be rationalized by an alternate pathway. This essentially requires that the rate determining step corresponds to the decomposition of the mixed anhydride. A mechanism such as this requires that acetic-citric anhydride be a relatively stable species which seems unlikely. It should be pointed out that direct hydrolysis of the mixed anhydride is also possible, but the slow rate of hydrolysis observed would again require that the mixed anhydride be a relatively stable species.

The catalytic effect of citrate buffer on the hydrolytic rate of the assumed citric anhydride has been observed with other anhydrides. An example of this is the catalytic hydrolysis of acetic anhydride by acetate ions. The most acceptable theory for this catalysis is a general base effect, since nucleophilic attack would result in an identical anhydride (3). A general base effect might be a satisfactory explanation in the citrate case, but it should be pointed out that attack of citrate ions on citric anhydride would produce an intermediate citric-citric anhydride which could undergo hydrolysis at a faster rate than citric anhydride.

Supportive evidence for the formation of citric anhydride rather than the mixed anhydride was obtained by using phthalate as the buffer rather than citrate. Addition of acetic anhydride to phthalate buffer produces a species which can be followed at a wavelength where there is no

interfering absorbance from the acetic anhydride. As shown in Figure 4, a species forms and subsequently undergoes hydrolysis. Applying the same kinetic treatment as was described for the citric case allows for separation of the individual rate constants. Figure 5 shows the rate of disappearance of this species as a function of phthalate concentration and pH. The proposed mechanism for this interaction is relatively rapid formation of phthalic anhydride, followed by a somewhat slower hydrolysis. The apparent pH dependency observed here is explained on the same basis as the acetic anhydride-citrate case i.e. at low pH values and low buffer concentrations the rate of formation is slow and therefore extrapolation of the terminal slope does not reflect the true hydrolytic rate. Above pH = 5.5 the limiting intercepts at zero buffer concentration value giving an uncatalyzed velocity constant of 9.3×10^{-3} $sec.^{-1}$ which is in good agreement with reported work (4).

Because of the sparingly soluble nature of phthalic anhydride it was possible to isolate it from the reaction mixture. Addition of excess acetic anhydride to the phthalate buffer gave a copious precipitate which redissolved upon standing. Isolation and characterization of this precipitate showed it to be phthalic anhydride, lending support to the hypothesis that the mixed anhydride has only a transitory existence in systems containing acetic anhydride and various carboxylic buffers.

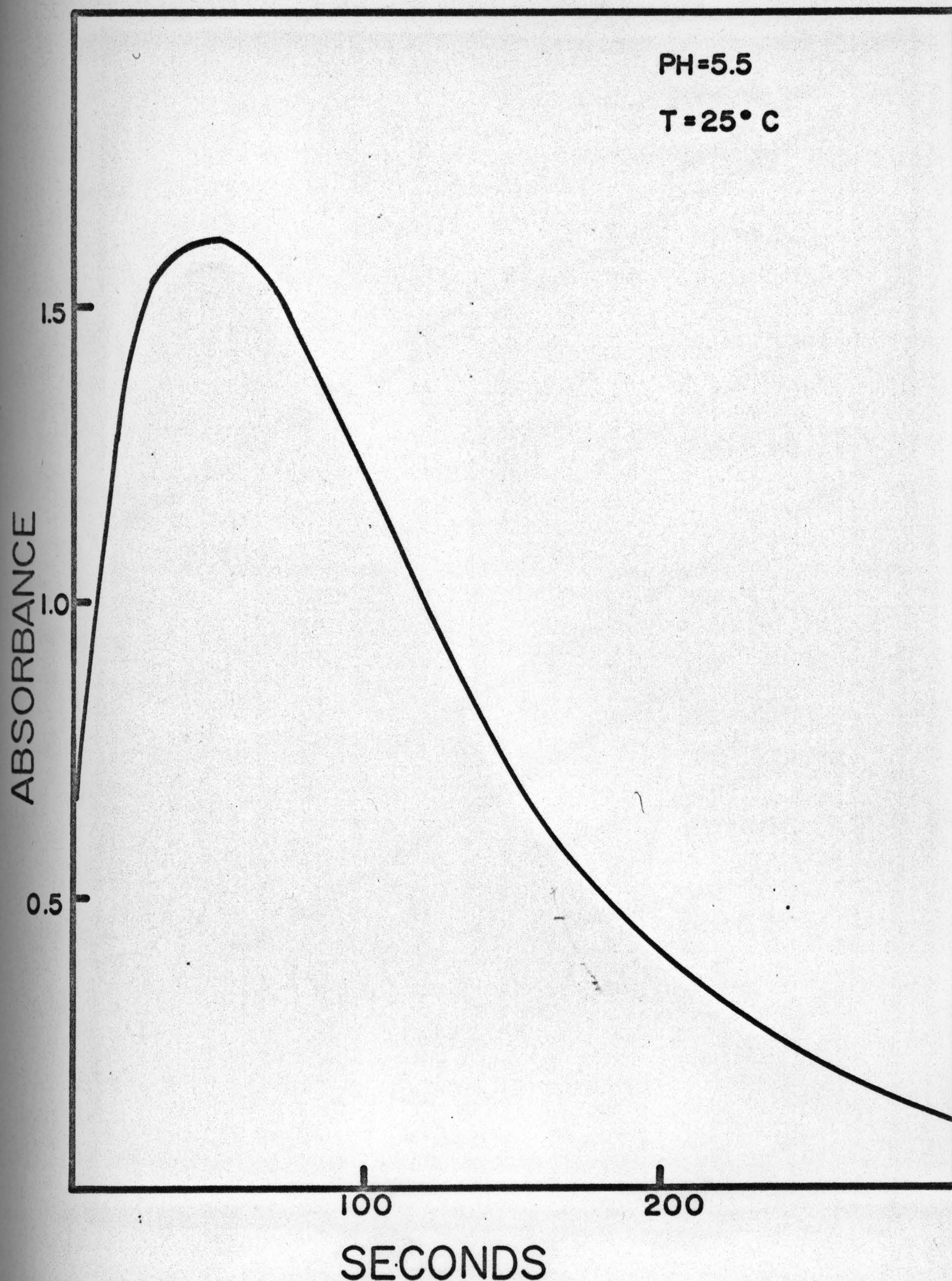

Figure 4. Absorbance-Time profile, for acetic anhydride, initial concentration 4.5 x 10^{-3} molar, in 0.5 molar phthalate buffer. The absorbance change was followed at 315 mu.

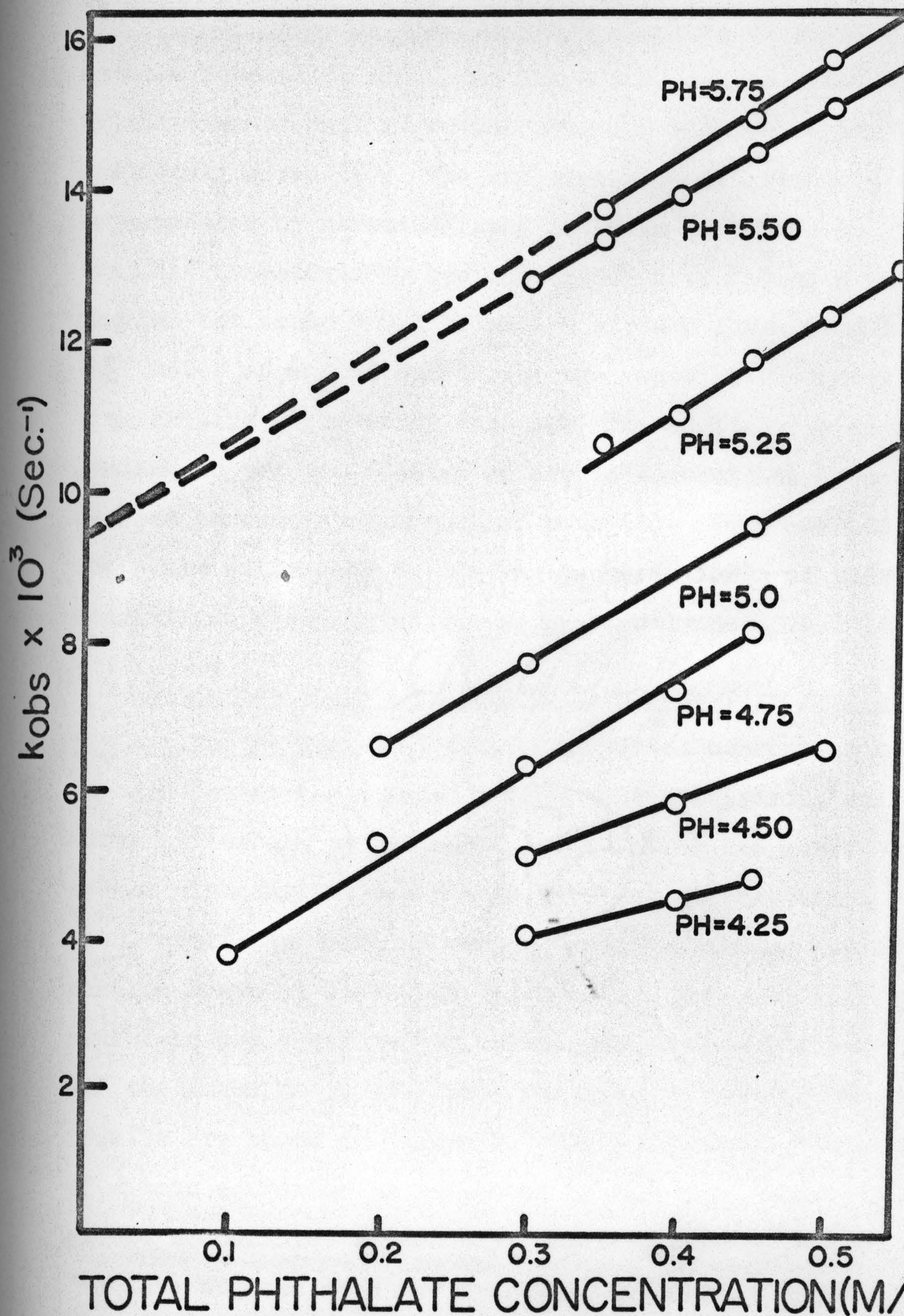

Figure 5. Observed rate of hydrolysis of the species formed from the interaction of acetic anhydride in phthalate buffer at various pH values and different buffer concentrations.

Chromatographic Studies.--The presence of a citrate species possessing anhydride properties can be demonstrated by addition of aniline to the reaction mixture as has been previously noted (5). The anilides that are produced can be separated by chromatography as shown in Figure 6. The typical chromatography run demonstrated in Figure 6 was carried out using a 0.3 M, pH = 5 citrate buffer and 8.0 x 10^{-5} moles of acetic anhydride; the reaction was quenched with aniline after forty seconds. The double peak corresponding to the two isomers of citric monoanilide is again in good agreement with earlier work (5). Verification that the peaks correspond to the two isomeric forms of citric monoanilide was carried out as previously described (1).

Determination of the Citric Anhydride-Time Profile by the Aniline Method.--The citric anhydride concentration in a mixture containing initially only acetic anhydride and citrate buffer can be estimated by quenching the reaction system with aniline and determining the concentration of the total citric monoanilide products. The anilides formed from samples drawn at different times during the course of the reaction were separated by column partition chromatography and the total yield of citric monoanilide determined. The results are shown in Figure 7. The indicated concentration of citric anhydride as reflected by the total yield of citric monoanilide apparently increased initially with time. The data suggest that the hydrolysis of citric anhydride resulted in a decrease in the amount of the anilides formed

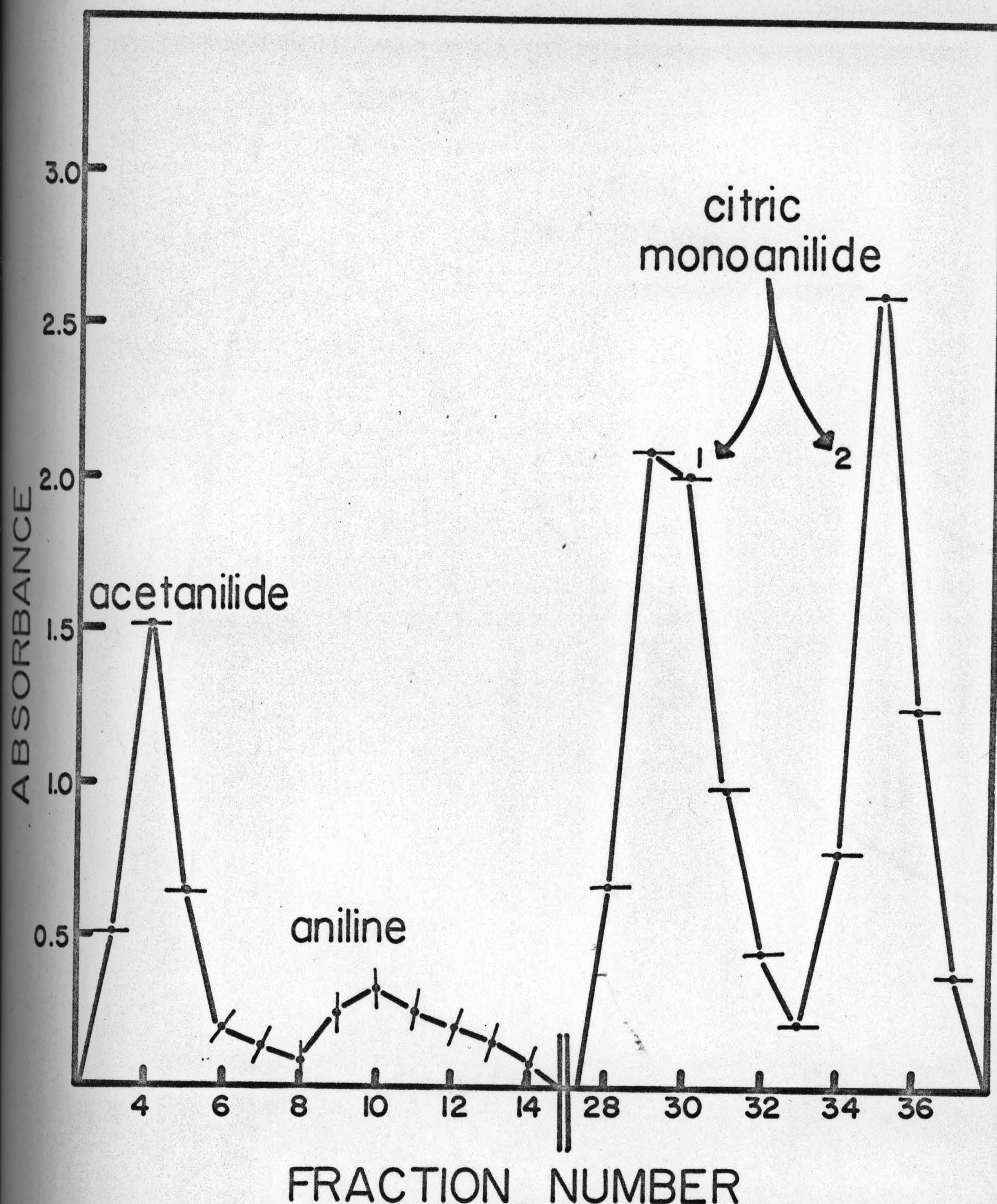

Figure 6. Typical chromatogram for the anilides formed by addition of aniline to the acetic anhydride-citrate system. The reaction was quenched with aniline after 40 seconds. The eluate was extracted with 0.1 N NaOH and the alkaline solution analyzed spectrophotometrically at 241 mu.

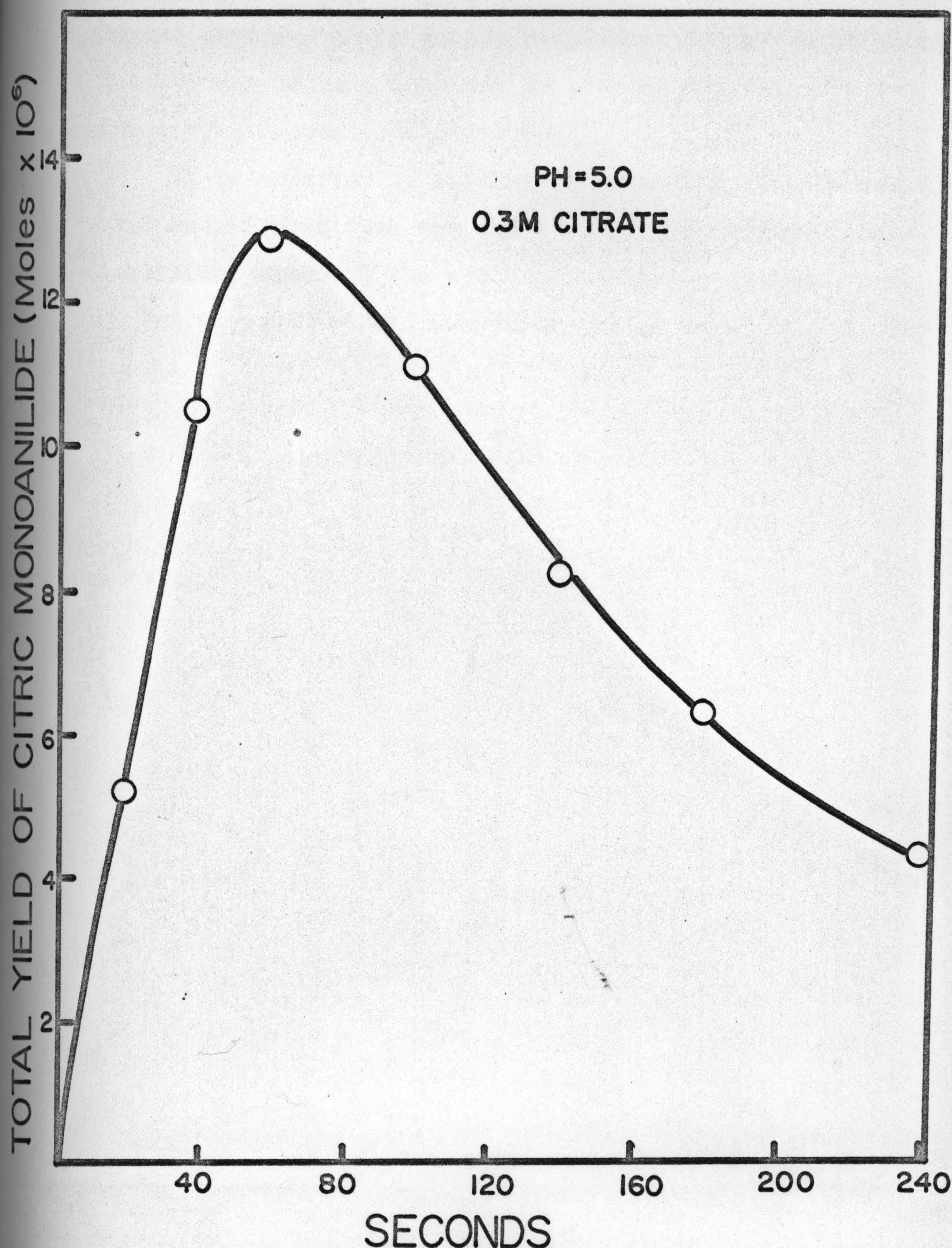

Figure 7. Total yield of citric monoanilide for the acetic anhydride-citrate system. The various points were obtained by withdrawing samples from the reaction mixture at various times and quenching the reaction with aniline. The total citric monoanilide present was then determined.

in the terminal phase of the reaction. The yield of citric anilide was calculated based on a molar absorptivity for the citric monoanilide of 11,000.

It is apparent in this system that the anilide could have been formed from the mixed anhydride or from citric anhydride, although the spectrophotometric evidence presented supports citric anhydride rather than a mixed anhydride.

DISCUSSION

These studies indicate that although the irreversible interactions of acetic anhydride with citrate differ markedly in character from the reversible reaction observed for the glutaric anhydride-citrate system, the apparent kinetic behaviors are surprisingly similar. The primary observation, aside from the irreversibility of the system, seems to be that the acetic anhydride reaction with citrate proceeds at a substantially slower second order rate than that observed for the cyclic anhydride.

The experimental observations again strongly suggest intermediate formation of a reactive citric anhydride species. Although the compound was not isolated in these studies, the data suggests that acetic anhydride can be employed to produce substantial concentrations of citric anhydride which, in turn, can be conveniently employed for synthesis of citric acid derivatives in aqueous solutions.

EXPERIMENTAL

Reagents and Equipment.--Commercial acetic anhydride was purified by distillation. Dioxane was purified according to Vogel (6). Aniline was purified by repeated distillation and was stored under nitrogen prior to use. All other chemicals were of analytical or reagent grade.

All pH measurements and adjustments were made with a Beckman Zeromatic pH meter with an expanded scale.

Kinetic Procedure for the Reaction of Acetic Anhydride with Various Buffers.--Fifty microliters of a 1.08 molar solution of acetic anhydride in dioxane were introduced together with 6 mls. of the buffer into a 2 cm. photometer cell. The reaction was allowed to proceed and was followed directly on a Cary model 11 M.S. recording spectrophotometer. All solutions were equilibrated at 25 $\pm$ 0.1° C. prior to use.

The dioxane concentration employed in these runs was determined to have a negligible effect on the rate constants.

Chromatographic Separation of Reaction Mixture.--The chromatographic columns were prepared as outlined in a previous communication (5). The reaction mixture was prepared by adding 15 mls. of the appropriate citrate buffer to 300 microliters of a 1.08 molar acetic anhydride in dioxane solution. The reaction was allowed to proceed the requisite period of time and was quenched with 4 mls. of a 0.33 molar

aqueous aniline solution. The pH was adjusted to 3.13 with HCl and a 5 ml. sample was placed on the column with 5 gms. of silicic acid. The eluting solutions have previously been described (1).

Blank determinations were made following the same procedure as above using: 1) acetic acid, citrate buffer and aniline solution, 2) acetic anhydride, water and aniline solution.

Preparative Chromatography of the Reaction Mixture.--A chromatographic column was prepared as previously reported (1). The reaction mixture was prepared by adding 100 mls. of pH = 5.0, 0.3 molar citrate buffer to 4 mls. of a 1.08 molar acetic anhydride in dioxane solution. At the end of 60 seconds the reaction mixture was quenched with 27 mls. of a 0.33 molar aqueous aniline solution. The reaction mixture was placed on the column in the previously outlined manner and the eluate was collected and treated as reported (1).

Characterization of Compounds.--Acetanilide: was recrystallized from methanol, m.p. 114-115° C. Commercial acetanilide was recrystallized from methanol and gave a m.p. 114-115° C. A mixture of the known and unknown sample gave no depression of the melting point. In addition, both known and unknown samples gave identical infrared and ultraviolet absorption spectra.

Citric Monoanilide-1: was recrystallized from chloroform, m.p. 137-138° C. Elemental analysis gave C = 54.09, H = 4.92, N = 5.22 (required C = 53.94, H = 4.87, N = 5.23). Equivalent weight determined by direct titration against sodium hydroxide solution was found to be 134.5 (calculated 134). Infrared and ultraviolet absorption spectra was characteristic of an anilide. N.M.R. spectrum strongly suggested this to be the symmetrical isomer; 2-hydroxy, 2-N phenyl carbamide-1,3 propane dicarboxylic acid.

Citric Monoanilide-2: was recrystallized from chloroform, m.p. 127-128° C. Equivalent weight determined by direct titration against sodium hydroxide solution was 135 (calculated 134). Elemental analysis gave C = 53.98, H = 4.92, N = 5.19, required C = 53.95, H = 4.87, N = 5.23. Infrared and ultraviolet absorption spectra was characteristic of an anilide. N.M.R. spectrum suggested this to be the unsymmetrical isomer; 2-hydroxy, 1-N phenyl carbamide, 2,3 propane dicarboxylic acid.

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Section 2

Carbonation of Aqueous Solutions

Part 1

Carbonation of Aqueous Solutions with Acid Anhydrides. Slow Acidification in Homogeneous Systems

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Part 2

Carbonation by Carbonic Anhydrides.

Synthesis and Evaluation of Ascorbic Carbonate, Anhydro Bis-O-Carboxy Tartaric Acid, and Anhydro-O-Carboxy Citric Acid

INTRODUCTION

The basic process of chemical carbonation of aqueous solutions has undergone relatively little change from the classical organic acid-sodium bicarbonate system that has been practiced for generations despite the fact the system has a myriad of problems associated with it. A possible solution to one such problem has been discussed in a recent publication (1) which has suggested the approach of using a latentiated acidifier to generate the organic acid in these systems. The rationale behind this change is based on the fact that in classical effervescent systems, the organic acid dissolves more rapidly than the sodium bicarbonate and therefore the subsequent reaction occurs at the saturated interface of the solid sodium bicarbonate particles. The solid bicarbonate thus serves as a nucleation point for loss of carbon dioxide to the atmosphere. Utilization of a latentiated acidifier would introduce a lag time to allow for dissolution of the bicarbonate particles before the acid portion becomes available, thus allowing for a homogeneous reaction in solution. This leads to a greater degree of supersaturation with respect to carbon dioxide than can be achieved by the conventional additives. Other problems associated with the classical effervescent system which cannot be solved by the above approach include, the saline taste that occurs due to salt formation, rate of

reaction of the carbonating components, and the added weight of the organic acid which is necessary in the carbonation process.

The present study was undertaken to prepare compounds that when placed in water hydrolyze to produce carbon dioxide. This approach to carbonation in addition to having the desirable advantage of producing carbon dioxide in a uniform manner throughout the bulk of the solution, would have the added advantage of elimination of the saline taste that is present in classical effervescent systems. This technique of carbonating aqueous solutions was suggested by Feldman and Foltz (2). Unfortunately their disclosure was limited to a patent on the preparation of anhydro-O-carboxy acids and no experimental evaluations were made.

In the present study three compounds have been synthesized: 1) ascorbic carbonate, 2) bis-O-carboxy anhydride of tartaric acid, and 3) anhydro-O-carboxy citric acid. Bis-O-carboxy anhydride of tartaric acid has been previously prepared (2), and was synthesized at this time only as a reference compound, whereas ascorbic carbonate and anhydro-O-carboxy citric acid presumably have not previously been described. Both ascorbic acid and citric acid have wide pharmaceutical applications and as such could serve as the basis for potential carbonating agents of pharmaceutical preparations.

Davies (3) has reported a method for preparing anhydro-O-carboxy acids from alpha hydroxy acids. The method involves reaction of phosgene (carbonyl dichloride) with the acid, employing dioxane as the solvent. As recently pointed out (2), the process has the disadvantages of long reaction time and low yield of product. It has been suggested (2), that because of the long reaction time, interactions between solvent and reagent can occur to form complexes that interfere with the reaction. The same authors recommend the use of tetrahydrofuran as the solvent to minimize the formation of undesirable side products. Both of these procedures utilize alpha hydroxy acids as the acid precursors. In this study the latter method utilizing tetrahydrofuran was employed for the synthesis.

DISCUSSION

To serve as carbonating agents suitable in aqueous solutions for pharmaceutical purposes, potential carbonating compounds should meet certain criteria. Evaluation of these compounds should be based not only on the physical-chemical properties of the compound, but also on dosage acceptability from a product standpoint.

It is highly desirable to utilize compounds which when placed in aqueous solution hydrolyze to yield physiologically acceptable hydrolysis products. In addition, the hydrolysis products should impart an acid pH to the resultant solution and confer an acceptable taste.

Obviously the total amount of carbon dioxide liberated by hydrolysis should be sufficient to produce a saturated or supersaturated product without employing large quantities of the carbonating agents. Similarly, the ratio of acid to carbon dioxide that is produced on hydrolysis should be such that an excessive amount of acid is not present in solution.

It is evident from the standpoint of patient acceptability and safety that the carbon dioxide acid anhydride species react relatively quickly when dissolved in water. It is suggested that all of the carbonic acid be released within an approximate time limit of one minute. On the other hand, overly reactive compounds would be expected to

present problems during formulation. The hydrolytic half life of potential carbonating agents between one and ten seconds may be expected to meet the above requirements.

It is desirable that the compound be a non-hygroscopic crystalline material.

Evaluation of anhydro-bis-O-carboxy tartaric acid

Hydrolysis of the bis-O-carboxy anhydride of tartaric acid in aqueous solution was evidenced by copious evolution of carbon dioxide upon introduction of the compound into water. The remaining hydrolysis product, tartaric acid, was identified by isolation and determination of its melting point and optical activity of the resulting solution which corresponded to that of the acid. When 100 mg of the solid anhydride was added to 10 mls of water at 0° C, it dissolved quickly to yield an effervescent clear solution. The solution tasted as expected.

The theoretical amount of carbon dioxide that can be generated by the bis-O-carboxy anhydride of tartaric acid is 43.5% by weight. The amount actually found by loss in weight in water corresponded to 43.4% by weight of carbon dioxide.

The apparent half life of hydrolysis determined by spectrophotometric changes in aqueous solution at 25° C was of the order of one to two seconds. This relatively rapid hydrolysis rate, together with the large content of carbon dioxide was reflected in the relative hygroscopicity

of the compound. Upon exposure to an atmosphere of 60 to 70% relative humidity the material became quite sticky, however, if the relative humidity was maintained at lower levels, for example 10%, the compound could be handled quite easily.

Evaluation of ascorbic carbonate

Hydrolysis of ascorbic carbonate also yielded carbon dioxide in large amounts as evidenced by the copious evolution of gas when it was placed in water. The hydrolysis also yielded ascorbic acid as determined by its optical rotation in aqueous solution. Ascorbic acid, like tartaric acid, can be biologically tolerated in large doses. Solutions resulting from the hydrolysis of ascorbic carbonate were acid in nature and possessed an acid taste.

The theoretical amount of carbon dioxide that can be liberated by ascorbic carbonate is 21.8% by weight. The actual per cent by weight of carbon dioxide generated, as determined by the method of Higuchi, *et al.* (1) was found to be 22.0%.

The apparent hydrolytic half life of ascorbic carbonate in aqueous solution at 25° C was found by spectrophotometric change to be of the order of five seconds. This is somewhat slower than the anhydro-bis-O-carboxy tartaric acid, but still within the ten-second time limit. Perhaps because of its lower solubility as well as its

somewhat slower rate of hydrolysis, ascorbic carbonate appeared to be less hygroscopic than anhydro-bis-O-carboxy tartaric acid under similar conditions.

Evaluation of anhydro-O-carboxy citric acid

Hydrolysis of anhydro-O-carboxy citric acid yielded carbon dioxide and citric acid. The theoretical percentage by weight of carbon dioxide is 20.2% for anhydro-O-carboxy citric acid. The actual amount found by loss in weight in water corresponded to 20.0%. The higher molecular weight of the anhydride would make this compound a less efficient source of carbon dioxide than the other two.

The apparent half life of hydrolysis of anhydro-O-carboxy citric acid, as determined by spectrophotometric changes in aqueous solution at 25° C, was of the order of five to six seconds. This compound appeared visually to be the least hygroscopic of all three compounds.

These compounds have been shown to be hydrolyzed at an acceptable rate to a biologically tolerated organic acid. Although anhydro-bis-O-carboxy tartaric acid contains almost double the quantity of carbon dioxide over that of ascorbic carbonate, and anhydro-carboxy citric acid, it appeared to be the most susceptible to moisture. It is felt that all three compounds may find use in pharmaceutical formulations under the proper conditions.

EXPERIMENTAL

Reagents

Tetrahydrofuran was purified by refluxing with calcium hydride for two hours, followed by distillation; only the middle fraction of the distillate was collected.

All other chemicals used were of reagent or analytical grade quality.

Preparation of ascorbic carbonate

Eighty-eight gms (0.455 moles) of anhydrous ascorbic acid was placed in 500 mls of anhydrous tetrahydrofuran. The resulting suspension, which was cooled by means of an ice bath to 0-5° C, was added slowly to 350 gms of phosgene in 250 mls of tetrahydrofuran. The reaction mixture was maintained at 0-5° C for one hour and subsequently allowed to equilibrate to room temperature (15-20°C). The reaction solution was maintained at room temperature for 12 hours and was then subjected to reduced pressure at room temperature to remove excess phosgene and tetrahydrofuran. Addition of chloroform to the amber colored oil gave a white precipitate which was recrystallized from ethyl acetate.

The melting point showed slow decomposition as the temperature was raised above 150° C. Molecular weight found by direct titration against sodium hydroxide solution

was 202 (calculated 202). Elemental analysis gave C = 41.43, H = 2.92 (theoretical, C = 41.58, H = 2.97).

Preparation of anhydro-bis-O-carboxy tartaric acid

One hundred and seventy-five gms of liquid phosgene were added to 200 mls of anhydrous tetrahydrofuran cooled to 0-5°C by means of an ice bath. Two hundred mls of anhydrous tetrahydrofuran containing 31.25 gms (0.208 moles) of anhydrous tartaric acid was then added to the cooled and stirred phosgene-tetrahydrofuran mixture. The resultant solution was maintained at 0-5°C for an additional hour and was then allowed to equilibrate to room temperature (15-20°C). The mixture was maintained at this temperature for 18 hours. The solution was then subjected to reduced pressure at a temperature between 10-20°C to remove excess phosgene and tetrahydrofuran. To the resulting viscous yellow oil was added 50 mls of anhydrous ethyl ether. Upon cooling the solution in a dry ice-acetone bath, a white crystalline solid was obtained, which was washed with cold ethyl ether and dried under reduced pressure.

The melting point was inconclusive in that the material slowly decomposed in the temperature range 125-180°C. Molecular weight determined by direct titration against standard sodium hydroxide solution was 202 (calculated 202). Elemental analysis gave C = 35.52, H = 1.09 (theoretical C= 35.64, H = 0.99). Supportive evidence of

purity was obtained from the loss in weight by carbon dioxide evolution in water.

Preparation of anhydro-O-carboxy citric acid

Fifty gms (0.26 moles) of anhydrous citric acid were dissolved in 200 mls of anhydrous tetrahydrofuran. To this solution, which was cooled by means of an ice bath to 0-5°C, was added 99 gms (1 mole) of phosgene dissolved in 100 mls of tetrahydrofuran. The resultant solution was maintained at 0-5°C for an additional hour and was then allowed to equilibrate to room temperature (15-20°C). The mixture was maintained at this temperature for 12 hours. The solution was then subjected to reduced pressure at room temperature to remove excess phosgene and tetrahydrofuran. To the resultant viscous yellow oil that is obtained was added 75 mls of anhydrous ethyl ether. Cooling of the solution in an acetone-dry ice bath gave copious amounts of anhydro-O-carboxy citric acid.

The melting point was inconclusive in that slow decomposition occurs. Molecular weight determined by direct titration against sodium hydroxide solution was found to be 216 (calculated 218). Elemental analysis gave C = 38.53 , H = 2.76, (required C = 38.53, H = 2.75).

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APPENDIX A

Release of a Drug from a Dosage Form

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APPENDIX B

The Theoretical Formulation of Sustained Release Dosage Forms

The approach most often used in the kinetic treatment of biologic data is that involving formulation of a mathematical model, the comparison of this model with *in vivo* findings, and finally the adjustment of the model and its constants to accommodate the *in vivo* results. Once a suitable model has been established, the complete interdependency of the model's parameters can be examined,[1] subject to the suitability of the model chosen. These parameters may be divided into two types, those under the control of the formulator, i.e., the dosage form, release pattern and rate, etc., and those that are imposed upon the model by the system studied, i.e., the absorption, distribution and excretion (A.D.E.) pattern for the drug in the body. In the past, the major emphasis has been placed on the A.D.E. parameters with relatively little attention being given to the dosage form release rate and pattern, not only because these studies are more difficult to carry out, but also because initially it was of particular concern to study the

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1. Testing of the effect of various parameters in a model can be carried out very effectively on an analog computer, since variables can be changed and the effect read out immediately with a subjective appraisal being made even if complete mathematical solutions are unavailable. Utilization of the analog computer for this purpose is well documented (1, 2, 3), therefore the required computer technology will not be stressed here. The circuits used in this study are given in the appendix.

suitability of various models as simulations of the body's A.D.E. capabilities. It seemed reasonable at that time to assume that in cases where the drug has been administered by injection or orally in some readily available form, e.g., drug in solution, the effect of the dosage form release pattern might safely be neglected; for non-readily available forms, particularly sustained release dosage forms, this assumption cannot be made as the dosage form release pattern and its rate undoubtedly do play <u>major</u> roles in the blood concentration <u>vs</u> time curve obtained, indeed it is the exploitation of this effect that makes sustained release possible. In addition to the rate and pattern of release from the dosage form, consideration must also be given to the effect of the relative amounts of the administered initial and maintenance dose on the resultant concentration in the blood. In the face of the recent barrage of studies supporting the suitability of the simpler kinetic models as descriptions of A.D.E. phenomena, attention should now be paid to those aspects of the kinetic path less amenable to analysis; the dosage form release pattern and administered dose fractions. Indeed, a knowledge of the effects of these controllable parameters is essential in order to formulate sustained release dosage forms having particular blood level characteristics.

Both Wiegand and Taylor (4,5) and Wagner (6) have shown that the percent released vs time data reported in the literature for many sustained release preparations follow apparent first order rate equations. Similarly, others (7,8) have shown that some sustained release preparations release drugs by apparent zero order processes. From an experimental standpoint it would appear that these two mechanisms might adequately describe the rate of release for the majority of existing sustained release dosage forms, and A.D.E. equations involving both of these release patterns have already been described (5,6,9).

In order to obtain a constant blood level for some desired period of time, from a sustained release dosage form, Nelson (10) has stated that a constant (zero order) rate of release from a dosage form is desired and has developed an approximate equation for calculating the amounts of sustained and initial drug forms required, based upon this assertion.

Utilizing essentially the same model as that of Nelson (10) but assuming first order release, Wiegand and Taylor (1) have reported computer drawn curves showing the effect of altering the first order release constant at a constant rate of absorption and elimination. In addition, they have also shown the effect of variation in the elimination constant on the blood concentration curve at a constant first order rate of release from the dosage form and a constant rate of absorption. Computer drawn curves

showing the effect of the fraction of the initial and maintenance dose at a constant rate of absorption, elimination and first order rate of release from the dosage form, have also been described (2), again using the same model.

In a recent paper supporting Nelson's assertion, Beckett and Rowland (9) have further claimed that first order release from a dosage form <u>cannot</u> give the 'idealized' blood concentration-time curve.

Unfortunately experience suggests that the majority of sustained release formulation techniques produce formulations that release drug at roughly a first order rather than zero order rate. In order to adequately compare these two availability patterns as to their potential to produce suitable sustained action forms, a complete investigation of the effects of the various parameters in the models is essential. <u>Part</u> of such a study has been done for the first order release case (1), but to the authors' knowledge a study of the effect of design parameters for a formulation having zero order release characteristics has not been reported.

For both types of release mentioned above, it is desirable to calculate the total (and ratio of initial to maintenance) dose necessary to obtain a blood concentration-time curve most closely approximating the 'idealized' case. Nelson (10) has given a method for calculating the maintenance dose of a constant rate of release dosage form, based

on the biological half life of the drug and the dose required to give the desired blood level, assuming however that the blood level <u>begins</u> at the concentration desired. The assumptions of these equations have been criticized recently (9), but completely corrected equations were not given. For first order release from the dosage form, Wiegand and Taylor (4) have presented equations for calculation of the total dose remaining in a dosage form <u>in vitro</u>. These equations while useful, cannot predict which combination of rate constant, fraction in initial form, and fraction in the maintenance form, will give a particular blood level.

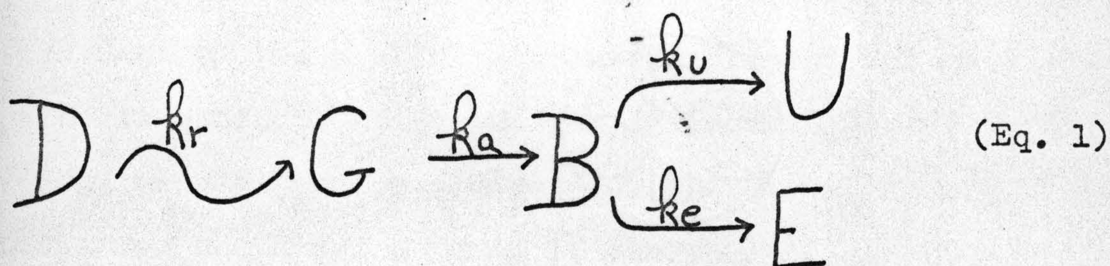
In order to calculate the dosages required, methods must be available to obtain the optimum release rate constant. For zero order release, one author (10) feels it is the product of the elimination constant and the dose required to give the desired blood level, while another (9) feels it is the product of the elimination constant and the desired blood level itself. For first order release, there appears to be no suitable method available for obtaining the desired rate of release constant, or the fractions of initial and maintenance dose required.

In an effort to summarize the work in this area, the present investigation was designed to completely characterize the standard model for sustained release dosage forms. In addition, it is our purpose to report the analog

computer, and where possible, mathematical solutions of the equations describing absorption, distribution, elimination, and availability relationships with the overall goal of devising complete equations suitable for calculation of the doses and rate constants to give a desired blood level based on the type of release pattern employed or available. It will be apparent to the pragmatists among our readers that the ease with which a given type of release can be formulated must always be a consideration and the value of considering only those parameters within the reach of the experimenter will be appreciated.

General Consideration of the Model

In this study, the following model (after Teorell (11)) has been adopted, portions of which have been found to adequately describe actual biological processes.



where D = concentration of drug remaining in the dosage form

C = concentration of drug at the sites of absorption

B = concentration of drug in the fluids of distribution (for purposes of simplicity referred to as blood concentration)

U = concentration of drug in the urine or other permanent drug sink

E = concentration of drug metabolized

kr = rate constant for release of drug from the dosage form, where the superscript 0 and 1 indicate the apparent order of release. The wavy arrow is used with kr to indicate that the precise form of the release is a variable also.

ka = rate constant for absorption

ke = rate constant for elimination of unchanged drug

ku = rate constant for elimination via all other routes.

For purposes of simplicity ke and ku have been combined into one rate constant kd (where kd = ke + ku).

In using a model such as this, a problem arises in the treatment of the various components present from the standpoint of concentrations and compartments. Recent articles (9) have used amounts and concentrations interchangeably, but since the driving force in kinetic equilibria is concentration, amount can be used in its place to describe the kinetic relationships between compartments only when the volumes in each compartment are the same or when the assumption is made that the whole process takes place in the same compartment and volume, Since the one compartment-

one volume idea is a useful and common, but tacit assumption, perhaps a brief explanation is necessary. The concept is more easily understood if the relationship of equation 1 is viewed as a chemical reaction involving four steps and taking place in a single given volume of solution, i.e., a beaker. The model then becomes volume independent; the concentrations obtained have units of moles per unit volume or grams per unit volume; amounts and concentrations are interchangeable. Since the volume in each compartment of the body is different, conversion from concentrations as described by the equations, to amounts in the body compartments, then requires a knowledge of the relative compartment volumes and assumes complete uniformity within each compartment. Such a change is not of concern if fractions of total dose <u>only</u> are to be considered.

In this study the one volume-one compartment idea has been adopted for simplicity and thus the results are subject to the above assumptions. In addition, it is assumed that the equilibria (which must exist) for each component lies far to the right, so that reverse reactions are negligible, the drug is completely absorbed, and that after release it is immediately available.

The concentration of drug at the absorption site at time zero is the initial dose (Di) and is equal to the fraction in the initial or in the immediately available dose (Fi) times the total dose given (W), i.e., drug being

in solution or in some rapidly dissolving drug form. The concentration of drug in the dosage form at time zero is the maintenance dose (Dm) and is that fraction of dose (Fm) required to maintain an optimum and as nearly as possible a constant concentration in the blood for a given length of time times the total dose given (W). It is proposed that release from the maintenance portion of the dosage form can be described by either zero or first order kinetics.

Results and Discussion

Figure 1a illustrates the blood concentration vs time computer curve obtained for an immediately available dose using the model (Eq. 1), based on representative values of ka = 2.0 hr^{-1} and ke = 0.2 hr^{-1}. Figures 1c and 1d are representative blood concentration curves for maintenance forms releasing drug by zero and first order kinetics respectively.[2] Figure 1b illustrates the desired or 'ideal' curve for a sustained release dosage form, which includes both initial and maintenance dose. The design of a suitable sustained action dosage form thus depends on finding the combination of 1a and 1c or 1a and 1d that

2. The computer curves shown in this report were obtained using an Applied Dynamics AD-24-PB computer, a Moseley model 2D-2AM x-y recorder with a type F-1 photo electric curve follower, and an Electro-Instruments model 101-1518 x-y recorder.

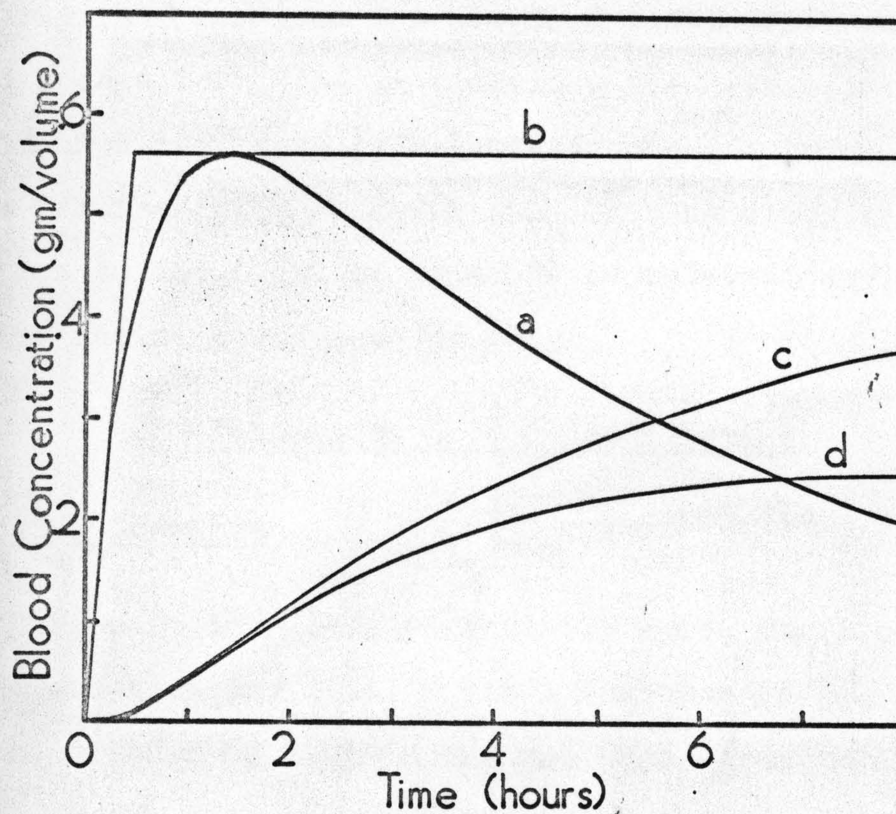


Figure 1. Comparable blood level vs. time curves obtained for a) an immediately available dose, b) an 'idealized' sustained action formulation, c) zero order release maintenance form, d) a first order release maintenance form.

produces the curve 1b, if such a combination exists, or as close an approximation as is possible.

The effect on the blood concentration vs time curve due to variation of the release rate constant as well as the effect of varying Fi and Fm are discussed under the appropriate headings for zero and first order release from the dosage form, and in addition, the mathematical relationships necessary to calculate both the total dose and the required rate constant such that as close an approximation to the ideal as possible is obtained are discussed under their appropriate headings.

Release by Zero Order Kinetics

General Concepts

From an immediately available dose, the blood concentration at any time, t, is a function of ka, ke, and concentration of drug in the gut (Eqs. 1 and 2).

$$Bt = \frac{D_i\ ka}{kd-ka}\ (e^{-kat} - e^{-kdt}) - \qquad \text{(Eq. 2)}$$

where Bt is the concentration of drug in the blood at any time, and all other symbols represent quantities previously defined. The peak concentration and time to arrive at the peak are also functions of these parameters. The equation for the peak time (Tp) being:

$$Tp = \frac{2.3}{ka-kd}\ (\log \frac{ka}{kd}) \qquad \text{(Eq. 3)}$$

To obtain a constant blood level, one suspects, and can show mathematically, that a constant rate of availability from the dosage form is desired and once this desired rate is estimated (kr^0), the required maintenance dose (Dm) may be found as the product of kr^0 and the time over which sustained action is desired (h).

$$Dm = kr^0 \times h \qquad \text{(Eq. 4)}$$

The desired rate of availability (kr^0) can be roughly estimated, from the equations for the model, to be:

$$kr^0 = kd \times Bd \qquad \text{(Eq. 5)}$$

where Bd is the desired blood level. The rational for this estimate can be shown by considering the differential equation for the blood level obtained from a sustained action dosage form having both an initial and a zero-order maintenance form. From the general equations of the model in (Eq. 1) one can obtain the relationship:

$$dB/dt = kr^0 - kd(Bd) - e^{-kat}(kr^0 - ka(Di)) \qquad \text{(Eq. 6)}$$

a constant blood level would require that $dB/dt = 0$ therefore,

$$kr^0 = kd(Bd) - e^{-kat}(kr^0 - ka(Di)) \qquad \text{(Eq. 7)}$$

if ka is very large, i.e., the absorption phase is not at all rate limiting,

$$kr^{0} = kd(Bd) \qquad \text{(Eq. 5)}$$

where Bd is the blood level to which the sustained action is aimed. Note that the assumption made to obtain Eq. 5 is in essence that the blood level equals Bd at time zero ($ka = \infty$). This is of course not the true situation and while the use of equation 5 produces a reasonably flat blood level curve, it is not the desired blood level used in the calculation, but a somewhat higher one even if ka is made very large. Variations in kr^{0} indicating this result are shown in Figure 2 using the absorption and excretion constants of ref (9). The kr^{0} calculated for a Bd = 0.56 is $kr^{0} = 0.096$ (the actual Bd obtained using these values can be seen to be about 0.59).

If the initial dose (Di) is varied while a constant kr^{0} is used, a family of curves such as those in Figure 3 are produced. It would appear that by a proper selection of Di and kr^{0}, a curve corresponding to the 'idealized' one should be possible, but it is not. The 'idealized' curve with a plateau slope equal to zero over the time period required cannot be obtained with a maintenance dose releasing drug in this fashion, although the slope is sufficiently close to zero to be considered 'ideal.' This can be seen in Figures 2 and 3 but can more easily be demonstrated by noting that the derivative of the equation describing the blood level-time relationship (Eq. 6) has a real solution at $dB/dt = 0$ (see Eq. 7),

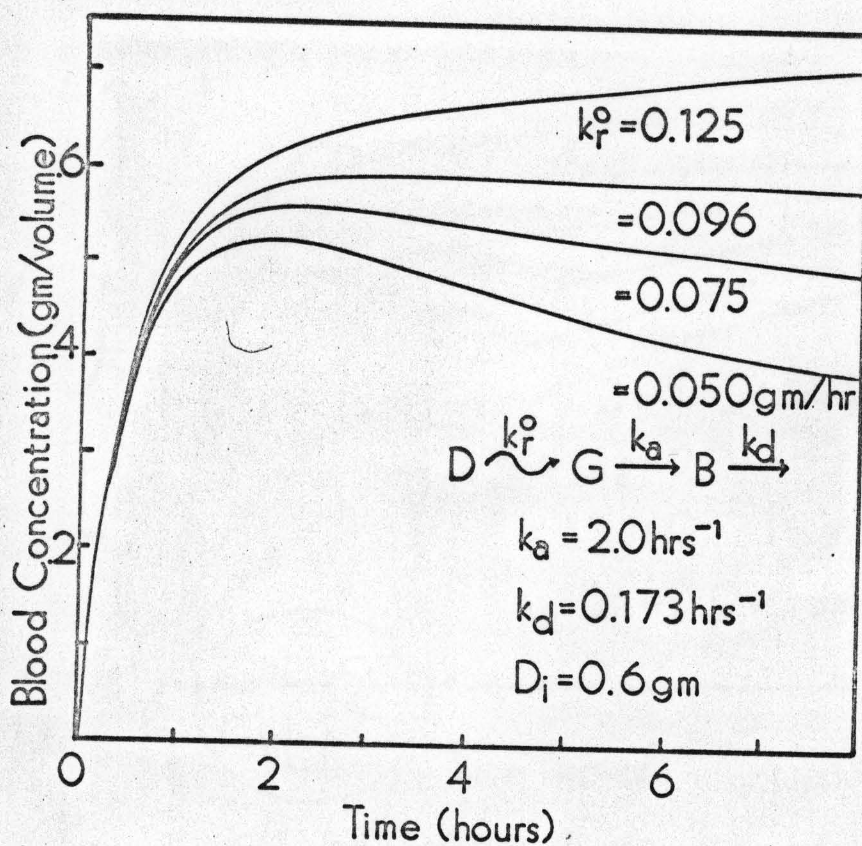


Figure 2. Blood level vs. time curves showing the effect of variation in the zero order release constant (using the A.D.E. constants from reference 9).

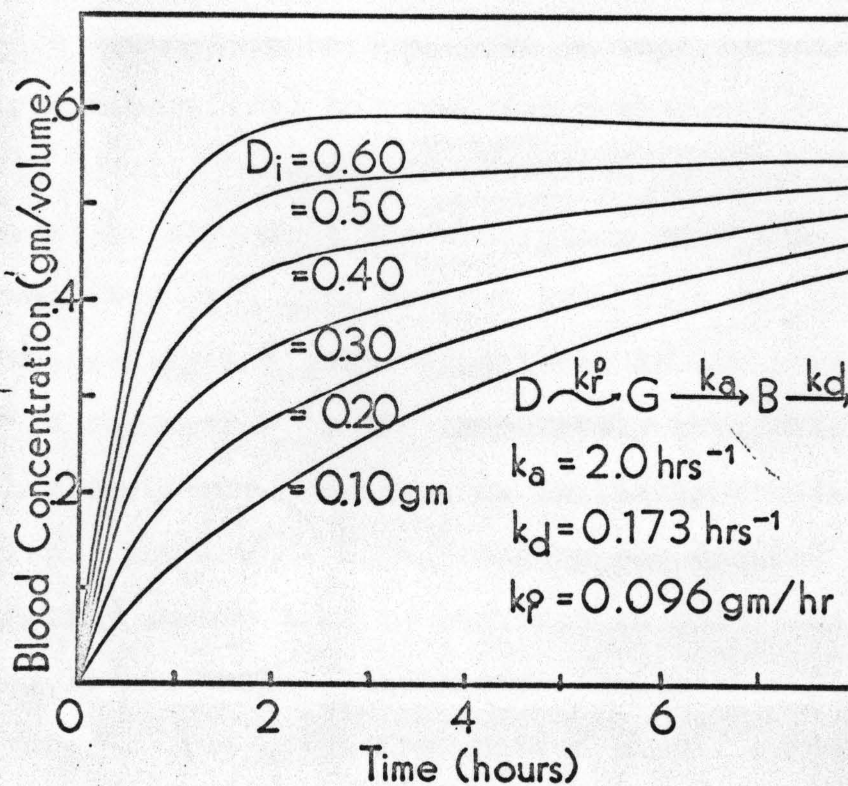


Figure 3. Blood level vs. time curves showing the effect of variation in the initial dose (using A.D.E. constants from reference 9).

denoting a true maximum value for this equation (it is of course different than that of the immediately available dose), unless one is able to assume that the time to reach a <u>maximum</u> blood level was zero (and ka $=\infty$).

The initial dose (Di) of a sustained release preparation cannot be assumed to be identical to that immediately available dose needed (Db) to produce a peak equal to the desired blood level. Because the sustained portion of the dose also provides some drug for absorption over this early interval, too much drug becomes available for absorption and consequently a higher blood level is obtained than is desired. A correction on the immediately available dose is needed then such that less drug is initially available for absorption. While this dose produces the desired blood level, a slightly longer time is required to reach the desired blood level; both of the above considerations are shown in Figure 4. The correction needed should concern the time interval from time zero until absorption of the initial dose is complete, but as mathematically, absorption is never complete, for calculation purposes this may be assumed to correspond to the time to achieve the peak height, and simple subtraction of the quantity yielded by the maintenance dose in this interval produces a suitable correction.

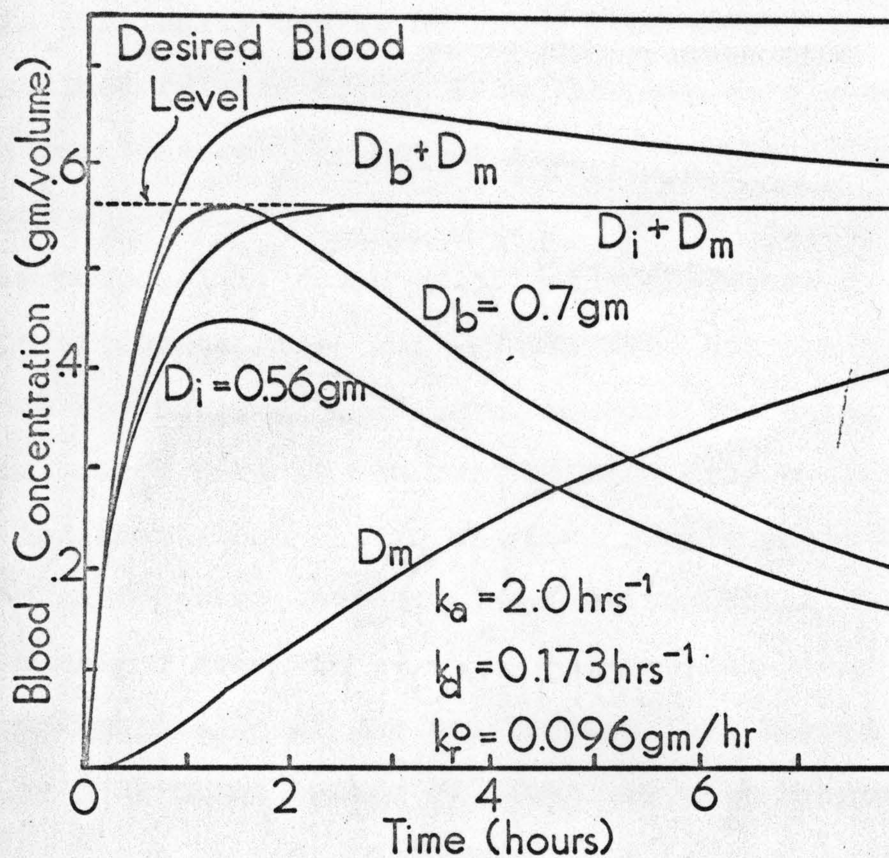


Figure 4. Blood level *vs*. time curves showing the achievement of the desired blood level by adjustment of the initial dose provided. The blood levels that would be obtained for the initial dose required to obtain the desired level when alone (D_b), when in the presence of the maintenance dose (D_i) and those obtained with a maintenance (D_m) dose alone are also shown.

This correction is equal to $kr^0 \times Tp$ where Tp is defined in Eq. 3,[3] so that

$$Di = Db - (kr^0 \times Tp) \qquad \text{(Eq. 8)}$$

This difficulty can be overcome more easily by using a sustained release dosage form that begins its release of the maintenance drug <u>not</u> at time zero, but at the point where absorption of the initial dose is virtually over. This proposal is shown graphically in Figure 5. For this type of dosage form, the initial dose and the time to reach the desired blood level remains the same, since the maintenance dose is not contributing drug over this time period (note that in the previous sample where both started together, adjustment of Db to obtain Di resulted in a slight delay in reaching the desired blood level (see Figure 4). If the maintenance form begins release of drug at times before or after the peak height time, the curve will tend to approach the desired blood level concentration at a rate which is dictated by the release constant of the maintenance form as shown in Figure 6.

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3. This simple method for making the correction is of course only an approximation. The exact calculation would involve the solution of the complete equation for the blood level obtained from a zero order sustained action dosage formulation for the initial dose Di, at some time after the expected peak. As no <u>mathematically</u> flat blood level <u>vs</u> time line is ever obtained using this formulation method, the equation is not soluble explicitly and the approximation given becomes the most desirable method for calculation of the correction.

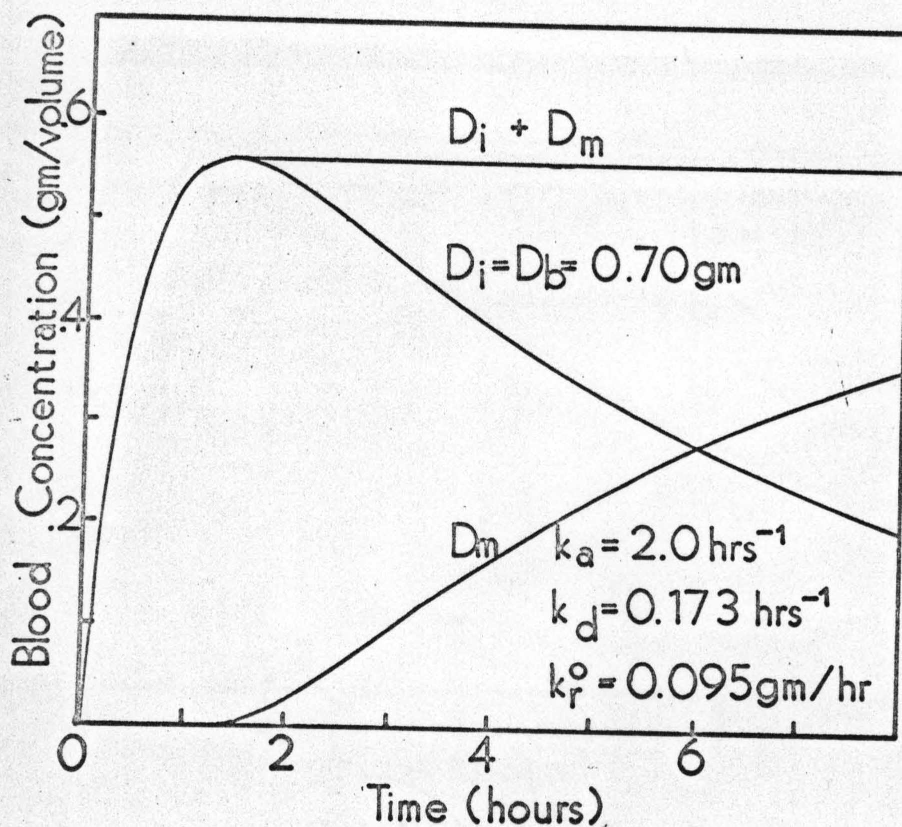

Figure 5. Blood level vs. time curves showing nearly ideal sustained action obtained by a delayed start of the maintenance dose. The blood levels that would be obtained for the initial dose (D_i) and maintenance dose (D_m) are also shown.

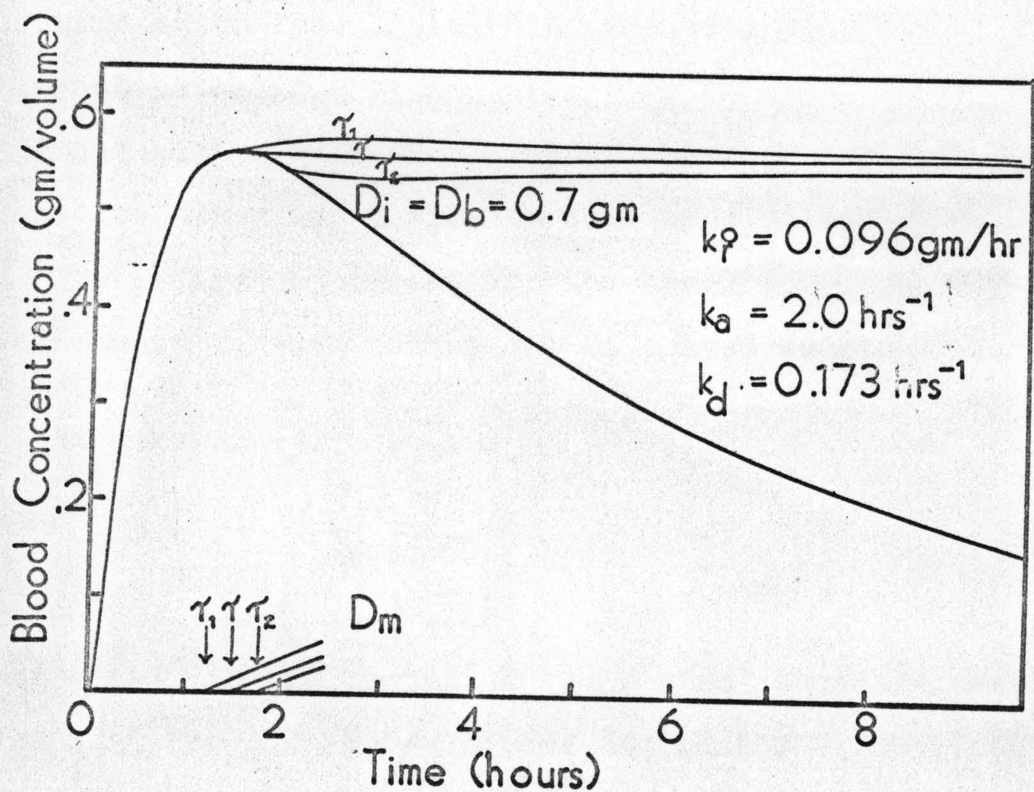

Figure 6. Blood level *vs*. time curves showing the effects of various selections of starting time for the maintenance dose.

While a delayed start appears to be a difficult complication, sustained action medication forms capable of providing it are currently available, for example cored tablets, in which the core is commonly the maintenance dose that does not become available until some time after the initial dose has been absorbed. While not actually designed with this in mind, cored sustained-action tablets of superior action may well be assumed to owe their action to this type of behavior.

The general equations for the blood level vs time relationship in such a case can be solved to yield:

$$Bt = kr^{0}u(t-\tau)\ \frac{1}{kd}\left(1-e^{-kd(t-\tau)}\right) + \frac{1}{ka-kd}\left(e^{-ka(t-\tau)}-e^{-kd(t-\tau)}\right) + \frac{Di\ ka}{ka-kd}\left(e^{-kdt}-e^{-kat}\right) \qquad \text{(Eq. 9)}$$

where $u(t-\tau)$ is the so-called 'unit step' function whose value is 0 for all values of its argument $\leqslant 0$ and +1 for all others. By making this substitution, one can see that before $t = \tau$, Bt describes the expected immediately available dose curve while after $t = \tau$ the maintenance dose adds its effect onto whatever is left at that time. The value of such a dosage form is more apparent from the computer curves than from the equation.[4]

4. In the interests of complete precision, it must be pointed out that the blood level curves obtained from this type of dosage form are not mathematically straight either as absorption of Di is mathematically "eternal." They represent major improvements on Eq. 7 and 8 and also give complete mathematical linearity of Bd only when $ka = \infty$, as discussed later.

Calculation of the Desired Zero Order Rate Constant

Previous publications (for example (10)) have directed that the rate constant necessary for sustained release be set equal to kd times the dose required to produce Bd. This has been criticized recently (9) and as found in this study the criticism is valid. The correct kr^0 is the product of the elimination constant and the desired blood level (Bd).[5] The zero order rate constant necessary can be obtained in another fashion also, utilizing the method of Stelmach, Robinson, and Eriksen (3), where using the desired blood concentration vs time curve as a computer input voltage, the dosage release vs time curve required is produced as an output. The slope of the dosage release curve can be seen to equal to the zero order rate constant required. This was tested using the plot of Figure 5 as an input, with results shown graphically in Figure 7.

The rate constant kr^0 used for Figure 5 had been set equal to 0.096 gm/hr; the slope of the line in Figure 7 calculated by the computer as being required to produce the blood level curve (labeled Di + Dm) in Figure 5, was found to be equal to 0.096 gm/hr.

The linearity of the blood concentration-time curve with a delayed start can be shown by changing the time

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5. The use of the dose required to produce the desired blood level yields the same result as the blood level itself only if the one compartment-one volume model is used and ka is assumed very large. In this case Bd = W.

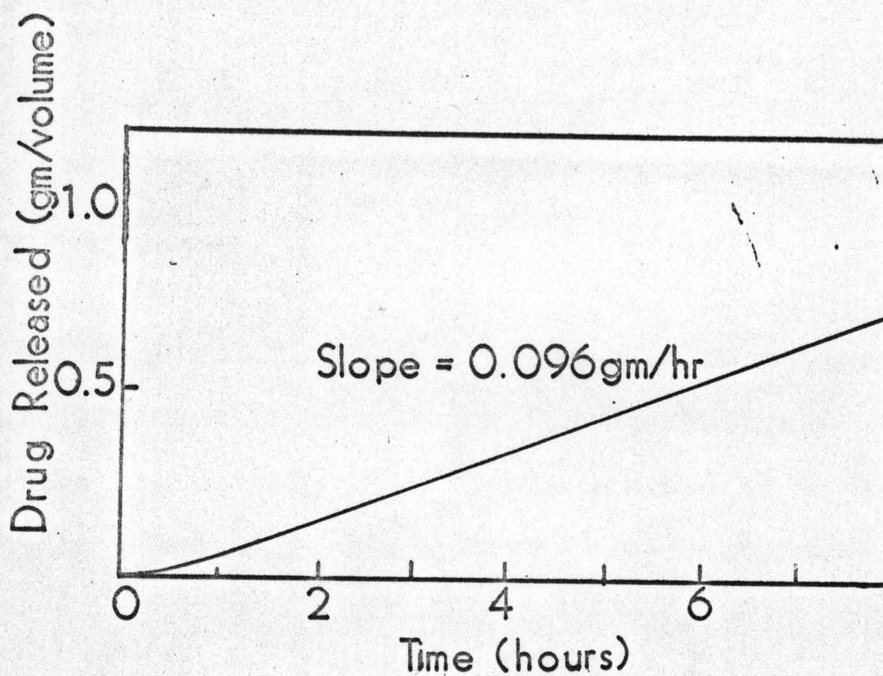

Figure 7. Dose release curve necessary to produce an 'idealized' blood level vs. time curve such as that shown in Figure 5 (labeled $D_i + D_m$).

variable in equation 9 to describe only times <u>after</u> the peak time of the immediately available portion, so that

$$\pi = t - \tau$$

and

$$\text{when } \pi = 0,\ B_\pi = B \text{ peak} = Bp$$

under these conditions, equation 9 becomes,

$$B_\pi = kr^0 \ \frac{1}{kd}\left(1-e^{-kd\pi}\right) + \frac{1}{ka-kd}\left(e^{-ka\pi} - e^{-kd\pi}\right)$$

$$+ \frac{Di\ ka}{ka-kd}\left(e^{-kd(\pi+\tau)} - e^{-ka(\pi+\tau)}\right) \qquad \text{(Eq. 9a)}$$

setting the first derivative of B_π with respect to π equal to zero produces an expression for B_π independent of time (and thus flat), only if $e^{-ka\pi}$ is assumed to be zero ($ka \longrightarrow \infty$) and $ka \gg kd$. Under those restrictions,

$$\frac{Di\ ka}{ka-kd}\ e^{-kd\tau} = kr^0 \left[\frac{1}{ka-kd} + \frac{1}{kd}\right] \qquad \text{(Eq. 9b)}$$

and as the left side of Eq. 9b is an approximation for the desired blood level which is produced by the immediately available dose Di (where Di = Db), one again finds,

$$kr^0 \cong Bpkd \cong Bdkd \qquad \text{(Eq. 5)}$$

Note that the blood concentration after the peak time (B_π) is a constant and identical to the peak blood level if and only if $ka = \infty$, as one might expect.

Calculation of the Total Dose for Release by Zero Order Kinetics

As pointed out previously (10) a dosage form releasing drug at a rate equal to the rate at which drug is eliminated will give a very nearly constant blood level, but differentiation must be made between a dosage form releasing drug from time zero and one releasing drug at the peak height time, in the calculation of total dose.

A) Release from time zero

For a maintenance form releasing drug from time zero, the following equations hold,

$$W = Di + Dm \qquad \text{(Eq. 10)}$$

where $Di = Db - (Tp \times kr^0)$ in this equation $(Tp \times kr^0)$ is the concentration of drug contributed by the maintenance form that represents the correction on the initial or immediately available dose,

and $Dm = kr^0 \times h$

Therefore

$$W = Db - (Tp \times kr^0) + kr^0 \times h \qquad \text{(Eq. 11)}$$

where W = total dose

Di = initial dose

Dm = maintenance dose

Db = dose required to give the desired blood level, when given in an immediately available form

Tp = peak height time

kr^0 = zero order rate of release constant

h = total desired time for sustained action in hours.

B) Delayed start maintenance dose

When the maintenance dose begins release of drug at the peak height time, the equation for total required dose (W) becomes

$$W = Db + kr^{0} \times (h - Tp) \qquad \text{(Eq. 12)}$$

and,

$$Di = Db$$

$$Dm = kr^{0} (h - Tp)$$

where the symbols have the same significance as above.

Release by First Order Kinetics

General Concepts

The relationship between the initial and maintenance dose of an absorption, distribution, and excretion model with first order availability is shown in Figure 8 for various values of ka (and fractions of dose as maintenance form, Fm) at constant kr' and kd. As expected, ka influences the curve very little but primarily <u>before</u> the peak height time; the intersection points remaining essentially in the same place as Fm and ka change. The common intersection point for various fractions of dose has been recognized and reported previously by Krüger-Thiemer and Eriksen (2). Mathematically, the intersection point lies at the peak time (Tp) for the maintenance dose alone, representing a solution of the equation,

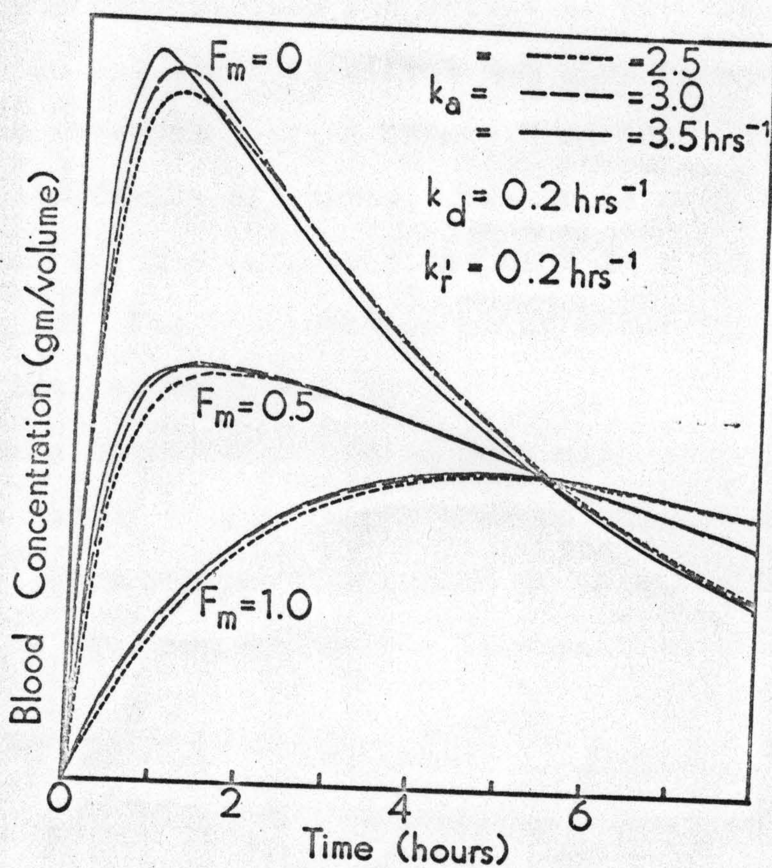

Figure 8. Blood level vs. time curves showing the same intersection point despite variation in absorption rate constant (ka) for several maintenance dose/initial dose ratios.

$$kr' \, (kd-ka) \, e^{-kr'Tp} = kd \, (ka-kr') \, e^{-kdTp}$$

$$+ \, ka \, (kr'-kd) \, e^{-kaTp} \qquad \text{(Eq. 13)}$$

as has also been noted previously (2).

Figure 9 demonstrates the effect of altering kr' at a constant kd and ka; this effect has also been suggested by previous workers (5). An interesting point may be noted in this family of curves, when ka is much larger than kr' and kd, the intersection point is a function only of kr' and kd. The intersection point occurring at later times for smaller values of kr'.

The same observation can be made mathematically by letting ka become enough larger than kd and kr' that its exponential term may be disregarded at an early time and then solving equation 13 for the intersection point of Tp:

$$Tp = \frac{2.3}{kr'-kd} \log \frac{(kr' \, (ka-kd))}{(kd \, (ka-kr'))} \qquad \text{(Eq. 14)}$$

or if ka $\gg$ kd and kr',

$$Tp \cong \frac{2.3}{kr'-kd} \log \left(\frac{kr'}{kd}\right) \qquad \text{(Eq. 15)}$$

It becomes rapidly apparent with the computer that no combination of rate constants and/or doses will produce a flat, constant blood level using a first order availability model. Mathematically, this can be shown also by considering the solution for the maximum of the equation for the blood level produced by our three step model with first order availability:

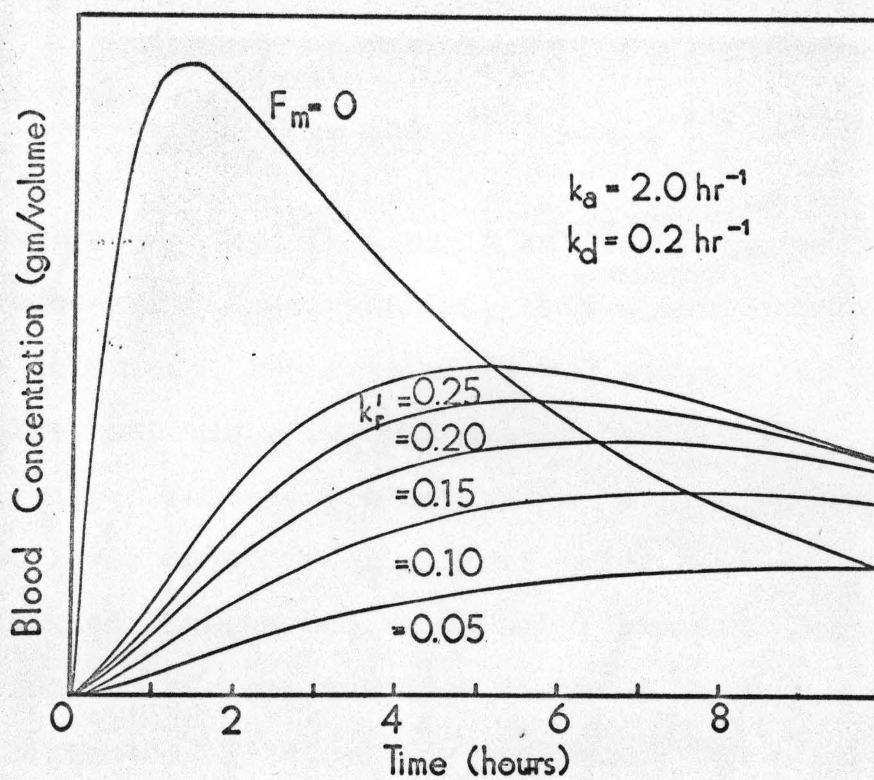

Figure 9. Blood level vs. time curves showing the change in intersection point for various first order release rate constants.

$$Bt = \frac{Dmkakr'}{(kd-kr')(ka-kr')}\left(e^{-kr't} - e^{-kdt}\right)$$

$$+ \frac{Dika - \frac{Dmkakr'}{ka-kr'}}{(kd-kr')}\left(e^{-kat} - e^{-kdt}\right) \qquad \text{(Eq. 16)}$$

(after Wiegand and Taylor (5)), and

$$\overset{o}{B}t = \frac{Dmkakr'}{(kd-kr')(ka-kr')}\left(+kd\ e^{-kdt} - kr'\ e^{-kr't}\right)$$

$$+ \frac{Dika - \frac{Dmkakr'}{ka-kr'}}{(kd-kr')}\left(kd\ e^{-kdt} - ka\ e^{-kat}\right) \qquad \text{(Eq. 17)}$$

Although the actual solution for t at $\overset{o}{B}t = 0$ can only be found by successive approximation, this equation obviously has three solutions, two trivial ($t = 0$ and ∞) and one real; a plot that has a maximum cannot be flat.

A sustained release product having a satisfactorily flat blood level curve using a first order release pattern can be designed however and that design depends upon the proper selection of both the dose fraction in each form and the maintenance dose release constant. The closest approach to the 'idealized' blood level can be found by computer experimentation to require a combination of initial and maintenance dose such that the intersection (Tp, see equations 14 and 15) occurs at a time equal to or greater than the desired sustained action interval (h) (Figure 10). In addition, the further past the desired time for sustained release this point lies, the more combinations of Fi and Fm are available that will give

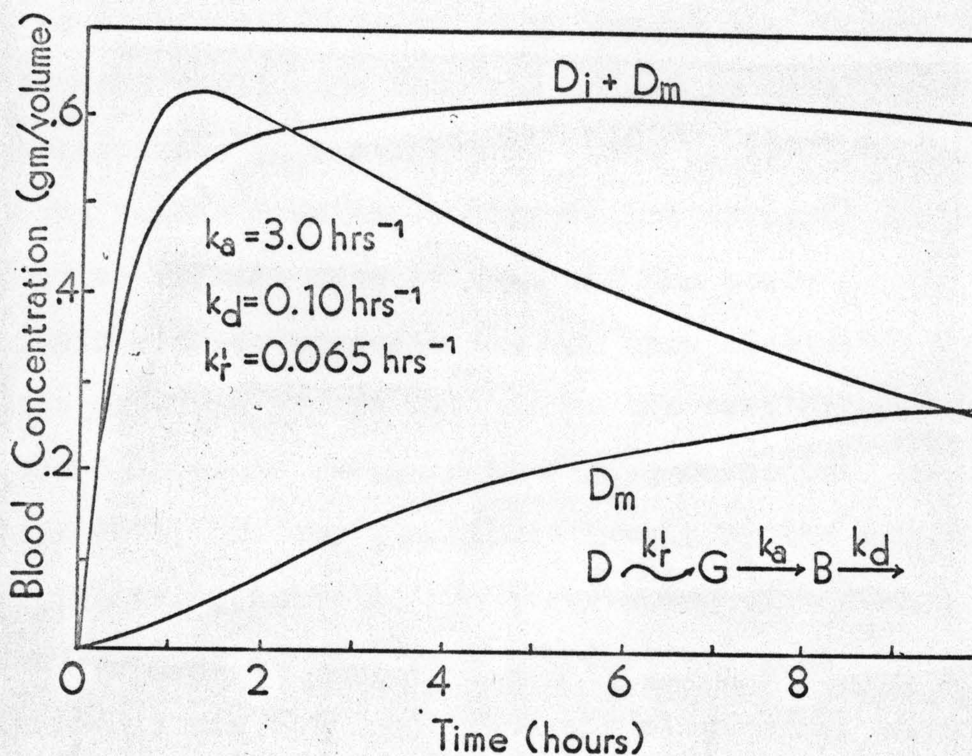

Figure 10. Blood level vs. time curve showing the degree of sustained action obtainable with first order release. The blood levels vs. time curves obtained from the initial (Di) and maintenance (Dm) doses are also shown.

the desired type of blood concentration curve. From the intersection (Tp) equation (Eq. 14) it is observed that the smaller the value of kd the larger the value of kr' may be and still produce a satisfactory dosage form. For cases where kd is large however, the value of kr' necessary to give an intersection point at or beyond the desired time can be very small, and thus the necessary dose present in the maintenance form may become quite high.

The rather remarkable improvement afforded in the zero order release case by delaying the start of the maintenance dose, suggests its use here also with the results shown in Figure 11. As in the zero order case, the blood level is obtained at a rate determined by the initial dose, and the subsequent levels by the maintenance dose. Quite opposite to the observation with first order release started at time zero however, the most "uniform"[6] blood levels are obtained, when the elimination rate (kd) is high, by making kr' high too. The generally "sustained" shape of this curve suggests that the delayed start principle might be extremely useful for sustained release here too, especially for drugs with large elimination (kd) constants.

The general equation for the blood level *vs* time relationship when a delayed start of release and first

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6. A "uniform" or "sustained" blood level in this case represents a non-horizontal blood level oscillating about some average value.

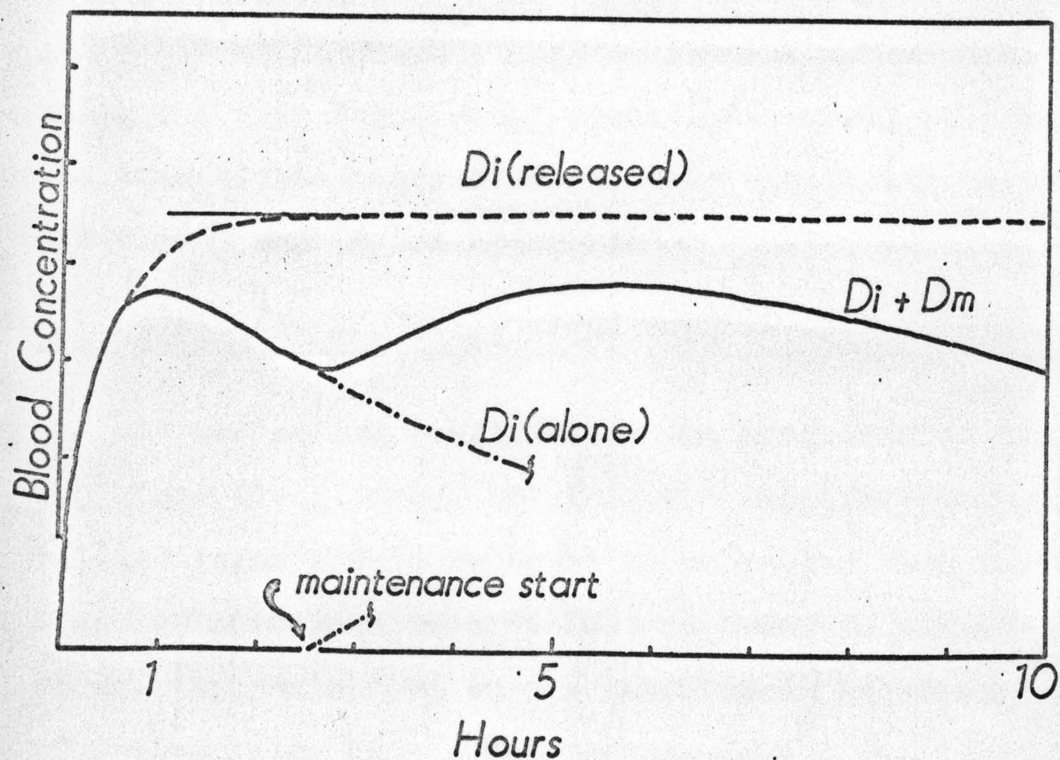

Figure 11. Blood level vs. time curves showing the degree of sustained action obtained by a delayed start of a first order release maintenance dose. Portions of the blood level vs. time curves obtained from the initial (Di) and maintenance (Dm) doses are also shown.

order kinetics is used is,

$$Bt = \frac{Dmkr'}{(ka-kr)} u(t-\tau) \left[\frac{ka}{(kd-kr')} (e^{-kr'(t-\tau)} - e^{-kd(t-\tau)}) + \frac{ka}{(ka-kd)} (e^{-ka(t-\tau)} - e^{-kd(t-\tau)}) \right] + \frac{Dika}{(kd-ka)} \left[e^{-kat} - e^{-kdt} \right] \quad \text{(Eq. 18)}$$

using the same "unit step" concept discussed before and the time of the delayed start. The similarity in form to equation 9 and 16 are apparent.

Calculation of the First Order Rate of Release Constant

It was stated earlier that the intersection points of the blood level curves obtained for immediate and sustained release forms should be equal to or longer than the desired time for sustained release (h), in order to achieve the closest approximation to the 'idealized' blood concentration-time curve when a maintenance dosage beginning immediately is used. As this intersection point depends essentially on kd and kr' (if ka is assumed large) and kd is a parameter over which the formulator has no control, kr' must be altered to move this intersection point to the desired time. With ka and kd given, Eq. 14 (or 15) may be used to solve for the rate constant required, to make the intersection point equal to, or greater than, the desired sustained action time (h). Alternatively as has been suggested for zero order release, calculation of the

necessary first order availability constant can be carried out using the desired blood level curve and the computer method of Stelmach, Robinson, and Eriksen (3).

When a delayed start is used, two approaches to the desired kr' are possible; the maintenance dosage form can provide drug in an approximately zero order fashion (but _actually_ first order) with an overall rate of kdBd, or the peak of the blood level produced by a maintenance dose alone may be positioned at a point between the initial dose peak and the desired sustained action time.

In the first case, the sustained portion should begin releasing at the Tp of the initial portion. The drug released from the dosage form in t hours may be set equal to the drug lost over the same interval assuming constant blood level,

$$Dm\,(1-e^{-kr't}) = kdBdt \qquad \text{(Eq. 19)}$$

and the approximation relating the release rate and the administered dose,[7]

$$kr'Dm \cong kdBd = kr^{0} \qquad \text{(Eq. 20)}$$

results. Using the maximum dose that the patient will swallow or that can be accommodated in the dosage form, kr' can be estimated; for any satisfactory degree of

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7. This is the "rate in equals rate out" equation of Nelson (10) and again implies $ka = \infty$ and its applicability one can also verify by solving the maintenance only portion of Eq. 16 for Bt in the case where ka and kd $\gg$ kr'.

sustained action a first order release form will require roughly 10x the dose required for a zero order form.

In the second case, a more rapid release rate may be used and a later time of onset tolerated producing, in general, a more practical dosage form. Experimentation on the computer suggests that for the A.D.E. constants normally found, the point where 99% of the initial dose has been absorbed is the most suitable onset point for the maintenance dose.

$$\tau = \frac{4.6}{ka} \qquad \text{(Eq. 21)}$$

The kr' required to obtain a maintenance dose generated peak at roughly the midpoint between the initial dose blood level peak and the desired sustained action time (h) may be estimated solving equation 14 or 15 for kr' at

$$Tp = \frac{h-\tau}{2} \qquad \text{(Eq. 21a)}$$

Calculation of the Total Dose for Release by First Order Kinetics

The total dose required for adequate sustained release (W) will again be described by Eq. 10 and may be approximately solved for both methods of first order release.

C) Release from time zero

$$Di = Db - D_{correction} \qquad \text{(Eq. 22)}$$

as before, a correction ($D_{correction}$) for Db is required which may be thought of as equal to the amount of drug contributed to the blood by the maintenance dose, during the phase controlled by the initial dose, and which can be estimated using reasoning similar to that used in deriving Eqs. 19 and 20.

$$D_{correction} = Dm\ (kr'Tp) \qquad \text{(Eq. 23)}$$

Thus we find Eq. 22 to be approximately[8]

$$Di = Db - Dm \times kr' \times Tp \qquad \text{(Eq. 24)}$$

The maintenance dose required to keep the blood level at approximately the desired value can be calculated by equating the desired "rate in" (kdBd) with the actual "rate in"

$$kr'Dm\ e^{-kr't} = kdBd$$

$$Dm = \frac{kdBd}{kr'-(kr')2t} \cong \frac{kdBd}{kr'} \qquad \text{(Eq. 25)}$$

The approximation is sufficiently accurate for most purposes, though the first is better, the time when the desired blood level is reached being used for t.

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8. The approximations made here are similar to those made for the zero order case (see footnote 6). The exact solution is similarly not possible and experimentation convinces us that for practical situations, this approximation is suitable.

The total dose (W) then is,

$$W = Db - Dmkr'Tp + \frac{kdBd}{kr'} \qquad \text{(Eq. 26)}$$

Delayed Start Maintenance

The immediately available dose (Di) completely dictates the attained blood level as described for the case of release by zero order process; but the maintenance dose required for a satisfactorily "uniform" blood level depends on the delay time, τ, and the placing of the maintenance peak.

A precise delay time value is not critical; it may be calculated easily from Eq. 21, and kr' calculated from Eq. 14 or 15 as mentioned before (to place the "maintenance peak" at roughly the midpoint of the desired sustained action time h, Eq. 21(a)).

The maintenance dose required to produce a secondary blood level peak equal to the first may be calculated as the maintenance dose (alone) required to produce a peak sufficient to increase the blood level remaining from the initial dose to the desired value. At the desired time for the secondary peak Tp*, the residual blood level is,

$$Br = \frac{Di\ ka}{ka-kd} \left(e^{-kdTp^*} - e^{-kaTp^*}\right) \qquad \text{(Eq. 27)}$$

and the peak blood level from the maintenance dose is that calculated from equation 16 at $t = (Tp^* - \tau)$ and

$Di = 0$. If it is assumed as before that at the time involved e^{-kaTp} becomes negligible, both these equations can be simplified and Dm easily calculated. Under these circumstances,

$$Br \cong \frac{Di\ ka}{ka-kd} (e^{-kdTp^*}) \qquad \text{(Eq. 28)}$$

Then

$$Dm = \frac{kd}{kr'} (Bd-Br)e^{+kr'(Tp-\tau)} \qquad \text{(Eq. 29)}$$

Figure 10 shows the computer generated blood level produced for the A.D.E. constants used before, calculated for such a delayed first order start. In addition, the drug delivered from the dosage form is also shown to indicate the separation of starting times.

Summary and Conclusions

The general phenomena involved in the A.D.E. kinetics of many drugs are found to be described by rather simple "overall" expressions and these phenomena according to the descriptive equations that happen to fit, i.e. absorption is described as "first order," etc.; while there is little real doubt that such a naive approach is incorrect, the fact that such simple equations CAN adequately describe the concentrations of biologic interest, should be of real use (if not importance) in the formulation of effective sustained action dosages. As discussed in this report, however, even these "simple" equations cannot be mathematically solved to produce explicit solutions for the

dosage fractions and rate constants required, but instead suitable approximations must (and can) be made that permit useful solutions for the single sustained release dose case. These approximations and the assumptions upon which they have been made have not always been explicitly described when (and if) they have been published before and the present authors felt sufficient benefit would accrue from collecting them both in one place that this has been done for the two theoretical cases described before,

1. simultaneous start of initial and "zero order" sustained dosages,
2. simultaneous start of initial and "first order" sustained dosages.

The methods for calculating the dose fractions of both the initial and the sustained portions as well as the rate constant most suitable for producing a sustained blood level are described fully.

Secondarily but of interest in order to complete the picture, the effect of delaying the start of the sustained portion of the dosage form has been shown to produce in one case (3. below) the best theoretical sustained action blood level picture available and in the other (4. below) a novel and perhaps not unuseable blood level situation.

3. initial release followed by "zero order" sustained dosage after some delay

4. initial release followed by "first order" sustained dosage after some delay.

The aim and the result of the analysis in this report has been to show the theoretically available blood level situations resulting from a *single* complete (sustained *and* initial) dosage form designed to produce the most constant blood level over the desired sustained action time and but for the irksome (though real) vagaries of the human gastro-intestinal tract would describe the blood level-time pictures actually observed.

Appendix

Figures 12 and 13 show the computer circuits used in simulating the solutions shown in this work. Of prime concern and utility here is the "non-linear delay input" by programmed relay shown in Figure 12. The remaining programs required (immediate start, zero order, and delayed start, first order) have been published and discussed previously (reference 3).

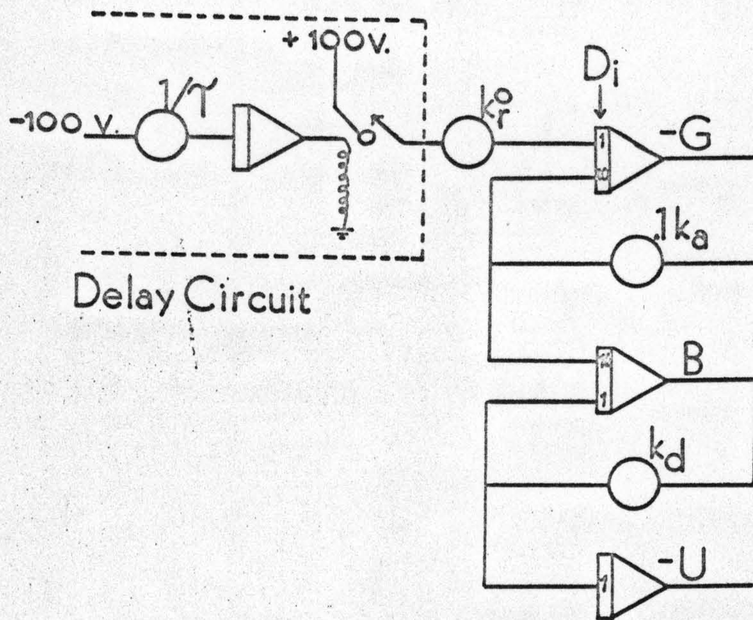

Figure 12. Scaled analog computer program for the system shown in equation 1, using a zero order, delayed start maintenance dose.

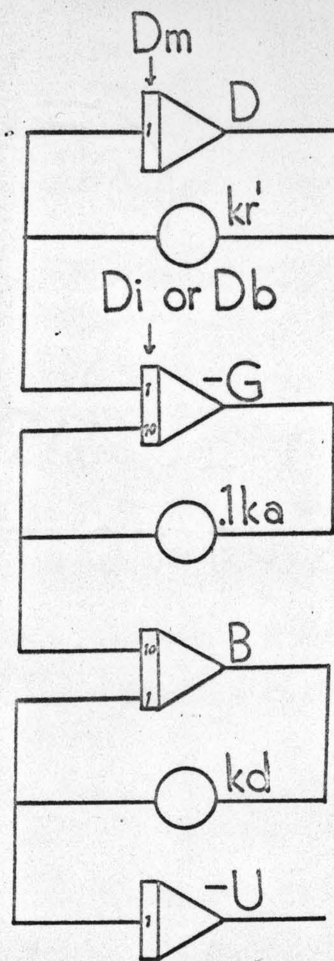

Figure 13. Scaled analog computer program for the system shown in equation 1, using a first order, immediate start maintenance dose.

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SUMMARY

Many organic acid derivatives find wide usage in pharmaceutical systems. A thorough understanding of the chemistry of these compounds is essential for proper utilization for pharmaceutical purposes. The present study has been concerned with: 1) investigation of the interaction between acid anhydrides and polycarboxylic acids in aqueous solution, such as may occur at ambient temperature and under autoclaving conditions; and 2) application of anhydro carboxy derivatives of these acids to pharmaceutical dosage forms.

Anionic forms of succinic and citric acid were found to interact reversibly and rapidly with glutaric anhydride in aqueous solution at room temperature to produce species which undergo subsequent hydrolysis. The species formed from citrate was reacted *in situ* with aniline and the resulting products isolated by column chromatography. Results of the chromatographic study and spectrophotometric observations on the reacting system suggested a mechanism based on the initial formation of a mixed anhydride which cleaved to produce an anhydride of the attacking anionic species. Spectrophotometric investigations have shown that the rate of formation and subsequent hydrolysis of the presumed citric anhydride were dependent upon the pH and the buffer concentration. These interactions were assumed to be highly reversible through intermediate

formation of a mixed anhydride.

Spectrophotometric determinations were also carried out on the above system in which acetic anhydride replaced glutaric anhydride, the reaction in this instance being presumed to be essentially irreversible. The linear anhydride apparently again reacted with citrate ions in aqueous solution to form the presumed citric anhydride species which underwent rapid hydrolysis. The rate of the initial reaction and the rate of the subsequent step depended as expected on the citrate concentration and pH. Results of chromatographic studies on products obtained by reaction with aniline at different phases of the reaction were as expected.

An investigation was also conducted on the possible application of organic acid derivatives such as anhydrides to formulations of effervescent dosage forms. Glutaric anhydride was used as a model latentiated acidifier to aid in achieving a superior degree of carbonation when used with sodium bicarbonate. The physical chemical basis of the formulation depended in this approach on essentially total dissolution of the bicarbonate salt and the latentiated acidifier prior to homogeneous formation of any free carbonic acid. The system achieved a markedly greater degree of supersaturation with respect to carbon dioxide than was possible by the conventional method of dissolving sodium bicarbonate and a solid acid in water.

Another possible application of anhydro carboxylic acids to effervescent dosage forms was based on availability of compounds which when placed in aqueous solution hydrolyze, yielding only carbon dioxide and an organic acid. A similar approach was recently suggested (1) in a patent which appeared subsequent to initiation of this program. Three compounds were prepared for this purpose; ascorbic carbonate, anhydro-bis-O-carboxy tartaric acid, and anhydro-O-carboxy citric acid. The bis-O-carboxy anhydride of tartaric acid has been previously prepared, while anhydro-O-carboxy citric acid and ascorbic carbonate were presumed to be new compounds. The half lives of hydrolysis in aqueous solution of all three compounds were relatively short being of the order of a few seconds. Anhydro-bis-O-carboxy tartaric acid contained the greatest percentage by weight of carbon dioxide, followed by ascorbic carbonate and finally anhydro-O-carboxy citric acid. Evaluation of the compounds, from a pharmaceutical point of view, has shown them all to be potentially useful in pharmaceutical formulations.

The complete interpretation and use of absorption, distribution and elimination data depend upon an understanding of the type and mechanism of release of the drug from the dosage form and then on the ability to utilize this information to predict its effect *in vivo*. An analog computer program suitable for using blood concentration *versus* time data as an input and producing the *in vivo*

dosage form availability *versus* time pattern as its output has been developed, tested on synthetic problems, and used to analyze published blood data for a sustained-release form and a multiple-dosage regimen of sulfaethylthiadiazole and solution, capsule, and tablet forms of acetylsalicylic acid. In addition, the result of a mathematical and an analog computer analysis of the kinetic relationships governing the rate of release of drugs from sustained release dosage forms have been reported. Two types of release have been considered, those described by zero order, and those that can be described by first order kinetics.

(1) Feldman, J. R. and Foltz, R. L., United States Patent #3,218,338 Nov. 16, 1965.