# Dissolution Behavior of Polymorphs of Chloramphenicol Palmitate and Mefenamic Acid

ARMANDO J. AGUIAR and JOYCE E. ZELMER

**Abstract** □ Relative rates of dissolution and solubilities of three polymorphic forms of chloramphenicol palmitate in 35% tertiary butanol–water mixture and two polymorphs of mefenamic acid in dodecyl alcohol were measured. The thermodynamic relationships involving the transition of the metastable polymorphs to the stable one were examined. The heats of solution, enthalpy, entropy, free energy differences, and transition temperatures were calculated. The relative stability of the polymorphs in the dissolving media is shown. The significance of free energy differences between the polymorphs and their absorptivity as reflected by blood levels in humans is discussed.

**Keyphrases** □ Chloramphenicol palmitate polymorphs—dissolution, tertiary butanol-water □ Mefenamic acid polymorphs—dissolution, dodecyl alcohol □ Thermodynamic values—chloramphenicol palmitate, mefenamic acid polymorphs dissolution □ UV spectrophotometry—analysis

The importance of polymorphic forms of relatively insoluble drugs in regard to their biological availability and stability has been discussed (1–4) and recently reemphasized (5). Measurements of thermodynamic properties such as heats of solution, entropy, and enthalpy relationships of the polymorphs often aid in the proper selection of the desired crystalline modification of the drug.

The present communication deals with the solubility and dissolution behavior of three polymorphs of chloramphenicol palmitate and two of mefenamic acid [*N*-(2,3-xylyl) anthranilic acid]. The thermodynamic relationships involving the transition of the metastable modifications to the stable one are examined. It is postulated, from a comparison of the *in vivo* absorption data in humans and the thermodynamic parameters, that when the free energy differences between the polymorphs are small, there may be no significant difference in their absorptivities as measured by blood levels. When the differences are large, they might affect the absorption profiles.

## THEORETICAL CONSIDERATIONS

The Nernst equation (6), which relates the rate of concentration increase to the solubility of a dissolving solid, is commonly written as:

$$\frac{dc}{dt} = \frac{AD}{Vh}(C_s - C_t) \qquad \text{(Eq. 1)}$$

where $A$ is the area of the dissolving interface of the solid, $D$ is the diffusion coefficient of the solute in the solvent, $V$ is the volume of the solvent, $h$ is the thickness of the diffusion layer and $C_s$ and $C_t$ are concentrations at saturation and at time $t$, respectively.

Equation 1 reduces to:

$$\frac{dc}{dt} = \frac{AD}{Vh}C_s \qquad \text{(Eq. 2)}$$

for the experimental conditions where $C_s \gg C_t$. Since $D$ is a property of the solute molecule and the solvent, it is independent of the solid state form. The experimental conditions can also be selected such that $A$, $V$, and $h$ in Eq. 2 can be maintained the same in measuring the dissolution rates of different polymorphic forms. The dissolution rate then is directly proportional to $C_s$, the saturation solubility, and differences in the solubility of the polymorphs can be related to their free energy differences.

## EXPERIMENTAL

**Apparatus and Procedure**—The method used in studying the solubility profiles of the polymorphs followed closely that of Shefter and Higuchi (4), who showed that the method, though simple, gave reproducible results.

A weighed sample of the polymorph, approximately three times the concentration necessary to saturate the solution was added rapidly to a fixed volume of the solvent, 350 ml. for the dissolution of mefenamic acid and 400 ml. for chloramphenicol palmitate, maintained at a constant temperature. A stopwatch was started and 3-ml. samples were withdrawn from the system at measured time intervals. The samples were filtered through Millipore filters (pore size 0.45 $\mu$) using a Swinney syringe adapter.

The concentration of mefenamic acid was measured spectrophotometrically at 350 m$\mu$, after dilution in 95% methanol–0.01 *N* HCl solution. A spectrophotometer (Beckman DU-2) was used for the analysis. The concentration of chloramphenicol palmitate was also measured spectrophotometrically at 278 m$\mu$ using a recording spectrophotometer (Carey model 11).

**Materials used**—The dissolution and solubility determinations of polymorphic forms of chloramphenicol palmitate were carried out in 35% tertiary butyl alcohol[1]–water mixture. Dodecyl alcohol[2] was chosen as the solvent for the studies with mefenamic acid. These solvents were selected because of the relative ease of obtaining measurable concentrations of the drug in the medium. Aqueous systems such as buffers and tertiary butanol–water mixtures were not suitable for use with mefenamic acid because of the polar nature of the drug.

Polymorph I of mefenamic acid was prepared by saturating 50 ml. of acetone with the drug. The undissolved drug was filtered off and the saturated acetone solution was cooled slowly by packing ice around the flask containing the solution. The flask was left in this medium overnight, and the crystals were harvested the next day by filtration. The sample was washed three times with water and dried at room temperature.

Polymorph II of mefenamic acid was prepared by dissolving 30 g. of mefenamic acid in 50 ml. of *N*,*N*-dimethylformamide. The solution was warmed on a steam bath to 60° and stirred to facilitate dissolving. When all the mefenamic acid was in solution, the hot dimethylformamide containing the drug was poured rapidly into a beaker previously cooled to −40°. The cooling medium was a bath of acetone and dry ice. The solution was maintained at this temperature until most of the mefenamic acid had crystallized out. The beaker was then transferred to a water bath maintained at room temperature. When the crystalline yield had warmed sufficiently to be fluid enough to pour, the crystals were filtered using a medium-porosity sintered-glass funnel. The crystals were then washed three times with water and allowed to dry at room temperature overnight.

Two lots of chloramphenicol palmitate were employed in the studies. One was synthesized from 93% pure palmitoyl chloride, the other from 99.8%. The methods utilized for preparing Polymorphs *A* and *B* of chloramphenicol palmitate were essentially those described by Tamura and Kuwano (7). Polymorph *C* was prepared by a procedure (8) similar to that used for making Polymorph II of

[1] Tertiary butyl alcohol m.p. 24–25°. Eastman Kodak Co., analytical grade.

[2] Eastman Organic Chemicals, Distillation Products Industries, Division of Eastman Kodak Co.

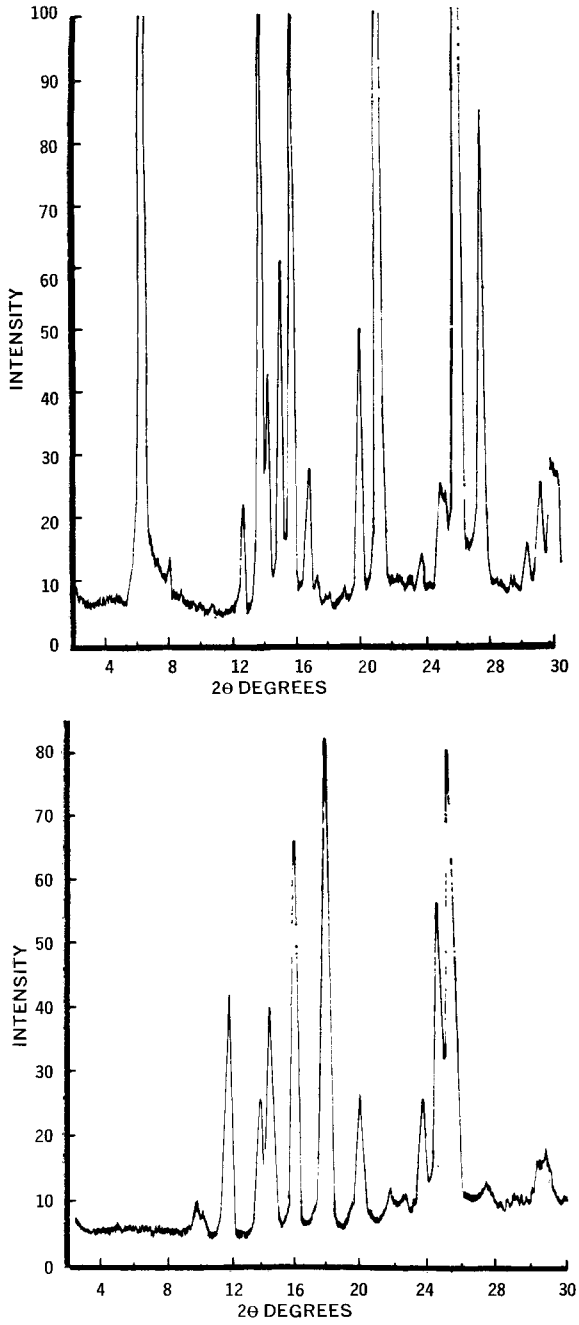


**Figure 1**—*X-Ray diffractograms for Polymorphs* I (*top*) *and* II (*bottom*) *of mefenamic acid.*

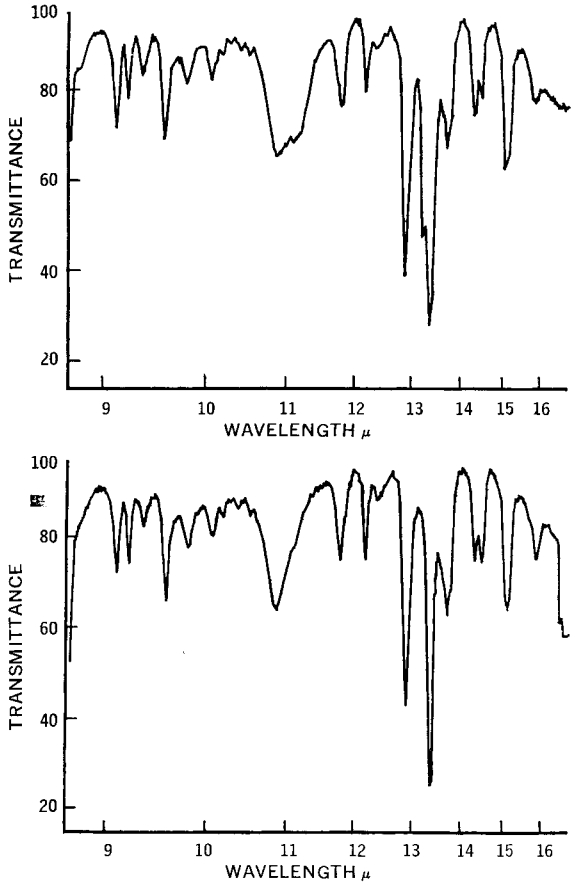


**Figure 2**—*IR spectra of Polymorph* I (*top*) *and Polymorph* II (*bottom*) *of mefenamic acid.*

mefenamic acid, except absolute methanol was employed instead of dimethylformamide.

The X-ray diffraction patterns and the IR spectra of Polymorphs I and II of mefenamic acid are shown in Figs. 1 and 2. The diffraction patterns and spectra for Polymorphs *A*, *B*, and *C* of chloramphenicol palmitate were reported previously (5).

## RESULTS AND DISCUSSION

The solubility and dissolution behavior of Polymorphs *A* and *B* of chloramphenicol palmitate at 30 and 38° are shown in Fig. 3. These plots show the concentration attained in solution for each polymorphic form as a function of time in the presence of an excess of the solid phase and under essentially constant agitation. It is apparent from the data that Polymorph *B* has a faster dissolution rate than Polymorph *A* and yields solutions approximately four times more concentrated at equilibrium. Although part of the greater dissolution rate and higher saturation concentrations of the metastable Polymorph *B* may be due to geometric factors, it is evident that the higher free energy content of this form plays a significant role.

The solubility and dissolution curves for Polymorph *C* of chloramphenicol palmitate at 30, 20, 15, and 6° are shown in Fig. 4. Qualitatively, it is apparent that the dissolution rate and the concentration in solution at equilibrium obtained with this form are intermediate between those of Polymorphs *A* and *B*. The higher thermodynamic activity associated with Polymorph *C* apparently is the major contributing factor causing the initially greater dissolution rate observed for this modification. After the maximum concentration plateau is reached, there is an apparent first-order decline in the amount of drug dissolved. The limiting value of this decrease was found to be the solubility of Polymorph *A*. The complete reversion to Polymorph *A* at this stage was confirmed by X-ray diffraction

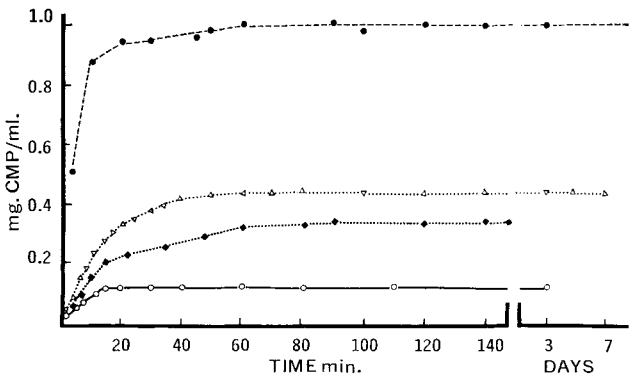


**Figure 3**—*Dissolution curves for Polymorphs* A *and* B *of chloramphenicol palmitate in 35% tertiary butanol and water at 30 and 38°. Key: Polymorph* A, *30°*, ○—○; *Polymorph* B, *30°*, △· · ·△; *Polymorph* A, *38°*, ◆- - -◆; *Polymorph* B, *38°*, ●- - -●.

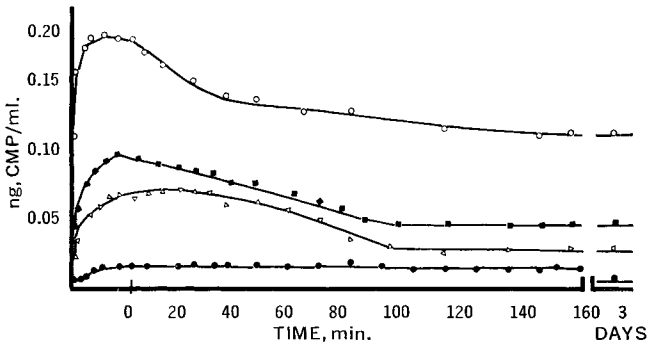


**Figure 4**—*Dissolution curves for Polymorph C of chloramphenicol palmitate in 35% tertiary-butanol and water at 30, 20, 15 and 6°. Key: 30°, ○—○; 20°, ■—■; 15°, △—△; 6°, ●—●.*

analysis of powders isolated after the lower plateau levels were reached.

The solubility profiles obtained with polymorphs prepared from 99.8% pure chloramphenicol were identical to the ones obtained with the less pure material.

It is apparent from the dissolution behavior that the maximum values obtained were good approximations of the true solubility of these crystals. Therefore, it would be possible from measurements made at several temperatures to calculate the thermodynamic quantities involved in the transitions of the metastable forms to the stable one. Measurements over the temperature range 6 to 38° are plotted in the classical van't Hoff fashion in Fig. 5. It is apparent that in the temperature regions studied a straight line relationship exists. The values for the heat of solution for each crystalline form, calculated from the slopes, are given in Table I.

At constant temperature and pressure, the free energy difference $\Delta G_T$ between the Polymorphs $A$ and $B$ is given by:

$$\Delta G_T = RT \ln \frac{C_s \text{ Polymorph } A}{C_s \text{ Polymorph } B} \qquad \text{(Eq. 3}a\text{)}$$

and for Polymorphs $A$ and $C$

$$\Delta G_T = RT \ln \frac{C_s \text{ Polymorph } A}{C_s \text{ Polymorph } C} \qquad \text{(Eq. 3}b\text{)}$$

These equations relate the solubility, $C_s$, of two polymorphic forms at a particular temperature, $T$, to the free energy differences. The values calculated for the free energy differences between Polymorphs $B$ and $C$ with respect to $A$ at 30° (303° K) are shown in Table I. The free energy difference is a measure of the free energy change in the conversion of the Polymorphs $B$ or $C$ to $A$.

The enthalpy change, $\Delta H$ for the transition of Polymorph $B$ to $A$ and Polymorph $C$ to $A$ can be obtained by subtracting the heats of solution derived for the stable form from that of the metastable modification. Thus, the enthalpy change in the transition from Polymorph $C$ to $A$ (*i.e.* $\Delta H_{C \to A}$) is −4592 cal./mole. That from $B$ to $A$ is −6352 cal./mole.

At a particular temperature, $T$, the entropy for the transition of Polymorph $B$ to $A$ and $C$ to $A$ can be evaluated by the following

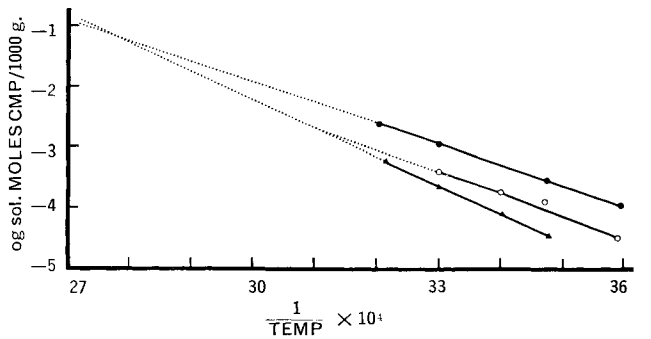


**Figure 5**—*The van't Hoff type plot for Polymorphs* A, B, *and* C *of chloramphenicol palmitate. Key: Polymorphs* A →; B ●—●; *and* C ○—○.

**Table I**—Thermodynamic Values Calculated for Polymorphs $A$, $B$, and $C$ of Chloramphenicol Palmitate

| Polymorph | Transition Temp. (°C.) to Form $A$ | Heat of Solution, kcal./mole | $\Delta G_T$, cal./mole[a] | $\Delta S_{303}$ e.s.u. | $\Delta S_{trans}$ e.s.u.[a] |
|---|---|---|---|---|---|
| $A$ | — | 21.8 | — | — | — |
| $B$ | 88 | 15.4 | −774 | −18 | −17 |
| $C$ | 50 | 17.2 | −465 | −13 | −14 |

[a] Calculated for the conversion to Polymorph $A$.

relationships:

$$\Delta S_T = \frac{\Delta H_{B \to A} - \Delta G_T}{T} \qquad \text{(Eq. 4}a\text{)}$$

$$\Delta S_T = \frac{\Delta H_{C \to A} - \Delta G_T}{T} \qquad \text{(Eq. 4}b\text{)}$$

The values computed for the reversion of Polymorph $B$ to $A$ and $C$ to $A$ are included in Table I.

At the transition temperatures for the reversion of Polymorphs $B$ to $A$ and $C$ to $A$, the free energy difference, $\Delta G_T$, is equal to zero and the entropy change, $\Delta S_{trans}$, can be calculated using Eq. 4*a* and 4*b* neglecting the $\Delta G_T$ term. The entropy changes at the transition temperature are given in Table I.

All the thermodynamic relationships discussed are based on the assumptions that Henry's law is obeyed and the values obtained are independent of the solvent used. In order to test the latter hypothesis, dissolution experiments were carried out in tertiary butanol–water systems containing 45 and 55% of the alcohol. The values derived were very similar to the ones presented for the 35% tertiary butanol–water system. The 35% mixture was chosen because it provided a suitable solubility range and ease of analysis.

The dissolution behavior of Polymorphs I and II of mefenamic acid in dodecyl alcohol at various temperatures is shown in Figs. 6 and 7. It is apparent from the data that Polymorph II has a higher saturation solubility than Polymorph I and the difference is apparently related to the higher free energy content of the metastable Polymorph II.

The data also stress the instability of Polymorph II of mefenamic acid in the dissolving medium. For example at 50° (Fig. 7) after the maximum concentration is reached, there is an apparent first-order decline in the amount of drug dissolved. The limiting value was found to be the solubility of Polymorph I. Similar behavior is also

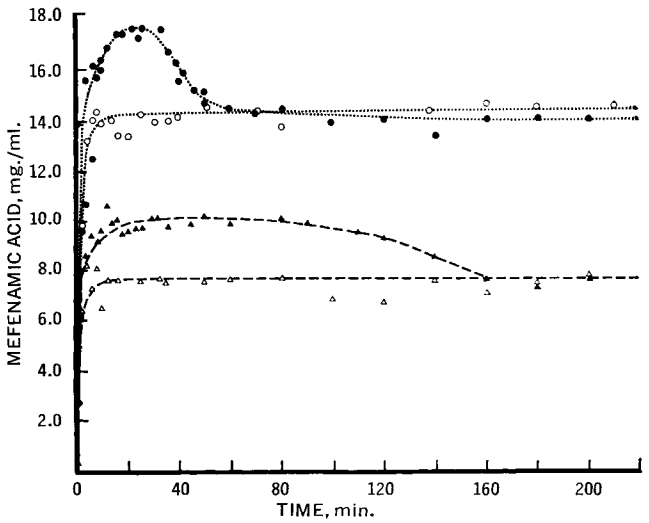


**Figure 6**—*Dissolution curves for Polymorphs* I *and* II *of mefenamic acid in dodecyl alcohol. Key: Polymorph* I *30°*, △- -△; *Polymorph* II *30°*, ▲- -▲; *Polymorph* I 50°, ○- -○; *Polymorph* II *50°*, ●- - -●.

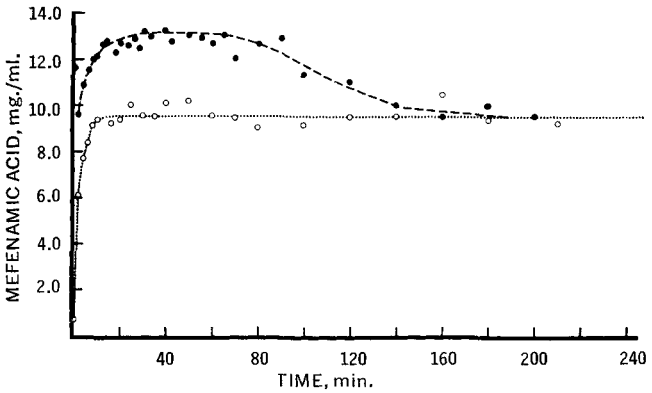


**Figure 7**—*Dissolution curves for Polymorphs* I *and* II *of mefenamic acid in dodecyl alcohol at 40°. Key: Polymorph* I, O- - -O; *Polymorph* II, ●—●.

evident at 30, 35 and 40°. The complete reversion of Polymorph II to I was confirmed by X-ray diffraction analysis of the powder isolated after the lower plateau levels were reached.

From the van't Hoff plot shown in Fig. 8, the transition temperature was estimated to be about 89°. The heat of solution calculated from the slopes was 6.7 kcal./mole for Polymorph I and 5.7 kcal./mole for Polymorph II. The free energy difference $\Delta G_T$ at 30°, between Polymorphs I and II of mefenamic acid calculated using the relationship shown in Eq. 3*a*, was found to be −251 cal./mole. The enthalpy change $\Delta H$ for the transition of Polymorph II to I was estimated to be −1031 cal./mole. At 30° the entropy for the transition of Polymorph II to I, evaluated from Eq. 4, was found to be −2.6 e.s.u. The entropy change at the transition temperature was −2.8 e.s.u.

The thermodynamic values calculated for Polymorphs I and II of mefenamic acid are summarized in Table II.

An analysis and comparison of the thermodynamic data presented for polymorphs of chloramphenicol palmitate and mefenamic acid is of some interest. For example, although the Polymorph II of mefenamic acid has a higher saturation solubility than Polymorph I, the difference between their heats of solution is small. On the other hand, the difference between the heats of solution of Polymorphs *A* and *B* of chloramphenicol palmitate is fairly large. Similarly the free energy difference between Polymorphs *A* and *B* of chloramphenicol palmitate is −774 cal./mole while the difference between the polymorphs of mefenamic acid is only −251 cal./mole.

The absorption studies in humans of Polymorphs *A* and *B* of chloramphenicol palmitate have been reported recently (5). The studies showed that suspensions containing Polymorph *B* of chloramphenicol palmitate gave blood levels approximately ten times higher than suspensions of Polymorph *A*. The differences in the human absorption shown by the polymorphic forms of chloramphenicol palmitate may involve the free energy difference between Polymorphs *A* and *B* of the drug. The relatively large (−774 cal./

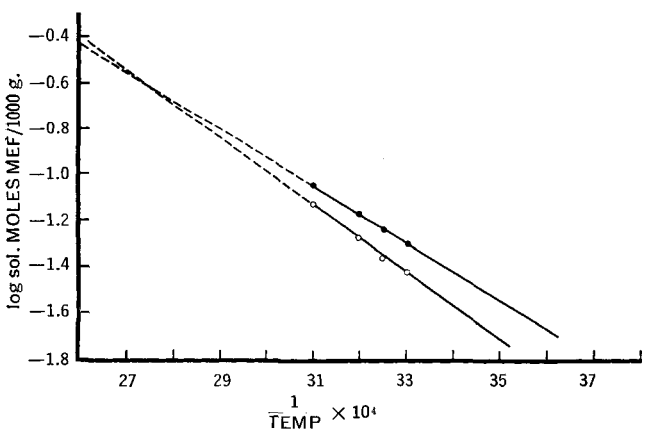


**Figure 8**—*The van't Hoff type plot for Polymorphs I and II of mefenamic acid in dodecyl alcohol. Key: Polymorph* I, O—O; *Polymorph* II, ●—●.

**Table II**—Thermodynamic Values Calculated for Polymorphs I and II of Mefenamic Acid

| Polymorph | Transition Temp. °C. to Polymorph I | Heat of Solution, kcal./mole | $\Delta G_T$ at 30°C. cal./mole[a] | $\Delta S_{303}$ e.s.u.[a] | $\Delta S_{trans.}$ e.s.u.[a] |
|---|---|---|---|---|---|
| I | — | 6.7 | — | — | — |
| II | 89 | 5.7 | −251 | −2.6 | −2.8 |

[a] Calculated for the conversion to Polymorph I.

mole) free energy difference between Polymorphs *A* and *B* of chloramphenicol palmitate could manifest itself in the significantly higher and faster absorption observed with Polymorph *B* in comparison to Polymorph *A*. If this hypothesis is valid, one would predict from the smaller free energy difference (−231 cal./mole) observed between Polymorphs I and II of mefenamic acid that there should be no significant difference in their absorption.

To test the above hypothesis, the absorption profiles of Polymorphs I and II of mefenamic acid were evaluated in a crossover comparative trial using 12 male normal human subjects. The subjects were administered a single oral 500-mg. dose of each polymorph of mefenamic acid. The drug was given in capsules. The blood level data presented in Fig. 9 show that Polymorphs I and II of mefenamic acid gave almost identical blood levels, with peak times at about 1.5 hr. and peak levels between 5 and 6 mcg./ml.

It has been postulated (9) that the packing in the crystal of an organic compound is determined by its molecular geometry, and the closest packed arrangement has the minimum free energy. The basic parameter affecting the free energy is apparently the packing density achieved by inserting the atoms of one molecule among those of its neighbor. Furthermore, it has been assumed (9) that the internal energy of an organic crystal depends mainly on the packing density and the symmetry of the molecules affects only the entropy.

The larger free energy differences and high entropy values of polymorphic forms of chloramphenicol palmitate can perhaps be ascribed to the difficulty of obtaining close packing of the molecules in the crystal lattice and lack of symmetry. The comparatively large fatty acid chain in the chloramphenicol palmitate molecule apparently prevents a close packing arrangement in the crystal lattice. In contrast, the mefenamic acid molecules being much smaller and more symmetrical are apparently able to pack closer in the crystal lattice. The free energy difference of the polymorphs of mefenamic acid is therefore much smaller as is the entropy change noted.

## SUMMARY AND CONCLUSION

Measurements of dissolution behavior of polymorphic forms of relatively insoluble drugs are a convenient way of measuring the thermodynamic parameters. Knowledge of the thermodynamic

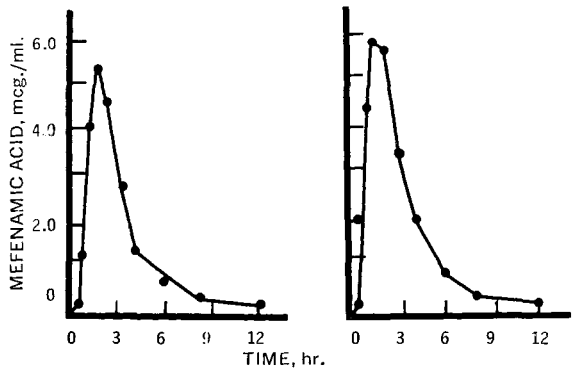


**Figure 9**—*Mean plasma levels following a single 500-mg. oral dose of Polymorphs* I *(left) and* II *(right) of mefenamic acid administered as capsules to 12 male normal human subjects.*

values allows a rational selection of the more energetic polymorphic forms of these drugs for pharmacological absorption studies, and also gauges their probable stability in various dosage forms.

It is suggested that large differences in the free energy content of the polymorphs, as was demonstrated in the case of chloramphenicol palmitate may affect significantly the absorption and resulting blood levels. On the other hand, a small difference as was seen with mefenamic acid does not appear to affect the absorbability of the drug.

It is hoped that future studies with polymorphs of other drugs will allow a closer correlation between the free energy differences and drug availability of the polymorphs.

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# Quantitative Determination of Dantrolene Sodium and Its Metabolites by Differential Pulse Polarography


**PHILLIP L. COX*, J. P. HEOTIS†, DONALD POLIN, and GORDON M. ROSE**



**Abstract** □ A differential pulse polarographic method has been developed for the simultaneous determination of dantrolene sodium and its nonreduced and reduced metabolites. The compound and its metabolites are extracted from urine or plasma with ethyl acetate and the ethyl acetate removed by evaporation. The compounds are dissolved in a small quantity of *N,N*-dimethylformamide and diluted with 0.2 *M* pH 4 acetate buffer. Dantrolene sodium plus the reduced and nonreduced metabolites are quantitatively determined by the polarographic reduction of the azomethine linkage at a cell potential of −0.86 v. The nitro compounds are quantitatively determined as dantrolene equivalents by the reduction of the nitro group at a cell potential of −0.26 v. The difference between the two determinations represents the reduced metabolites. Levels as low as 0.1 mcg./ml. can be determined by the reduction of the nitro group or the azomethine linkage.



**Keyphrases** □ Dantrolene Na and metabolites—analysis □ Plasma, urine—dantrolene, metabolite determination □ Polarography, differential pulse—analysis


Dantrolene sodium, 1-{[5-(*p*-nitrophenyl)furfurylidene]amino}hydantoin sodium salt hydrate, was reported by Snyder *et al.* (1) as a muscle relaxant of potential clinical usefulness. The compound is currently undergoing clinical investigation.

Research in these laboratories has shown that dantrolene sodium is metabolized by nonreductive and reductive pathways (Fig. 1). *Via* the latter route the nitro group is reduced to the amine (F-405), and in some animals, including man, the amine is acetylated (F-490). By the nonreductive pathway the compound is metabolized to a metabolite designated *A*. Metabolite *A* spontaneously degrades to Compound *B*. Both Metabolite *A* and Compound *B* retain the nitro group and the azomethine linkage.

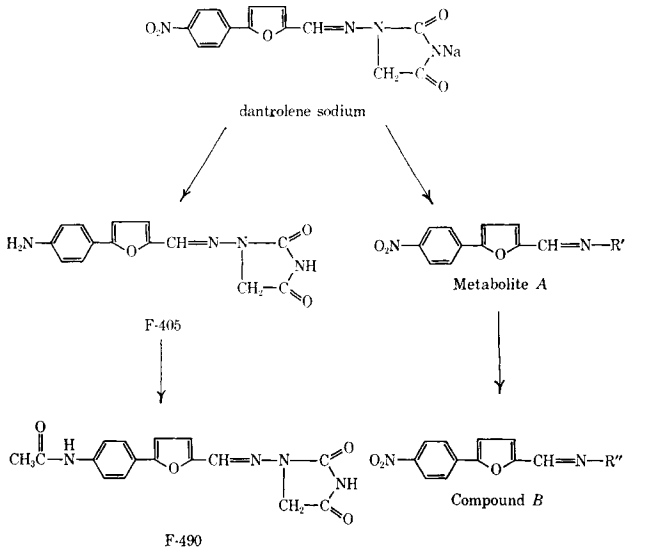


**Figure 1**—*Metabolic pathway of dantrolene sodium.*