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# EFFECT OF THE CRYSTALLIZATION PROCESS AND SOLID STATE STORAGE ON THE PHYSICO-CHEMICAL PROPERTIES OF SCALE-UP LOTS OF CI-936

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## ABSTRACT

N-(2,2-diphenylethyl)adenosine, designated as CI-936, is a novel, orally active antipsychotic agent. Depending on the manufacturing process, the drug substance exists in more than one crystalline form. Three lots of the drug were characterized by thermal analysis, scanning electron microscopy, X-ray diffraction, and dissolution. Two of these lots were found to be crystalline while the third was amorphous. The physical properties of the crystalline forms appear to change during storage under ambient conditions. The amorphous form, inspite of being in a high energy state, was not affected by storage. The absolute bioavailability of the amorphous form in dogs is more than 90%. In contrast, the

other two crystalline lots demonstrated lower and unpredictable oral absorption profiles.

## INTRODUCTION

During the last several years, a number of adenosine receptor agonists have been synthesized and tested in animals for antihypertensive or antipsychotic activity based on the presumption that adenosine is a mediator for many pharmacological responses[1-3]. One such compound, designated as CI-936, synthesized as part of drug discovery efforts, was found to be orally active in reducing the locomotor activity in rodents. The results of animal studies showed that CI-936 is an effective antipsychotic agent without causing the extra pyramidal side effects that are commonly observed with agents of this therapeutic class. This article describes some of the unique physicochemical properties observed in the course of development of this compound.

In drug discovery and development, it is a common practice to continuously refine, improve and modify the bulk chemical manufacturing process as the new chemical entity is progressing through the various stages of preclinical testing. During such optimization procedure, the properties of the drug

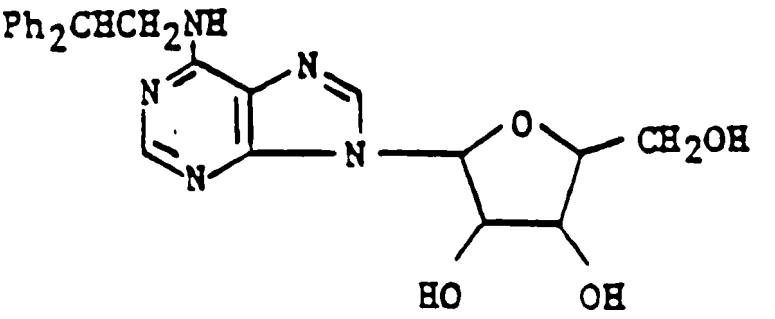
produced from successive batches usually differ, in many respects, from the very first lot made in the laboratory. The preparation of scale-up lots can yield bulk drug material that differ markedly in pharmaceutical properties; including bioavailability of the formulated drug. A review of the literature revealed that while a number of articles and publications dealing with preformulation assessment of various pharmaceutical compounds are available, there is a paucity of information on the physicochemical properties of different lots of bulk pharmaceutical produced during the scale-up and refinement of the synthetic process[4-6]. Moreover, the vast majority of published references deal with drug materials specially prepared in the laboratory under controlled experimental conditions. The objective of the current studies is to characterize some unique physicochemical properties of different lots of CI-936 received during the scale-up of the bulk drug.



## Chemical Structure and Basic Properties

The chemical structure of CI-936 is N-(2,2 diphenylethyl) adenosine as shown in Scheme 1.

The pka of this drug determined in 50 % methanol is 2.9. The compound exhibits a UV absorbance maxima at 267 nm in 0.1 N




Scheme 1. Structure of CI-936

hydrochloric acid and at 270 nm in 0.05 M pH 7.5 phosphate buffer. The absorptivity values at these wavelengths are 42.5 and 46.8, respectively. Solubility studies indicated that the compound has limited aqueous solubility below pH 3. It is practically insoluble in distilled water and in aqueous fluids above pH 4.

## EXPERIMENTAL

### Materials

The different lots of CI-936 bulk chemical examined have been designated A, B and C. The lots were prepared by the Chemical Development group and were used as received. Lot A was prepared by dissolving the compound in methanol,

concentrating the solution to a gummy mass, followed by the addition of distilled water to precipitate the compound. Lot B was prepared by recrystallization of Lot A from methanol. Lot C was obtained by adding distilled water to a solution of the compound in methanol. All the chemicals used in preparing the dissolution media were reagent grade.

## Procedures

The three lots of CI-936 were characterized using: 1) thermal analyses including differential scanning calorimetry (DSC), thermogravimetric analysis (TGA) and hot-stage microscopy, 2) X-ray powder diffractogram analysis, 3) Infrared (IR) spectroscopy, 4) dissolution studies including bulk powder and intrinsic dissolution determinations.

## Differential Scanning Calorimetry (DSC)

A Perkin Elmer DSC-2C differential scanning calorimeter, in conjunction with a Model 3600 data station, was used to obtain thermal curves and record the enthalpies and temperatures associated with the transitions. The sample powder was weighed and sealed in an aluminum pan and placed in a platinum sample holder. A heating rate of 5° C/min was

employed from 30° to 150° C with continuous nitrogen purge. The system was calibrated using Indium (m.p. 156.6° C) as the reference standard.



## Thermogravimetric analysis (TGA)

Gravimetric changes of samples were measured using a Perkin Elmer TGS-2 thermogravimetric analyzer equipped with a System 4 microprocessor controlled heater. Thermal curves were obtained at a heating rate of 10° C/min from 30° to 150° C in a nitrogen atmosphere.

## Thermomicroscopy

A polarizing microscope fitted with a Mettler FP2 hot-stage was used to observe phase transitions of the samples. A small amount of sample was placed on sample stage and heated from 40° to 140° C at a rate of 2° C/min. The transitions were examined at a magnification of 100X.

## X-ray Powder Diffraction Analysis

X-ray powder diffraction patterns were obtained using a Phillips automated diffractometer controlled by a Zenith 150

microcomputer with CuK$\alpha$ (K=1.5418 Å) radiation monochromatized with a graphite crystal. The patterns were recorded from 2 to 40 degrees ($2\theta$) in steps of 0.02 degree.

Infrared spectroscopy (IR)

IR spectra were obtained on a Perkin Elmer Model 283B spectrometer. A thin disk of the drug was prepared by grinding a 2% CI-936 sample in potassium bromide followed by compression on a carver press. The disc was placed in the sample beam and the spectrum recorded from 4000 to 200 $cm^{-1}$.

Bulk Powder Dissolution Rates

Fifty milligrams of each lot were hand filled in No. 3 size clear gelatin capsule. Dissolution experiments were conducted using USP dissolution method II at a paddle rotation speed of 80 rpm. The studies were conducted in 900 ml of 0.1 N HCl or 0.05 M pH 7.5 phosphate buffer and a bath temperature of 37 ·C. An average of three replicates was used to determine the dissolution rate.

Samples (5 ml) were withdrawn from the dissolution vessels at preset time intervals and replaced by an equivalent

volume of fresh dissolution medium automatically. Filtered samples were assayed spectrophotometrically at the absorbance maximum of 268 nm.



### Intrinsic Dissolution Rates

Four hundred milligrams of each of the three lots were compressed into a disk having a surface area of 5.07 $cm^2$ using a carver press at a pressure of 8000 psi. The disk was rotated at 50 rpm using the same dissolution conditions described above.

## RESULTS AND DISCUSSION

### Differential Scanning Calorimetry(DSC)

DSC thermograms of the three lots are given in Figures 1 and 2 which show, respectively, the thermograms of freshly prepared samples and the results obtained on samples stored under ambient conditions for 15 months. Fig.1a shows two endothermic peaks near 120° and 137° C which indicate that Lot A is composed of a mixture of two forms with different melting points. This conclusion is consistent with the results obtained from hot-stage microscopy which will be

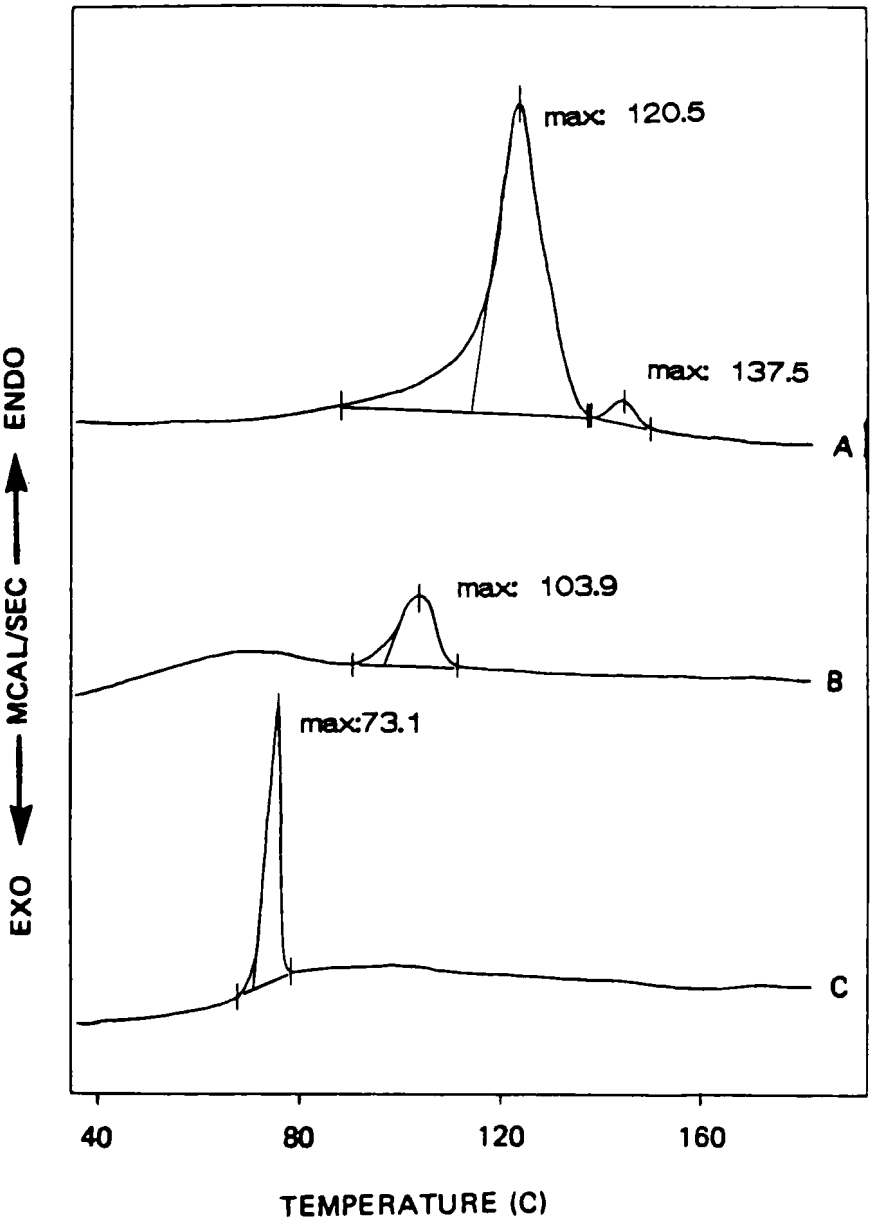



FIGURE 1

Initial DSC Curves of Lots A, B and C of CI-936.

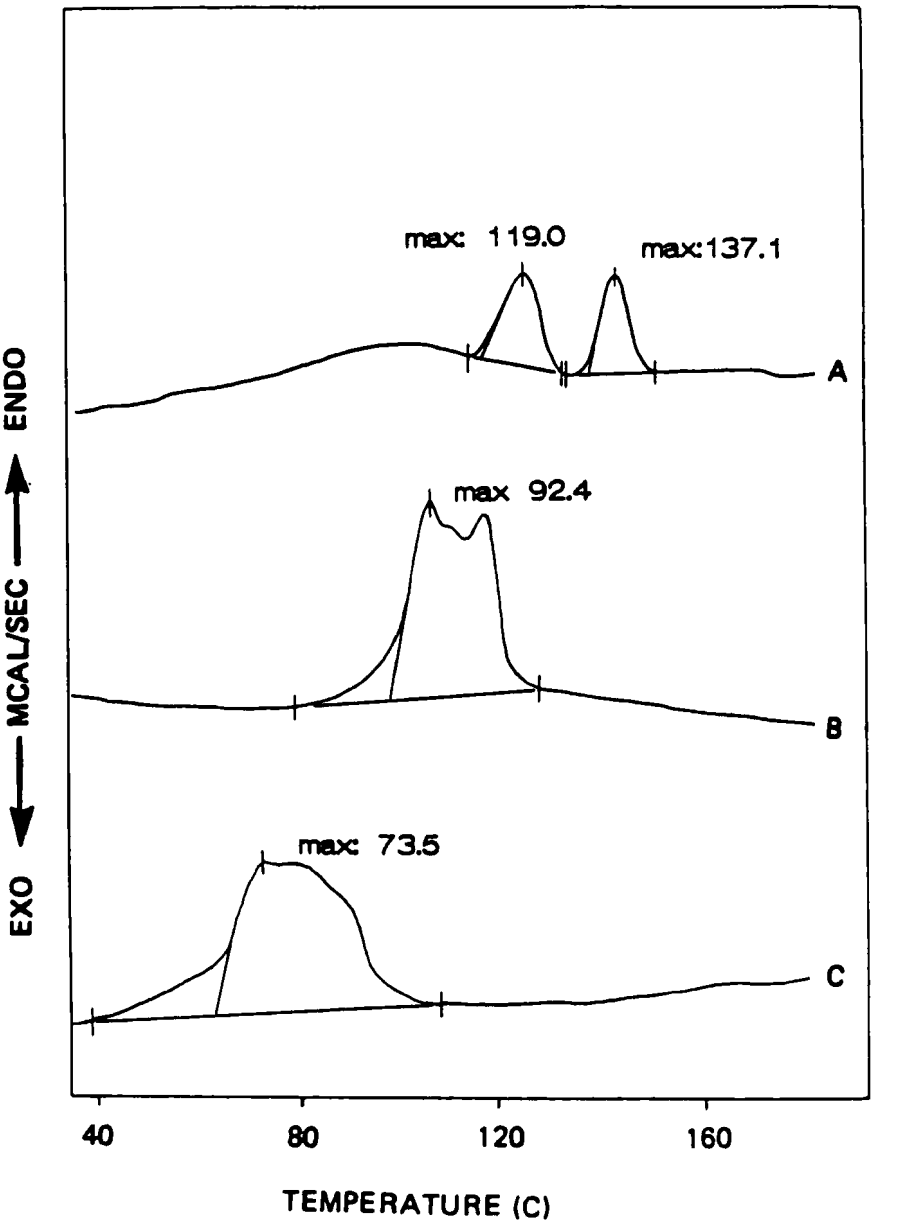



FIGURE 2

DSC Curves of Lots A, B and C of CI-936; the Samples Were Stored under Ambient Conditions for 15 Months.

described later. During storage for 15 months, Lot A underwent gradual transformation; the peak height near 137° C increased while that of the first peak at 120° C decreased (Fig.2a). Lot B initially exhibited a peak at ca. 104° C (Fig.1b); after 15 months, the endothermic peak became broad and was shifted between 90° to 110° C (Fig.2b). The three small peaks on top may indicate that Lot B was converted to at least two polymorphs with different melting points during the 15 month storage period. The thermogram of a fresh sample of Lot C exhibited a sharp endothermic peak at 73° C which could be due to dehydration and was confirmed by TGA studies. Upon storage, Lot C exhibits a broader peak suggesting that dehydration occurs over a broader temperature range. Because Lot C (the amorphous form) existed in a high energy state and only a small energy change was involved in the melting process, no peak was evident at the temperature at which the solid melts. The three batches of samples were characterized as received, and no attempt was made to separate the polymorphs observed in the DSC thermograms.

It was interesting to note that the thermograms obtained were dependent on the way samples were enclosed in the aluminum pan. In the preceding studies, samples stored for 15 months were placed in a plain aluminum pan and loosely crimped

with a flat cover (standard pans received from Perkin-Elmer). When the experiments were repeated with the samples tightly sealed in a concave pan having a convex cover (volatile pans obtained from Perkin-Elmer), each lot of CI-936 produced endotherms that were different from those observed earlier. The two peaks seen at 120° and 137° C for Lot A (Fig.1a and 2a) coalesced to a single peak at 129° C (Fig.3a). The multiple peaks for Lot B disappeared and a single peak was observed between 70° to 82° C (Fig.3b); the peak position varied from one run to another. Similar behavior of change in the peak position was observed for Lot C at around 65° C (Fig.3c). It appears that the change in peak positions is attributable to vaporized water which was released from the lattice structure of the sample and trapped in the pan. Similar observations were reported in a drug-excipient interaction study of enalapril maleate[7] where different thermal curves were obtained using standard and volatile pans. The thermal response of samples enclosed in volatile pans was influenced by the retained moisture in the excipient. Since thermograms generated using volatile pans in this study were affected by the presence of water and the results were not reproducible, all further studies were carried out using the standard pans.

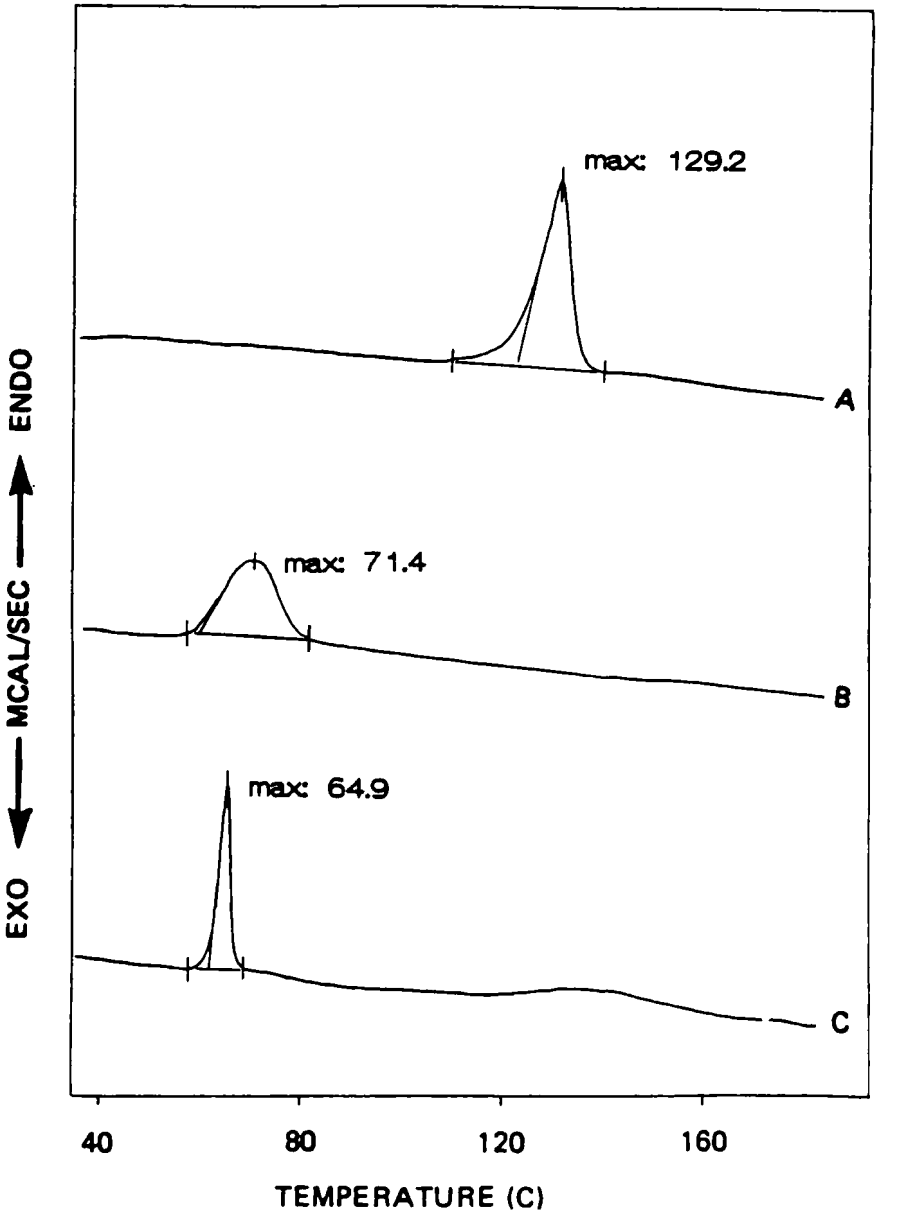



FIGURE 3

DSC Curves of Lots A, B and C of CI-936; Samples Were Sealed in the Volatile Pans.

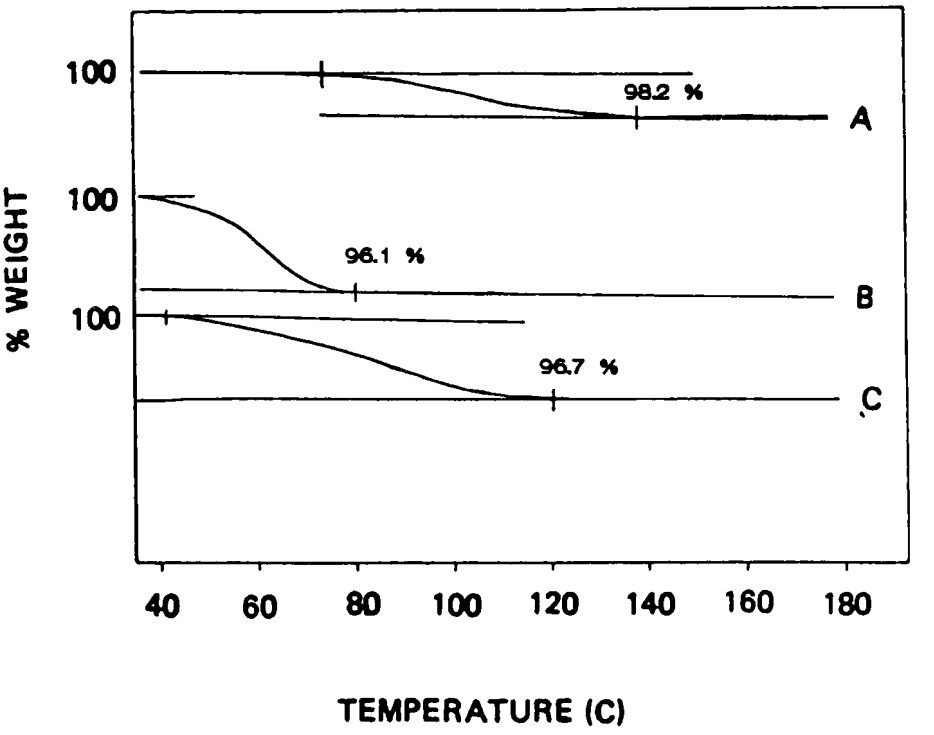



FIGURE 4

TGA Thermal Curves of Lots A, B and C of CI-936.

Further studies revealed that when the three lots were heated to fusion temperature, allowed to cool to room temperature, and then re-scanned, the thermal curves for all three lots were identical. A minor peak at 89 °C was observed with the baseline shifted upward after the peak. It appears that all the lots were converted to the amorphous form during the heating and cooling process.

## Thermal Gravimetric Analysis(TGA)

Thermal curves obtained from the TGA represent weight change as a function of time. The amount of weight change for a given sample was determined directly from the y-axis of the thermograms. Figure 4 shows that all sample lots absorb moisture to different degrees and dehydrate at different temperatures. The data indicate that the equivalent of about 0.4, 0.9 and 0.7 mole of water were associated with each mole of Lots A, B and C, respectively. These values are in agreement with the water content of the samples as determined by the Karl-Fischer titration method.

## Thermomicroscopy

Using a polarizing light microscope equipped with a hot-stage, the melting range as well as the polymorphic transitions of the three lots were determined. As observed under the microscope, Lot A contained irregular crystals and exhibited a wide range of color spectrum when examined between crossed polars. The melting range was between 120° to 135° C which is consistent with the wide endothermic peaks observed in the DSC thermogram. Lot B existed in rod-like crystals or irregular chips and exhibited birefringence when observed

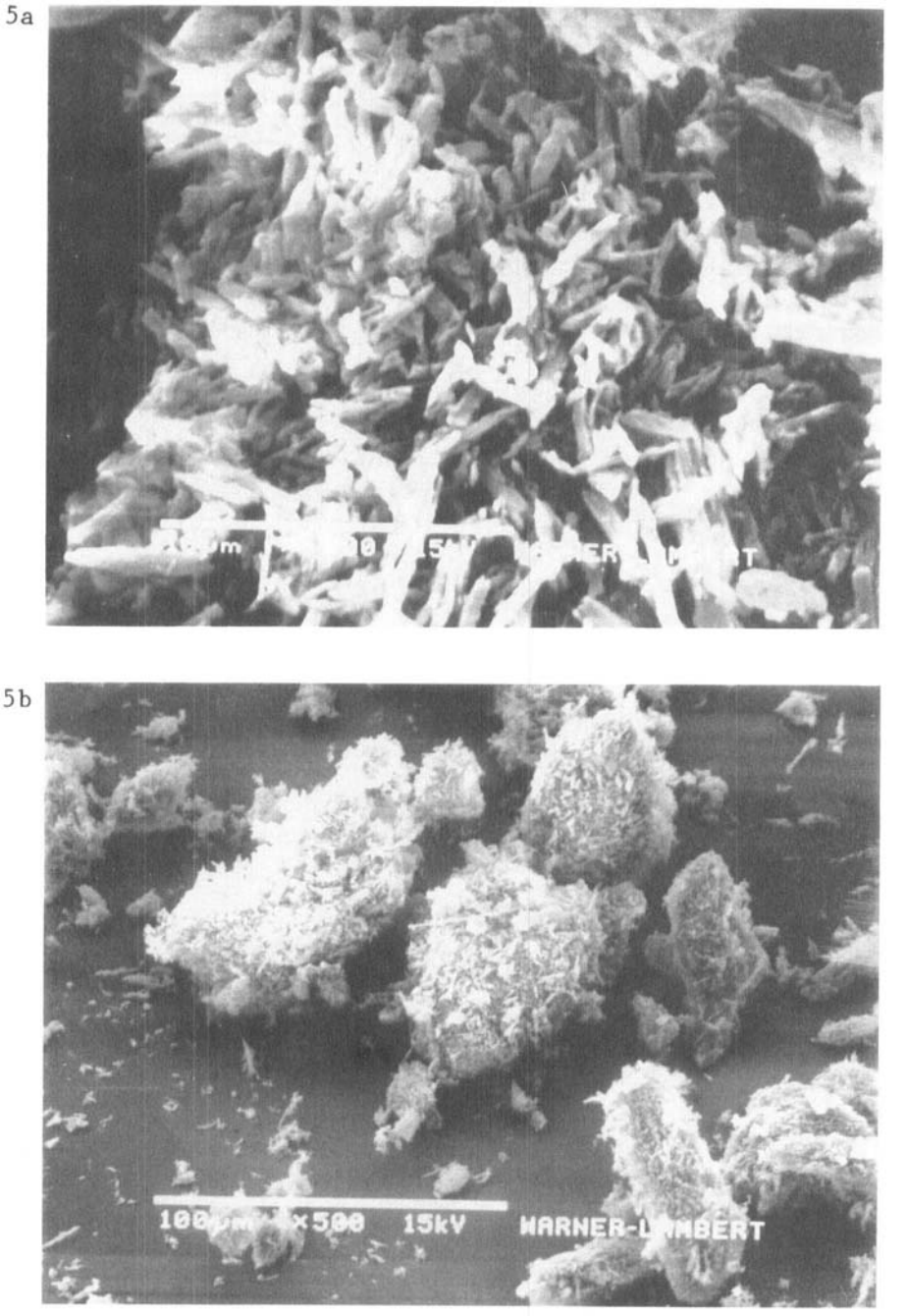
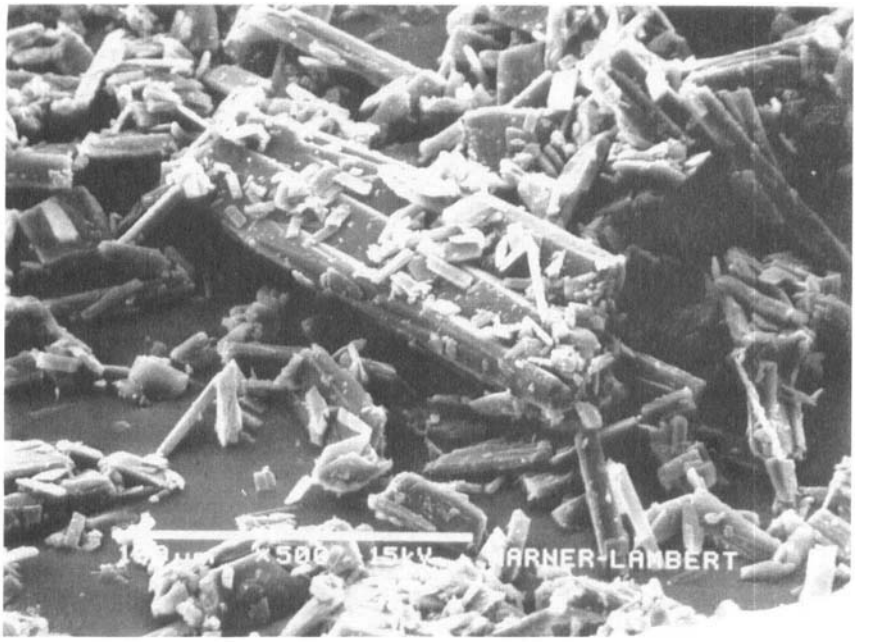



FIGURE 5

Scanning Electron Micrographs of CI-936. 5a, Lot A (5000 X); 5b, Lot A (500 X).

5c




5d

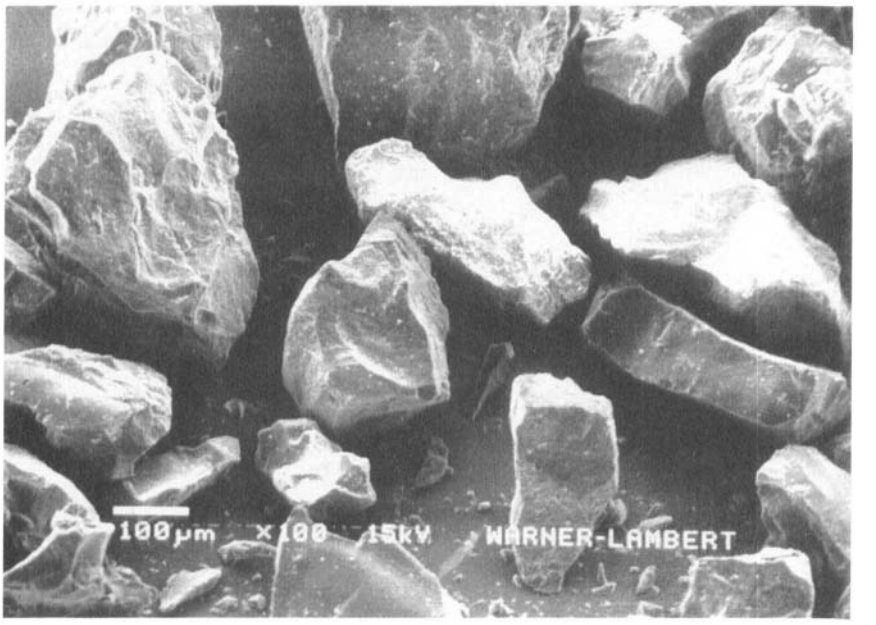



FIGURE 5 (Continued)

Scanning Electron Micrographs of CI-936. 5c, Lot B (500 X); 5d, Lot C (100 X).

between crossed polars. The colors of the crystals changed during heating on the hot-stage. The crystals melted at about 103 to 106 °C. Lot C contained a significant amount of chunks scattered among irregular aggregates. The sample melted from 103 to 106 °C which is similar to the melting range of Lot B.

## Scanning Electron Microscopy(SEM)

Scanning electron micrographs of the three lots are illustrated in Figure 5. Different magnification factors were used for each form to obtain clear pictures of the particles. Lot A is composed of very small rod-like crystals (Fig.5a) which are loosely packed into large fluffy aggregates ranging in size between 30 and 100 microns (Fig.5b). Lot B contains a mixture of small chips and large rods of ca. 50 microns (Fig.5c). Lot C is block-like in shape (Fig.5d) and the particle size (ca. 300 microns) is significantly larger than the other two lots.

## X-Ray Diffraction

Figure 6 shows the X-ray powder diffraction patterns of the three lots of CI-936. The data indicate that Lot C has only a broad background scattering between $2\theta$ of 4 and 34

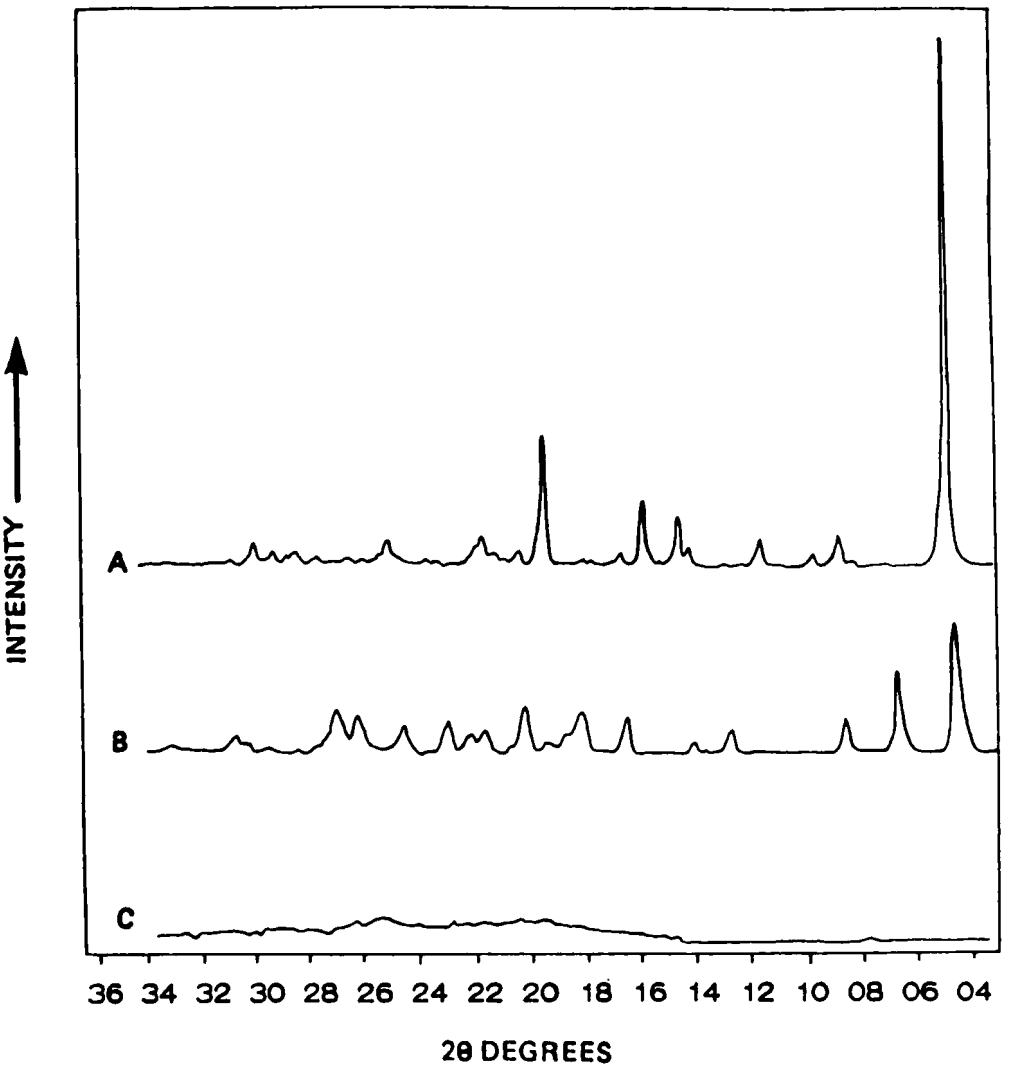



FIGURE 6

X-Ray Powder Diffractograms of CI-936 of Lots A, B and C.

degrees and is basically non-crystalline. Lot A and B are moderately crystalline with sharp and well defined peaks. Peak positions and intensities for these two forms are different as shown in Table 1. The differences in molecular arrangements distinguish the two crystal polymorphs.



## Infra-red(IR)

Figure 7 shows the IR spectra of the three lots. The three spectra are different. Fig.7a shows that Lot A has a characteristic band at 3300 to 3400 $cm^{-1}$ corresponding to thehydrogen bonding. This may suggest the presence of water of crystallization associated with Lot A. This interpretation is consistent with the higher and wide temperature range required to evaporate water from the sample as seen in TGA (Figure 4a). A closer examination of the finger-print region of the spectra indicates that there are indeed differences between the three lots. This may be attributed to differences in the crystal structure and the presence of both bound and unbound water in the samples.

## Bulk powder dissolution rate

The dissolution studies were conducted undere sink conditions. The dissolution profiles of representative powder

TABLE 1

X-ray Diffraction Data for CI-936

| Lot A | | Lot B | |
|---|---|---|---|
| 'd' space | intensity | 'd' space | intensity |
| 20.028 | 99 | 17.673 | 99 |
| 13.598 | 60 | 9.825 | 9 |
| 10.402 | 26 | 8.934 | 8 |
| 6.907 | 17 | 7.499 | 9 |
| 6.281 | 9 | 6.194 | 8 |
| 5.405 | 26 | 5.985 | 13 |
| 4.901 | 30 | 5.539 | 16 |
| 4.418 | 34 | 5.277 | 8 |
| 4.133 | 17 | 4.529 | 26 |
| 4.022 | 13 | 4.332 | 8 |
| 3.900 | 26 | 4.077 | 10 |
| 3.648 | 22 | 3.548 | 9 |
| 3.427 | 30 | 3.048 | 8 |
| 3.326 | 34 | 2.978 | 9 |
| 2.921 | 13 | | |

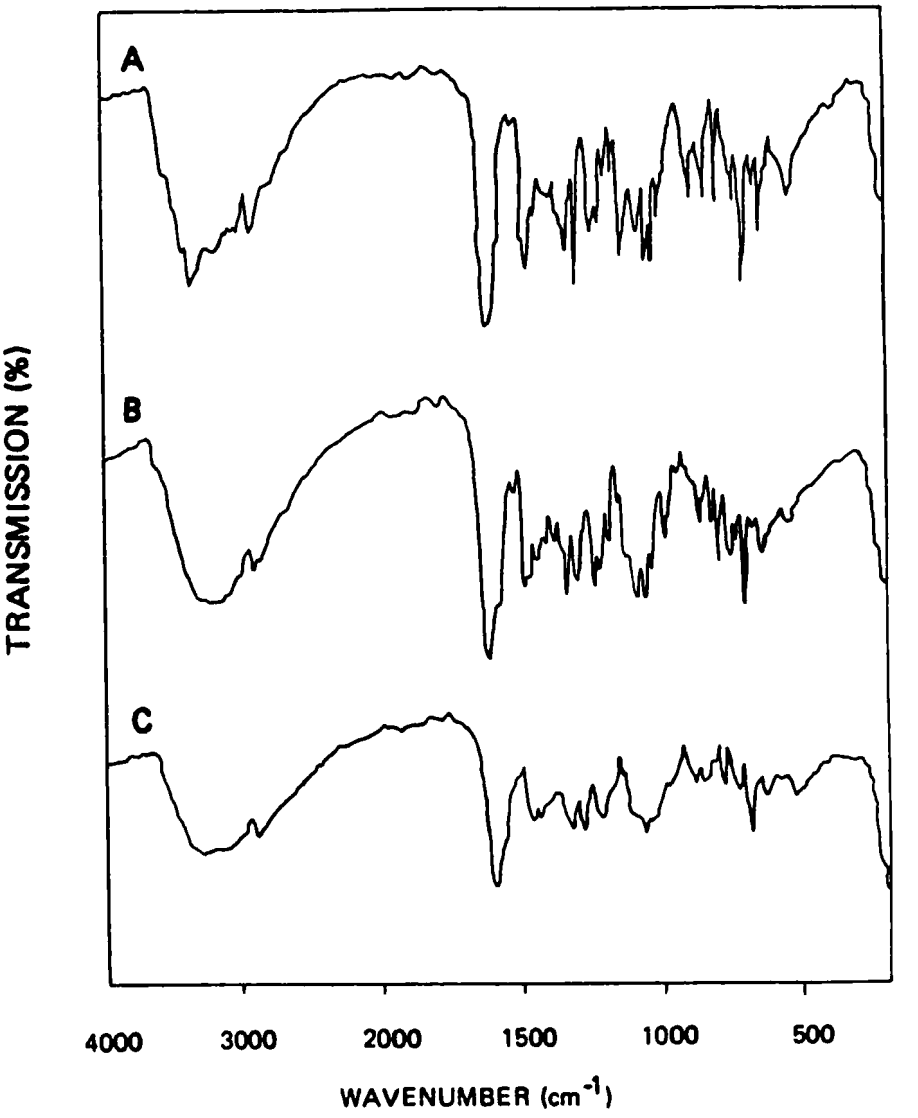



FIGURE 7

Infra-red Spectra of CI-936 of Lots A, B and C.

samples of the three lots of the drug are given in Figure 8. All the lots tested dissolved relatively fast in 0.1 N hydrochloric acid (Figure 8a). The dissolution rates were significantly slower in pH 7.5 phosphate buffer (Figure 8b). As expected, Lot C, the amorphous form, dissolved in both media faster than the other two lots which, being crystalline, dissolved at slower rates. Differences in the dissolution rates of lots A and B could be attributed to differences in crystalline form and variations in particle size.



## Intrinsic dissolution rate

The intrinsic dissolution rates of the three lots of CI-936 were measured to determine if the differences in bulk powder dissolution rates are due to variations in crystal form. Disks of constant surface area were prepared for the three lots and their intrinsic dissolution profiles are shown in Figure 9. Since sink conditions were maintained at all times, the dissolution rate of the drug in a dissolution medium can be described by the following equation[8]:

$$dM/dt = DSCs/h$$

where M is the mass of drug dissolved in time t, dM/dt the mass rate of dissolution, D the diffusion coefficient of the solute in solution, S the surface area of the exposed solid,

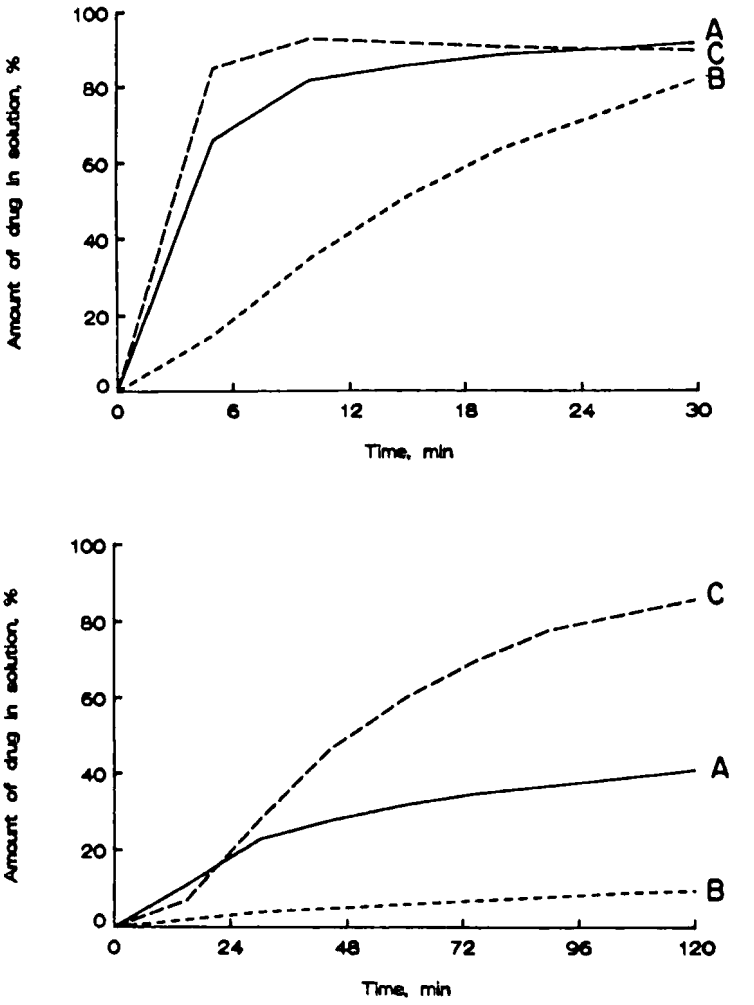



FIGURE 8

Bulk Powder Dissolution Profiles of Lots A, B and C of CI-936. 8a, 0.1 N HCl as the dissolution medium; 8b, pH 7.5 phosphate buffer as the dissolution medium.

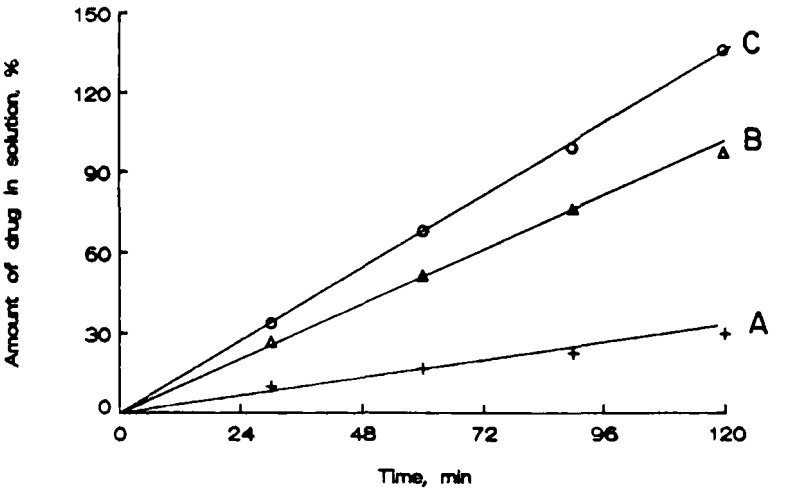



FIGURE 9

Intrinsic Dissolution Profiles of Lots A, B and C in 0.1 N HCl.

h the thickness of the diffusion layer and Cs the solubility of CI-936. Integration of the above equation from time 0 to t yields:

$$M = DSCs/h \times t$$

Since D, S and h remain relatively constant during the dissolution study, a plot of the amount dissolved versus time gives a straight line; the slope of which is used along with the surface area to determine the intrinsic dissolution rate (DCs/h, per unit square centimeter). Figure 9 shows that the intrinsic dissolution profiles appear to be linear up to 120 minutes. The slopes of the dissolution profiles were thus

determined by linear regression through the origin[9]. The calculated intrinsic dissolution rate constants of Lots A, B and C in 0.1 N HCl are 0.0453, 0.148, and 0.200 mg/cm²/min, respectively.Unlike bulk drug dissolution, Lot B shows a faster intrinsic dissolution rate than Lot A when the effect of particle size is eliminated. Lot C, which is mostly amorphous, has the most rapid dissolution rate in 0.1 N HCl as expected. The apparent differences in dissolution rates obtained from different scale-up lots are of concern, because the potential batch-to-batch variation in type and ratios of crystalline forms may lead to differences in biological performance of the drug. Moreover, if polymorphic interconversion occurs upon storage, stability problems could also be anticipated during the shelf-life of the dosage form.

Different scale-up lots of CI-936 were characterized using different techniques. It appears that the scale-up lots of CI-936 exist as a mixture of solid phases. Lots A and B are mostly crystalline while Lot C is predominantly composed of the amorphous form. The amorphous form did not undergo any transformation in three years of storage under ambient condition. In a pilot study using dogs, Lot A was about 70% bioavailable with average blood levels about 50% lower than the amorphous form. Bioavailability results of Lot B were

similar to Lot A with less reproducibility, probably due to particle size variations and agglomerations in different studies. In the same study, an absolute bioavailability of more than 90% was obtained when the amorphous form (Lot C) was dosed orally in dogs.

It is obvious from these studies that scaling up of synthetic bioactive agents from the laboratory to production can lead to unpredictable and sometimes undesirable results both during formulation development and in vivo evaluation. Repeated physicochemical characterization of various lots throughout the product development phase is essential to control the stability of the dosage form. This will ensure reproducible therapeutic performance of the biological active material.

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