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DIFENOXIN HYDROCHLORIDE POLYMORPHISM

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SUMMARY

Methods for the preparation of two difenoxin hydrochloride polymorphic forms are described. X-ray diffraction patterns and IR spectra for the two forms along with a quantitative IR procedure are also presented.

Solubility studies demonstrated that, of the two difenoxin hydrochloride polymorphs, form I was more soluble than form II. The apparent higher solubilities of the hydrochloride salt forms compared to the base were due to a super-saturation phenomena, as the hydrochloride salt forms converted to the base in aqueous media.

The solubilities of difenoxin hydrochloride lots containing known proportions of form I to form II reflected the differences in the solubilities of the pure forms. Likewise, the dissolution of difenoxin hydrochloride from tablets was related to the ratio of form I to form II.

Micronization proved to be a successful method for improving the dissolution of tablets prepared from a difenoxin hydrochloride lot with an approximately 2 : 1 form I : form II ratio. Micronization of the difenoxin hydrochloride at this form I : form II ratio made its dissolution from tablets equivalent to that of 100 mesh pure form I.

INTRODUCTION

The existence and importance of multiple crystalline forms of pharmaceutically interesting compounds is a frequently reported phenomenon and has been extensively reviewed in the pharmaceutical literature (Carstensen, 1973; Gibaldi, 1976; Kennon and Storz, 1976; Shami et al., 1976). Particularly relevant to this communication are descriptions of the relationships between polymorphism and bioavailability or dissolution (Aguiar et al., 1967; Allen and Kwan, 1969).

This communication describes part of a development effort to ascertain the importance of difenoxin hydrochloride, i.e. 1-(3-cyano-3,3-diphenylpropyl)-4-phenylisonipecotic acid hydrochloride, polymorphic forms and how they were processed.

Emphasis was placed on selecting a form of difenoxin hydrochloride early in development so as to minimize the need for extensive polymorph-related biopharmaceutical and clinical investigations at a later date.

In this report, the preparation, identification, and quantitation of the difenoxin hydrochloride forms are described. The differences in the solubilities of the forms and how they influence the dissolution of difenoxin hydrochloride from tablets are discussed. Finally, the effect of micronization on the dissolution of difenoxin hydrochloride from tablets is described.

MATERIALS AND METHODS

Preparation

The forms are numbered in order of discovery.

Form I. Difenoxin hydrochloride was dissolved in 3 : 1 isopropanol : water by refluxing. The solution was filtered and then refluxed briefly to dissolve any crystals which had formed in the filtrate. The solution was allowed to cool at room temperature for 16 h and then was stored at 4°C for 16 h. The resulting solid was filtered and then dried in vacuo at 90°C for 24 h.

Form II. Difenoxin hydrochloride was dissolved in 9 : 1 methanol : dimethylformamide by refluxing. The solution was filtered and then reheated briefly to dissolve any crystals which had formed in the filtrate. This solution was cooled rapidly, with agitation, in an ice bath and the cold mixture was stored at 4°C for 18 h. The resulting solid was filtered, air-dried and then dried in a steam oven for 18 h.

Identification

Identification was made by X-ray diffraction and supported by IR spectra.

X-ray diffraction. X-ray diffraction patterns were obtained on a diffractometer [1]. Instrument variables were set as follows: 0.6° entrance (aperture), 0.4° receiving slit, copper Kα radiation, 40 kV, 18 mA, nickel β filter, 1×10^3 cps full scale, 0.4 s timing.

IR spectra. IR spectra of the forms of difenoxin hydrochloride were determined using a Grating Spectrophotometer [2]. Mineral oil mulls using a 1000 X 1 slit program, 4.2 gain, 11.00 attenuator speed, 16 min scan time, 6 suppression, 0.4 A source current were employed.

Quantitation

IR spectra of forms I and II, which were observed to be contaminants of each other, were obtained on known ratios. A mull consisting of 10 mg of difenoxin hydrochloride plus 0.03 ml of mineral oil was placed between potassium bromide discs with a 0.025 mm spacer. Seven mixtures between 100% and 30% of polymorph I with polymorph II were prepared. The spectrum between 950 cm^{-1} and 645 cm^{-1} was recorded for each mixture with the percent transmission scale expanded two times. The percent transmission was

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[1] Crystalloflex IV, ES Industries, Marlton, N.J. 08053, U.S.A.

[2] Model 521, Perkin Elmer Corporation, Norwalk, Conn. 06856, U.S.A.

measured at the peaks at 873 cm^{-1} (form I), 845 cm^{-1} (form II), 660 cm^{-1} (internal reference peak) and at the valley at 650 cm^{-1}. The percent form I in each sample was correlated to the percent transmissions expressed as the relationship $(T_{845} - T_{873})/(T_{650} - T_{660})$.

Thermal stability

Attempts to thermally induce transitions from one form to another in either the dry state or in suspension on the hot stage [3] of a microscope have failed due to the evolution of hydrogen chloride with liberation of the base. However, samples have been stored in the dry state for many months up to 80°C without chemical decomposition and without change in form.

Solubility

The dissolution rate of difenoxin hydrochloride in water, in simulated gastric fluid TS, USP, with or without pepsin, and in citrate–phosphate buffers, pH 2–8, was so slow that other media were sought for dissolution testing. After examination of several possible media, a 1% tartaric acid solution was selected. Consequently, 1% tartaric acid solution was employed in solubility and dissolution experiments.

In the first solubility study, the solubility of difenoxin hydrochloride polymorphic forms and difenoxin base were compared. Difenoxin base was prepared by dissolving a sample of the hydrochloride salt in ammonium hydroxide solution, evaporating the solvent, washing the recovered solid with distilled water and drying to a constant weight.

All difenoxin hydrochloride and difenoxin base samples were passed through a 100 mesh screen. Microscopic examination of all lots of difenoxin hydrochloride and difenoxin base showed them to have approximately the same particle dimensions.

An excess of 100 mesh difenoxin hydrochloride or difenoxin base was added to 50 ml of 1% tartaric acid. The containers were rotated at 45 rpm for 120 h at 37°C on a rotating bottle apparatus [4]. Samples were removed at 24, 48, and 120 h and filtered through a 0.45 μm mixed cellulose ester filter [5]. The assay procedure was an adaptation of the assay procedure described under *Dissolution.*

A second solubility study was conducted to determine the behavior of difenoxin hydrochloride lots which were crystallized as polymorphic mixtures. These lots were composed of the following form I to form II ratios as determined by quantitative IR analysis – 30 : 70, 68 : 32, 70 : 30, 96 : 4. The mixture rich in form II was prepared via the preparative procedure for form II, but with room temperature cooling. The mixtures rich in form I were prepared via the preparative procedure for form I, but with rapid (refrigerated) cooling. One lot composed of pure form II and one lot composed of pure form I were also included in this study. Samples were removed at 10, 20, 30, 40, 50, 60 and 120 min.

As in the case of the first solubility study, 1% tartaric acid solution at 37°C was used as the solvent and the assay procedure was an adaptation of the assay procedure described under *Dissolution.*

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[3] Model FP-2 Hot Stage, Mettler Instrument Corp., Princeton, N.J. 08540, U.S.A.

[4] National Formulary, 13th Ed., American Pharmaceutical Association, Washington, 1970, p. 882.

[5] No. HAWP 04700, Millipore Corp., Bedford, Mass 01730, U.S.A.

Dissolution

The tablet dissolution experiments compared the dissolution of 100 mesh difenoxin hydrochloride supplied as pure form I, pure form II, a 69 : 31 ratio of form I to form II and a 62 : 38 ratio of form I to form II as determined by quantitative IR analysis.

Also studied was the dissolution of difenoxin hydrochloride in a 69 : 31 ratio of form I to form II which had been micronized in an air attrition mill [6]. The mean particle diameter of the micronized sample was 4.0 μm as determined by an air permeability specific surface analyzer [7]. Infra red analysis of the micronized sample demonstrated that its polymorphic form ratio did not change as a result of micronization.

The equivalent of 8.0 mg of difenoxin base was added, in the form of 0.5 mg tablets, to 750 ml of 1% tartaric acid maintained at 37.5 ± 0.5°C in a 1 liter round bottom flask. The system was stirred at 50 rpm with a 3 in. semi-circular polytetrafluoroethylene paddle [8]. The bottom of the paddle was positioned 5 cm from the bottom of the flask. Samples (25 ml) were taken at 10, 20, 30, 45 and 60 min. Following each sample removal, fresh dissolution fluid warmed to 37.5 ± 0.5°C was added to the flask. Samples were filtered through 0.45 μm mixed cellulose ester filters. Twenty ml aliquots of the filtrate were combined with 20 ml portions of chloroform and 6 ml portions of 1 N HCl. Following extraction, 15 ml of each chloroform layer was combined with 10 ml of a saturated pH 4 solution of methyl orange. After shaking the mixture, the aqueous layers were discarded. Finally, 5 ml of the chloroform layers were combined with 5 ml of 5% HCl in methanol. The solutions were assayed spectrophotometrically at about 522 nm versus a 1 : 1 : chloroform : 5% HCl in methanol blank.

RESULTS AND DISCUSSION

X-ray diffraction

The X-ray diffraction patterns for the difenoxin hydrochloride forms are illustrated in Fig. 1.

IR spectra

The IR spectra for the difenoxin hydrochloride forms are illustrated in Fig. 2.

Quantitation

Fig. 3 illustrates the linear relationship between form I content and transmission.

Solubility

Results of the first solubility study employing the two hydrochloride forms plus the sample of difenoxin base are listed in Table 1. After 24 h, the solubility of form I was greater than form II, while the solubility of the base was less than from either of the hydrochloride salt forms.

As the experiment proceeded, the difenoxin in solution from the salts decreased to the

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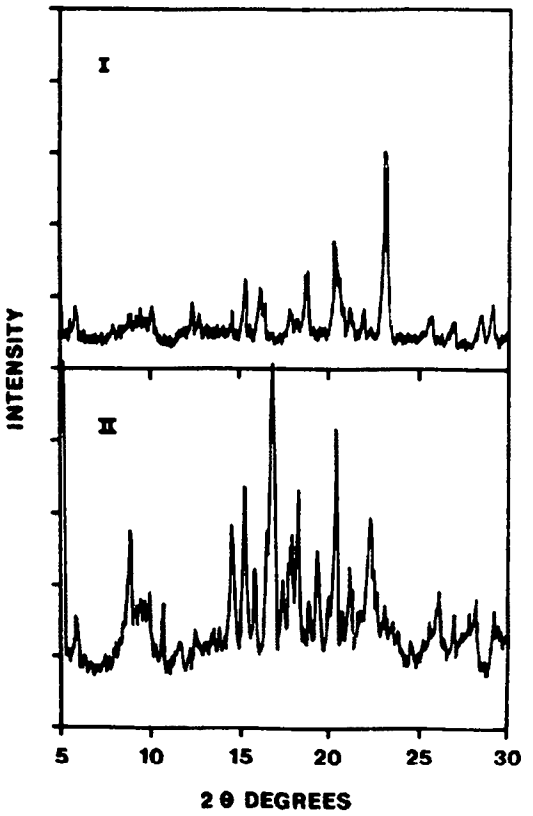
[6] Gem-T Research Model Jet Mill, G.W. Helme Co., Helmetta, N.J. 08828, U.S.A.
[7] Sub-Sieve Sizer, Fisher Scientific Co., Pittsburgh, Pa. 15219, U.S.A.
[8] No. 9510T-104, Lab Glass, Inc., Vineland, N.J. 08360, U.S.A.



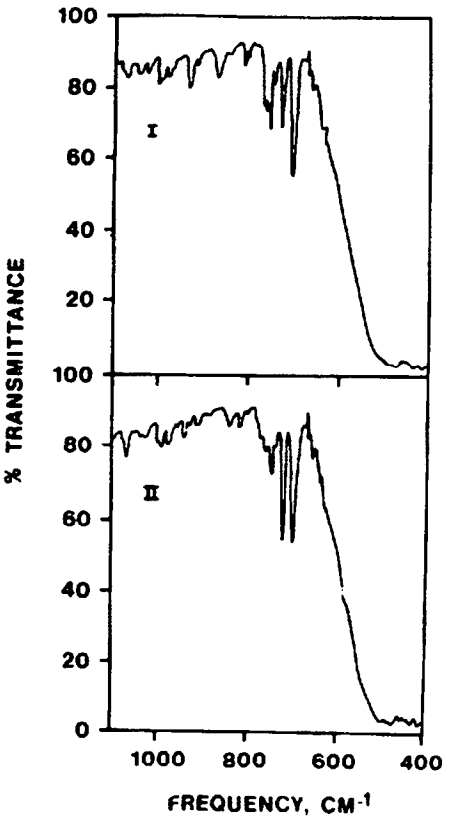


Fig. 1. X-ray diffraction patterns of difenoxin hydrochloride polymorphic forms.

Fig. 2. IR spectra of difenoxin hydrochloride polymorphic forms.

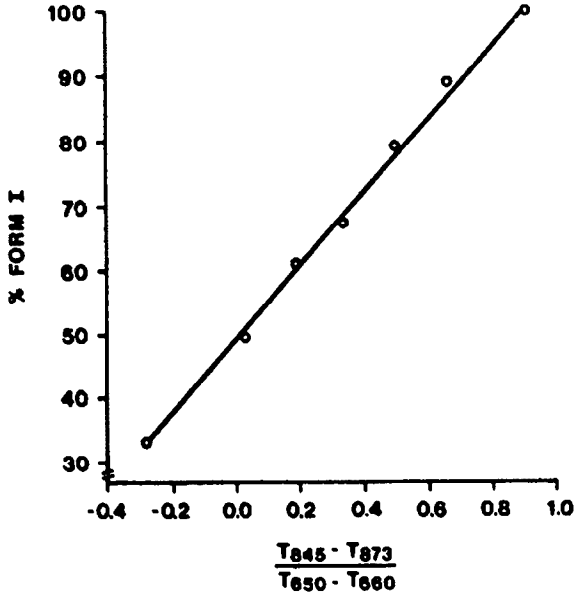


Fig. 3. Correlation of percent polymorph I with IR transmittance.

TABLE 1

SOLUBILITY OF 100 MESH SAMPLES OF DIFENOXIN HYDROCHLORIDE FORMS AND DIFENOXIN BASE IN 1% TARTARIC ACID (mg BASE/100 ml)

Hours	Hydrochloride forms or base		
	Form I	Form II	Base
24	4.5	3.1	2.5
48	2.2	2.0	2.2
120	1.9	1.7	1.8

point where it was equivalent to the base. The data indicated that the higher solubility from the salts is a super-saturation phenomenon since eventually the solubility of difenoxin from the salts declined to the level of the base. The conclusion that the higher solubility of the salts was a super-saturation phenomenon was substantiated when air dried samples of pure forms I and II which had been shaken for 1 h in a 1% tartaric acid solution (containing 1% polysorbate 80 to accelerate recrystallization) were submitted for chloride analysis [9]. The results demonstrated the conversion of the salt to the base for both forms. Form I exhibited a change of chloride content from an initial concentration of 7.69% to 0.54% following polysorbate 80 mediated recrystallization. Form II showed a change from 7.69% chloride to 0.81% chloride after polysorbate 80 mediated recrystallization.

Additional evidence of conversion of the salt forms to the base was obtained when the IR spectra in mineral oil of samples from the chloride analysis experiments were compared to the spectrum for the base prepared by treating the hydrochloride salt with ammonium hydroxide solution.

The peak solubility for each lot in the second solubility study was plotted versus form I content in Fig. 4. All but one of the lots followed the trend of increased peak solubility with increased form I content. On the assumption that a straight line relationship existed, a correlation coefficient (95% probability) of 0.849 was calculated. The reason for the deviation is unexplained. Possibly, the deviation was related to particle surface area, a variable which was controlled only through sieve sizing.

Dissolution

The means of dissolution data obtained from four lots of difenoxin hydrochloride prepared as tablets are illustrated in Fig. 5. These lots represent form I assays of 100%, 69%, 62% and 0%. Form II was the other form present. Lot C was prepared into a single batch of tablets and is represented by the means of two determinations. Lots A, B and D were prepared as two batches of tablets and are represented by the means of four determinations (two determinations per batch).

The dissolution results indicated that the dissolution method was sensitive enough to

[9] Micro-Analysis, Inc., Wilmington, Dela. 19808, U.S.A.

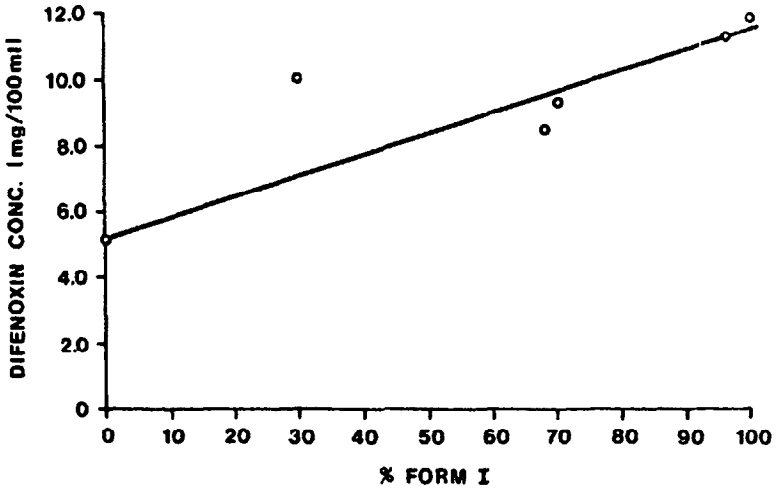


Fig. 4. Relationship of peak solubility to form I content from difenoxin hydrochloride recrystallizations in 1% tartaric acid.

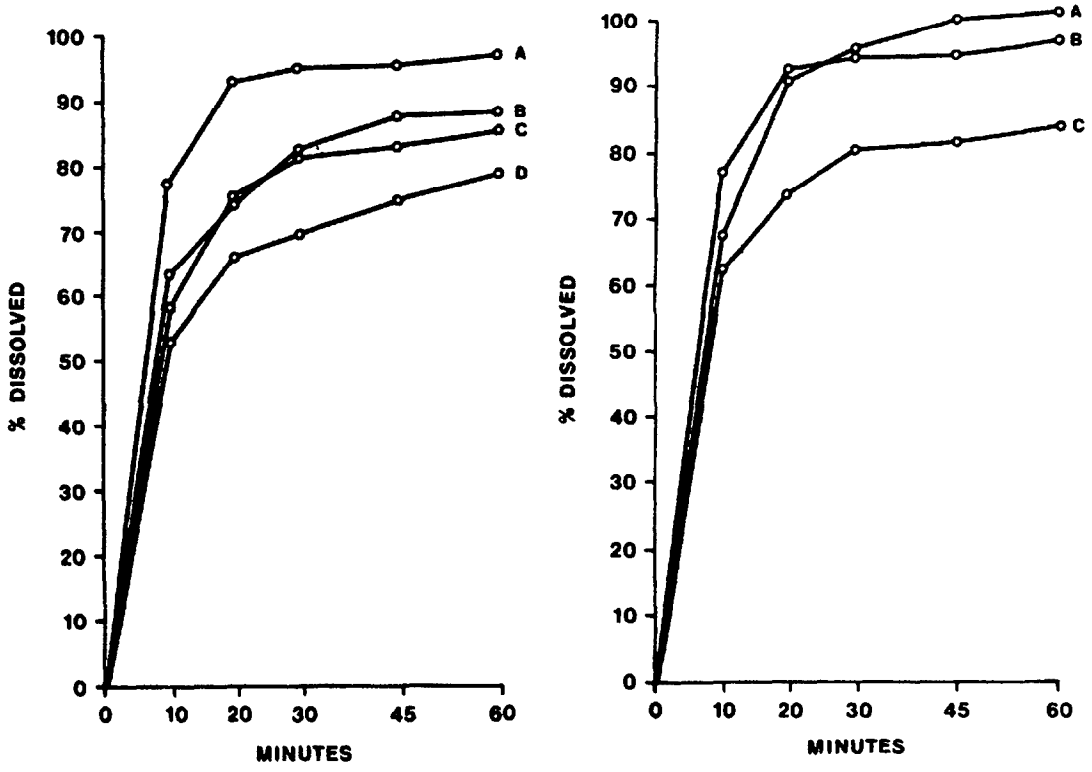


Fig. 5. Effect of form I content on tablet dissolution. Key: A, 100%; B, 69%; C, 62%; D, 0%.

Fig. 6. Effect of particle size on difenoxin hydrochloride dissolution. Key: A, 69% form I, micronized; B, pure form I, 100 mesh; C, 69% form I, 100 mesh.

distinguish between large differences in polymorphic content. However, the relationship was not linear, and it would be difficult to observe differences in dissolution with lots having concentrations of form I less than 62%. The lack of linearity was most probably due to the influence of sink effects, i.e. different effects for the different forms due to their different solubilities.

The tablet dissolution of micronized difenoxin hydrochloride containing the 69 : 31 form I to form II ratio is illustrated in Fig. 6. X-ray diffraction and IR analysis confirmed that the form I to form II ratio had not changed during the micronization process. The data from the 100 mesh material of the same lot as well as the data from 100 mesh 100% form I are also included for comparison. The results indicate that, as anticipated, micronization improved the tablet dissolution profile of difenoxin hydrochloride to where it became equivalent to 100 mesh 100% form I. Apparently, the dissolution difference due to difference in crystal structure (up to 31% form II in form I) can be essentially eliminated by micronization. It is not known if samples with less than 69% form I will show the same degree of dissolution improvement upon micronization.

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