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Complexation of Acetaminophen with Methyl Xanthines

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Abstract □ The interaction, in aqueous solution, of acetaminophen with caffeine, theophylline, and theobromine was investigated. Caffeine and theophylline form isolatable crystalline 1:1 complexes with acetaminophen, while theobromine apparently does not complex with acetaminophen. The caffeine–acetaminophen complex was found to exist in three forms, differing only in the degree of solvation. The solubility and dissolution rates of some of the caffeine–acetaminophen and theophylline–acetaminophen complexes were determined.

Keyphrases □ Acetaminophen—complexation with methyl xanthines, particularly caffeine and theophylline □ Caffeine–acetaminophen complexes—isolation, solubility, dissolution rates, stability constants □ Theophylline–acetaminophen complexes—isolation, solubility, dissolution rates, stability constants □ Complex formation—acetaminophen with methyl xanthines □ Analgesic availability—effect of complexation

Many studies have shown that caffeine and related xanthines are capable of interacting in aqueous media with a variety of drugs through complexation (1).

In a recent search of the literature relative to the possible influence of complexation on the availability of analgesics, it became obvious that the effect of the interaction of acetaminophen with the methyl xanthines had not been reported.

Although acetaminophen and caffeine are present in many commercial analgesic products (2), the role or purpose of caffeine as a therapeutically active ingredient may be doubtful. However, such combinations may exhibit physical–chemical interactions of pharmaceutical interest and importance from a formulation standpoint.

EXPERIMENTAL

Equipment[1]—Constant-temperature water baths were used which maintained the temperature at 25 ± 0.1°. Tablets for the dissolution studies were prepared using a Carver press and a flat-face punch and die with a diameter of 1.27 cm. (0.5 in.). The dissolution apparatus consisted of a 500-ml., two-necked round-bottom flask immersed in a constant-temperature bath. The dissolution medium (350 ml. of water) was stirred through the central neck of the flask using a stirring motor[2] and an L-shaped glass stirrer located 2.5 cm. above the bottom of the flask. The stirring rod was fashioned from a 7-mm. glass rod, as shown in Fig. 1.

Table I—Amounts of Substrate and Ligand Used in the Various Solubility Studies

Substrate	Ligand
Acetaminophen, 2 g.	Caffeine, 0–3.1 g.
Acetaminophen, 1.5 g.	Theophylline, 0–2.4 g.
Theobromine, 0.03 g.	Acetaminophen, 0–0.32 g.

Materials—Caffeine, theophylline, theobromine, and acetaminophen were recrystallized from distilled water, dried to constant weight, and shown to be anhydrous by differential scanning calorimetry and/or NMR. Melting points were found to be in good agreement with literature values (3). All water used was distilled from acid permanganate solution in an all-glass apparatus. The NMR spectra were obtained in deuterated dimethyl sulfoxide (97.5% isotopic purity). All other reagents were of analytical or reagent grade.

Methods—*Solubility Study*—A certain quantity of substrate, in excess of its aqueous solubility, was placed in 30-ml. glass, screw-capped vials together with increasing but accurately weighed amounts of ligand (Table I) and 20 ml. of distilled water. The vials were sealed and fixed on a rotating shaft in a constant-temperature bath and equilibrated for 48 hr. at 25 ± 0.1°. Aliquot portions of the

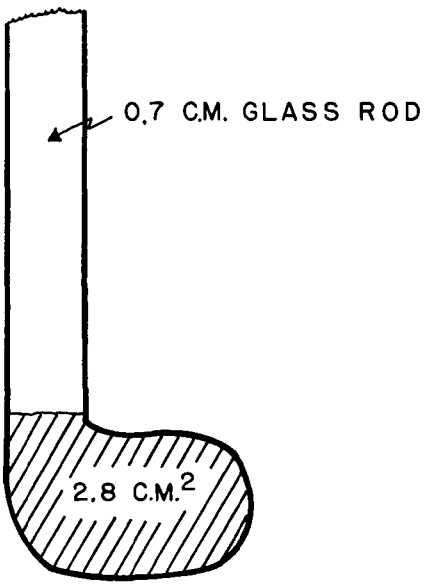

Figure 1—*Shape and dimensions of glass stirrer used in dissolution rate studies.*

[1] Spectrophotometric measurements were made using a Cary model 14 or 15 spectrophotometer. Differential scanning calorimetry was performed using a Perkin-Elmer model DSC-1B differential scanning calorimeter. The Varian model T-60 was used for obtaining NMR spectra.

[2] Inframo model RZR64.

Table II—Molar Absorptivities of the Various Components at 25° and the Conditions under which They Were Determined

Components	Absorptivities, 1 cm.$^{-1}$ M^{-1}					
	257.5 nm. in pH 12 Aqueous Solution	276.5 nm. in Chloroform	248.5 nm. in Methanol	271 nm. in Methanol	272 nm. in Methanol	273 nm. in Methanol
Acetaminophen	10,380	—	13,300	3700	3230	2970
Caffeine	—	9600	2,640	—	—	9240
Theophylline	—	—	3,140	9680	—	—
Theobromine	—	—	2,680	—	9260	—

supernatant liquid were removed using a pipet (whose tip had been wrapped with glass wool) and analyzed by the procedures described later. In the acetaminophen–caffeine system, glass wool (or a tiny crystal of the complex) was added to induce crystal formation prior to sampling.

Isolation of Complexes—The complexes were isolated during the solubility studies. The acetaminophen–caffeine complex did not precipitate spontaneously during solubility studies, and it was necessary to induce crystallization by introducing a small piece of glass wool into the solutions and allowing them to remain at 25° for about 1 hr. The resulting precipitate was then separated by filtration. The damp residue melted over a range of 40–45°. When the precipitate was allowed to air dry for several hours and then placed in a vacuum oven at 40° and a pressure of 2 mm. of mercury for 24–48 hr., the resulting anhydrous material melted at about 145°. The preparation of the monohydrate acetaminophen–caffeine complex (melting point ~85°) is described below.

In the case of the acetaminophen–theophylline complexes, precipitation occurred spontaneously during the solubility studies. The precipitate was removed by filtration, and the damp precipitate melted at about 55°. Complete drying in a vacuum oven resulted in the anhydrous complex which melted at ~192–195°.

Dissolution Rate Study—All dissolution studies were performed according to the following method.

Approximately 300 mg. of the material to be tested was compressed at 2270 kg. (5000 lb.) into a tablet. The weight and surface area of the tablet were then determined. The tablet was then placed in the 350 ml. of water contained in the dissolution apparatus and the time was noted. One- or two-milliliter samples were withdrawn at the appropriate times through the side arm of the flask. A pipet whose tip was wrapped in glass wool was used to remove the sample. The samples were analyzed as described below.

Analytical Procedure—The analysis of all compounds was done by spectrophotometric measurements after appropriate treatment of the various samples. The absorptivities of the various compounds under the conditions at which they were measured are given in Table II.

1. Acetaminophen–caffeine—In the acetaminophen–caffeine system, caffeine was separated from acetaminophen by adjusting the pH of the aqueous solution of the compounds to 12 with 0.1 *N* NaOH solution [pKa of acetaminophen was determined as 10.15, pKa of caffeine is 14 (3)] and extracting with chloroform. Aliquots of the aqueous portion, containing the sodium salt of acetaminophen, were diluted with an equal volume of pH 12 buffer, and the absorbance was measured at 257.5 nm. The aliquot of chloroform extract, containing caffeine, was diluted with an equal volume of chloroform and the absorbance was measured at 276.5 nm. (Table II).

2. Acetaminophen–theophylline—Multicomponent analysis by spectrophotometry (4) was used in this system. The absorptivities (Table II) were obtained from Beer's law plots which were made by dissolving known weights of sample in methanol. The concentrations of acetaminophen and theophylline were determined by taking 1 ml. of aqueous sample, diluted 1:5000 or 1:10,000 with methanol, and measuring the absorbances at 248.5 and 271 nm.

3. Acetaminophen–theobromine—Multicomponent analysis (4) by spectrophotometry was also used in this system. The concentrations of acetaminophen and theobromine were determined at 248.5 and 272 nm. after appropriate dilution of the samples.

Structure of Complexes—To determine the degree of hydration of the acetaminophen–caffeine complex which was precipitated, and to demonstrate that the acetaminophen–caffeine complexes differed only in the degree of hydration, the following experiment was carried out.

A sample of the appropriate complex was place in an open vial, and the vial was then placed in a vessel containing a saturated aqueous barium chloride solution and excess barium chloride. This system was then sealed and placed in a water bath at 25°. The activity of water on the saturated barium chloride solution was reported (5) to be 0.90. The experimental design did not permit direct contact beteen the barium chloride solution and the sample, but it did allow water to be transferred *via* the vapor phase. The melting behavior, as reflected by differential scanning calorimetric recording, was checked at various times under the conditions employed. When no further change was observed for the "freshly filtered sample" (after about 3–4 weeks), a portion of the sample was weighed and then dried completely and reweighed. The loss in weight was attributed to water loss. The complex was shown in this way to contain about 6.4 molecules of water. The NMR spectrum also showed 6 moles of water/mole of the 1:1 acetaminophen–caffeine complex.

RESULTS AND DISCUSSION

The complexation of acetaminophen with each of the compounds (caffeine, theophylline, and theobromine) was studied at 25° in

Table III—Initial Dissolution Rate of Acetaminophen, Caffeine, and Theophylline from Tablets at 25° in Water[a]

Tablet Composition	Initial Dissolution Rate[b] of Components, M cm.$^{-1}$ min.$^{-2}$ × 10^5		
	Acetaminophen	Caffeine	Theophylline
Pure acetaminophen	0.90	—	—
Pure caffeine	—	1.10	—
Pure theophylline	—	—	0.50
Hexahydrate complex of acetaminophen–caffeine	0.67	0.67	—
Monohydrate complex of acetaminophen–caffeine	2.75	2.75	—
Anhydrous complex of acetaminophen–caffeine	2.50	2.50	—
Anhydrous complex of acetaminophen–theophylline	0.66	—	0.76
Physical mixture of[c] acetaminophen–caffeine	3.80	3.80	—
Physical mixture of[d] acetaminophen–theophylline	0.72	—	1.07

[a] Stirring rate was 120 r.p.m. [b] Based on initial surface area of tablet. [c] Coprecipitate (see text for further discussion). [d] Mixture of powdered acetaminophen and theophylline.

Table IV—Summary of Data for the Complexation of Acetaminophen with Caffeine, Theophylline, and Theobromine at 25° in Water

System	Evidence of Complexation	Stoichiometry of Complex (Acetaminophen–Xanthine) Chemical	Stoichiometry of Complex (Acetaminophen–Xanthine) Calculated	Initial Slope (Fig. 1)	$K_{1:1}$ (l. mole)	Solubility[a] of Complex (mole l.)
Acetaminophen–caffeine	Yes	1	1.10	0.838	59.4	~0.1
Acetaminophen–theophylline	Yes	1	1.03	0.614	16.1	~0.05
Acetaminophen–theobromine	No	—	—	—	—	—

[a] Estimated from increase in total acetaminophen concentration in Fig. 2.

water using the solubility method (1). The equilibrium phase solubility diagrams obtained for the acetaminophen–caffeine and acetaminophen–theophylline systems are shown in Fig. 2. In both of these systems, the solubility of the substrate (acetaminophen) was found to be increased by the addition of the ligand. Furthermore, both systems deposited crystalline complexes at higher concentration of ligand. These complexes were isolated, dried, and analyzed. Both were found to contain 1 mole of ligand/mole of substrate. The acetaminophen–theobromine system, however, did not give any evidence of complexation. Even when theobromine was employed as the substrate in the presence of a 12-fold excess of acetaminophen, there was no detectable changes in solubility and no crystalline complex was formed. These results may have been due in part to a lack of sensitivity of the analytical method employed; but it is safe to say that if any complexation did occur, it was negligible relative to that observed for the acetaminophen–caffeine and acetaminophen–theophylline systems.

Calculation of the stoichiometry of the acetaminophen–caffeine and acetaminophen–theophylline complexes based on the data in the plateau region (1) of the respective diagrams in Fig. 2 were in good agreement with those obtained by isolation and analysis of the crystalline complexes as indicated in Table II. The slightly greater value of the stoichiometric ratio of acetaminophen to caffeine in the complex as found from calculation based on the solubility diagram suggests that complexes consisting of two or more molecules of acetaminophen per molecule of caffeine exist in solution but are not precipitated. On the basis of the formation of the 1:1 complex, the stability constants for both systems were calculated (1). These are listed in Table IV along with the approximate solubility of each complex as calculated from the increased solubility of acetaminophen for each system. The stability constants are in general agreement in both rank and magnitude with those found for similar systems (1).

An interesting observation made in the studies involving acetaminophen and caffeine was that the precipitation of the complex was not spontaneous. When sealed vials containing acetaminophen and caffeine in four- to fivefold excess of their equilibrium solubility were equilibrated at 25°, a clear solution resulted which did not produce a precipitate over periods of up to 2 weeks. Only when glass wool or a crystal of complex was introduced did crystallization occur.

In the process of isolating and determining the stoichiometry of the crystalline complexes, it was noted that the melting point of the solids changed with time. This was especially noticeable with the acetaminophen–caffeine complex. When the freshly filtered solid was air dried for 1–2 hr. and then heated in a sealed capillary, the solid (AC-I) melted at about 42–50°. When allowed to air dry for about 24–48 hr., the resultant material (AC-II) melted at about 75–80°. If the solid was exhaustively dried in a vacuum oven at 40° and about 2 mm. of mercury, the resulting anhydrous solid (AC-III) melted at about 145°. Analysis of all three solids demonstrated that each contained acetaminophen and caffeine in an equimolar ratio and that the difference in melting points was due to water of hydration.

In the case of AC-II, spectrophotometric analysis, weight loss on drying, and NMR showed the complex to be a monohydrate. AC-III was found to be anhydrous. The water content of AC-I was not easily determined, however, due to the rapidity of water loss. To determine the composition of AC-I and to establish whether or not the hydration and dehydration were reversible, the following experiment was carried out. Samples of AC-III, AC-II, and the freshly filtered complex (presumably wet AC-I) were equilibrated at 25° with an aqueous solution in which the water activity was 0.9 (3). The resulting changes in the samples were followed by differential scanning calorimetry as a function of time.

The appearance, shape, and location of endotherms at various times of equilibration are shown schematically in Fig. 3. As indicated, all samples exhibited only a single endotherm before

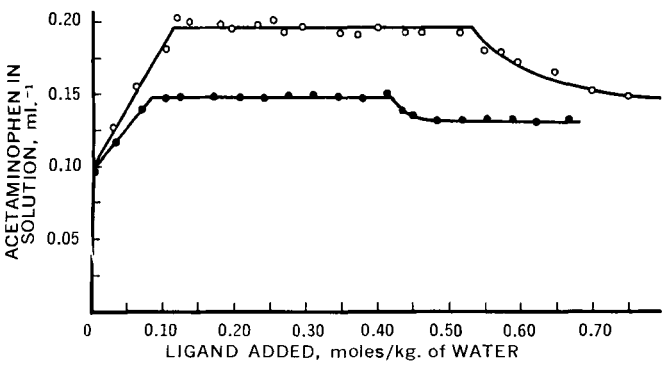

Figure 2—*Solubility of acetaminophen as a function of caffeine* (○) *or theophylline* (●) *added.*

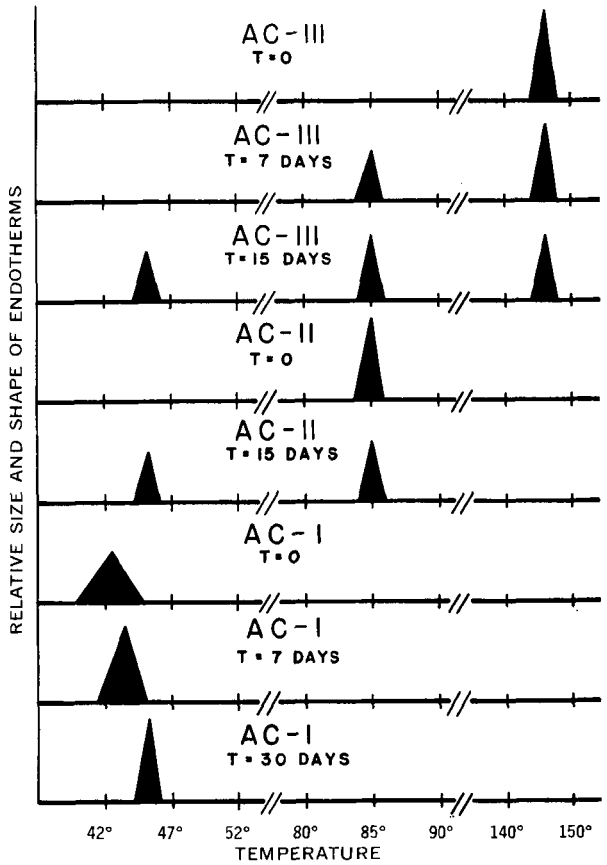

Figure 3—*Schematic diagram of the changes in differential scanning calorimetric recording of samples of AC-III, AC-II, and wet AC-I as a function of the time of equilibration at 25°. (See text for further discussion.)*

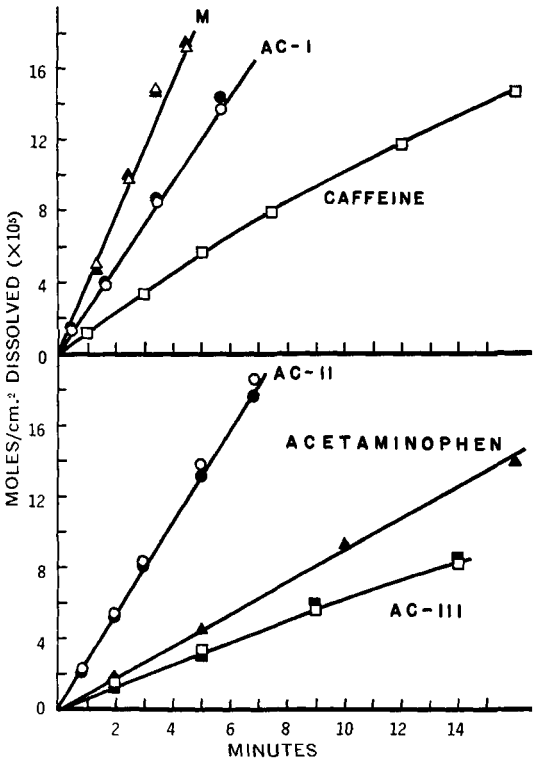

Figure 4—*Dissolution in water at 25° of acetaminophen (closed symbols) and caffeine (open symbols) from tablets of AC-III, AC-II, AC-I, caffeine, acetaminophen, and an equimolar mixture of acetaminophen and caffeine (M).*

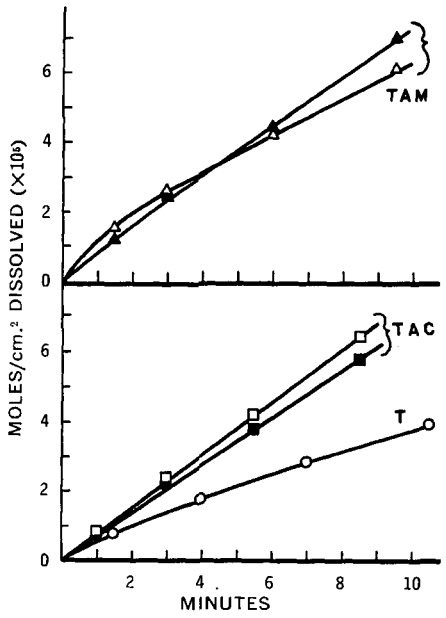

Figure 5—*Dissolution in water at 25° of acetaminophen (closed symbols) and theophylline (open symbols) from tablets of anhydrous theophylline (T), an anhydrous theophylline–acetaminophen complex (TAC), and an equimolar physical mixture of acetaminophen and theophylline (TAM).*

equilibration. With AC-III and AC-II the endotherms were quite sharp, in contrast with those seen for AC-I. As the time of equilibration increased, both AC-II and AC-III developed additional endotherms corresponding to more hydrated species. AC-I, on the other hand, appeared to lose water as evidenced by: (*a*) the decrease in the broadness of the single endotherm, and (*b*) the increase in the temperature corresponding to the maximum of the endotherm. After 4 weeks of equilibration of AC-I, there were no additional changes in the differential scanning calorimetric readings. The material which melted at about 50° was analyzed for water content by integration of NMR spectra and by loss of weight on drying. Both methods showed AC-I to contain six molecules of water associated with each mole of complex.

Thus, the equilibration studies confirmed the fact that the complexes differ only in degree of hydration and, furthermore, that all of the species are in equilibrium. In addition, the presence of only three distinct endotherms suggested that the anhydrous, monohydrate, and hexahydrate species are the only species involved in the equilibrium.

Similar changes in the melting point of the acetaminophen–theophylline complex with drying were observed. The initially isolated crystalline material melted at about 55°. After air drying for 24–48 hr., it exhibited a melting point of about 130°. Complete drying in a vacuum oven resulted in a solid which melted at 192–195°. This high melting solid was found to be anhydrous. However, no attempt was made to determine the composition with respect to water of the other, lower melting, acetaminophen–theophylline complexes.

Since acetaminophen does complex with both caffeine and theophylline, the effects of the complexation on the dissolution rate of each compound were investigated. These dissolution studies were carried out at 25° in water as described in the *Experimental* section. The initial dissolution rates obtained for acetaminophen, caffeine, and theophylline from various systems are summarized in Table III. The dissolution curves for each system were followed for periods of times during which at least 30% of the material in the tablet had dissolved.

Figure 4 shows the dissolution curves obtained for tablets of AC-I, AC-II, AC-III, and an equimolar mixture of acetaminophen and caffeine prepared by evaporation of a methanolic solution of acetaminophen and anhydrous caffeine. In all cases where acetaminophen and caffeine were present in equimolar amounts, the rate of dissolution of both species occurred at about the same rate to within ±5%. For this reason the solid curves in Fig. 4 were drawn to approximate the dissolution behavior of both species from a given tablet.

Inspection of the dissolution curves in Fig. 4 and the initial dissolution rates in Table III shows that the rate of release of acetaminophen and caffeine was greatest from the equimolar mixture prepared by precipitation from methanol solution. Although it was anticipated that the anhydrous complex would dissolve more rapidly than either acetaminophen or caffeine alone, it was rather surprising that the equimolar mixture of acetaminophen and caffeine dissolved at a rate about 70% greater than that for the anhydrous complex. The reason for these results is not clear. It was felt that the equimolar mixture prepared by precipitation may have been a more energetic solid than would be obtained with a simple physical mixture. To determine if similar results could be obtained from a true physical mixture, equimolar quantities of acetaminophen and caffeine were powdered and intimately mixed. The resulting mixture was compressed into tablets and an attempt was made to determine the dissolution rates. These attempts were not successful since the tablets almost immediately began to disintegrate when placed in the dissolution medium. Because of the changes in surface area associated with disintegration, the dissolution rates obtained could not be compared with those obtained for the other system where the tablet remained intact.

An additional unexpected result was the greater dissolution rate of AC-II relative to AC-III. Related work on hydrated and anhydrous systems showed the solubility and dissolution rate of the anhydrous solid to be greater than the hydrated form at 25° (6, 7).

It seems reasonable in the present case that the dissolution rates obtained reflect the relative solubilites of AC-II and AC-III in water at 25°. Thus, by using the initial dissolution rate together with the heats of solution of AC-II and AC-III, which were calorimetrically determined[3] to be about 10.1 and 6.5 kcal./mole, respectively, a transition temperature of about 20° was calculated. Thus, at temperatures of less than about 20°, the dissolution rate of AC-III would be expected to be greater than the dissolution rate of AC-II.

[3] These values were determined (at 25°) in these laboratories by S. Lindenbaum. A solution batch calorimeter was used.

Although the results shown in Fig. 2 and Table IV clearly show that the presence of caffeine and acetaminophen in equimolar amounts has a significant effect on the dissolution rate of acetaminophen (and caffeine), they still left some question as to whether or not the presence of smaller ratios of caffeine to acetaminophen, such as are found in many commercial analgesic preparations (2), would have any significant physical effects on the release of acetaminophen from such tablets. Therefore, tablets containing one part anhydrous caffeine (30 mg.) and 10 parts acetaminophen (300 mg.) were prepared. The dissolution behavior of such tablets was then compared to pure acetaminophen tablets (300 mg.). The tablets containing the mixture began to disintegrate within 2 min. and dissolved completely within 50 min. The pure acetaminophen tablets, on the other hand, dissolved without disintegration and required more than 100 min. for complete dissolution.

Whether or not these results can be extrapolated to commercial tablets that contain various additives such as binders and diluents is doubtful. However the results do suggest that caffeine has a substantial effect on the release of acetaminophen from tablets. Therefore, in any formulation attempts or changes, the possible effects of the presence of caffeine in such a system should be considered.

The dissolution rates of theophylline and acetaminophen from various systems are shown in Fig. 5. It is obvious that in these systems the dissolution rates of acetaminophen and theophylline from the same tablet are not as similar as in the acetaminophen–caffeine systems. Furthermore, there is no significant difference between the anhydrous complex and the equimolar physical mixture which, in this case, was prepared by blending of powders. The initial rate of dissolution for theophylline from the physical mixture and the pure theophylline tablet should be noted. The theophylline dissolves approximately 100% faster from the physical mixture than from pure theophylline tablets. This increase in dissolution rate, together with the neutral to slightly acid pH of the resulting solution, suggests that the anhydrous complex may have some utility in the formulation of more rapidly dissolving oral dosage forms of theophylline which could replace, in some cases, the highly alkaline theophylline salts presently employed.

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DRUG STANDARDS

Desorption of Belladonna Alkaloids from Antacids for Analysis

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Abstract □ The desorption of belladonna alkaloids from antacids for analysis was investigated. The antacids studied were dried aluminum hydroxide gel, calcium carbonate, magnesium carbonate, magnesium oxide, and magnesium trisilicate. It appears that the alkaloids are strongly adsorbed only on the surface of magnesium trisilicate. The complete desorption of alkaloids requires boiling the complex with concentrated hydrochloric acid and then using a dye method for the analysis.

Keyphrases □ Belladonna alkaloids—desorption from antacids for analysis □ Desorption, belladonna alkaloids—from antacids, analysis □ Antacids—adsorption of belladonna alkaloids, desorption for analysis □ Magnesium trisilicate—adsorption of belladonna alkaloids, desorption for analysis

The pharmaceutical industry is marketing some liquid antacid preparations in combination with belladonna alkaloids. One typical product of this nature was brought to the authors' notice for the development of an assay procedure for *l*-hyoscyamine. During the investigations, *l*-hyoscyamine was found to be strongly adsorbed onto the surface of one or more of the ingredients. An effective method was required to desorb the alkaloids for analysis. The purpose of this paper is to report the details of these investigations.