In Situ Crystalline Transformation of a New Antihypertensive Determined by Photomicrographic and Dissolution Methods

Keyphrases ☐ Crystalline transformation *in situ*—antihypertensive salt ☐ Solubility differences—crystalline forms ☐ UV spectrophotometry—analysis ☐ Photomicrographs—antihypertensive salt crystals ☐ X-ray diffraction patterns—identity ☐ IR spectrophotometry—identity

Sir:

The applications of microscopic techniques in pharmaceutical research are numerous. It has been used to study polymorphism, solvation, particle shape, particle-size distribution, double salt formation, crystal growth in suspension, and for the identification of particulate contaminants in parenterals. In this communication, the authors would like to present a simple microscopic technique for investigating, observing, and recording an *in situ* crystalline transformation.

During the preformulation investigation of a new investigational compound for its various physical and chemical parameters, the equilibrium solubility and intrinsic dissolution profiles are routinely studied. The equilibrium solubility of a monohydrochloride salt of a new antihypertensive in 0.1 *N* hydrochloric acid at 37° was found to be 0.74%. However, in observing the residual solid phase under the microscope, it was found that the original hexagonal crystals of the compound had completely disappeared and were transformed into rod-shaped crystals.

As a result of these findings, certain adaptations were made in designing the experimental procedures for determining the intrinsic dissolution characteristics of this particular drug substance. An excess of the drug beyond its equilibrium solubility was introduced into exactly 100 ml. of 0.1 *N* hydrochloric acid maintained at 37°. The drug and the dissolution medium were stirred by an overhead stirrer operating at 500 r.p.m.

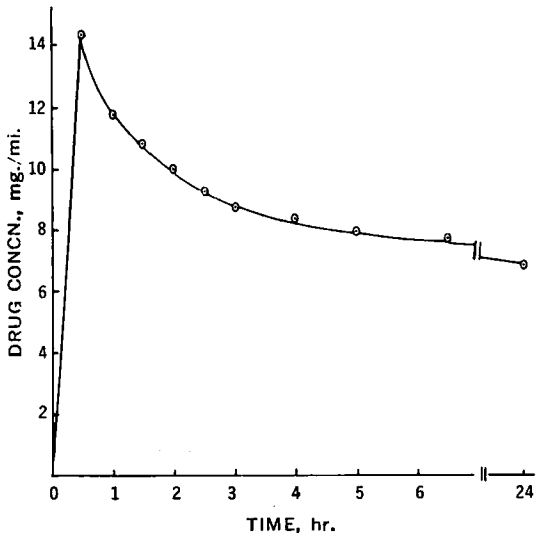

Figure 1—*Intrinsic dissolution profile of a new antihypertensive drug in 0.1* N *hydrochloric acid at 500 r.p.m.*

and placed 2 cm. below the surface of the liquid. At prescribed time intervals, aliquot samples were withdrawn and replaced with the same volume of fresh medium kept at 37°. The samples were immediately filtered through a filter (Millipore) using filter paper having a pore size of 0.45 μ. The filtrates were properly diluted and assayed with a spectrophotometer (Beckman DB) at 282 mμ

The intrinsic dissolution profile obtained is depicted in Fig. 1. It is evident from the plot that after an apparent rapid dissolution and attainment of a peak concentration of the drug in solution, the results are a pronounced decline of drug in solution with time. In general, this is attributed to the formation of a less-soluble species such as a hydrate of the drug. The development of hydrates was discussed extensively by Shefter and Higuchi (1). However, the possibility of an *in situ* crystalline transformation of the drug during dissolution has not been reported.

During the course of determining the intrinsic dissolution profile, the precipitates retained on the filter

Table I—Physical Characteristics of a New Antihypertensive Before and After Intrinsic Dissolution Profile Determination

Sample[a]	Treatment	Melting Point[b], °C	Photomicrograph[c]
A	Control sample before dissolution study	218–221	Hexagonal-shaped crystals of about 50 × 60μ
B	Stir for 30 min. at 500 r.p.m., the remaining solid material is vacuum-dried at 40° for 24 hr.	224–225	Short, rough-edged, pointed rods of about 5 × 15μ
C	Stir for 60 min. at 500 r.p.m., dried as in Sample B	224–225	As in Sample B
D	Stir for 24 hr. at 500 r.p.m. dried as in Sample B	225–226	As in Sample B
E, F	Contact with HCl without agitation for 15 (E) and 30 (F) min. by the proposed photomicrographic method	224–225	As shown in photo No. 4 of Fig. 3 for Sample E

[a]Corresponds to the numbering of samples in Table II and Fig. 2. [b]Corrected values as determined with the Thomas-Hoover capillary melting point apparatus. [c]Polaroid camera attached to Zeiss microscope at 100-fold magnification.

Table II—Data on X-ray Diffraction Pattern of a New Antihypertensive

Angle of Total Reflection (2θ)					
Sample A[a]	Sample B	Sample C	Sample D	Sample E	Sample F
	6.5 (w)	6.5	6.6	6.5	6.5
7.0					
	7.3 (w)	7.5	7.4	7.3	7.5
10.2	10.5	10.5	10.7	10.7	10.7
11.2	11.7 (w)				
	12.0 (v.w)	12.0	12.0	12.0	12.0
14.0	13.4	13.5	13.7	13.5	13.6
14.8 (w)	14.7	14.6	14.8	14.7	14.8
16.2 (w)	16.2	16.2	16.3	16.2	16.3
	17.4	17.5	17.5	17.4	17.5
18.2 (w)					
	19.5 (w)	19.5	19.5	19.5 (w)	19.5 (w)
20.0 (w)	20.3	20.5	20.5	20.2	20.3
21.5					
21.8	21.8	21.8	21.8	21.8	21.8
24.8	24.6	24.6	24.6	24.6	24.6
26.8					
28.5	27.3	27.2	27.3	27.2	27.2
29.6	29.0	29.0	29.0	29.0	28.9
30.3					
	31.3	31.2	31.4	31.3	31.3
32.2	32.5 (v.w.)			32.5 (w)	32.5 (v.w.)
33.2	33.1	33.1	33.0	33.1	33.1
34.2	34.3	34.3	34.4	34.3	34.2

[a] Treatment of the sample is presented in Table I.

papers after 30 min. (Sample B, Table I), 60 min. (Sample C), and 24 hr. (Sample D) postdissolution study were collected. Small quantities of the precipitates were transferred carefully onto the microscopic slides, suspended in mineral oil, and examined under a microscope (Zeiss) equipped with a camera (Polaroid). Photomicrographs were taken at 100-fold magnification.

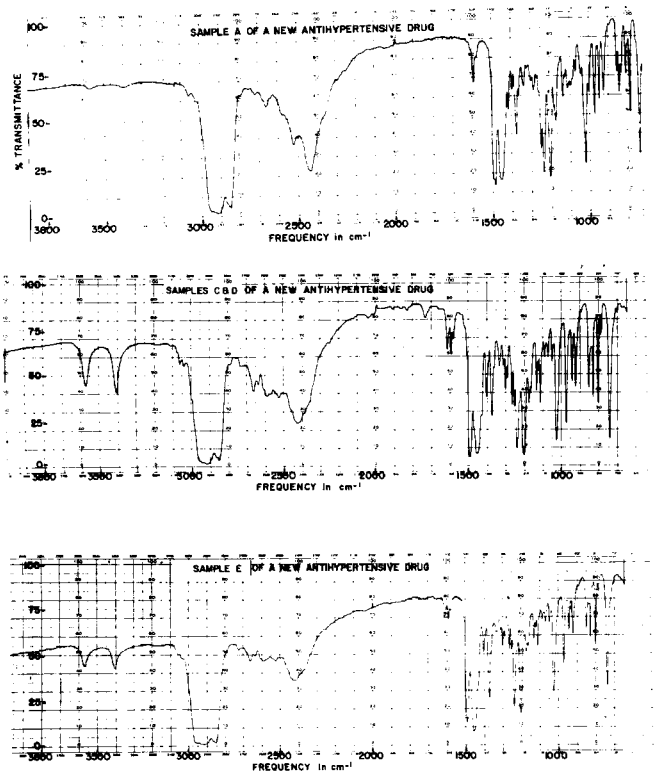

Figure 2—*Infrared spectra of a new antihypertensive drug before and after dissolution profile determination. Treatment of the sample is presented in Table I.*

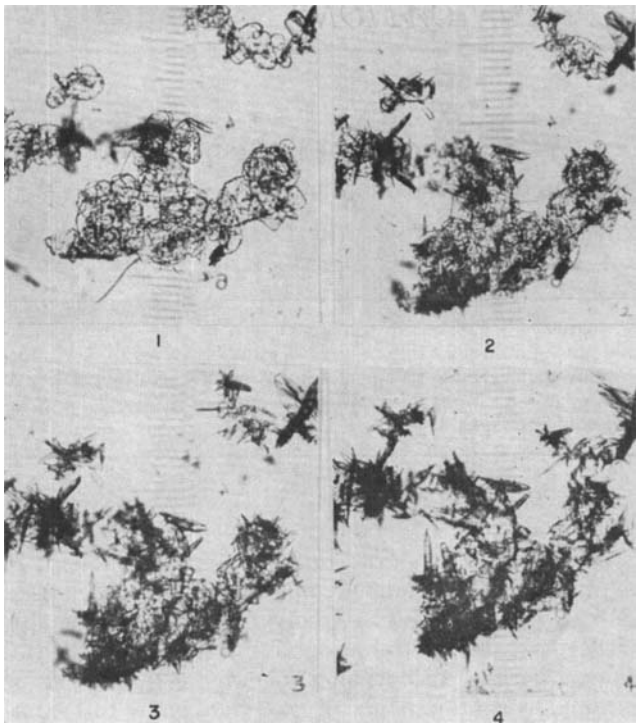

Figure 3—*Series of photomicrographs taken at 1* (1), *5* (2), *10* (3), *and 15* (4) *min. after the introduction of a quantity of 0.1* N *hydrochloric acid onto a sample of the antihypertensive drug on a microscope slide.*

The residual quantities of the precipitates were dried under vacuum at 40° for 24 hr. and the melting points, infrared spectra, and X-ray diffraction pattern of these precipitates were determined. The results obtained are shown in Tables I and II and Fig. 2, together with the data for the control sample before dissolution study (Sample A). Examination of the photomicrograph, melting point, infrared spectra, and X-ray diffraction pattern of Sample B as compared with that of Sample A, indicates that the compound has undergone *in situ* crystalline transformation within 30 min. postdissolution since further changes in various physical characteristics of the subsequent samples (Samples C and D) were not detectable up to 24 hr. Furthermore, the results depicted in Tables I and II and Fig. 2 clearly demonstrated that the new antihypertensive compound undergoes *in situ* crystalline polymorphic transformation rather than hydrate formation. The original hexagonal crystals (Sample A) are transformed into another crystalline polymorph and appeared as short, rough-edged, pointed rods (Sample B) within 30 min. By repeating the intrinsic dissolution profile study, it was found that the data of various physical characteristics of the excess drug in the dissolution medium collected a few minutes postdissolution study are identical to that of Sample B.

This observation leads us to conclude that the rate of *in situ* crystalline transformation is fairly rapid under the experimental conditions employed. In order to observe this phenomenon, the following photomicrographic technique was employed: a few milligrams of drug were scattered on a microscope slide and a cover glass was placed over the drug. The powder was properly

focused with 100-fold magnification on the microscope. Then a drop of 0.1 *N* HCl was introduced onto the microscope slide along the side of the cover glass. After a slight adjustment of the focus and the intensity of the light source, time-lapse photomicrographs were taken. The photomicrographs in Fig. 3 demonstrate a series of time-lapse photomicrographs taken of the same microscopic field without any stirring or agitation. After the solvent was in contact with the crystal for approximately 1 min., the hexagonal shape of the drug was still recognizable. After 10 min. of contact time, progressive transformation of the hexagonal to the rod-shaped crystals was observed and recorded with the photomicrographs. Under the experimental conditions employed the complete transformation into the rod-shaped crystals was observed to take place within 15 min.

In order to prepare enough quantity of samples for physical characterization, an excess of the drug beyond its equilibrium solubility was introduced into approximately 20 ml. of 0.1 *N* hydrochloric acid contained in a Petri dish at 22°. The drug and the medium were kept on the bench without agitation to mimic the condition of the microscopic slide. After the drug was in contact with the medium for 15 (Sample E) and 30 (Sample F) min., portions of residual solid materials were filtered rapidly through a filter, dried under vacuum at 40° for 24 hr. and characterized again by means of photomicrograph, melting point, infrared spectra, and X-ray diffraction pattern, as compiled in Tables I and II and Fig. 2. Inspection of physical characteristics of Samples E and F, as compared with that of Samples B, C, and D, indicated that the *in situ* crystalline transformation observed by dissolution method at 500 r.p.m. for a few minutes is similar to that attainable by photomicrographic or Petri dish methods without agitation at the end of 15 min. A detailed report on this investigation and the *in vitro* to *in vivo* relationship for this compound will be presented in the future (2).

The photomicrographic technique employed in this study for observing and recording the *in situ* crystalline transformation of the drug is simple, rapid, and straightforward. Meaningful results can be obtained within a short period of time.

(1) E. Shefter and T. Higuchi, *J. Pharm. Sci.*, **52**, 781(1963).
(2) S. Lin and L. Lachman, to be published.

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Antibacterial Activity of *Solanum carolinense* L.

Keyphrases □ *Solanum carolinense*—antimicrobial extract □ Antimicrobial spectrum—*S. carolinense*

Sir:

In a screening procedure for antibacterial activity of several crude botanical materials, small pieces of air-dried fruit of *Solanum carolinense* L. (horse-nettle) produced marked inhibition zones on blood-agar plate cultures of recent hospital isolates of *Pseudomonas* species. Lysis of red cells occurred around the samples placed on the plates. This study has been undertaken to define the antimicrobial spectrum and to isolate the active constituents of this plant. This preliminary report summarizes the antimicrobial spectrum of aqueous extracts of *S. carolinense*.

Frisby *et al.* (2) have reported inhibitory activity against *Mycobacterium* species in aqueous extracts of the leaves of *S. carolinense*. A galenical preparation of *S. carolinense* was recognized in NF V. It was formerly used in the treatment of epilepsy.

Extracts were prepared by macerating air-dried ground fruit (25 g.) for 24 hr. with 100 ml. 1% acetic acid. The resulting extract was filtered and ethanol (100 ml. of 95%) added to the filtrate and the resulting precipitate removed by filtration and discarded. The filtrate was shaken with three successive 50-ml. portions of chloroform. The hydroalcoholic layer was then brought to pH 9 with 10% NaOH and again shaken with three successive 50-ml. portions of chloroform. The hydroalcoholic fraction was brought to pH 7 with 10% acetic acid and evaporated to dryness on antibiotic sensitivity disks in an oven at 60°. The resulting disks were placed on agar plates streaked with the test organisms. Sabouraud dextrose agar,[1] Sauton's agar (1), and trypticase-soy agar [1] were used for the cultivation of the fungi, the *Mycobacterium* species, and the remaining bacterial species, respectively. Disks were also placed on blood-agar plates to detect any remaining hemolytic activity. Widths of inhibition zones were measured from the edge of the disks to the edge of the region of visible microbial growth. Zones were measured when sufficient confluent growth appeared on the surface of the agar.

The sensitivities of the responding test organisms

[1] Difco Laboratories, Detroit, MI 48201