Application of Differential Thermal Analysis To Compatibility and Stability Problems in Penicillin-Stearic Acid Mixtures

HAROLD JACOBSON and GEORGE REIER

Abstract □ Differential thermal analysis (DTA) was used to identify stearic acid as the inactivating component in an unstable formulation containing sodium dicloxacillin and several excipients. Analyses were also performed on mixtures of stearic acid with potassium penicillin G, with sodium oxacillin monohydrate, and with ampicillin trihydrate. The resulting thermograms indicated the former two mixtures to be incompatible and the latter mixture to be compatible. The results as obtained by DTA gave excellent correlations with those obtained by an 8-week, 50° stability test.

Keyphrases □ Penicillin-stearic acid formulations—stability □ Stearic acid effect—pencillins, stability □ Differential thermal analysis—penicillins

Differential thermal analysis (DTA), a relatively old analytical tool, has recently gained favor in the study of compounds of interest to the pharmaceutical industry. Brancone and Ferrari (1) demonstrated the utility of the technique to detect crystal solvates, identify structural configurations, determine purity, detect crystal polymorphism, and determine the presence of eutectics. Guillory (2) used the technique to determine the heat of transition of methylprednisolone and sulfathiazole. Reubke and Mollica (3) have used a modification of DTA, differential scanning calorimetry (DSC), to determine the purity of various compounds.

Simon (4) has utilized this technique as a tool for the rapid evaluation of interactions of drugs with excipients in preformulation stability studies. He pointed out a possible interaction of triampyzine sulfate with magnesium stearate. The authors have also found DTA useful in preformulation stability studies and wish to present a report on the interaction of some penicillins with stearic acid.

The stability of a formulation depends, among other factors, on the compatibility of the active component with the other ingredients in a mixture. Unless incompatibility is glaringly evident (*e.g.*, the formation of an eutectic melting below room temperature) it is necessary to carry out a stability study that usually requires weeks or months. Utilizing DTA, however, it is possible to obtain significant data rapidly. Although DTA will not soon, or perhaps ever, replace the classical stability program involving long-time observation, it can provide an early alert to compatibility problems and indicate the most favorable directions to pursue for a successful formulation.

An experimental multicomponent capsule containing sodium dicloxacillin monohydrate as the active ingredient was found to suffer a 20% and an 8% decrease in potency after storage for 3 months at 50° and for 6 months at 22°, respectively. This report will describe how DTA was used to identify stearic acid, present as an excipient, as the inactivating agent A study then was also made of the compatibility of stearic acid with several different penicillins.

EXPERIMENTAL

The thermal analyses were made employing a differential thermal analyzer,[1] using 2-mm. micro sample tubes. A heating rate of 15°/min. was used with a differential temperature sensitivity of 1°/in. The measurements were made in the presence of air.

Mixtures of the various ingredients, prepared with mortar and pestle, and portions of each individual material were slugged in a 2.54-cm. (1 in.) die under 5,000 p.s.i. pressure and reduced through a No. 24 screen. This procedure was designed to approximate the conditions used in preparing the original capsule. In the latter instance, slugs were made of a mixture on a tableting machine, and subsequently reduced through an appropriate screen. The mixtures made contained sodium dicloxacillin monohydrate (hereafter referred to as dicloxacillin) with each of the following USP excipients or mixtures of excipients: lactose, magnesium stearate, stearic acid, lactose plus magnesium stearate, and, finally, lactose plus magnesium stearate plus stearic acid. The percentages used of each of these materials is shown in Fig. 1.

In addition, mixtures containing 5% stearic acid were also made using the same procedure as described above with each of the following penicillins: sodium oxacillin monohydrate, potassium penicillin G, ampicillin trihydrate, and a repeat with

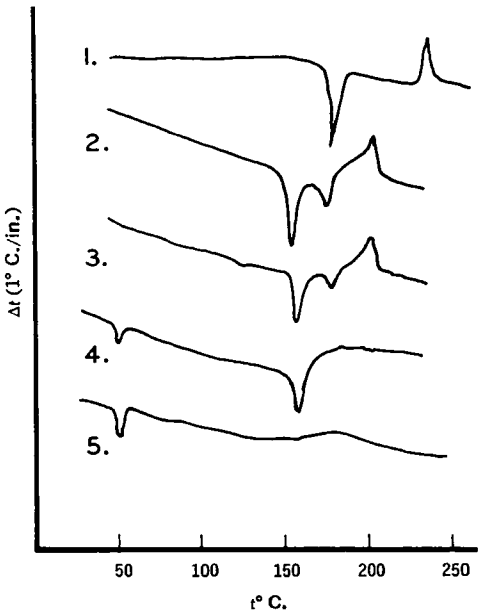

Figure 1—*Thermograms of sodium dicloxacillin and mixtures thereof. Key: 1, dicloxacillin; 2, 76% dicloxacillin +24% lactose; 3, 73% dicloxacillin, 23% lactose, 4% magnesium stearate; 4, 71% dicloxacillin, 22% lactose, 4% magnesium stearate, 3% stearic acid; 5, 93% dicloxacillin, 7% stearic acid.*

[1] DuPont 900.

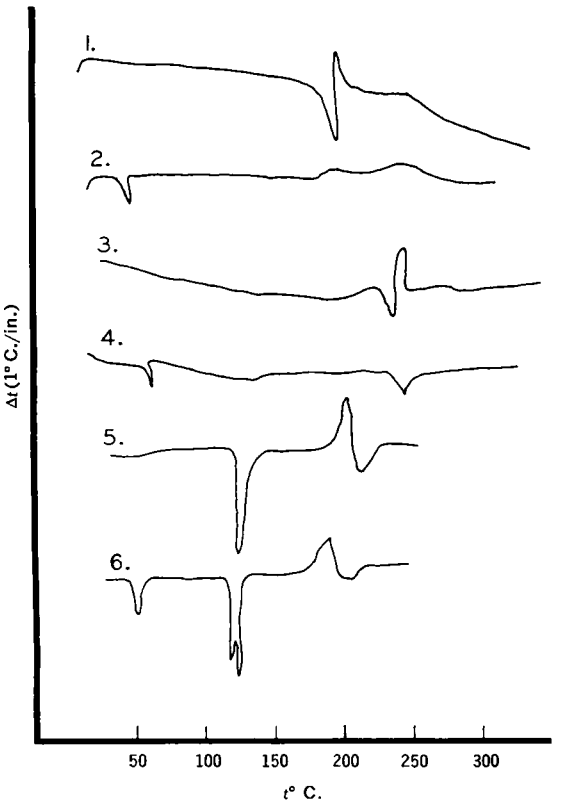

Figure 2—*Thermograms of penicillins and mixtures thereof with 5% stearic acid. Curves 1 and 2 refer to sodium oxacillin monohydrate and the mixture thereof with stearic acid, respectively; Curves 3 and 4 to potassium penicillin G and the mixture with stearic acid, respectively; Curves 5 and 6 to ampicillin trihydrate and the mixture with stearic acid, respectively.*

dicloxacillin. These mixtures, in addition to being examined by DTA, were also tested for stability. They were placed in capped vials and stored at 50° for 8 weeks. The potency was determined by the hydroxylamine assay (5).

RESULTS AND DISCUSSION

Figure 1 illustrates the significant thermograms. Trace 1 of this figure is that of dicloxacillin. The endotherm observed at 178° represents the desolvation of this material, and the exotherm at 233° represents oxidative and thermal degradation.

The thermogram of the dicloxacillin-lactose mixture shown in Trace 2 of Fig. 1 combined the features characteristic of the thermograms of each component indicating no interaction under these conditions. The first endotherm of Trace 2 is attributable to lactose. A thermogram of pure USP lactose shows endotherms at 148° and 218°. At the latter temperature decomposition begins. This ensuing degradation of lactose is the probable cause of the depression of the exotherm of the mixture (Trace 2) to a lower temperature than that found with pure dicloxacillin. Nevertheless, it is evident that the characteristic features of the dicloxacillin are preserved.

The minimal effect of the addition of magnesium stearate to the above mixture is shown in Trace 3 of Fig. 1. A thermogram of pure magnesium stearate gives an endotherm at 125°. At the concentration in which it is present in the mixture and at the sensitivity setting of the instrument, this endotherm is barely discernible.

The incorporation of stearic acid into the mixture (Trace 4) has a profound influence upon the characteristic thermogram features of dicloxacillin. The endotherms present are those due to stearic acid and lactose; those due to the dicloxacillin have been obliterated. Finally, in Trace 5 there is a mixture of only dicloxacillin and stearic acid, and again the obliteration of those features attributable to the dicloxacillin is observed. A thermogram

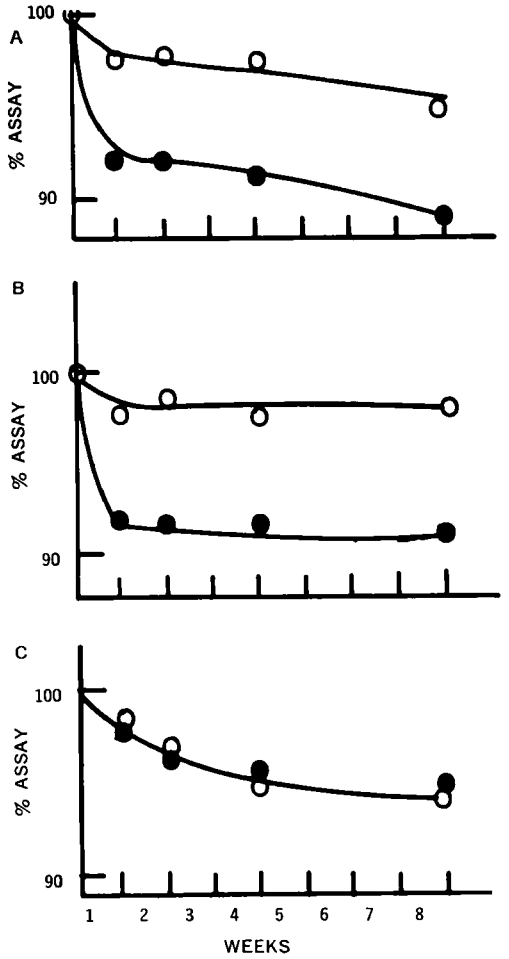

Figure 3—*The potencies of several penicillins as a function of time at 50°C. Key: ○, single penicillin species; ●, penicillins mixed with 5% stearic acid. A, Na oxacillin·H_2O; B, K pen. G; C,1 ampicillin·$3H_2O$.*

of pure stearic acid gives an endotherm at 50° and the material is essentially stable when heated to 285°, a temperature well beyond that where the other features of the thermogram are obtained. The thermogram of stearic acid obtained after cycling between room temperature and 285° is identical to the initial one.

It is clear that stearic acid was the inactivating agent in this formulation, and that furthermore, it would be possible to predict the incompatibility between stearic acid and dicloxacillin from an examination of the thermograms in Fig. 1.

The application of DTA in this manner would be severely limited if, upon mixing, an eutectic or other mixed melting-point entity were formed. Such mixtures, which are readily detectable by DTA, give rise to thermograms characteristic of the new entities. They would not be combinations of the thermograms of the simple components. It would be difficult to state if these new physical forms would be deleterious to stability, and, consequently, the interpretation of the thermogram becomes uncertain. However, the persistence of the stearic acid endotherm at the low concentrations in which it was present in all the mixtures studied excluded this possibility. Further support is given by the essentially unchanged melting point of the stearic acid in the mixtures from that observed with pure stearic acid.

The effect of stearic acid upon sodium oxacillin monohydrate, potassium penicillin G, and ampicillin trihydrate is shown in Figs. 2 and 3. The thermal patterns indicate the formation of unstable mixtures when either the sodium oxacillin monohydrate or potassium penicillin G is mixed with stearic acid. However, the thermal pattern for the ampicillin trihydrate-stearic acid mixture shows no significant alterations, and this mixture is presumed to be stable. The correlation with the results of the stability analysis was excellent as can be seen from the assay curves of Fig. 3.

The potencies of the former two mixtures have decreased significantly faster than either of the respective pure species, whereas no difference in assay was observed between that obtained for the pure ampicillin trihydrate and its mixture. The results for dicloxacillin confirmed the original finding. The stability assay showed that the potency of the dicloxacillin stearic acid mixture decreased to 85% whereas pure dicloxacillin decreased to 94%.

Although part of the information gained in this work came as confirmation of an already observed unstable experimental formulation, it did identify the inactivating component. In the latter part of the study, however, data have been obtained that can now be used as a guide for future formulating trials. Since the completion of this study, it has become routine to examine new systems with DTA to provide data relevant to the compatibility of the several materials present in a formulation, and thereby a more rational approach to early formulation designs.

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Differential Agar-Diffusion Bioassay for Cytotoxic Substances

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Abstract □ A differential agar-diffusion bioassay for antitumor compounds was developed based on the cytocidal effects of the test compound on Eagle's KB cells, Earle's L cells, and L-1210 cells in an agar medium. The three cell strains were grown in suspension culture in Waymouth's medium MB 752/1 supplemented with calf serum. Paper disks dipped in test solutions were applied to seeded agar plates and after 18-hr. incubation the cytocidal effects were determined by flooding the surface with the vital stain, 2,6-dichlorophenolindophenol. The diameters of the nonreduced zone around the paper disks were measured. The KB cells were sensitive to more compounds than the Earle's L cells or the L-1210 cells in a survey of 30 compounds with demonstrated antitumor activity *in vivo*. The agar plates were also used for preparing bioautographs of paper chromatograms and thin-layer chromatograms.

Keyphrases □ Cytotoxicity—screening method □ Agar diffusion bioassay, differential—cytotoxic activity □ 2,6-Dichlorophenolindophenol—viable cell detection

The use of an agar-diffusion test with mammalian cells to screen for potential antitumor agents is based either on the loss of the capacity of mammalian cells in agar suspension to reduce an indicator dye to suitable oxidation-reduction potential in the presence of the test compound (1–3) or the inhibition of cell growth in the agar media in the presence of the toxic agent (4, 5). The authors have examined the former method as a screen for compounds of potential interest as antitumor agents and have used cells from three sources: Eagle's KB strain of human epidermoid carcinoma cells (6); Earle's L cells (mouse fibroblast, NCTC 929) (7), and L-1210 leukemia cells in tissue culture (8). A number of compounds were cytotoxic to only one or two of these three cell lines. This differential cytotoxicity may be useful in screening compounds in the future.

METHODS

Earle's L cells and Eagle's KB cells were grown in suspension culture using Waymouth's medium MB 752/1 supplemented with 10% (v/v) calf serum; 0.3 g./l. 4000 cps. methylcellulose,[1]; 1 g./l. nonionic polymer,[2]; and 1 g./l. anionic surfactant[3] in 250-ml. conical flasks on a rotating shaker as previously described (9). The L-1210 cells were grown in this medium without shaking as the cells remained in suspension under these conditions when incubated at 37°. The cells were grown in the shaken culture for 3 days at 37° and then harvested by centrifugation. The cells of each culture were then suspended in fresh medium so that the cell count was 4×10^6 cells/ml. Fifty milliliters of this cell suspension was added to a flask containing 37 ml. of calf serum, 1 g. of glucose, and 3 g. of melted agar (in 120 ml. water) and the resulting suspension poured into a 3-qt. Pyrex baking dish and allowed to solidify. Schleicher and Schuell 12.5-mm. paper disks (holding 0.1 ml. of solution) were dipped in the test solutions and placed on the agar surface. The baking dishes were loosely covered with an aluminum cover and incubated at 37° for 18 to 20 hr. The disks were removed and the agar surface was flooded with 0.05% solution of 2,6-dichlorophenolindophenol and allowed to stain for 5 min. After the dye had been poured off the plates were placed in a 37° incubator for 40 to 60 min. Under these conditions the viable cells reduced the dye while the dead cells did not. The diameters of the zones of toxicity were measured and the appearance of halos or diffuse edges noted.

RESULTS AND DISCUSSION

A series of 27 compounds which were found to inhibit tumor growth in experimental animals and/or man or to have cytotoxic activity (as measured by inhibition of growth of KB cells) were

[1] Methocel, Dow Chemical Co., Midland, Mich.
[2] Pluronics, Wyandotte Chemical Co., Wyandotte, Mich.
[3] Darvan No. 2, R. T Vanderbilt Co., Inc., New York, N. Y.