

Synthesis, Isolation, and Some Chemistry of Citric Acid Anhydride

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Abstract □ A crystalline anhydride of citric acid has been produced for the first time. The compound was shown to be the unsymmetrical monomolecular anhydride. Details of the preparation are presented along with some of its chemistry. The rate of hydrolysis of the anhydride was found to decrease over the range pH = 1–6. Possible uses in pharmaceutical formulations are suggested.

Keyphrases □ Citric acid anhydride—synthesis, characterization □ Hydrolysis—citric acid anhydride □ Aniline—citric acid anhydride—reaction products □ Column chromatography—separation □ UV spectrophotometry—analysis

Until recently the cyclic anhydrides of hydroxy polycarboxylic acids such as malic, tartaric, citric, and related acids were only theoretically possible. During the past few years, however, relatively small quantities of solutions of tartaric and malic anhydrides in inert solvents were prepared by the action of thionyl chloride on the silver salts of the respective acids (1, 2). The products however, were never isolated and only little could be learned of the chemical and physical properties of these anhydrides. *In situ* synthesis of these and other anhydrides in aqueous solutions have also been recently reported (2–5).

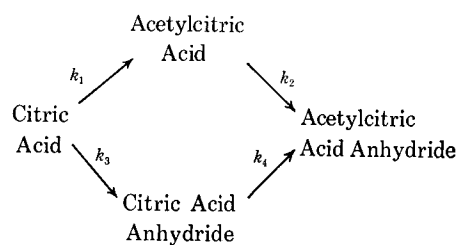
No one seems to have made any concerted attempt to produce anhydrides of hydroxy acids in significant quantities. This is probably due in large part to the fact that the usual techniques for synthesis of anhydrides often results in esterification or elimination of hydroxy groups present depending on the synthetic method used. The present report is concerned with details of successful synthesis, isolation, and determination of some of the characteristics of citric acid anhydride. Some preliminary aspects were reported in an earlier communication (6).

The reaction of acetic anhydride and citric acid has been shown to produce largely acetylcitric anhydride when heated at about 120° for several hours (7). This product would appear to form as the result of two consecutive reactions, one of which is the acetylation of the alcohol group of citric acid while the other is the transfer of anhydride character from acetic anhydride to citric or acetylcitric acid. Depending on which is the more facile step, the synthesis of acetylcitric anhydride from citric acid in the presence of excess acetic anhydride could come about by intermediate formation of either acetylcitric acid or citric acid anhydride as shown in Scheme I.

If the route involving k_3 and k_4 was kinetically preferred, it was considered possible to prepare primarily the citric acid anhydride by judicious choice of reaction conditions such as temperature, concentration of reactants, and reaction time.

EXPERIMENTAL

Experiment and Reagents—A recording spectrophotometer (Cary model 14) was used for UV measurements. NMR spectra



Scheme I

were obtained at 37° (Varian model A-60A spectrometer). A dry box (Lab-Con-Co.) was used for procedures requiring a low humidity environment. The thermometer (Brooklyn Thermo Co. ASTM) used in the cryoscopic determinations was graduated in tenths of a degree with a total range of –8–32°. The length and internal diameter of the chromatographic columns used were 26 and 2 cm., respectively. All pH-stat measurements were done on an autotitrator (Radiometer TTT11) used in conjunction with a titrator (SBR2c), an auto-buret (ABU1b) equipped with a titration assembly (TTA3), and a pH meter (PHM26).

The aniline used was distilled from zinc dust prior to use. All other chemicals used were of analytical or reagent grade and were employed without further purification.

Procedures—Synthesis and Isolation of Citric Acid Anhydride—A heterogeneous mixture consisting of 183 g. (0.953 moles) of powdered anhydrous citric acid, 90.3 g. (1.50 moles) of glacial acetic acid, and 194.4 g. (1.90 moles) of acetic anhydride was heated at 36–38°, with good stirring, in a closed vessel until all of the citric acid had dissolved (19–20 hr.). The solution was then filtered through glass wool. The reaction vessel rinsed with 500 ml. of hot chloroform which was then added to the filtered solution. The resulting solution was stirred and allowed to cool, and within half an hour a viscous oil began to separate. Continued stirring resulted in conversion of the oil to a white crystalline solid which was removed by filtration in a low humidity atmosphere. The precipitate was washed with 700 ml. of warm chloroform and dried for 24 hr. over potassium hydroxide pellets at a pressure of less than 1 mm. of mercury and a temperature of about 45°. The white crystalline solid melted at 120–123° and was shown by NMR spectra and other physical measurements to be citric acid anhydride. The yield of the reaction was about 40%.

Equivalent weight found by direct titration with aqueous sodium hydroxide was 58.3 (calculated = 58.0). The molecular weight obtained from cryoscopic measurements in dioxane was 183 (calculated = 174). Elemental analysis showed 41.09% C and 3.54% H which was in good agreement with the calculated values of 41.39% C and 3.47% H.

Chromatographic Separation of the Products of the Reaction of Aniline and Citric Acid Anhydride—The column was prepared by packing on the bottom of the column a slurry consisting of 4 g. of silicic acid, 6 ml. of chloroform, and 4 ml. of 4 N aqueous sulfuric acid (the purpose of this acid plug was to retain on the column any excess aniline present in the sample). On top of the acid plug was packed another slurry consisting of 30 g. of silicic acid, 45 ml. of chloroform, and 30 ml. of 0.5 M, pH = 3.13, aqueous sodium phosphate buffer. The sample solution was prepared by mixing 100 μl. of a 1.0 M solution of citric acid anhydride in tetrahydrofuran with 10 ml. of 0.16 M aqueous aniline. The resulting solution was acidified to pH = 3.13 and 5 ml. of the solution was mixed with 5 g. of silicic acid and 8 ml. of chloroform. The resulting slurry was then packed on the column and eluted with the following volumes of water-saturated eluants:

0–110 ml. 0.0% butanol in chloroform
110–220 ml. 1.5% butanol in chloroform

220–390 ml. 10.0% butanol in chloroform
390–550 ml. 30.0% butanol in chloroform

The eluate was collected in 15-ml. fractions and the absorbance of each was determined at 265 $m\mu$ using water-saturated chloroform as the reference solution.

Cryoscopic Determination of Molecular Weight—The cryoscopic measurements were carried out according to a routine procedure (8). Dioxane was the solvent chosen since it does not react with anhydrides and the freezing point and molal freezing point depression factor were suitable. A solution containing 1.50 g. of citric acid anhydride in 8.36 g. of dioxane exhibited a freezing point depression of 4.78°. Appropriate calculations based on the molal freezing point depression constant of 4.9°(9) gave an apparent molecular weight of 183 (calculated = 174).

Kinetic Measurements of the Hydrolysis of Citric Acid Anhydride in Aqueous Solutions—*Spectrophotometric Measurements*—One hundred microliters of 0.55 *M* citric acid anhydride in tetrahydrofuran was added to 15 ml. of the desired aqueous solution (previously equilibrated at 25 ± 0.1°) in a spectrophotometer cell fitted with a stopper. Mixing was accomplished by rapid inversion of the closed cell, taking care not to disperse air bubbles throughout the sample. The sample was then quickly placed in the cell compartment and the absorbance changes were followed at wavelengths of 230 to 250 $m\mu$ depending on the absorbance of the aqueous solution used.

pH-stat Measurements—Kinetic measurements were carried out using aqueous sodium chloride solutions of the desired ionic strength as the solvent and either 0.2 or 0.4 *M* sodium hydroxide solutions adjusted with sodium chloride to an ionic strength equal to that of the solvent. The solutions were equilibrated at 25 ± 0.1° in a water-jacketed cell. Various quantities of titrant were added to the solvent solution before the addition of the sample in order to allow the neutralization of the free acid present. This was necessary for the pH-stat to “catch-up” with the reaction. The sample solution, which was 0.5 *M* citric acid anhydride in tetrahydrofuran, was added to the solvent in quantities of 0.2–0.5 ml. by use of a hypodermic syringe.

Measurements Using pH-stat and Spectrophotometer Jointly—This technique was employed in the region of pH = 2.4–5.0. The rates were determined spectrophotometrically utilizing the pH-stat to maintain pH. Details will be presented in a later publication.

RESULTS AND DISCUSSION

Synthesis and Isolation of Citric Acid Anhydride—Synthesis and isolation of citric acid anhydride was attempted first by mixing equimolar amounts of citric acid and acetic anhydride and heating the mixture at 60–80° until a homogeneous solution was formed. NMR spectrum of the resulting solution diluted with deuterioacetone indicated the presence of a large amount of acetylcitric anhydride and lesser amounts of citric acid and another species suspected to be a citric acid anhydride. When temperatures of 35–48° were used total dissolution of the citric acid failed to occur over a period of several days.

Since the appearance of any new anhydride species in such a system would be subjected to second-order kinetics, the concentration of acetic anhydride was progressively increased until a 2:1 molar ratio of acetic anhydride to citric acid was reached. It was apparent from NMR spectra of the reacting mixture at various times that initially the citric acid anhydride species was being formed but with the continued heating necessary to effect total dissolution of the citric acid, acetylation of the anhydride was occurring and was resulting in appreciable quantities of acetylcitric anhydride. The initial reaction thus appeared to be dissolution controlled. In an attempt to increase the solubility of the citric acid, various amounts of glacial acetic acid were added to the reaction mixture. It was found that an approximately 2:1:1 molar ratio of acetic anhydride, citric acid, and glacial acetic acid, respectively, when mixed together and heated at 35–40° with stirring in a closed container until dissolution had occurred resulted in the production of mostly citric anhydride. Precipitation of the anhydride with chloroform, washing the filtered material with the same solvent and drying resulted in the isolation of citric anhydride with apparently little contamination.

Characterization of Citric Acid Anhydride—Although elemental analysis, equivalent weight obtained by direct titration of aqueous

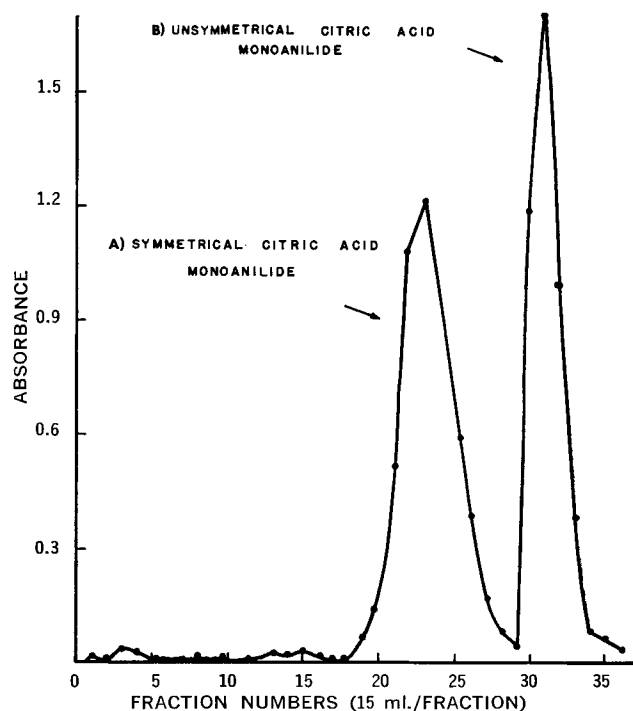
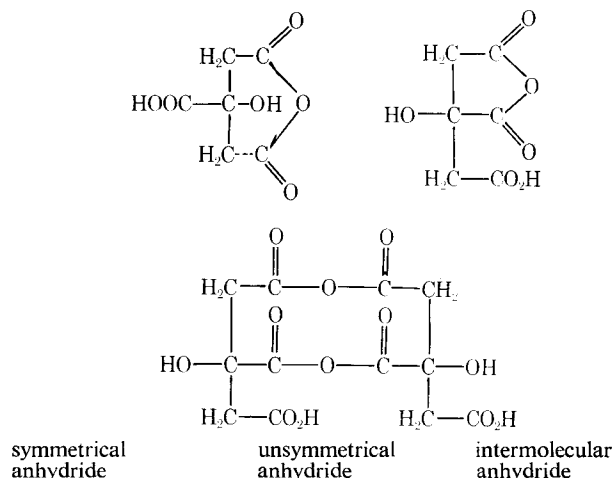


Figure 1—Chromatogram of the anilides formed by adding 100 μ l. of 1.0 *M* citric acid anhydride to a solution containing 5 ml. of water and 5 ml. of 0.32 *M* aqueous aniline.

solutions of the anhydride, and equivalent weight determined by titration of solutions in which compound had been added to aqueous aniline (10), gave consistent results, it was not possible to distinguish among the following possible anhydride structures.¹



In order to determine whether the compound was the intermolecular or the intramolecular anhydride the freezing point depression of the compound was determined in dioxane solution. The molecular weight obtained by calculations based on these measurements was found to be about 183 which most closely agreed with the molecular weight of 174 for the intramolecular anhydrides.

When the anhydride was reacted with aniline and the products separated by chromatography (see Fig. 1 and Scheme III), two anilides were found and they were apparently the symmetrical (Compound A) and the unsymmetrical (Compound B) citric acid monoanilides (4). The ratio of the total absorbances of the unsymmetrical isomer to the symmetrical was found to be 0.85 which is in good

¹ One might also postulate the possible lactones of citric acid as likely candidates, but these can easily be ruled out mainly on the basis of the products found in the reaction of aniline with the compound.

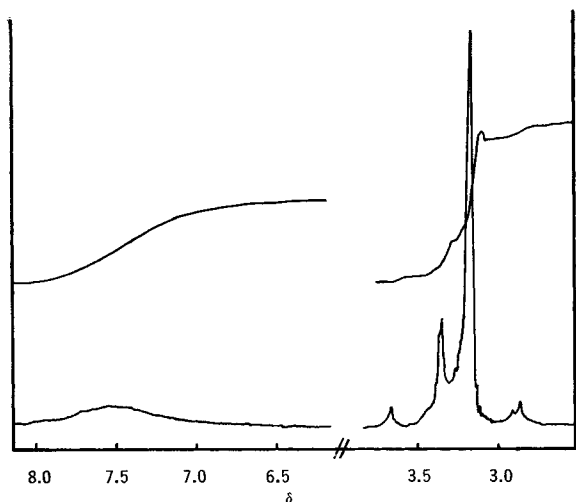


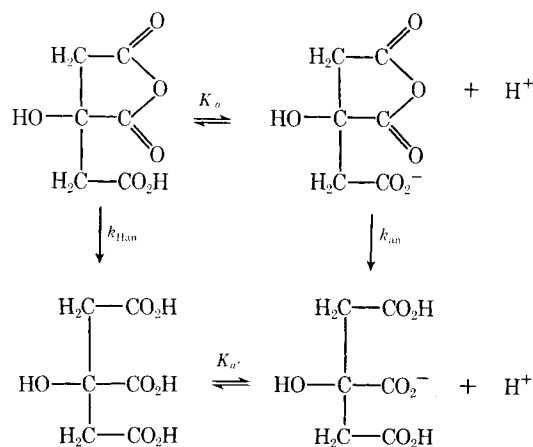
Figure 2—Reproduction of the important features of a typical NMR spectrum of citric acid anhydride in deueroacetone. The internal standard was TMS.

agreement with earlier results obtained by addition of aniline to aqueous solutions in which citric acid anhydride was produced by interaction of acetic (3) or glutaric (2) anhydride and citrate ions. Since both monoanilides were produced in similar amounts, it appeared that the unsymmetrical anhydride must at least be the predominant species.

NMR studies also supported this structural assignment. A typical NMR spectrum is shown in Fig. 2. Inspection of the spectrum and its integration curve led to the conclusion that the anhydride structure was the unsymmetrical one. The coupling constant for the doublet at $\delta = 3.68$ and 3.38 was calculated to be 19 c.p.s. which is in good agreement with similar calculations by Ericksen for malic anhydride (1).

Hydrolysis of Citric Acid Anhydride²—The pH profile for the reaction over pH range 1–6 is shown in Fig. 3. It is obvious that the hydrolytic behavior of this compound differs markedly from that of carboxylic acid anhydrides such as acetic or glutaric which show no effect of pH on hydrolytic rate in this range.

The citric acid anhydride however, is markedly different from most other anhydrides in that it contains an ionizable functional group. The decrease in hydrolytic rate with increasing pH rate is apparently due to the ionization of the free carboxylic acid group, which results in a negatively charged anhydride which apparently increases the energy of the transition state formed upon attack of the nucleophilic water molecule. A proposed scheme for the hydrolysis is shown below.



Scheme II

From Scheme II the following equation can be derived to express

² During the hydrolytic studies it was found that the citric acid anhydride contained some reactive impurity. From NMR and kinetic measurements it was concluded that the impurity consisted of acetyl-citric anhydride.

the observed rate constant as a function of pH and the dissociation constant K_a of the carboxylic acid group of the anhydride

$$K_{\text{obs.}} = \frac{k_{\text{Han}} + (k_{\text{an}} K_a)/[\text{H}^+]}{1 + K_a/[\text{H}^+]}$$

Using the above equations, and values of k_{Han} , k_{an} , and $\text{p}K_a$ to be $68 \times 10^{-3} \text{ sec.}^{-1}$, $18 \times 10^{-3} \text{ sec.}^{-1}$, and 2.9, respectively,³ the smooth curve in Fig. 3 was generated and is in good agreement with experimental results. The observed rate constant of $18 \times 10^{-3} \text{ sec.}^{-1}$ at $\text{pH} = 5$ was in fair agreement with earlier estimates of about 11×10^{-3} (4, 5).

Effects similar to those seen for the hydrolysis of citric acid anhydride have also been observed for tricarballic anhydride (11) and could probably be expected for any anhydride which contained an ionizable functional group.

The value, $\text{p}K_a = 2.9$ for citric acid anhydride, which is found from Fig. 3 is somewhat lower than would be expected for this type of acid even at an ionic strength of 0.5. However, because of the presence of the hydroxy group and carbonyl groups of the anhydride one might attribute the relatively strong acidity to some intramolecular stabilization of the anion of the anhydride.

Effect of Temperature on Hydrolytic Rate—Since the hydrolytic rate presumably depends on the extent of ionization of the anhydride, it would be expected that the effect of temperature would be different at pH values where the extent of ionization was markedly changed. Therefore, the activation energy was determined from spectrophotometric measurements at $\text{pH} = 1$ in 0.1 *N* hydrochloric acid ($I = 0.5$ with sodium chloride) and at $\text{pH} = 5$ ($I = 0.5$) using the pH-stat. The results are in agreement with the supposition and are shown in Fig. 4. The activation energy was calculated to be about 10.7 kcal./mole for the hydrolysis of the acid form of the anhydride, and about 12.9 kcal./mole for an anionic form of the anhydride.

Effects of Ionic Strength—While it was found that ionic strength did not grossly influence the hydrolytic rate in the region of $\text{pH} < 2$ and $\text{pH} > 5$, there was a tendency for the rate to decrease as ionic strength increased over the range of $\text{pH} = 2$ –5. This can generally be accounted for by a secondary salt effect on the dissociation of the carboxylic acid group.

Comparison of Aniline Addition to Malic and Citric Acid Anhydrides—The reaction of citric acid anhydride with excess aniline in aqueous solution and the relative yield of products is shown in Scheme III.

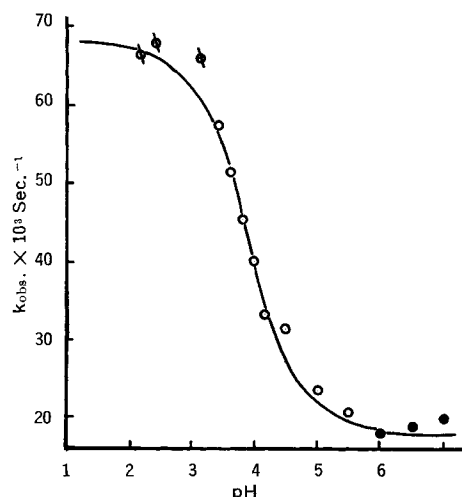
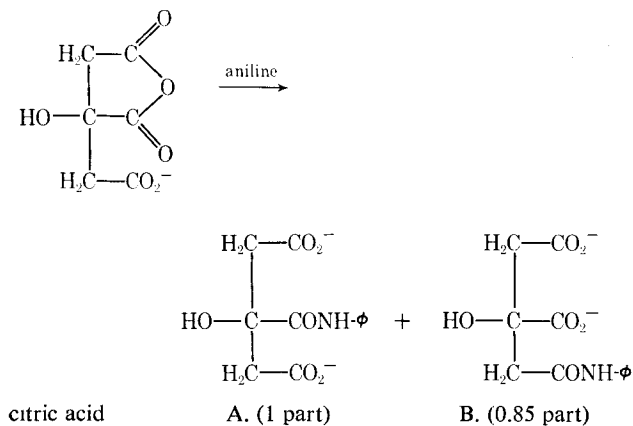


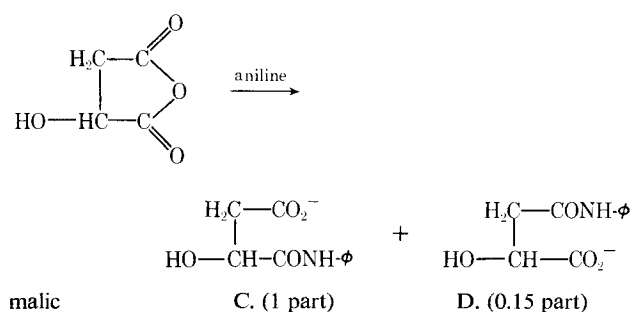
Figure 3—The pH rate profile of the hydrolysis of citric acid anhydride at 25° and ionic strength = 0.5. The smooth curve correspond to the calculated profile based on $\text{p}K_a = 2.9$, $k_{\text{Han}} = 68 \times 10^{-3} \text{ sec.}^{-1}$ and $k_{\text{an}} = 18 \times 10^{-3} \text{ sec.}^{-1}$. Key: ϕ , spectrophotometric measurements in aqueous HCl solutions; \circ , spectrophotometric measurements using pH-stat to maintain pH; \bullet , pH-stat measurements.

³ k_{Han} was taken as the maximum value of $k_{\text{obs.}}$ and k_{na} as the minimum value of $k_{\text{obs.}}$ over the range of $\text{pH} = 1$ –6. The $\text{p}K_a$ value was chosen to give the best fit to the data in Fig. 3.



Scheme III

A similar study of the reaction of malic anhydride with aniline has been made (2, 12) and the scheme and relative yield of products is shown in Scheme IV.



Scheme IV

In both schemes all acid species are shown to be in the neutralized form and this is to be expected in the presence of excess aniline.

Compounds A and C are similar differing only in the substitution of a methylene carboxy group on the hydroxy substituted carbon. The same is true for Compounds B and D.

The proportions of the products from each reaction was quite different. The greater production of C with respect to D has been explained on the basis of the greater electrophilic nature of the α -hydroxy carbonyl of malic anhydride.

While a similar situation would be anticipated with citric acid anhydride, the product distribution is much different, *i.e.*, B/A = 0.85. This may be largely explained on the basis of the electronic effects of the ionized carboxy group which would be expected to decrease the rate of addition of aniline at the α -hydroxy carbonyl carbon due to creation of a tetrahedral transition state with two negative charges.

Molecular models suggest the effects on the transition state would be much less for attack on the carbonyl group which is further removed from the ionized carboxyl group. Thus it would be expected that the attack of aniline on the carbonyl group which results in A would be retarded more than the attack at the carbonyl group leading to B, and therefore the ratio of products B/A in the citric acid case should be significantly greater than the ratio of D/C found for malic acid.

This explanation agrees well, quantitatively with the results found for the water hydrolysis of citric acid anhydride where the rate decreases between two and threefold in going from the free acid to the anionic form of the anhydride.

While no studies of the aniline reaction with citric acid anhydride at strongly acid pH values were undertaken, it would be expected that the relative yield B/A would be appreciably higher under such circumstances.

Solubility Properties of the Anhydride—While no quantitative solubility studies were carried out, it was observed that the compound is more soluble in water, alcohols, diethyl ether, tetrahydrofuran, dioxane, acetic acid, and acetone than in the less polar organic solvents such as chloroform, benzene, carbon tetrachloride, and petroleum ether.

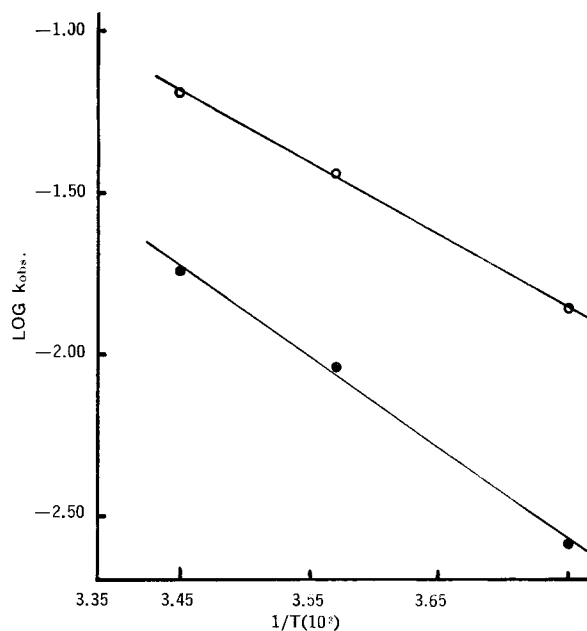


Figure 4—Temperature dependence of k_{obs} for the hydrolysis of citric acid anhydride at ionic strength of 0.5. Key: O, pH = 1; ●, pH = 5.

Suggested Uses of Citric Acid Anhydride—The ready dissolution of the anhydride in water and the effect of pH on the hydrolytic rate, in addition to wide acceptance of the product acid strongly suggests this compound for use in formulations for spontaneous carbonation of aqueous solutions. An earlier paper (13) has investigated the use of glutaric anhydride as a latent acidifier in such systems claiming more effective and longer-lasting carbonation. While the taste of glutaric acid leaves much to be desired, the usefulness of such an approach appears to be sound. Citric acid anhydride in the presence of the bicarbonate used in such formulations would be in the anionic form and as such would hydrolyze with a half-life of about 4 min. in ice water. This slow hydrolysis would allow the necessary time for the total dissolution of the bicarbonate to occur. As the pH of the solution decreased the half-life for hydrolysis would also decrease to less than 1 min. Thus a significant portion of the carbon dioxide would be produced in homogeneous solution which is the ideal situation for retaining the gas in the solution.

Another apparent use would seem to be as a food or drug desiccant since the anhydride reacts rapidly with moisture and the product acid is completely biologically acceptable.

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Epoxy Resin Beads as a Pharmaceutical Dosage Form I: Method of Preparation

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Abstract □ The preparation of various epoxy resins containing acidic and basic curing agents was investigated. These resins were prepared in bead and bulk forms. The effects of the types of curing agents and their concentrations on the solubility of the resins in artificial gastrointestinal juices have been studied. The addition of basic and acidic curing agents in the resins was observed to influence their solubility in acidic and alkaline buffer, respectively. The method of preparation of these resins into bead forms was developed in the presence of drugs.

Keyphrases □ Epoxy resin beads—dosage forms □ Acids, bases—epoxy resin curing agents □ Drug-epoxy resin beads—preparation □ UV spectrophotometry—analysis

Many synthetic and natural polymers have been developed and used in pharmacy for various purposes. However, little attention has been paid to the use of

epoxy resins in dosage forms. Epoxy compounds cured with amine can be used to incorporate the drugs by coating (1), by embedding (2), and by extrusion molding (2, 3). Drugs can furthermore be incorporated in the bulk or beads of water-insoluble monomers during polymerization (4).

In the present work epoxy compound was condensed with either basic or acidic curing agent to prepare resins in bulk as well as in bead form and three drugs were embedded in the beads. Furthermore, the possibilities of these resins for enteric coating and sustained-release dosage forms have been shown.

EPOXY COMPOUNDS

The epoxy compounds are prepared by heating epichlorohydrin and 2,2'-bis-*p*-hydroxyphenyl propane at 99–119° for 3.5 hr. in

