# Interactions Between Pharmaceutical Compounds by Thermal Methods

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**Abstract** □ A critical evaluation of the utility of thermal methods for the detection of possible interactions occurring between solid components of pharmaceuticals is given. Differential thermal analysis (DTA), the cooling curve method, and the thaw-melt method are used to obtain data from which phase diagrams are constructed for a number of binary systems. DTA is shown to be a versatile technique, demonstrating several advantages over the more classical methods of thermal analysis. Systems in which interactions were detected and stoichiometries determined include deoxycholic acid–menadione (2:1), quinine–phenobarbital (1:1), theophylline–phenobarbital (2:1), caffeine–phenobarbital (1:1), and atropine–phenobarbital (1:1). Systems in which no interactions were detected include aspirin–phenobarbital, phenacetin–phenobarbital, diphenylhydantoin–phenobarbital, and acetaminophen–phenobarbital.

**Keyphrases** □ Pharmaceutical compounds—interactions □ Phenobarbital interaction—deoxycholic acid, theophylline, quinine, caffeine, atropine □ X-Ray powder patterns—compound interaction □ Differential thermal analysis (DTA)—interaction determination □ Cooling curve, thaw-melt methods—DTA comparison

Reports of physical and chemical interactions occurring between medicinal ingredients, and between medicinal ingredients and adjuvants in solid dosage forms have been appearing with increasing frequency. Given the large volume of data on the formation of drug complexes in solution, it is not surprising that similar molecular associations should occur in the solid state. While such interactions could presumably be eliminated if drug entities were incorporated into separate dosage forms, using demonstrably nonreactive fillers, it seems unlikely that the trend toward multicomponent tablets and capsules will soon be reversed.

Pharmaceutical analysts, recognizing the fact that interactions within solid dosage forms may be responsible for observed modifications in therapeutic response, have intensified their search for methods of detecting interactions. French and Morrison (1) were among the first to examine this problem. Using IR spectroscopy they detected complexes of phenobarbital and quinidine (or hydroquinidine) in three of four commercial products tested. Only one of the products contained uncomplexed phenobarbital. Troup and Mitchner (2), using TLC, isolated three acetylated phenylephrine degradation products from tablet formulations containing phenylephrine and aspirin. Acetaminophen (3) has been shown to undergo a similar acetylation reaction.

Diffuse reflectance spectroscopy (DRS) has been employed by Lach and Bornstein to study interactions between adjuvants and a variety of drugs (4) and dyes (5). Recently, it was suggested (6) that differential thermal analysis (DTA) might prove useful as a technique for the routine screening of combinations of pharmaceuticals for potential interactions.

DTA, and a related technique, differential scanning calorimetry (DSC), have been employed in the pharmaceutical industry for the detection and estimation of impurities (7, 8), and for identification of polymorphic forms and solvates (7). In the present paper, however, it is the use of DTA for the detection of inclusion compounds, complexes, and molecular compounds which is of primary concern.

Chiu (9) has shown that DTA is a useful technique for the characterization of amines, because when a picrate can be formed *in situ* in the presence of excess amine, DTA affords a method for the simultaneous determination of the melting and boiling points of the amine, and the melting point of the derivative picrate. Milgram (10) determined the phase diagram of the 2-methylnaphthalene-*n*-heptane system using DTA, along with other techniques. Six different complexes were isolated, all of which melt incongruently. Joncich and Bailey (11) constructed a phase diagram of the anthracene–phenanthrene system using DTA and zone melting. Boeyens and Herbstein (12) used DTA to study donor-acceptor type molecular compounds formed between pyromellitic dianhydride and anthracene, pyromellitic dianhydride and diphenyl, and pyromellitic dianhydride and perylene.

Kung and Goddard (13) applied DTA to the study of molecular interactions between lauryl or myristyl alcohol and sodium lauryl or myristyl sulfate. The melting behaviors of these mixtures provided evidence for the existence of association complexes of the alcohols and sulfates. Later (14), they prepared complexes exhibiting identical stoichiometries from aqueous and aqueous–ethanol solutions.

Visser and Wallace (15) studied the phase diagram of the benzoic acid–naphthalene system by DTA, and emphasized the point that this method is especially useful in the study of eutectics. They also employed DTA for detecting eutectic-forming impurities in *p*-toluenesulfonamide. Sekiguchi and his associates (16) have examined the urea–chloramphenicol and aminopyrine–barbital systems using a semimicro differential thermal analyzer which permitted visual observation of the sample. McAdie (17) has prepared a series of molecular compounds of urea and even-numbered normal paraffins, and has studied these molecular compounds using DTA. In addition, many papers have

appeared in the literature describing the use of DTA in the detection of inorganic complexes (18).

While the utility of DTA in the identification of complexes and inclusion compounds has been amply documented, very little attention has been devoted to its use for the routine screening of combinations of pharmaceuticals for potential interactions. In this paper DTA is employed as a screening tool, and its usefulness for this purpose is compared with two of the better known techniques of thermal analysis—the cooling curve method and the thaw-melt method.

Thermal methods for the detection of complex formation, molecular compound formation, or inclusion compound formation, are based on the construction of phase diagrams from thermal data. Such phase diagrams are of great value in the determination of the stoichiometry of the complex or complexes formed. In addition, they furnish an indication of the thermal stability of the complex, as well as the concentration range over which it can exist. Phase diagrams have been constructed using the cooling curve method, the thaw-melt method, differential thermal analysis, and thermomicroscopic techniques. The first three methods listed will be considered here.

The cooling curve method was first suggested by Tamman in 1903 (19). The cooling curve method suffers from a number of inherent disadvantages. It is rather time consuming, requires relatively large amounts of sample, and changes in slope can be missed, especially if cooling is accomplished rapidly.

The thaw-melt method, proposed by Rheinbolt in 1925 (20) requires only small amounts of sample, and is easier to use. It has been employed by a number of workers for the detection of interactions in a variety of organic systems. The principal drawback of this method is that it relies on a subjective observation, and thus, is not highly reproducible. Furthermore, since visual observation is required, one is restricted to the use of melting point determination techniques which permit visualization of the contents of the capillary tube. This places a practical limit on the upper temperature of measurements.

DTA is a thermal technique in which the heat effects associated with chemical or physical changes are measured as a function of time or temperature while a substance is being heated at a uniform rate. Physical changes such as crystalline transitions, fusion, evaporation and sublimation, and some chemical changes, such as oxidative decomposition and decarboxylation, can be observed. The heat absorption or liberation which occurs during the course of these changes can be measured by a differential method.

The sample to be investigated is placed in one chamber, and a thermally inert material is placed in another. A thermocouple is inserted in each chamber, and the sample and reference material are heated at a uniform rate. The temperature of the sample is continuously compared with that of the reference material, and the difference between the two temperatures, the temperature differential, $\Delta T$, is measured as a function of sample temperature or of time. If no physical or chemical change associated with heat absorption or liberation takes place in the sample, $\Delta T$ will be zero. When a transition begins to occur in the sample, the sample temperature will be different from that of the reference material, and either a positive or negative $\Delta T$ will be observed.

In DTA, even a very small heat effect can be detected. The temperature can be raised to 500° or higher, depending on the equipment used. This technique effectively overcomes the low-temperature restriction of the thaw-melt method, as well as the subjective observation limitation, and makes it possible to obtain binary-phase diagrams for a large number of organic compounds.

Pure compounds which do not exhibit polymorphism, do not contain solvent of crystallization, and do not decompose prior to melting, generally exhibit a single endothermic peak corresponding to the fusion temperature. The resolidified melt of a binary mixture corresponding to the eutectic composition will also exhibit a single peak. Other binary mixtures will typically exhibit more than one peak. As a rule, two peaks are observed: the first corresponds to the eutectic melting temperature, and the second, to the temperature at which complete liquefaction occurs. If molecular compounds are formed between the components, additional peaks will appear in the DTA thermograms.

## EXPERIMENTAL

**Reagents**—Caffeine (m.p. 235–237°), theophylline (m.p. 269–273°), acetaminophen (m.p. 168–170°), and quinine (m.p. 173–177°) were recrystallized from distilled water. Phenobarbital (m.p. 174–177°), phenacetin (m.p. 134–136°), aspirin (m.p. 135°), and diphenylhydantoin (m.p. 295–298°) were recrystallized from 95% ethyl alcohol. Atropine (m.p. 114–116°) was recrystallized from acetone. Deoxycholic acid[1] (m.p. 174–176°) and menadione[2] (m.p. 104–106°) were used as obtained from the suppliers. All reagents were thoroughly dried in a vacuum desiccator over calcium sulfate.

**Procedure**—When samples were examined by the cooling curve method, reagents in various proportions were weighed and thoroughly mixed using a mortar and pestle. The mixture was placed in a 2.4-cm. diameter test tube, fitted with a rubber stopper. A thermometer and loop-type metallic stirrer were inserted, and the contents of the test tube were heated in an oil bath until the temperature was approximately 20° higher than the melting point of the mixture. The test tube was transferred into another larger test tube, diameter 4.4 cm. The molten mixture was stirred manually, with an up-and-down motion, and the temperature was recorded at 5–10-sec. intervals. Approximately 10 g. of sample was used for each determination.

For the thaw-melt method, various combinations of binary mixtures were weighed and mixed thoroughly using a mortar and pestle. Each mixture was carefully melted, then resolidified with the aid of agitation. Approximately 2 to 5 mg. of finely powdered sample was put into an ordinary capillary melting point tube, and the latter was placed near the thermometer bulb in a silicone bath. The temperature of the bath was raised at a rate of 1°/min. using an electric heating coil. The silicone oil in the bath was kept in constant circulation with an electric stirrer. Thaw points and melting points were determined by visual observation with the aid of a magnifying glass.

Samples examined using DTA were prepared by two methods, hereafter referred to as the physical mixture method and the resolidified melt method. For the former, samples of binary mixtures were weighed, triturated until thoroughly mixed using a mortar and pestle, then subjected to DTA. In the resolidified melt technique, those samples which did not exhibit a tendency to sublime at temperatures below the melting point were thoroughly mixed and placed

[1] Matheson Coleman & Bell, East Rutherford, N. J.
[2] Mann Research Laboratories.

in a beaker. The beaker was heated in an oil bath until its contents melted.

Steps were taken to minimize sublimation in those cases where it occurred. Samples were placed, after mixing, in a small glass sublimation cup, which was then covered with a glass cover slip. The sublimation cup was heated on a Kofler hot stage until the sample melted.

After the sample was obtained in a molten state, it was cooled to room temperature to induce resolidification, regardless of the technique of melting. In some instances agitation or slight reheating was required to promote crystallization. The finely powdered resolidified sample was placed in a 2-mm. diameter capillary melting point tube. The amount of sample used varied from 2 to 5 mg. for each determination.

**Instruments**—A differential thermal analyzer, equipped with a standard cell attachment was used for DTA measurements.[3] For determinations in which visual observation of the sample was advantageous, a slitted heating block fitted with a Pyrex sleeve was used. Glass beads were used as the reference material, and the temperature was determined by use of chromel/alumel thermocouples. A heating rate of 10°/min. was employed in all of the experiments, with the exception of the diphenylhydantoin-phenobarbital system. A heating rate of 30°/min. was used in order to minimize the problem of phenobarbital sublimation.

A Y-axis sensitivity setting of 0.5°/in. was used to measure the differential temperature, $\Delta T$. Experiments were carried out in a nitrogen atmosphere in order to reduce the likelihood of oxidative decomposition.

Peak temperatures from the differential thermograms were taken as transition temperatures. These temperatures were corrected for the nonlinear temperature response of the chromel/alumel thermocouple. Corrected peak temperatures were plotted against corresponding compositions to obtain phase diagrams.

Various combinations of resolidified melts were analyzed by the X-ray powder-diffraction method. Samples were placed in a 0.3 to 0.5-mm. diameter capillary tube. A camera (Debye-Scherrer) with 114-mm. diameter was used, and Cu K$\alpha$ radiation was employed.[4] The powder patterns obtained in this study have been reproduced elsewhere (21).

## RESULTS AND DISCUSSION

**Deoxycholic Acid–Menadione**—Figure 1 illustrates DTA thermograms obtained from resolidified melts of pure deoxycholic acid, pure menadione, and mixtures of these two components. These thermograms were used to construct Fig. 2, the phase diagram for the deoxycholic acid–menadione system. This diagram indicates the existence of eutectic compositions corresponding to 0.12 and 0.96 mole fraction of menadione, with eutectic temperatures of 162 and 103°, respectively. No endothermic peak is observed at the liquidus line for the sample containing 0.9 mole fraction menadione. The liquidus line is rather steep in this region, and hence melting occurs over a range of temperatures without the appearance of a separate,

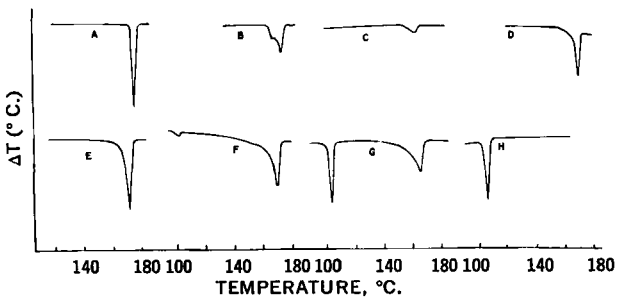


**Figure 1**—*DTA thermograms. Key;* A, *deoxycholic acid (DCA)*; H, *menadione (M)*; B, *0.05 mole fr. M*; C, *0.10 mole fr. M*; D, *0.30 mole fr. M*; E, *0.33 mole fr. M*; F, *0.50 mole fr. M*; G, *0.70 mole fr. M.*

[3] Du Pont model 900.

[4] The authors would like to thank Dr. Norman Baenziger of the Department of Chemistry, University of Iowa, for his assistance in obtaining and interpreting the powder patterns.

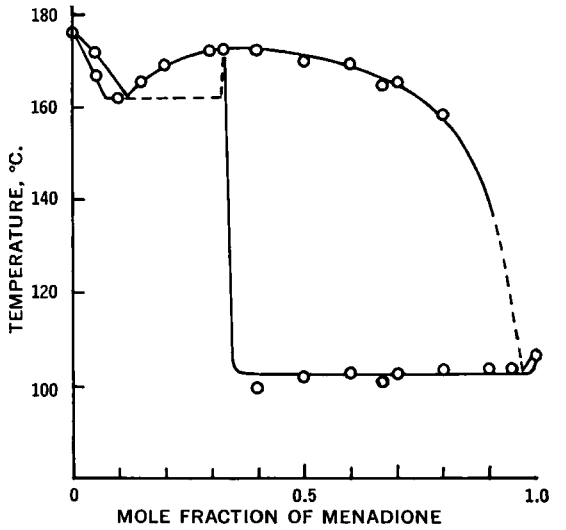


**Figure 2**—*Phase diagram for the deoxycholic acid-menadione system constructed from DTA data.*

sharp endothermic peak. A eutectic peak is observed at 162° for the composition 0.1 mole fraction menadione, but no eutectic peaks were obtained in the composition range 0.15 to 0.3 mole fraction menadione. Presumably, in this region, the liquidus line lies so close to the eutectic line that the latter cannot be readily distinguished. For this reason the eutectic horizontal is represented in the figure by a dashed line.

A phase diagram was also constructed (Fig. 3) from cooling curves. Points for this diagram were obtained by noting the temperature corresponding to a change in slope of the cooling curves and to the characteristic eutectic halts. Cooling curves could not be obtained for pure deoxycholic acid, and for the sample containing 0.9 mole fraction deoxycholic acid, since these samples solidify as glasses when cooled from the molten state, and their cooling curves fail to exhibit marked changes in slope. Cooling curves for the other compositions in this system permit the construction of a partial phase diagram. Eutectic halts, however, are not obtained for the majority of the samples examined, and hence, this technique is clearly inferior to DTA for the characterization of the deoxycholic acid-menadione system.

Points which can be obtained by the cooling curve method do closely correspond to those obtained by DTA, however, and substantiate the conclusion that a molecular compound is formed in this system. The stoichiometry of the complex, whose existence is deduced from the maximum in the phase diagram at 0.33 mole

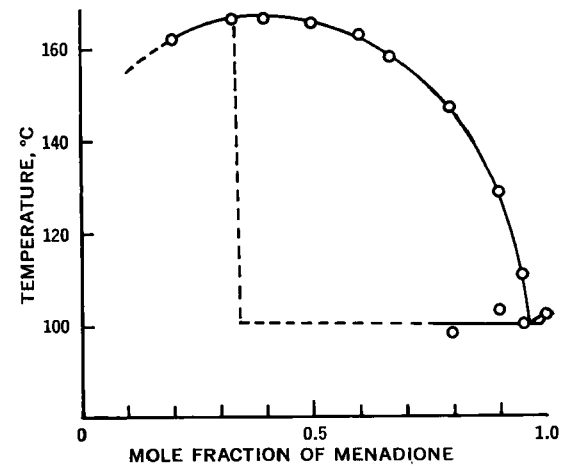


**Figure 3**—*Phase diagram for the deoxycholic acid-menadione system constructed from cooling-curve data.*

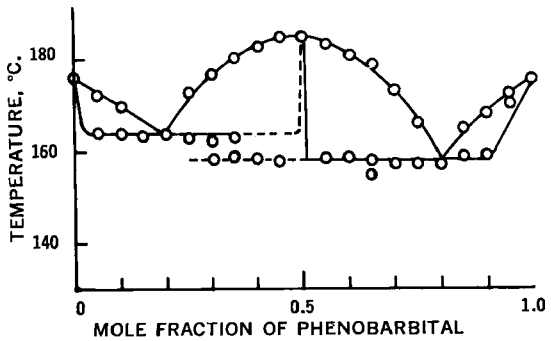


**Figure 4**—*Phase diagram for the quinine-phenobarbital system constructed from DTA data obtained from resolidified melts.*

fraction menadione (166°), is apparently 2:1 (deoxycholic acid–menadione).

X-Ray powder analysis provides supporting evidence for the stoichiometry of the complex. A powder pattern distinctly different from that of deoxycholic acid or menadione appears when the resolidified melt has a molar composition of 2:1. This pattern also is evident, but less distinct, in samples on either side of the maximum. The powder pattern for the 2:1 composition is virtually superimposable on that obtained from a sample of deoxycholic acid–menadione complex separated from 40% aqueous ethanol solution. The stoichiometry of this complex, determined by the solubility method, has been found to be 2:1 (22).

**Quinine–Phenobarbital**—A phase diagram of this system, constructed from DTA data obtained on resolidified melts, is shown in Fig. 4. It consists of two simple eutectic systems which meet at a maximum corresponding to the 1:1 composition. The eutectic temperatures are 164 and 158°, and the eutectics are formed at 0.2 and 0.8 mole fraction phenobarbital, respectively. The melting point of the 1:1 composition, 185°, is higher than the melting point of either phenobarbital or quinine. Only one endothermic peak is observed at this composition, suggesting complete, or virtually complete conversion to the complex.

An almost identical phase diagram is obtained from DTA thermograms of physical mixtures of the two components (Fig. 5). An additional broad endothermic peak is observed in most of the samples in the temperature range 122–130°. The positions and sizes of the peaks are variable. In each case, however, the endothermic peak is followed by a broad exothermic peak of approximately the same size, having a maximum located at a temperature approximately 13° higher than the minimum of the endothermic peak. It will be observed that extrapolation of the two extreme branches of the liquidus line results in an intersection in this temperature range.

It should be noted that quinine trihydrate dehydrates at 125°. The fact that no peak is observed in this region in the pure quinine sample effectively rules out the possibility that the endothermic peaks observed here in the other samples might be attributed to dehydration. Endothermic peaks are not observed in this temperature range in pure phenobarbital, nor are they observed in the resolidified melts. Consequently, these peaks are assumed to correspond to metastable eutectic liquefaction. Samples of the resolidified melts consist of mixtures of quinine and the complex, or of phenobarbital and the complex, depending on the proportions of the two components. The physical mixture samples, on the other hand, probably contain negligible amounts of the complex until partial liquefaction occurs at the metastable eutectic temperature. Following partial liquefaction, the interaction between the two components occurs more rapidly than it does in the solid state. The exothermic peak which follows a metastable eutectic peak has been attributed by Sekiguchi (23) to one of two phenomena: release of the heat of molecular compound formation, or solidification of the metastable liquid. DTA is used to advantage in this system, since metastable eutectic temperatures furnish confirmatory evidence of molecular compound formation, and are difficult to detect by visual observation.

The existence of a molecular compound in this system is further substantiated by powder patterns obtained from resolidified melts having varying compositions. A new diffraction pattern is observed in these mixtures, and appears to be most distinct at the 1:1 composition, where there is little evidence of the presence of free quinine or free phenobarbital.

A similar molecular compound has been reported by Busquet and Vischniac (24). The species they obtained from ethyl alcohol solutions is reported to melt at 182–183°. The melting point observed in the present study was 185°, identical with the melting point of the 1:1 complex isolated by French and Morrison (1) from alcohol solutions. The latter authors concluded from IR studies that phenobarbital exists as the phenobarbiturate ion in the molecular compound. The complex appears to be weak, however, since two separate spots appear, corresponding to phenobarbital and quinine, when the complex is analyzed by TLC.

**Theophylline–Phenobarbital**—Figure 6 is the phase diagram for this system, constructed from DTA data obtained from resolidified melts. The phase diagram can be divided into two eutectic systems, having eutectic temperatures of 248 and 169°. The compositions of the two eutectics correspond to 0.25 and 0.96 mole fraction phenobarbital, respectively. The intermediate maximum, at 250° and 0.33 mole fraction phenobarbital, suggests that two molecules of theophylline associate with one molecule of phenobarbital to form a molecular compound. The dashed lines in the diagram rep-

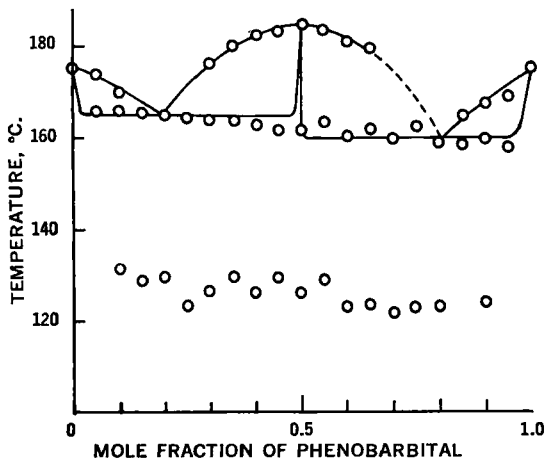


**Figure 5**—*Phase diagram for the quinine-phenobarbital system constructed from DTA data obtained on physical mixtures.*

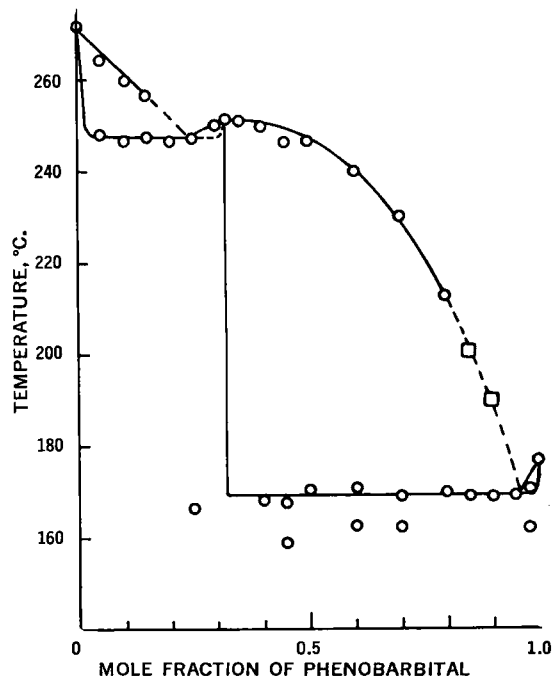


**Figure 6**—*Phase diagram for the theophylline-phenobarbital system constructed from DTA data.*

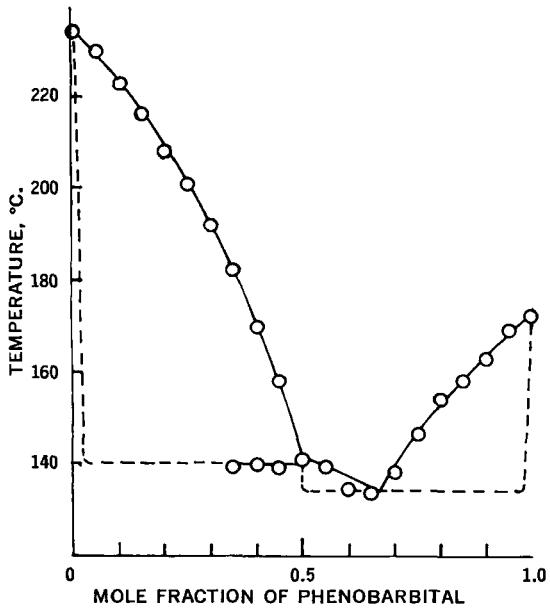


**Figure 7**—*Phase diagram for the caffeine-phenobarbital system constructed from cooling-curve data.*

resent extrapolations of available data. A portion of the eutectic horizontal, at 248° cannot be observed, presumably because of its proximity to the liquidus line. Furthermore, in the region 0.85 to 0.90 mole fraction phenobarbital, no endothermic peaks are observed corresponding to the liquidus line. The temperatures at which the last skeleton of crystal disappears in these samples can, however, be determined through the open slit in the DTA cell. Points on the phase diagram determined in this way are identified by square symbols.

In some samples endothermic peaks are observed in the 159–164° range. Experimental conditions, such as agitation on cooling, cause these peaks to disappear in some instances, and cause changes in their size in other instances. These peaks are assumed to be due to polymorphism in phenobarbital. Vegh *et al.* (25) have reported that 1% of sodium nitrate added to phenobarbital permits the observation of a polymorphic transformation at 163° in the latter compound.

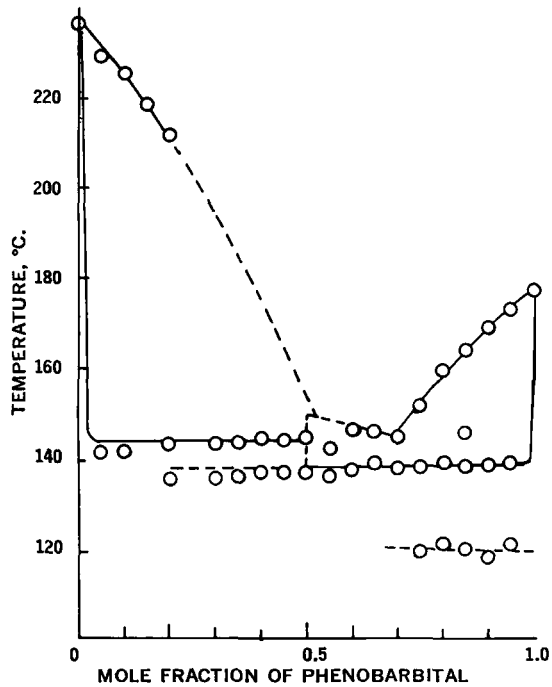


**Figure 8**—*Phase diagram for the caffeine-phenobarbital system constructed from DTA data.*

Others (26, 27) have reported that polymorphism in phenobarbital is sensitive to conditions of heating, and to the presence of other substances. Mesley (27) lists melting points of 13 phenobarbital polymorphs, including Form III, 167°; Form IV, 163°; Form V, 160°; and Form VI, 157°.

X-Ray powder patterns of solidified melts of mixtures of theophylline and phenobarbital reveal a new diffraction pattern, different from that of the pure components. This pattern appears to be most distinct at the composition 0.4 mole fraction phenobarbital. The powder pattern for the 0.33 mole fraction theophylline sample apparently exhibits the influence of a small excess of unreacted theophylline. On the basis of the other available evidence, however, it seems reasonable to conclude that the complex formed probably has a stoichiometry of 2:1 (theophylline–phenobarbital).

A molecular compound with this composition has been separated by Higgins and Dunker (28) from ethanol solutions of the two components. The melting point reported for this complex is 250.7–251.7°, in good agreement with the value, 252°, obtained in the present study. Higgins and Dunker regard this interaction product as a molecular compound rather than a salt, in view of the fact that two molecules of theophylline are in association with 1 mole of phenobarbital, the latter being a weak monobasic acid.

Ariesan *et al.* (29) on the other hand, reported a stable 1:1 complex, m.p. 254°, and an unstable 1 theophylline:2 phenobarbital complex, m.p. 244°. They constructed a phase diagram from thermomicroscopic observations. This diagram indicates that the eutectic horizontal extends from 0 to 40% theophylline, whereas in the present study the eutectic horizontal extends to a composition corresponding to 60% theophylline. There is no evidence in these data for the formation of an unstable eutectic.

**Caffeine-Phenobarbital**—This system was investigated using three thermal methods, the cooling curve method, the thaw-melt method, and DTA. Figure 7 represents the phase diagram for this system, obtained by the cooling curve method. It can be inferred from the diagram that an incongruently melting molecular complex with a composition of 1:1 is formed in the molten state. No eutectic halt is observed in the majority of compositions. It is reasonable, however, to designate the eutectic temperatures as 135 and 140° based on the eutectic halts that were obtained.

The DTA results (Fig. 8) are in marked contrast to those obtained by the cooling curve method. No liquidus line is observed in the region 0.45 to 0.7 mole fraction caffeine. Two distinct eutectic horizontals are shown at 138 and 143°. The eutectic horizontal at 138° is observed to extend beyond the 1:1 composition. The peaks on this extended segment, however, are small in comparison to those from which the 143° eutectic horizontal was constructed, and hence, the extension is represented by a discontinuous line. The phase diagram indicates that this system is not a simple eutectic, and offers

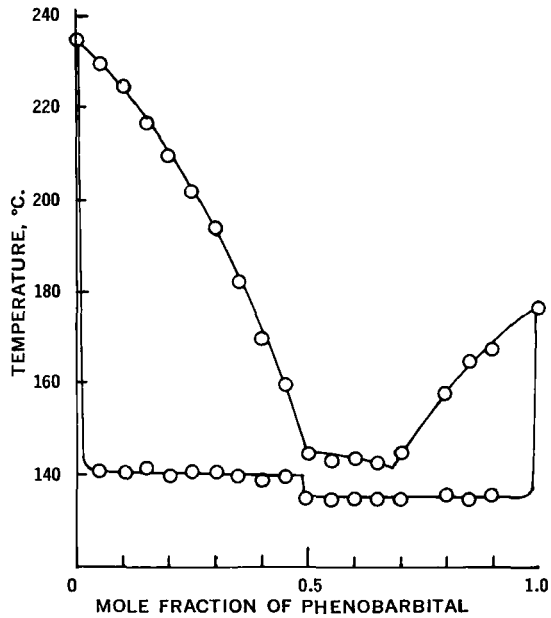


**Figure 9**—*Phase diagram for the caffeine-phenobarbital system constructed from thaw-melt data.*

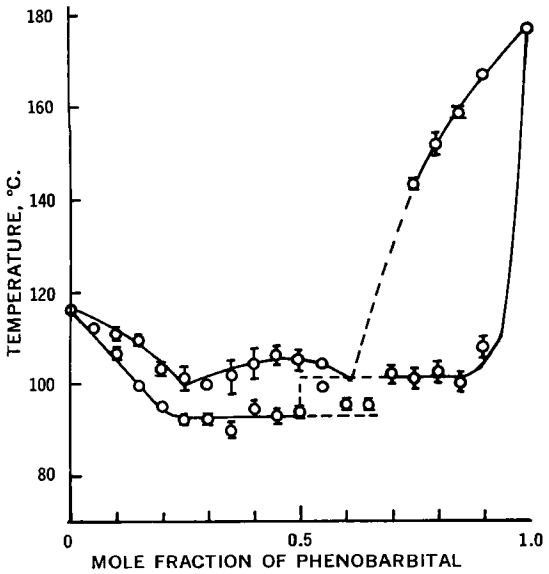


**Figure 10**—*Phase diagram for the atropine-phenobarbital system constructed from DTA data.*

evidence that a molecular compound is formed. It is not unreasonable, on the basis of the evidence, to postulate that the stoichiometry of the complex is 1:1.

Five samples, in the range 0.05–0.15 mole fraction caffeine, exhibit extremely small endothermic peaks at temperatures near 120°. The size of these peaks increases with increasing caffeine concentration. Larger endothermic peaks are observed at temperatures somewhat higher, 125–128°, when physical mixtures are examined. These endothermic peak are followed immediately by exothermic peaks, and hence, presumably represent metastable eutectic liquefaction, followed by reaction and solidification. The appearance of these peaks in the resolidified melt samples suggests that a small amount of phenobarbital must remain in the free form, even in the presence of a large excess of caffeine. Hence, when the sample is reheated, a second metastable eutectic is formed. This eutectic has a liquefaction temperature somewhat lower than the eutectic formed in the physical mixture samples. The complex formed in this system must be extremely weak.

The most complete phase diagram can be constructed from data obtained by the thaw-melt method (Fig. 9). Eutectic horizontals (135 and 141°) obtained by use of this technique are in good agreement with those drawn from the DTA data. The formation of a metastable eutectic, observed in the DTA study, cannot be detected by the thaw-melt method. Since formation of a metastable eutectic is confirmatory evidence for the formation of a molecular compound, the DTA technique still offers one advantage over the thaw-melt, in spite of the fact that it does not afford as complete a phase diagram.

All three methods indicate that the stable eutectic composition corresponds to 0.33 mole fraction caffeine, and all three methods suggest the formation of an incongruently melting complex, with a molar ratio of 1:1. Additional evidence for the formation of this complex is obtained from X-ray powder patterns. A pattern different from that of either phenobarbital or caffeine emerges, and is most distinct at the 1:1 molar ratio.

Higuchi and Lach (30) studied this system by the solubility method, and reported that a 2:1 (caffeine:phenobarbital) complex is formed at 30°, and a 1:1 complex is formed at 15°. The melting point of the 2:1 complex is given as 145–146°. The 1:1 complex was not isolated.

**Atropine-Phenobarbital**—When atropine is melted with phenobarbital and the mixture is cooled, a glassy material is formed upon resolidification. Consequently, the cooling curve method cannot be employed in the construction of phase diagrams for this system. Resolidified melts provide no useful information when analyzed using DTA. For this reason, the atropine-phenobarbital system can best be examined by subjecting physical mixtures of the two components to DTA. Results of this study are shown in Fig. 10.

Since the endothermic peaks obtained are very broad, ranges of temperatures instead of single peak temperatures are recorded in many cases. Sharp peaks are indicated on the diagram by circles; broader peaks are indicated by the range symbol.

Two eutectics were observed, at 93 and 101°, with eutectic compositions corresponding to 0.6 and 0.25 mole fraction phenobarbital, respectively. The phase diagram suggests the formation of a molecular compound having a molar ratio of 1:1.

To the authors' knowledge, the atropine-phenobarbital complex has not previously been reported. Atropine has, however, been observed to form complexes with ascorbic acid (31), with 8-nitrotheophylline (32), and with its own sulfate (33).

**Systems in Which No Interaction Is Detected**—Several other systems were also examined using DTA. In each case a complete phase diagram could be constructed from the data, and there was no evidence of molecular compound formation. The eutectic temperatures and eutectic compositions in these systems are as follows: aspirin-phenobarbital, 111–122°, 0.35 mole fraction phenobarbital; phenacetin-phenobarbital, 114°, 0.33 mole fraction phenobarbital; diphenylhydantoin-phenobarbital, 165°, 0.925 mole fraction phenobarbital; and acetaminophen-phenobarbital, 141°, 0.45 mole fraction phenobarbital. Phase diagrams for these systems are reproduced in Figs. 11–14. The additional horizontals observed in the latter two systems are assumed to be due to polymorphism in phenobarbital.

## SUMMARY AND CONCLUSIONS

Several thermal techniques including the cooling curve method, the thaw-melt method, and differential thermal analysis were employed to investigate their relative usefulness for the detection of interactions between pharmaceuticals. The existence of such interactions can be deduced from phase diagrams constructed from the thermal data.

The cooling curve method is a convenient technique for constructing phase diagrams, since it generally enables one to obtain reasonably complete data for the liquidus line. However, in order for this method to be useful, the sample must resolidify on cooling during fairly short time intervals. Some compounds, such as deoxycholic acid, become glassy after melting, and the cooling curve fails to provide any information about the eutectic temperature in the majority of samples. Stirring is required during the cooling process, and this becomes something of a problem when the melt is viscous. Relatively large amounts of sample are required in this method.

The thaw-melt method requires relatively simple and inexpensive equipment, and shares with DTA the advantage of requiring only small sample sizes. Typically, complete phase diagrams are obtained. Temperature measurements are obtained by visual observation, however, and subjective judgments are frequently required in the determination of thaw points. Phase diagrams obtained by this method may not be as reproducible as those obtained by DTA.

This is not to say that DTA is the perfect method for detecting molecular compound formation. In cases where the slope of the liquidus line is steep, the endothermic peak at the final melting point may be so broad that it is virtually indistinguishable from the base line. Heating blocks which permit visual observation of the sample are helpful in overcoming this difficulty. In some instances

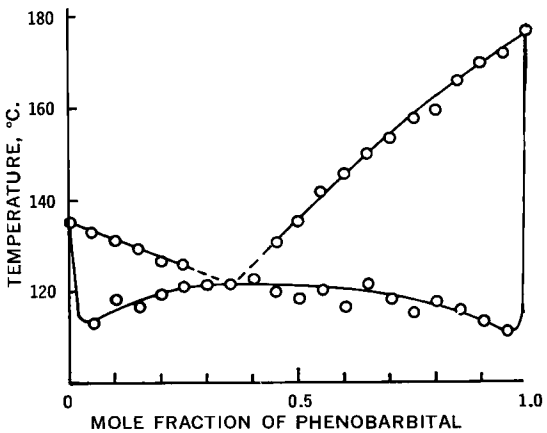


**Figure 11**—*Phase diagram for the aspirin-phenobarbital system constructed from DTA data.*

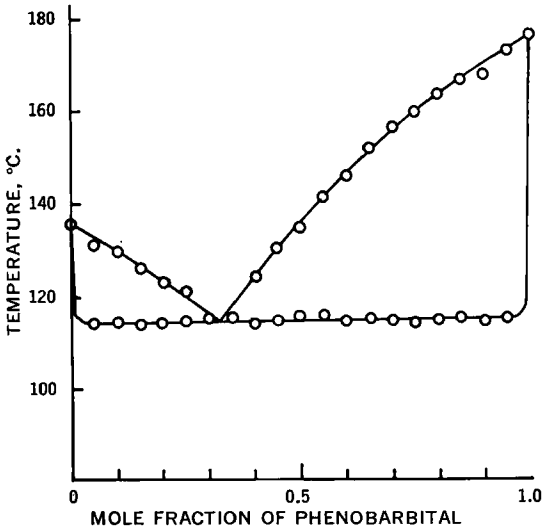


**Figure 12**—*Phase diagram for the phenacetin-phenobarbital system constructed from DTA data.*

experimentation is required in order to find a heating rate which will produce the best resolution of closely spaced transitions. Furthermore, since all chemical and physical transformations associated with heat loss or gain are represented by either exothermic or endothermic peaks, there may be some difficulty in interpreting the more complex thermograms. The concurrent use of thermogravimetric analysis, and the use of a heating block which permits visual observation of the sample will usually provide the additional information required to identify peaks in the thermogram.

Perhaps the most serious limitation of this method is the fact that many organic pharmaceutical compounds, including antibiotics and vitamins, decompose at or near the melting point, and cannot be studied by this method. This, however, is a limitation common to all thermal methods, and the advantages of DTA would seem to outweigh its disadvantages, making it a very versatile tool for routine investigations designed to detect possible interactions between pharmaceutical compounds.

While the formation of a complex in the melt of a binary system is not *a priori* evidence that an interaction will occur between the components in the solid state, it does suggest the need for a careful analysis of the system to ascertain the absence of such an interaction. Thermal changes which are observed when powdered solids are subjected to compression may be sufficient to facilitate reactions between components of a compressed tablet. Hanus and King (34) have shown that temperature increments induced by compression of powders may be appreciable. Such thermal effects can be expected to accelerate reactions between ingredients of a tablet formulation. It is entirely possible that similar reactions may occur (admittedly at a slower rate) in the absence of compression. Again,

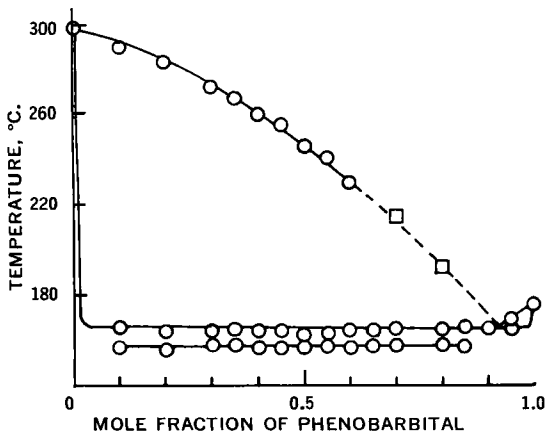


**Figure 13**—*Phase diagram for the diphenylhydantoin-phenobarbital system constructed from DTA data.*

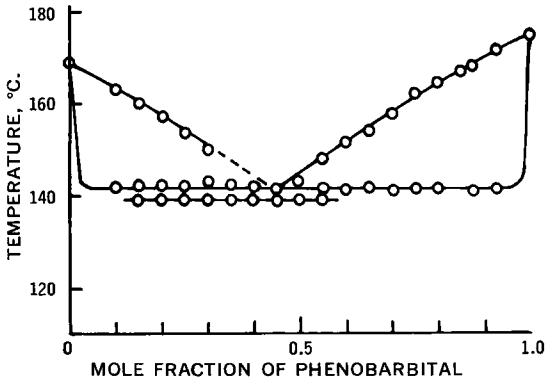


**Figure 14**—*Phase diagram for the acetaminophen-phenobarbital system constructed from DTA data.*

although no interaction may be detected initially in a finished formulation, it is possible that under conditions of storage, such an interaction may develop after a period of time.

When interactions between medicinal ingredients do occur, one can expect to observe considerable differences in physical properties such as dissolution rates, equilibrium solubilities, partition coefficients, *etc.* Since all these factors are known to influence the absorption and distribution of drugs, it should be apparent that a considerable amount of judgment must be exercised in the selection of combinations of medicinal agents to be incorporated into solid dosage forms. Control procedures should be instituted in order to provide assurance that no unexpected interactions are likely to occur between the components of the formulation. The DTA technique shows promise of being adaptable to the examination of finished dosage forms, as well as simpler binary mixtures.

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# Measurement of Acidity and Equilibria in Glacial Acetic Acid with the Glass-Calomel Electrode System

**THOMAS MEDWICK, GERALD KAPLAN,* and LOIS G. WEYER†**

**Abstract** □ This work describes the use of the glass-acetous fiber calomel electrode system in potential measurements in glacial acetic acid. Using the Nernst relationship and other equations describing equilibria in glacial acetic acid solution, the measurement of acidity, in terms of the activity of $H_2OAc^+$, and then the calculation of overall dissociation constants for some bases, salts, and perchloric acid have been carried out. The electrode system has been found to respond as predicted by theory. Most calculated values agree with literature constants. Discordant results are discussed. The choice of the acetous fiber calomel electrode is explained and other reference electrodes are treated. The use of electrochemical potentials, obtained from potentiometric titration curves, in the calculation of $K_B$ and $K_{BHClO_4}$ for bases is described. Results compare well with other methods. This procedure is recommended in the interest of increasing the specificity of titrations in acetic acid solvent. Based on sound fundamental principles, this work establishes a practical method for the determination of acidity in glacial acetic acid.

**Keyphrases** □ Acetic acid, glacial—acidity, equilibria measurements □ Glass-acetous fiber calomel electrode—acetic acid potential measurements □ Dissociation constants, overall—salts, bases, acid □ Potentiometric titration—acidity measure

Potentiometry has been a useful means for the study of equilibria in glacial acetic acid solvent. The chloranil electrode, introduced by Hall and Werner (1), was used by Bruckenstein and Kolthoff (2) to evaluate overall dissociation constants for selected acids, bases, and salts. The latter potentiometric study formed part of a series of papers thoroughly describing equilibria in glacial acetic acid (2–6).

The glass electrode, proven as an invaluable pH sensing device in water, has been used successfully in acetous solution. Higuchi *et al.* (7) used the glass-calomel cell to show that the glass electrode behaved according to theory in the study of salt phenomena. The general behavior of the glass electrode in acetous solution has been described by Cheng *et al.* (8). These workers suggested the use of this electrode for dissociation measurements. Measurements of dissociation constants using the glass electrode have been reported but agreement with constants obtained by other means is poor (9). Kolling *et al.* (10, 11) utilized the glass-calomel cell for determining dissociation constants for various bases and salts using a comparative potentiometric method. This procedure does not, however, involve the explicit measurement of solvated hydrogen ion activity.

This work reports the study of the use of the glass electrode to determine acidity, *viz.*, the activity of the solvated proton as $H_2OAc^+$, in glacial acetic acid. First the *Theory* section treats the theoretical basis for the use of direct or static potential measurements in the evaluation of acidity and dissociation constants; the necessary equations are presented. Then the theoretical basis for the use of titration or dynamic potential measurements in the calculation of dissociation constants is treated. Several compounds have been examined by these methods and the results are discussed. The ability of the glass electrode to function satisfactorily is reestablished. The use of the titration curve potentials seems particularly attractive in light of the rather widespread use of potentiometric titrations in glacial acetic acid (12–14).

## THEORY

When made with a hydrogen ion-sensitive electrode, potential measurements can be related to the hydrogen-ion activity