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Effect of Polymorphism on the Absorption of Chloramphenicol from Chloramphenicol Palmitate

By ARMANDO J. AGUIAR, JOHN KRC, JR., ARLYN W. KINKEL, and JOSEPH C. SAMYN

The effect of polymorphism on the availability of chloramphenicol from chloramphenicol palmitate was studied. *In vivo* absorption studies in humans, following single oral doses of the ester, indicate that the polymorphic state of chloramphenicol palmitate influences significantly the blood levels obtained. The use of enzymatic hydrolysis as an *in vitro* method for predicting blood levels is evaluated. The utility of X-ray diffractometry, melting point determinations, and infrared spectroscopy in qualitative and quantitative analysis of the polymorphic forms is discussed. In addition to the two previously reported polymorphs of this drug designated as polymorphs A and B, a third polymorph, form C, is described.

PHYSICAL PROPERTIES such as particle size, solubility, rates of solution, aggregation of primary particles, and wettability have been shown by various workers (1–7) to influence the absorption and therapeutic efficacy of relatively insoluble drugs. The polymorphic state of a drug can also be an important factor (8) since differences in the free energy of polymorphs influence some of these properties. Higuchi (9) has suggested that these differences may appreciably affect their physiological activity.

The present study is concerned with an evaluation of the effect of polymorphic state on the absorption of chloramphenicol from chloramphenicol palmitate. The study was also designed to determine the usefulness and limitations of commonly applied analytical techniques such as X-ray diffractometry, infrared spectroscopy, and melting point techniques in identifying and characterizing the polymorphs of chloramphenicol palmitate. Furthermore, the utility of enzymatic hydrolysis as an *in vitro* criterion for predicting *in vivo* blood levels is examined.

This report deals with the properties and absorption characteristics of polymorphs A and B of chloramphenicol palmitate. Data on the third polymorph (form C) will be presented in a subsequent publication.

Chloramphenicol palmitate was synthesized by Edgerton (10) as a tasteless derivative of chloramphenicol. Glazko *et al.* (11) showed that the intact esters are poorly absorbed from the intestinal tract and must first be hydrolyzed by the esterases in the small intestine before any significant absorption can take place. The rate of hydrolysis of the ester is governed by the rate of solution which, in turn, is dependent on factors such as primary particle size, state of aggregation of primary particles, but most important, as this study shows, on the polymorphic form.

PAST WORK ON THE POLYMORPHISM OF CHLORAMPHENICOL ESTERS

Milosovich (12), studying the physical stability of chloramphenicol palmitate suspensions, suggested the existence of two polymorphs of this drug. Subsequently, Tamura and Kuwano (13) and Maruyama *et al.* (14) reported their work on the two polymorphs and an additional amorphous glassy phase.

Almirante and his co-workers (15) showed that chloramphenicol stearate also exists in two forms, one of which gave good blood levels regardless of particle size. These authors also showed that the presence or absence of a wetting agent had no appreciable effect on the blood levels obtained.

Recently Menachemoff (16) reported that the hydrolysis time of chloramphenicol palmitate is the

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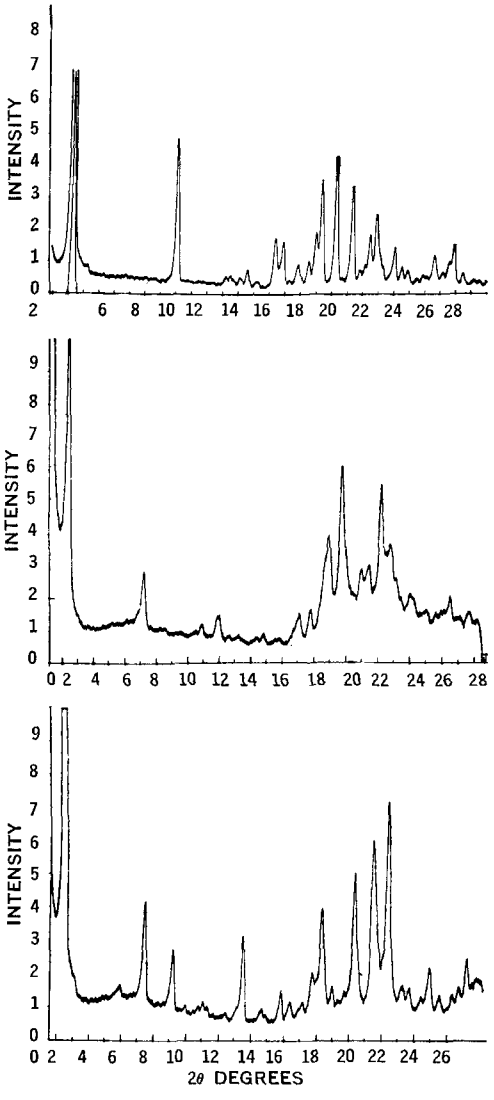

Fig. 1—X-Ray diffractograms for polymorphs A (top), B (middle), and C (bottom) of chloramphenicol palmitate.

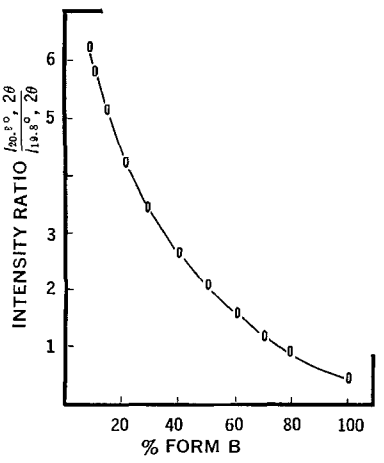

Fig. 2—Intensity ratio as a function of composition of forms A and B of chloramphenicol palmitate.

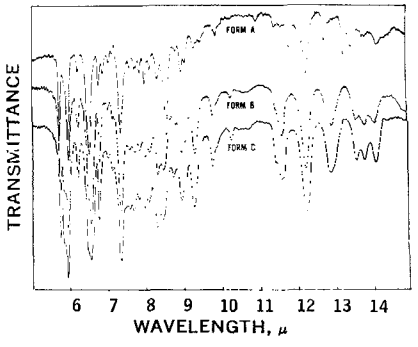

Fig. 3—Infrared spectra of polymorphs A, B, and C of chloramphenicol palmitate.

essential factor for the rate of absorption, and the amount of hydrolysis is related to the crystalline structure of the palmitate.

ANALYTICAL METHODS

The analytical methods commonly applied to confirm the existence of polymorphic states are X-ray diffractometry, optical crystallography, infrared spectroscopy, and fusion behavior (17–20). When polymorphs can be prepared separately and their physical characteristics established, distinguishing features can be accurately chosen for their identification and analysis in mixtures. In these laboratories, X-ray diffractometry provided a dependable method for initial identification and for quantitative analysis of polymorphs of chloramphenicol palmitate.

Once the characteristic diffraction criteria for the polymorphic forms were established by X-ray diffraction, it was found that other methods, such as infrared spectroscopy, could be conveniently used for routine determinations of the quantity of polymorph A present in any samples containing mixtures of the polymorphic forms.

X-Ray Diffractometry—The X-ray diffraction patterns for polymorphs A, B, and C of chloramphenicol palmitate are summarized in Fig. 1. It is evident that these patterns differ.

X-Ray diffractometry was used successfully to measure the quantities of polymorphs A and B present in chloramphenicol palmitate suspensions over the whole range of composition. The relative intensities of two diffraction lines—one at 19.8° (2θ, CuKα) associated with form B, and the other at 20.3° (2θ), associated with form A—were used. A working curve, in which composition was plotted against the ratio of intensities, $I_{20.3°}/I_{19.8°}$ (Fig. 2) was prepared by reading intensities of known mixtures of forms A and B.

It should be noted that an analysis of this type is based on the assumption of a binary system of A and B polymorphs. The presence of form C or amorphous material in the sample can cause errors. The interference of form C was obviated by applying a suitable correction to the intensity at 20.3° (2θ), determined on the basis of the intensity of form C at 9.2° (2θ). Amorphous chloramphenicol palmi-

Table I—Comparison of Values of Polymorph A Obtained by X-Ray and Infrared Procedures in Samples Containing Known Mixtures of Polymorphs A and B

% A (Theoretical)	% A (by I.R.)	% A (by X-Ray)
5	4	8
7	7	7
15	14	13
50	50	49
75	79	72

tate did not affect the analysis of A and B with regard to proportion, but only with regard to the absolute amount of each.

X-Ray Diffraction Instrumentation and Procedure—A Norelco diffractometer was used. The instrument was equipped with a flat specimen spinner, which rotated the packed powder in the plane of its surface. The sample was packed into an aluminum planchet having a depression 1.5 mm. deep and 22 mm. in diameter. The flat side of a spatula was used to pack the powder and smooth it so that a uniform level surface was presented to the X-ray beam. Coarse or lumpy powder was first ground in a mortar or mechanical blender.

The instrumental variables of the diffractometer were set as follows: (*a*) 1° divergence and scatter slits, and 0.006 in. receiving slits; (*b*) CuKα radiation, 35 Kv., 20 ma., Ni filtered; (*c*) detector: scintillation counter with pulse height discrimination. Intensities were measured by recording the time necessary to count a fixed number of pulses, usually 25,600.

Infrared Spectroscopy—The method used for the infrared determination of chloramphenicol palmitate polymorphs followed closely that of Maruyama *et al.* (14) using a Beckman IR-4 spectrophotometer.

Thus far, two infrared spectra of solid chloramphenicol palmitate have been encountered, one of which is specific for polymorph A, while the other shows minor variations for any of the "non-A" phases. The infrared spectra for polymorphs A, B, and C are shown in Fig. 3. It is readily apparent that while the spectrum for form A is characteristic, the spectra for forms B and C are indistinguishable.

The infrared procedure can therefore be used to determine quantitatively the concentration of polymorph A present in a sample of chloramphenicol palmitate. However, it cannot be used to either distinguish or estimate the quantity of polymorph B in the presence of C or other "non-A" phases.

To compare the accuracy of X-ray diffractometry and infrared spectroscopy in estimating quantities of polymorph A in chloramphenicol palmitate powders, known mixtures of polymorphs A and B were prepared and analyzed by these methods. The results are given in Table I. The values obtained are close and it is obvious that these methods can be used interchangeably for estimating the quantity of polymorph A.

Melting Point—The use of melting point determinations is of limited value for identification of polymorphic forms of chloramphenicol palmitate or as a dependable criterion of their purity. It was found that regardless of the techniques used for determining the melting point, form C was transformed into form B, and the melting point observed (about 86°) was that of form B. Similar transformations also occurred when attempts were made to determine the melting point of the amorphous phase.

The presence of impurities also affected the melting point. Analytical difficulties precluded the quantitative determination of homologous impurities in chloramphenicol palmitate. However, it was found that the extent and nature of such impurities had a marked and varying effect on the melting point.

Table II—Summary of Properties of Suspensions Used in the Absorption Studies

Designation	% Polymorph B Theoretical	% Polymorph B by X-Ray	Dispersion	Av. Particle Size, μ
K[a]	100	100	W.D.[b]	1
L	100	95	H.A.[c]	5
M	0	0	H.A.	5
N	25	24	H.A.	5
O	50	50	H.A.	5
P	75	75	H.A.	5
Q	100	99	H.A.	25

[a] Polysorbate 60 added. [b] Well dispersed. [c] Highly aggregated. [d] Microscopic evaluation.

ABSORPTION STUDIES

The absorption studies were carried out to: (*a*) evaluate and compare the blood levels obtained with suspensions of form B of chloramphenicol palmitate in the presence and absence of a surface-active agent, (*b*) determine the absorption properties of suspensions containing varying concentrations of polymorphs A and B, (*c*) ascertain the type of blood levels obtained from suspensions containing 100% polymorph B with a larger (25 μ) particle size.

Experimental—For the purpose of the absorption studies, experimental suspensions of chloramphenicol palmitate were prepared in an aqueous vehicle containing sweetening agents, gums, a preservative, and flavoring agents. For convenience of identification, these suspensions were labeled K, L, M, N, O, P, and Q.

Suspension K was prepared by suspending polymorph B with a uniform particle size of about 1 μ. The drug was well dispersed by adding polysorbate 60 to the vehicle. This preparation was used as a "reference standard" with which the absorption patterns of the other suspensions were compared.

Suspension L contained 100% polymorph B. No surface-active agent was added to the vehicle. The drug was highly aggregated. The average particle size, determined microscopically, was approximately 5 μ.

Suspension M was made from 100% form A chloramphenicol palmitate powder. The powder was prepared by dissolving the drug in hot xylene and allowing to cool overnight. The suspension was highly aggregated. The average particle size was about 5 μ. No surface-active agent was added to the vehicle.

[1] Marketed as Tween 60 by Atlas Powder Co., Wilmington, Del.

Suspension N, containing 75% of form A and 25% of form B, was prepared by mixing suspensions M and L in these proportions.

Suspension O was prepared by mixing equal quantities of suspensions L and M to obtain a mixture of 50% form A and 50% form B.

Suspension P, containing 25% of form A and 75% form B, was prepared by mixing 75 parts of L with 25 parts of M.

Suspension Q contained 100% form B with an average particle size of 25 μ. The drug was crystallized from ethanol. No surface-active agent was used, and the drug was highly aggregated in suspension.

The properties of the suspensions used in the absorption studies are summarized in Table II.

Methodology—To ascertain the effect of polysorbate 60 on the absorption of chloramphenicol from suspensions of chloramphenicol palmitate containing 100% polymorph B, a double-blind crossover type of study was used.

Six normal adult male human volunteers were used. These ranged in weight from 134 to 199 lb. with an average weight of 154 lb. Three of the subjects were given oral doses of suspension K, equivalent to 1.5 Gm. chloramphenicol, and three other subjects received the same dose of suspension L. After 1 week, each subject received the opposite suspension.

Blood specimens were taken at 0, 1, 2, 4, 8, 12, and 24 hr. after dosage. Urine collections were made for 24 hr. before medication, and at 0–2, 2–4, 4–6, 6–8, 8–12, and 12–24 hr. after dosage. Total urine volumes were measured for each collection period and a sample of each specimen was retained for analysis.

All samples were assayed for total nitro compounds, using Glazko's (21) colorimetric procedure. All values are reported in terms of chloramphenicol equivalents and are the average of the value obtained for six individuals in each instance.

Thirty normal male volunteers were used to study the absorption of chloramphenicol from suspensions containing varying quantities of polymorphs A and B (suspensions L, M, N, O, and P). Their weights ranged from 150 to 170 lb. Their average weight was 160 lb.

The volunteers were divided into groups of six individuals, and each group was given one of the suspensions. Each individual in the group received oral doses of the suspension equivalent to 1.5 Gm. of chloramphenicol. The procedure and intervals of time used to collect the blood and urine samples were the same as those described for the first study. The samples were also assayed in the same manner.

The absorption characteristics of chloramphenicol palmitate suspension Q were determined in five normal male volunteers. Their weights ranged from 143 to 220 lb. and their average weight was 182 lb. The dose, urine and blood collection periods, and assay procedure were identical to the ones described previously for the other suspensions.

RESULTS AND DISCUSSION

Results of blood levels obtained in the first study, designed to evaluate the absorption characteristics of polymorph B with and without polysorbate 60 (suspensions K and L), are shown in Fig. 4. This

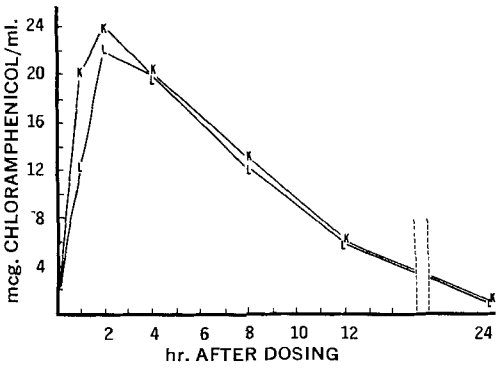

Fig. 4—Comparison of mean blood serum levels in normal humans, obtained with suspensions of 100% form B chloramphenicol palmitate. (K, with polysorbate 60; L, no surface-active agent.) Single oral dose equivalent to 1.5 Gm. chloramphenicol.

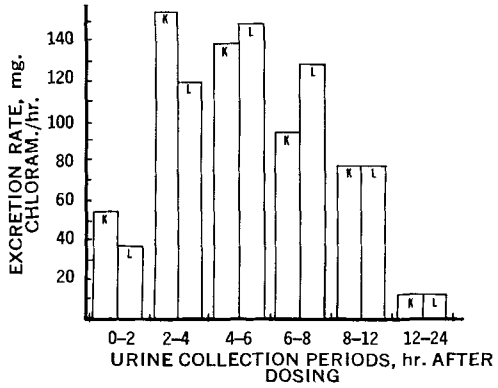

Fig. 5—Urinary excretion rate of total nitro compound (chloramphenicol equivalent) following single oral dose of 100% form B chloramphenicol palmitate suspension. (K with and L without polysorbate 60.)

plot of mean blood levels *versus* time shows that the suspension L without the surfactant appears to give comparable blood levels to suspension K with polysorbate 60.

The absorption from suspension L is, however, slightly slower initially as indicated by the lower plasma levels 1 hr. after dosage. However, no apparent differences occur at later time periods. The slower absorption is indicated also by the urinary excretion data shown in Fig. 5. The maximum excretion rate with suspension K was found in the 2–4-hr. collection period after the drug was administered, while the highest excretion rate with suspension L was found to be 4 to 6 hr. after dosage.

The delay in the absorption of suspension L can perhaps be ascribed to the slightly larger primary particle size (than that of suspension K), but it seems more likely that it is due to the highly aggregated state of the primary particles in the suspension. It is reasonable to assume that the presence of aggregates in the suspension in the initial stages will decrease the surface area presented for dissolution and hydrolysis of the ester. At later times, when the drug is dispersed in the gastric media, there is no

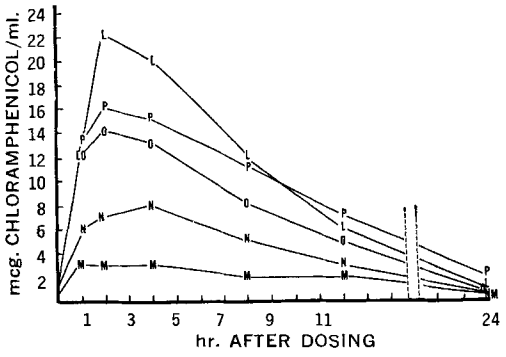

Fig. 6—Comparison of mean blood serum levels obtained with chloramphenicol palmitate suspensions containing varying ratios of A and B polymorphs, following single oral dose equivalent to 1.5 Gm. chloramphenicol. (Per cent polymorph B in the suspension: M, 0%; N, 25%; O, 50%; P, 75%; L, 100%.)

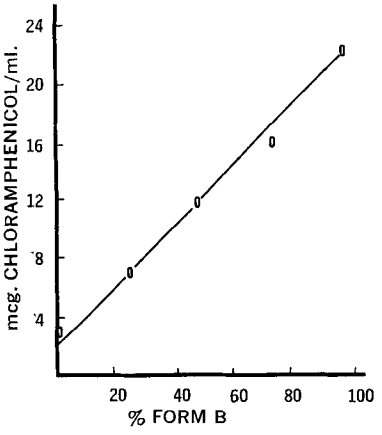

Fig. 8—Correlation of "peak" blood serum levels (2 hr.) vs. per cent concentration of polymorph B.

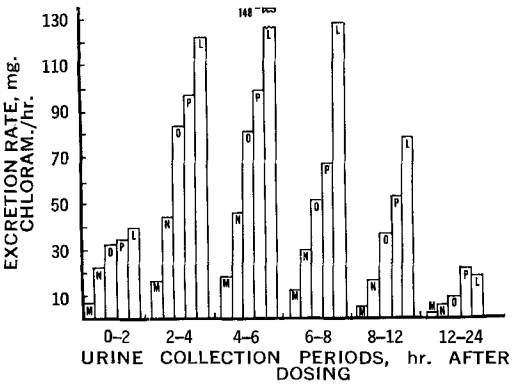

Fig. 7—Urinary excretion rate of total nitro compound (chloramphenicol equivalent) following single oral dose of chloramphenicol palmitate suspensions containing varying quantities of polymorphs A and B. Dose equivalent to 1.5 Gm. of chloramphenicol. (Per cent polymorph B in the suspension: M, 0%; N, 25%; O, 50%; P, 75%; L, 100%.)

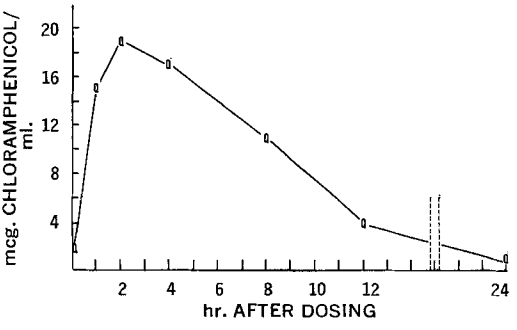

Fig. 9—Mean blood serum levels obtained with suspensions of chloramphenicol palmitate prepared from 100% polymorph B with average particle size of 25 μ.

longer any difference in the absorption patterns of the two suspensions.

The first study agrees with the observation of Almirante (15), who showed that good blood levels of chloramphenicol stearate could be obtained without polysorbate and the levels depend primarily upon the crystal form present.

Mean blood levels obtained in the second study intended to determine the absorption properties of chloramphenicol palmitate suspensions containing varying ratios of polymorphs A and B (suspensions L, M, N, O, P) are shown in Fig. 6. Urinary excretion data are given in Fig. 7.

In these single dose studies the highest mean blood levels were obtained with the suspensions containing only form B. The blood levels decreased proportionately as the concentration of form A increased. These results demonstrate that the levels are influenced by the type and concentration of the crystal polymorph present.

The differences in the absorption are even more evident from Fig. 8 where the absorption at 2 hr. ("peak" blood levels) is plotted *versus* per cent form B present in the suspension. A linear relationship apparently exists between the "peak" levels and concentration of polymorph B, the levels increasing in a direct relationship to the increase of polymorph B.

The results of the third study, shown in Fig. 9, indicate that good blood levels can be obtained with polymorph B having a larger particle size. Although the average primary particle size of the drug in suspension Q was approximately 25 μ, the absorption pattern is not significantly different from that observed with suspension L containing smaller particle size polymorph B.

It appears from these absorption studies that the polymorphic form is of much greater significance than the particle size. The greater availability of the metastable polymorph of chloramphenicol palmitate is apparently related to its greater thermodynamic activity.

Some measure of the difference in the free energy content between polymorphs A and B is obtained from solubility measurements carried out at different temperatures. From these measurements it was found that the heat of solution for polymorph A was 21.8 Kcal./mole, while that for polymorph B

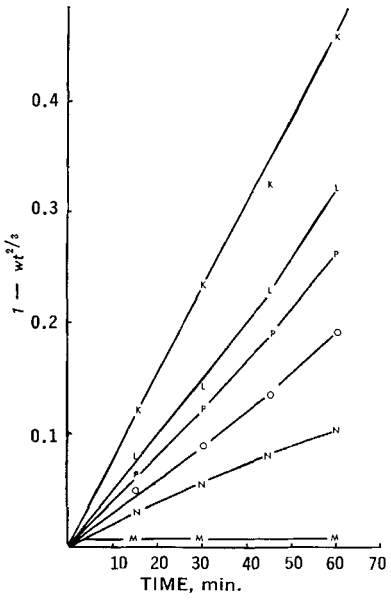

Fig. 10—Enzymatic hydrolysis rates of chloramphenicol palmitate suspensions plotted in accordance with Eq. 5. (K, 100% polymorph B with polysorbate 60) (L, 100%; P, 75%; O, 50%; N, 25%; M, 0%, polymorph B).

was 15.4 Kcal./mole. The details and results of these experiments are being presented in another publication.

USE OF ENZYMATIC HYDROLYSIS TO PREDICT BLOOD LEVELS

Glazko *et al.* (22) showed that chloramphenicol palmitate can be hydrolyzed *in vitro* at 37° by pancreatin at pH 4.5. They also indicated that the *in vitro* hydrolysis rate could be used to predict the development of blood levels in humans.

If proper conditions are selected for the experimental determinations of the hydrolysis rate, it can be shown that the rate of dissolution of the chloramphenicol palmitate particles in the suspension is a diffusion controlled process. Following Higuchi's work (23) on the dissolution rates of finely divided drug powders, one can then write Eq. 1 to describe the dissolution rate for a single particle as a function of time:

$$\frac{dw}{dt} = -\frac{4\pi D w^{1/3}}{\left(\frac{4}{3}\pi\rho\right)^{1/3}}\Delta c \qquad \text{(Eq. 1)}$$

where

Δc = concentration gradient from surface of particle to boundary of diffusion layer,
D = diffusion constant,
w = weight of single particle of chloramphenicol palmitate,
ρ = density.

For n particles in a uniform suspension of spheres, one then has:

$$\frac{dw_t}{dt} = \left(\frac{dw_1}{dt} + \frac{dw_2}{dt} + + + + \frac{dw_n}{dt}\right)$$

$$= -\frac{4\pi D\Delta c}{\left(\frac{4}{3}\pi\rho\right)^{1/3}}\left(w_1^{1/3} + w_2^{1/3} + + + + w_n^{1/3}\right) \qquad \text{(Eq. 2)}$$

Integrating Eq. 2, between the limits w_0 (total weight of particles at time zero) and w_t (total weight of particles at time t) one obtains:

$$-\int_{w_0}^{w_t}\frac{dw_t}{w_t^{1/3}} = \frac{4\pi D\Delta c n^{2/3}}{(4/3\pi\rho)^{1/3}}\int_{t=0}^{t=t} dt \qquad \text{(Eq. 3)}$$

which resolves to:

$$\frac{3}{2}\left(w_0^{2/3} - w_t^{2/3}\right) = \frac{4\pi D\Delta c n^{2/3}}{\left(\frac{4}{3}\pi\rho\right)^{1/3}}t \qquad \text{(Eq. 4)}$$

Now $w_0 = \frac{4}{3}\pi r_0^3\, nd$, where n = number of particles per 1 ml., and r_0 = initial radius of particles.

Equation 4 can be divided by the initial weight of chloramphenicol palmitate raised to the two-thirds power ($w_0^{2/3}$) and simplified to obtain Eq. 5.

$$\frac{w_0^{2/3} - w_t^{2/3}}{w_0^{2/3}} = \frac{2D\Delta c}{\rho r_0^2}t = Kt$$

or

$$1 - fw_t^{2/3} = Kt \qquad \text{(Eq. 5)}$$

where $K = \frac{2D\Delta c}{\rho r_0^2}$ and $fw_t^{2/3}$ = fraction total weight raised to the two-thirds power.

Therefore, a plot of $1 - fw_t^{2/3}$ *versus* t should give a straight line with a positive slope which is directly proportional to the solubility of chloramphenicol palmitate in the vehicle, and inversely proportional to the initial surface area of a single particle for a suspension containing one of the polymorphic forms.

In Fig. 10 representative data plotted according to Eq. 5 are shown. It is apparent from this plot that when dealing with suspensions containing either polymorph A or polymorph B (suspensions K, L, and M) the data appear to fit well with the theoretical concepts presented. The value of K can then be determined from the slope of these lines. When dealing with suspensions containing mixtures of polymorphs (suspensions P, O, and N) the lines initially appear to follow approximately Eq. 5. However, the authors have not been able to derive equations to deal quantitatively with suspensions containing such mixtures.

The experimental conditions can be selected to ensure that the determining factor in the initial rate of hydrolysis of chloramphenicol palmitate is the rate of solution or the drug. The factors which control rate of solution of chloramphenicol palmitate in suspensions are solubility of the different polymorphic forms and specific surface area.

Suspension K with 100% polymorph B well dispersed, having a particle size of about 1 μ, is used as a control. This control preparation is arbitrarily assigned as having a 100% hydrolysis rate. The values obtained with other suspensions are then compared with the control preparation and the results are tabulated as per cent relative hydrolysis rate with reference to the control preparation.

The rate of hydrolysis test values has paralleled blood level values with the exception of suspensions containing 100% polymorph B which are in a highly aggregated state. These suspensions gave lower hydrolysis rates but satisfactory blood levels.

Examples of the per cent relative hydrolysis rate values obtained with different suspensions are given in Table III together with the "peak" blood levels and the per cent mean urinary excretion 24 hr. after administration of the suspension.

From the data given in Table III it is apparent that the suspensions can be effectively deaggregated by the *in vivo* mechanisms of the gastrointestinal tract, thus increasing the surface area available for hydrolysis, which is not evident from the *in vitro* method.

It appears that although the *in vitro* enzymatic hydrolysis test could predict blood levels satisfactorily in most cases, certain refinements still have to be made in the technique to study the effect of aggregation, *etc.*, on the hydrolysis rates.

TABLE III—COMPARISON OF "PEAK" BLOOD LEVELS WITH THE PER CENT RELATIVE HYDROLYSIS RATE DETERMINED BY ENZYMATIC HYDROLYSIS TESTS

% Relative Hydrolysis Rate	Peak Blood Levels, mcg. CM/ml.	% Mean Urinary Excretion	% Polymorph B
85	15	69	100
65	22	93.6	95
50	16	69	75
35	14	49.4	50
18.21	8	29.5	25
16.9	9	29	7
14[a]	19	84.4	100
7.9[a]	18	67.7	100
1	3	9.7	0

[a] Suspensions with highly aggregated primary particles.

CONCLUSIONS AND SUMMARY

In using analytical methods for identifying polymorphs, careful consideration, as shown in this study, should be given to their usefulness and limitations. For identification and analysis of chloramphenicol palmitate polymorphs, X-ray diffractometry was found to be the most accurate method. The infrared spectra are useful in distinguishing form A from the "non-A" phases. Melting point *per se* is of limited value in identifying the polymorphs of chloramphenicol palmitate.

These studies demonstrate the influence of polymorphic state of chloramphenicol palmitate on its physiological availability. The suspension containing only form B gives higher blood levels following single oral doses than suspensions containing only form A. The blood levels decrease proportionately as the concentration of polymorph B decreased in the suspension.

Addition of a surface-active agent to the suspension has a very limited influence on the availability of chloramphenicol palmitate, and high blood levels are obtained in its absence.

It is also shown that increasing the particle size of chloramphenicol palmitate to 25 μ had little effect on the blood levels. The enzymatic hydrolysis test can be used for predicting blood levels from chloramphenicol palmitate suspensions if the particles are not highly aggregated.

This study emphasizes the effect of polymorphic form on the availability of chloramphenicol palmitate. In view of these findings, it would appear that other slightly soluble drugs should be carefully examined both for the existence and effect of polymorphs.

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