Differential Thermal Analysis as Screening Technique for Candidate Adjuvants in a Parenteral Formulation: Cephradine for Injection

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Abstract ☐ Differential thermal analysis (DTA) was used to study parenteral formulations of cephradine. Such studies showed that cephradine interacted with N-methylglucamine, tromethamine, and trisodium phosphate dodecahydrate, but it did not react with anhydrous sodium carbonate. The presence of an interaction does not necessarily indicate incompatibility, but it is an early warning of such a possibility. Finally, because DTA showed no new characteristic peaks, the absence of eutectics or of other specific phase entities could be presumed. Chemical analyses of various test formulations that had been stored at 23 and 50° gave excellent correlations with the results obtained earlier by DTA and showed that the interactions had been ones of incompatibility.

Keyphrases ☐ Adjuvants, parenteral formulations—use of differential thermal analysis as a screening technique, cephradine ☐ Parenteral formulations—drug-adjuvant interactions screened using differential thermal analysis ☐ Cephradine—suitability of adjuvants for parenteral formulation screened using differential thermal analysis ☐ Differential thermal analysis—use in determining suitability of adjuvants for cephradine parenteral formulation

Jacobson and Reier (1) discussed the application of differential thermal analysis (DTA) to problems of compatibility and stability that occur with mixtures of solid-state drugs and excipients, notably combinations of penicillin and stearic acid. The method is here extended to a study of parenteral formulations of cephradine, a new antibiotic of the cephalosporin class, coincidentally emphasizing the general utility of this rapid screening method.

In the formulation of a parenteral dosage form of cephradine, a stable, dry powder for reconstitution was sought which could be readily dissolved just prior to injection. Dürsch[1] suggested the use of a solubilizing adjuvant for the parenteral form of cephradine.

Several physiologically acceptable solubilizing adjuvants exist, and it became necessary to establish that the dry powder mixture of each adjuvant with cephradine exhibited stability under the usual storage conditions. Among the possible solubilizers, N-methylglucamine, tromethamine, trisodium phosphate dodecahydrate, and anhydrous sodium carbonate appeared useful.

EXPERIMENTAL

Blends of cephradine with the appropriate solubilizer were prepared, using the minimum amount of solubilizer that would give a complete solution in 1 min. or less when 250 mg. of cephradine was reconstituted with 1.2 ml. of water.

The tromethamine and trisodium phosphate dodecahydrate employed were reagent grade, the N-methylglucamine was USP grade (2), and the anhydrous sodium carbonate conformed to specifications in the British Pharmacopoeia (3).

The mole fractions of solubilizer in the respective blends were: N-methylglucamine, 0.445; trisodium phosphate dodecahydrate, 0.374; tromethamine, 0.564; and anhydrous sodium carbonate, 0.500.

Each solubilizer was passed through a 40-mesh screen before being blended with cephradine in a mortar and pestle. Containers for storage were 7.5-ml. siliconed, type III glass vials with butyl rubber stoppers. Samples were stored at 23 and 50°.

The thermograms of the freshly prepared mixtures were obtained on a thermal analyzer[2], using 2-mm. microsample tubes. A heating rate of 15°/min. was used, with a differential sensitivity of 1°/2.54 cm. The thermograms were obtained in the presence of static air.

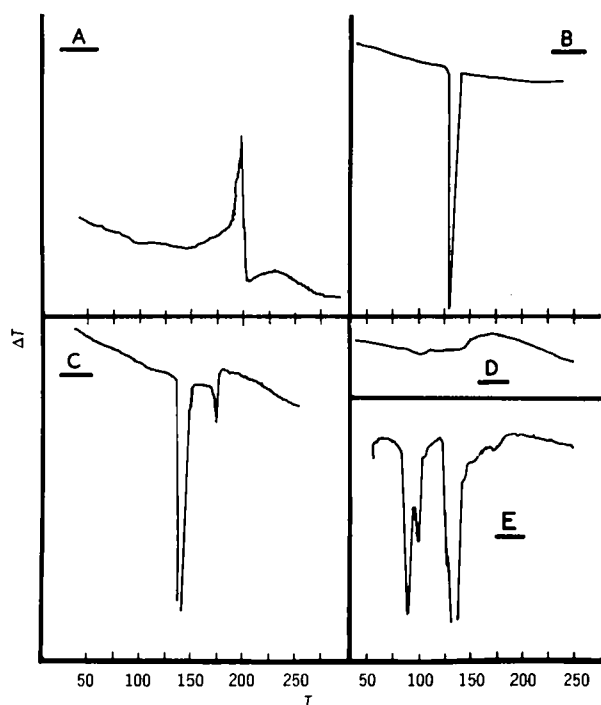The stability of cephradine in each blend was determined by an iodometric assay method (4).

Figure 1—*Thermograms of pure materials. Key: A, cephradine; B, N-methylglucamine; C, tromethamine; D, anhydrous sodium carbonate; and E, trisodium phosphate dodecahydrate.*
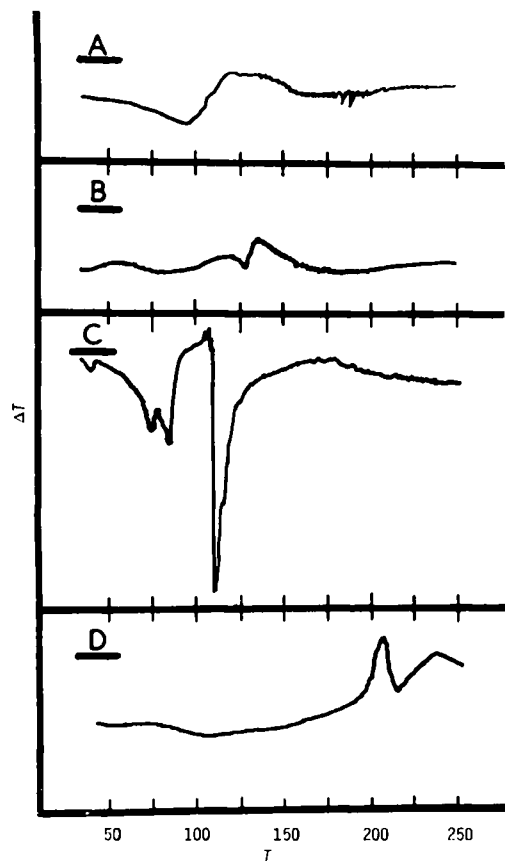
Figure 2—Thermograms of mixtures of cephradine with: (A) N-methylglucamine, (B) tromethamine, (C) trisodium phosphate dodecahydrate, and (D) anhydrous sodium carbonate.

RESULTS AND DISCUSSION

Thermograms of the pure materials are shown in Fig. 1. The exotherm observed with cephradine between 175 and 200° was due to an oxidative degradation. The endotherm exhibited by N-methylglucamine at about 129° was due to melting; those in tromethamine were due to a solid-solid phase transition (5) and to melting. Sodium carbonate had no characteristic features at temperatures of interest (up to about 350°). The first two endotherms of trisodium phosphate dodecahydrate (Fig. 1E) were due to desolvation, and the third was due to the boiling of released water.

Thermograms of the various mixtures are shown in Fig. 2. It was evident that interactions had taken place when cephradine was mixed with N-methylglucamine, tromethamine, or trisodium phosphate dodecahydrate. With the former two compounds, the features of both components of the mixture had been changed or obliterated. With trisodium phosphate dodecahydrate, only the features of cephradine were obliterated; those of the phosphate were largely intact and unchanged. In the thermogram of the mixture of cephradine and anhydrous sodium carbonate, the most significant feature is the retention of the cephradine exotherm at about 200°. It can be surmised that no interaction had taken place.

The presence of a physical or chemical interaction does not necessarily indicate incompatibility. It may indicate the formation of a eutectic mixture or the occurrence of a new phase with different thermal characteristics which are not necessarily deleterious to stability. However, the fact of an interaction is undeniable and gives an early warning for a more rational approach to the design of formulations.

In the examples examined in this work, DTA showed no new characteristic thermal peaks. Consequently, it could be presumed that eutectic mixtures or other specific phase entities, such as the formation of a new compound, were absent.

Figures 3 and 4 illustrate the potencies of the various blends as a function of time at 23 and 50°. The least stable blend (that with trisodium phosphate dodecahydrate) showed marked instability after
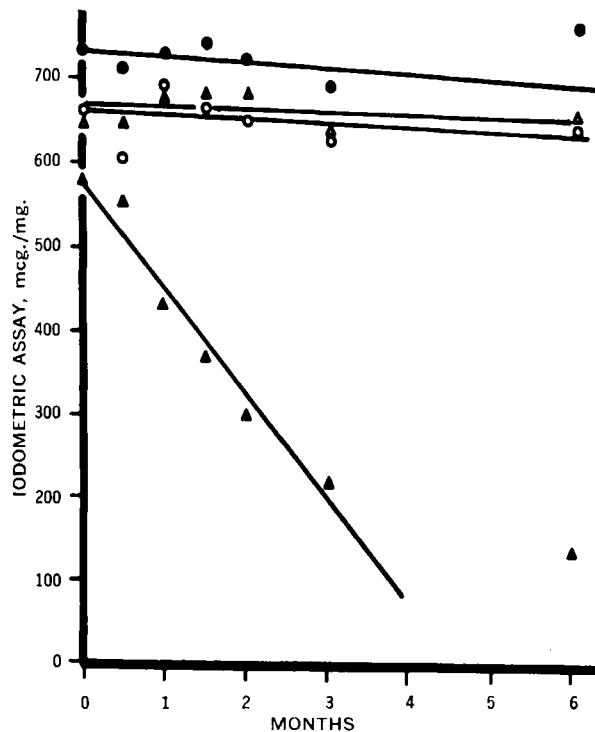

Figure 3—Potencies of blends of cephradine as a function of time at 23°. Key for adjuvants: ▲, trisodium phosphate dodecahydrate; O, tromethamine; △, N-methylglucamine; and ●, anhydrous sodium carbonate.

storage at room temperature for 1 month. The other blends did not show a decline in potency by chemical assay when stored at room temperature for 6 months. At 50°, the mixture of cephradine with sodium phosphate dodecahydrate exhibited complete loss of potency as determined by chemical assay after 2 weeks of storage, while testing for at least 1 month was required to make a judgment of instability for the other mixtures. By use of DTA, the cephradine
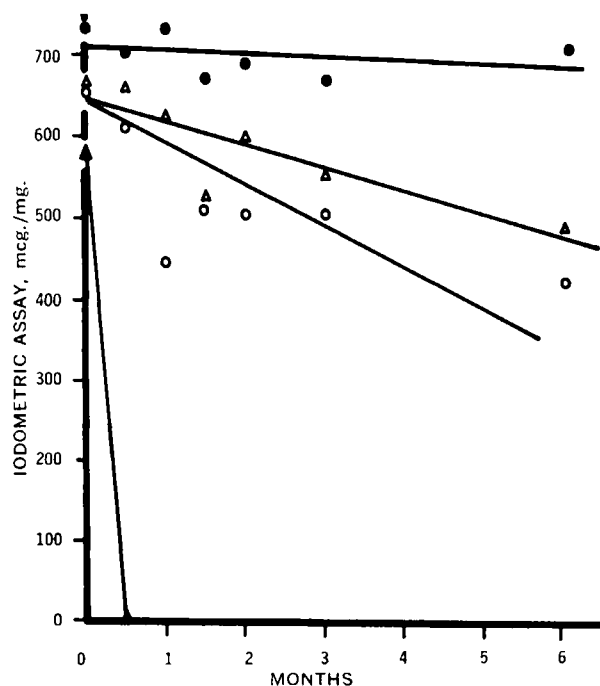

Figure 4—Potencies of blends of cephradine as a function of time at 50°. Key for adjuvants: ▲, trisodium phosphate dodecahydrate; O, tromethamine; △, N-methylglucamine; and ●, anhydrous sodium carbonate.

sodium carbonate mixture could be predicted to be the most stable blend after 1 day of testing. The prediction was clearly confirmed by results of a classical stability study requiring up to 6 months of storage at 50°.

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Drug–Albumin Interactions Using Spin Labeling

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Abstract ☐ Three spin labels were used to monitor possible drug-induced conformational changes in bovine serum albumin. The electron spin resonance spectra of labeled bovine serum albumin in all cases consisted of two subspectra corresponding to labels in a partially immobilized environment and a strongly immobilized environment. Only bovine serum albumin with the triazine spin label was suitable for quantitation of data. The strongly protein-bound acidic drug, phenylbutazone, caused a conversion of strongly immobilized sites to partially immobilized sites, an effect that was proportional to the amount of drug added. This probably was due to the initial drug binding inducing a conformational change in the bovine serum albumin, thereby exposing additional binding sites.

Keyphrases ☐ Drug–albumin interactions—use of three spin labels to monitor drug-induced protein conformational changes, electron spin resonance spectra ☐ Albumin–drug interactions—use of three spin labels to monitor drug-induced protein conformational changes, electron spin resonance spectra ☐ Spin labeling—monitoring drug-induced conformational changes in bovine serum albumin, three spin labels studied

The technique of spin labeling was recently applied to a number of macromolecular systems where conformational changes were suspected (1, 2). This report describes, for the first time, an attempt to monitor drug-induced changes in bovine serum albumin utilizing this method. Although various analogous systems have been studied similarly (3–5), the pharmacodynamically important drug–albumin system (6, 7) has not been investigated; preliminary data are reported here.

EXPERIMENTAL

Materials and Methods—The spin labels N-(1-oxyl-2,2,6,6-tetramethyl-4-piperidinyl)bromoacetamide and N-(1-oxyl-2,2,6,6-tetramethyl-4-piperidinyl)maleimide were purchased[1]. The spin label 2,2,6,6-tetramethyl-4-dichlorotriazineaminopiperidine-1-N-oxyl was synthesized according to a reported procedure (8). These spin labels are referred to here as bromoacetamide, maleimide, and triazine, respectively.

Prior to use, the crystalline bovine serum albumin[2] was dialyzed against several changes of glass-distilled water at 4° followed by lyophilization. Protein concentration was checked by the biuret assay (9) or by measurement of absorbance at 279 nm., using a = 0.667 (10). Spin-labeled derivatives of bovine serum albumin were prepared using techniques similar to those previously reported (8, 11). All solutions were prepared and labeled in phosphate buffer of pH 6.9 (μ = 0.0527). Electron spin resonance spectra were recorded on an X-band spectrometer, utilizing 100-kHz. field modulation and Fieldial control of the magnetic field. The titration of triazine spin-labeled bovine serum albumin with increasing amounts of phenylbutazone was performed at ambient temperature using a quartz flat cell and a syringe pump set up similarly to one described elsewhere (3). Each spectrum could be recorded under identical instrumental conditions, thereby facilitating direct comparison of successive spectra.

RESULTS AND DISCUSSION

The electron spin resonance spectra obtained with both maleimide and bromoacetamide spin-labeled bovine serum albumin did not change appreciably when interacted with phenylbutazone. This could be due to the fact that these two sulfhydryl group-directed labels were located at a site far removed from the binding locus for phenylbutazone. Another possibility is that these labels may have been present in too low a concentration to have their spectra detectably altered by the binding process. It is also possible that the sulfhydryl grouping may be in a region of the protein that results in the attached labels being too strongly immobilized for their spectra to be adequately perturbed by the binding process.

In contrast, the amino group-directed triazine spin label, which at pH 6.9 is linked solely to histidine residues (8), appears to contain a ratio of strongly immobilized sites amenable to quantitation. Figure 1A shows that the spectrum of triazine spin-labeled bovine serum albumin actually consists of two types of signals, a sharp three-line spectrum corresponding to a label at a partially immobilized site and a superimposed broadened spectrum characteristic of the label in a strongly immobilized environment. Figure 1B shows the effect of adding 24 moles of phenylbutazone/mole of labeled bovine serum albumin. This spectrum represents the endpoint in the titration, because this was the maximum amount of phenylbutazone added. Spectra of several intermediate ratios of phenylbutazone to labeled bovine serum albumin were also recorded. Figure 2 shows a plot of the relative intensities of the high field peak of the partially immobilized spectrum (peak 4) *versus*

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[1] Synvar Associates, Palo Alto, Calif.

[2] A grade, Calbiochem.