Dilatometric Behavior of Polymorphic Forms of Chloramphenicol Palmitate

ARMANDO J. AGUIAR*

Abstract □ The dilatometric behavior of two polymorphs of chloramphenicol palmitate was studied. The aspects investigated were (*a*) the melting behavior of Polymorphs *A* and *B*, and the delineation of their dilatometric profiles; and (*b*) the kinetics of phase transformation of Polymorph *B* to Polymorph *A*. The study emphasizes the complexity of the composition of Polymorph *B* of chloramphenicol palmitate. The studies dealing with the kinetics of phase transformation showed that the addition of increments of chloramphenicol stearate to a 99.9% chemically pure sample of Polymorph *B* of chloramphenicol palmitate slowed the rate of reversion to Polymorph *A* at 80°. Chloramphenicol heptadecanoate and nonadecanoate, stearic and palmitic acid, had no effect on the rate of transformation.

Keyphrases □ Chloramphenicol palmitate, polymorphic—dilatometric behavior □ Melting behavior—chloramphenicol polymorphs □ Dilatometric profiles—chloramphenicol polymorphs □ Transformation, chloramphenicol polymorphs—kinetics

Dilatometric techniques have been used extensively in the past to detect and analyze phase transitions, particularly in the field of fats and oils. For example, Vold (1) used these techniques to detect several allotropic forms of anhydrous sodium palmitate. Singleton (2) studied the melting dilation and polymorphism of alpha and beta tung oil. He also showed (3) that stearic acid gave two distinct polymorphs, depending on the solvent used, and in addition (4), demonstrated that monostearin was capable of existing in four crystalline modifications. Bailey (5) used dilatometric procedures to study the melting behavior of fats and devised a scheme to verify the purity of fats from their dilatometric profiles.

Ravin and Higuchi (6) carried out dilatometric studies with mixtures of carbowaxes, spermaceti, and cetyl alcohol. They also determined the effect of rapid temperature changes on the phase transitions of monostearin, distearin, and white petrolatum. Simonelli and Higuchi (7) and Wray and Higuchi (8) studied the mechanics and kinetics of melting and freezing of methyl stearate under various conditions.

The present report is concerned with dilatometric studies of Polymorphs *A* and *B* of chloramphenicol palmitate.[1] Specifically, two aspects were investigated: (*a*) the melting behavior of Polymorphs *A* and *B*, and the delineation of their dilatometric profiles; and (*b*) the effect of impurities on the kinetics of phase transformation of Polymorph *B* to *A* at 80°. The importance of polymorphism with regard to the human absorption of chloramphenicol from chloramphenicol palmitate has been shown by Aguiar *et al.* (9).

MELTING BEHAVIOR

Figure 1 illustrates the melting behavior of Polymorph *A* of chloramphenicol palmitate prepared from 99.9% chemically pure chloramphenicol palmitate. Within the interval LM the compound is completely solid. The expansion seen within this interval is a sum of the thermal expansion of the sample, the confining liquid (mercury), and of the glass. Melting of the sample begins at M and is complete at N. Using Bailey's (5) method, one can represent the melting dilation by the line NP, which is the vertical intercept at the melting point between the liquid line NO and the solid line LM projected. It is apparent that the melting range for this sample is small, beginning at 94.1° and is completed at 95.8°.

The melting behavior of Polymorph *A* prepared from a 94% chemically pure chloramphenicol palmitate is shown in Fig. 2. In this instance, melting begins at 88.2° and is completed at 93.6°, and reflects the influence of impurities present in the sample. It begins at a lower temperature and the melting span is greater than that seen in Fig. 1.

The dilatometric plot of Polymorph *B* prepared from the less pure sample of chloramphenicol palmitate is illustrated in Fig. 3. For convenience of interpolation, only the region between 60 and 90° is shown on an expanded scale. The complexity of Polymorph *B* is evident from this plot and it suggests that Polymorph *B* is a mixture of at least two phases. Krc (10) studying microscopical properties of Polymorph *B* noticed that samples of Polymorph *B* of chloramphenicol palmitate, such as those used in these dilatometric studies, when stored in bulk underwent birefringent changes with time. It has however been experimentally established that these birefringent changes are not a transformation of Polymorph *B* to *A*.

The section designated LM in Fig. 3 represents the thermal expansion of the mixture in the solid state, mercury, and glass. The lower melting fraction (called Polymorph *B'* for convenience) begins to melt at M at approximately 69°. The exact temperature is not known since it is possible that the point is approached as a smooth curve rather than the straight line shown on the plot. The section MN

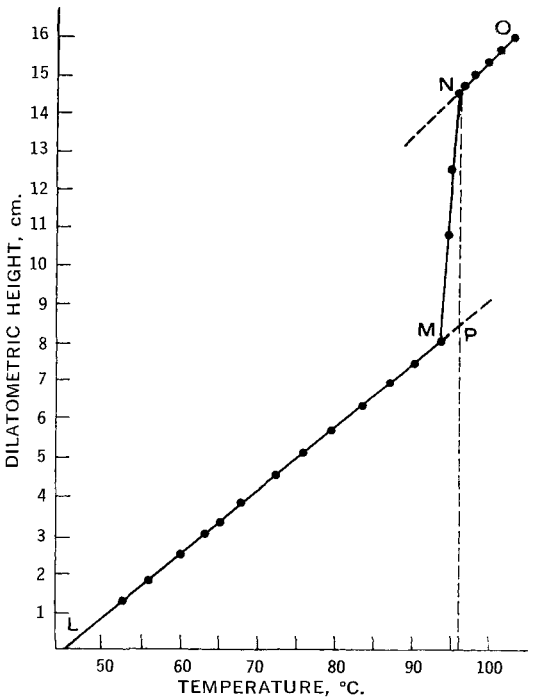

Figure 1—*Dilatometric curve showing the melting dilation of Polymorph A prepared from 99.9% chemically pure chloramphenicol palmitate.*

[1] Marketed as Chloromycetin palmitate, Parke, Davis & Co.

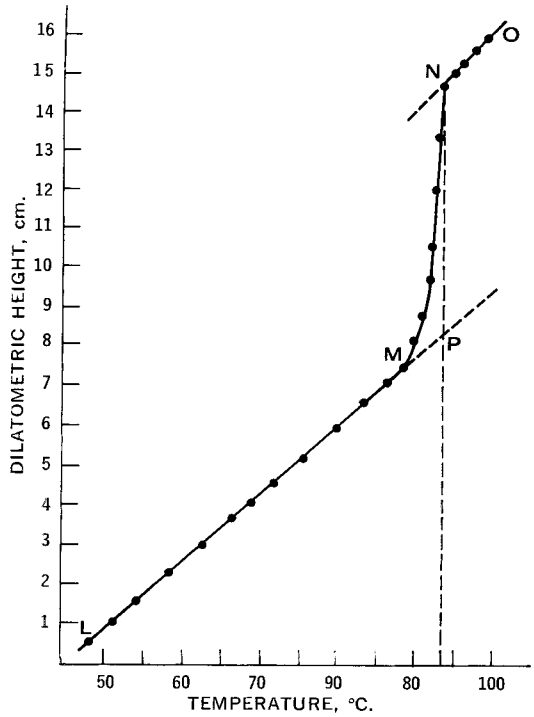

Figure 2—*Dilatometric curve showing the melting dilation of Polymorph* A *prepared from 94% chemically pure chloramphenicol palmitate.*

represents the melting of Polymorph *B'*, or its conversion to Polymorph *B*. At N the slope of the line changes indicating either that all of Polymorph *B'* has melted (or transformed) or Polymorph *B* also begins to melt, which is completed at P. The segment PQ represents the thermal expansion of the liquid.

The melting profile shown in Fig. 3 is much too involved to be handled with any pretense of exactness. For example, accurate calculations of the phase proportions cannot be made without knowing *a priori* the identity, proportions, and properties of the individual components in the sample. However, the curve serves to emphasize the complexity of the composition of Polymorph *B* of chloramphenicol palmitate, which has generally been considered as a single phase.

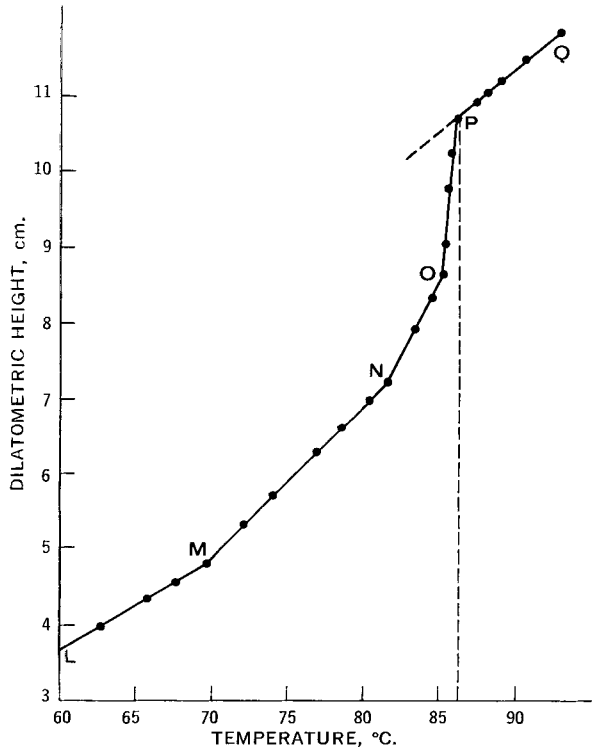

Figure 3—*Dilatometric curve showing the melting dilation of Polymorph* B, *prepared from 94% chemically pure chloramphenicol palmitate.*

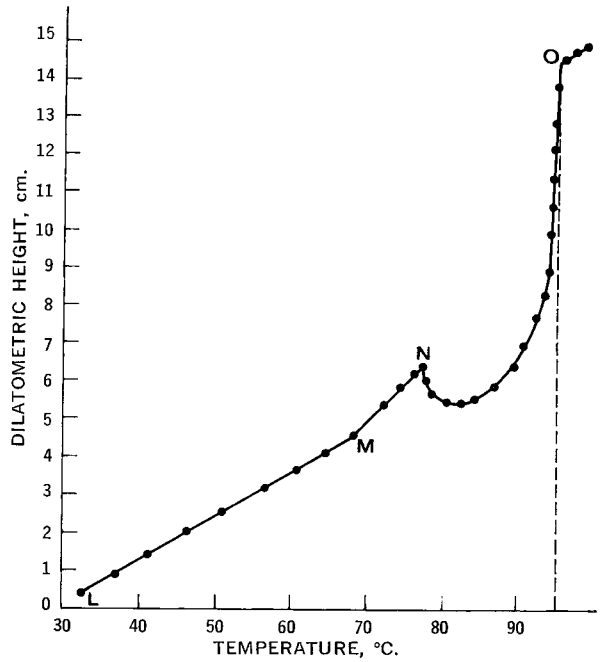

Figure 4—*Dilatometric curve of Polymorph* B *prepared from 99.9% chemically pure chloramphenicol palmitate, showing the transformation of Polymorph* B *to* A.

The melting behavior of Polymorph *B* prepared from 99.9% chemically pure chloramphenicol palmitate is shown in Fig. 4. Unfortunately, due to the instability of the phase, a complete profile could not be obtained even using a rapid rate of heating. This is evident from the plot where it is shown that on heating the sample, transformation to Polymorph *A* takes place at approximately 77°, and the sample melts at 96°. It should be pointed out that the polymorphic system of chloramphenicol palmitate is monotropic above 25° at normal pressures. Furthermore studies at temperatures lower than 25° have not revealed any transformation of Polymorph *A* to *B*. It is emphasized that the 77° is not a transition temperature for reversion of Polymorph *B* to *A*, but a temperature at which reversion of the metastable *B* polymorph to the stable modification *A*, is rapid enough to be followed easily.

A comparison of Figs. 3 and 4 emphasizes another aspect. Both the dilatometric curves show a definite break in the lines, going from L to M to N. This appears to indicate that the presence of at least two phases in what was originally thought of as a single form is not due to the presence of impurities but is indeed a true characteristic of the solid state.

KINETIC STUDIES

Most compounds (5) generally contract upon undergoing polymorphic transformations; therefore, it is possible to carry out precise kinetic measurements using a dilatometer. It was shown in the study dealing with the melting behavior that Polymorph *B*, prepared from 99.9% chemically pure chloramphenicol palmitate, transformed rapidly on heating to Polymorph *A*. This aspect was investigated quantitatively.

The results of a kinetic study to determine the transformation rate at 80° of Polymorph *B* to *A* (99.9% chemically pure) are shown in Fig. 5 as a plot of the ratio $H_{(t)}/H_{(0)}$ *versus* time where $H_{(t)}$ is the dilatometric height at time *t* and $H_{(0)}$ the height at time zero. This ratio is proportional to volume at time *t* (V_t) and time zero (V_0). It is apparent that the transformation begins at 80° after an induction period of approximately 70 min., and the sample is completely transformed after 260 min. On the other hand, a sample of Polymorph *B*

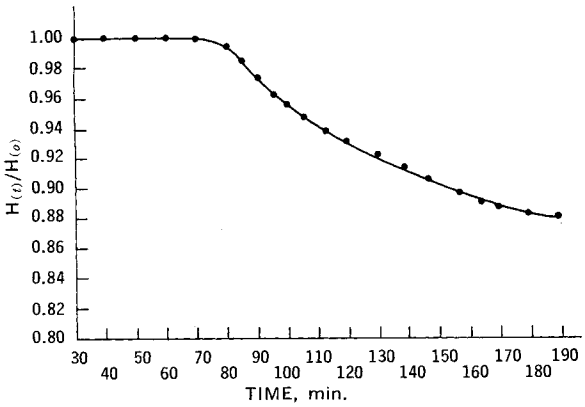

Figure 5—*Plot showing the transformation rate of Polymorph* B *to* A, *of 99.9% chemically pure chloramphenicol palmitate.*

prepared from 94% chemically pure chloramphenicol palmitate begins to transform at this temperature only after 8 days and is not complete after 30 days (Fig. 6). The selection of 80° for the kinetic studies was an arbitrary one, as the changes could be observed rapidly.

Since the impurities commonly present in chloramphenicol palmitate are esters of homologous acids (stearate, myristate) the effect on the transformation rate of intentionally adding varying concentrations of chloramphenicol stearate and chloramphenicol heptadecanoate and nonadecanoate to the 99.9% chemically pure sample was investigated. Known increments, 1, 2, 3, and 5% of the stearate, nonadecanoate, and heptadecanoate esters were added to the pure chloramphenicol palmitate sample. The mixtures were prepared by melting the components together and mixing to obtain a true solution of known composition. Polymorph *B* was prepared following the procedure described in the *Experimental* part of this paper.

The effect of adding, 1, 2, and 3% of chloramphenicol stearate on the transformation of Polymorph *B* of chloramphenicol palmitate is summarized in Fig. 7. The effect of 5% is shown in Fig. 6. It is evident that the induction period for nucleation of Polymorph *A* to start is lengthened as the concentration of the stearate increases. Furthermore as the concentration of the stearate increases, it also affects the transformation rate of Polymorph *A* as seen from the change of the slope of the curves. The increase in the induction period perhaps represents an interference with the nucleation mechanism. The presence of chloramphenicol stearate apparently delays the formation of nuclei of Polymorph *A*. A plot of induction time for nucleation of Polymorph *A* to occur *versus* concentration of chloramphenicol stearate is shown in Fig. 8.

An analogous situation was described by Gilpin (11, 12) who found that *ortho, para*-dichloro-diphenyl-trichloroethane and 1, 3, 5-

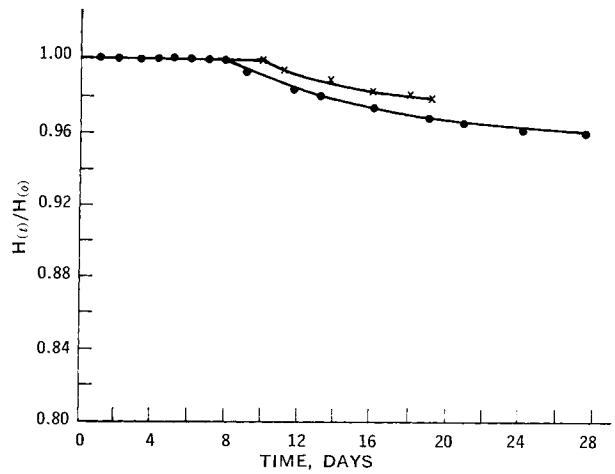

Figure 6—*Plot showing transformation of Polymorph* B *to* A *of chloramphenicol palmitate (approximately 94% chemically pure),* ●; *and effect on the rate of transformation of adding 5% chloramphenicol stearate to Polymorph* B *of 99.9% chemically pure chloramphenicol palmitate,* ×.

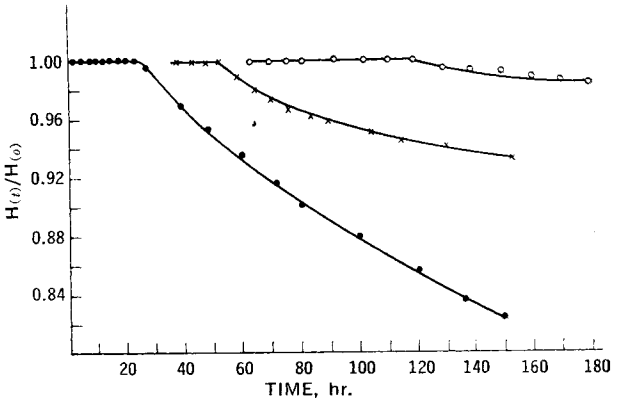

Figure 7—*Plot showing the effect of adding 1, 2, and 3% chloramphenicol stearate on the transformation rate of Polymorph* B *to* A *of 99.9% chemically pure chloramphenicol palmitate. Key:* ●, *1%;* ×, *2%;* ○, *3% chloramphenicol stearate added.*

triphenylbenzene decrease the rate of crystal growth of dichlorodiphenyl-trichloro ethane. Ferguson and Lutton (13) studying fatty acids and glyceride esters of fatty acids state that transformation of one polymorph to another is retarded by impurities; however, these authors did not carry out kinetic measurements.

Added increments (up to 5%) of chloramphenicol heptadecanoate and nonadecanoate failed to affect the transformation rate of Polymorph *B* of 99.9% pure chloramphenicol palmitate. Stearic and palmitic acids also had no effect. It appears therefore that for an impurity to be effective as a retardant, it must be not only closely related chemically, but also have a favorable spatial configuration to fit in the lattice arrangement of the crystal.

Ferguson and Lutton (13), studying the polymorphic transformations of fatty acid glycerides found that only the impurities prepared from members of the same homologous series were effective retardants. Smith (14) working with glyceride esters of long chain fatty acids found that compound formation occurs only between near members of a homologous series, and states that the second member of a binary system tends to slow up the transformation of one polymorphic form to another. The inhibition of the rate of transformation

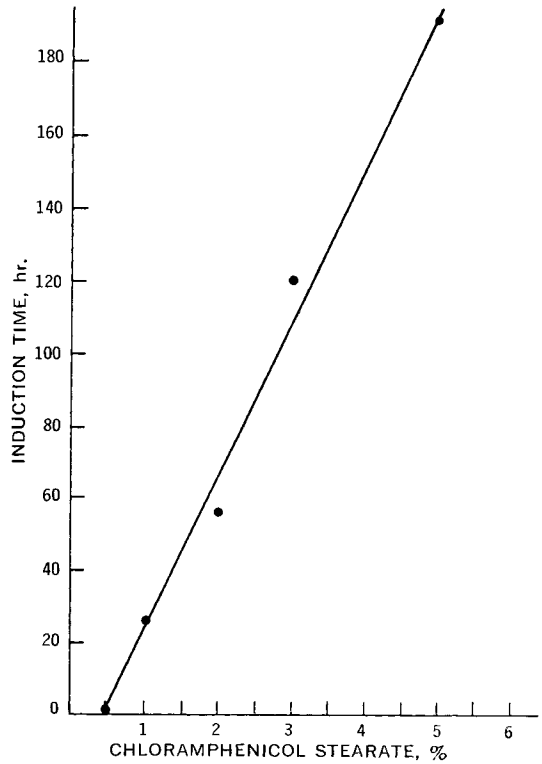

Figure 8—*Plot of "induction time" for nucleation of Polymorph* A *of chloramphenicol palmitate (99.9% chemically pure) versus concentration of chloramphenicol stearate.*

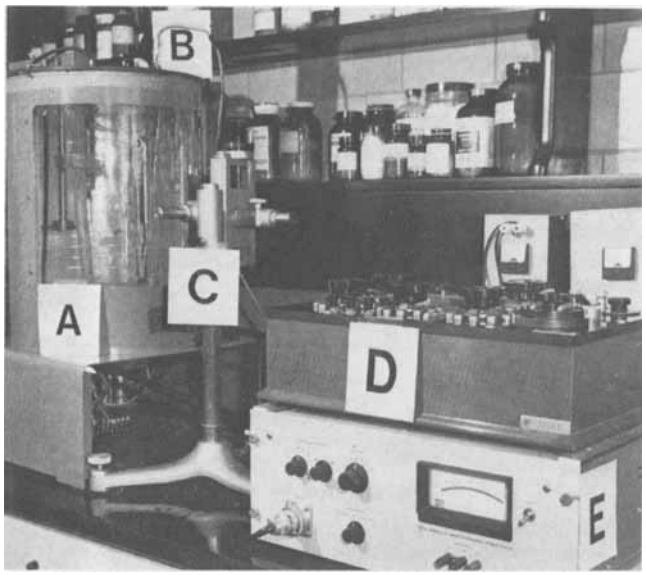

Figure 9—*Equipment used in the dilatometric studies.* A, *kinematic viscosity bath;* B, *platinum resistance thermometer;* C, *cathetometer;* D, *Mueller resistance Bridge;* E, *milli-microvoltmeter.*

of Polymorph *B* of chloramphenicol palmitate by chloramphenicol stearate and not by the heptadecanoate and nonadecanoate ester, nor by palmitic and stearic acids, indicates that the situation described for the glycerides could also apply in this instance.

The effect of chloramphenicol stearate in slowing the transformation rate of Polymorph *B* of chloramphenicol palmitate to Polymorph *A* can perhaps be ascribed to the entropy of specific crystal structures which in principle can be increased by a variety of randomizations of mutual orientation of molecules. Ubbelohde (15) describing this phenomena states that whenever the molecular interactions occur in the crystal lattice, they are often likely to appear as orientational transformations in the crystals. Transitions in solids of this kind may be highly sensitive to impurities in solid solutions. On the other hand it is conceivable that the orientation of an impurity such as the chloramphenicol stearate in the crystal lattice of chloramphenicol palmitate can increase the energy barrier necessary for inducing the nucleation of the thermodynamically stable Polymorph *A*.

EXPERIMENTAL

Instrumentation—The volumetric dilatometer used in this study was identical to the one described by Ravin and Higuchi (6). A 2-mm. constant-bore capillary was used for studies dealing with melting behavior. For the kinetic studies, a 1-mm. constant-bore capillary was used to increase the accuracy of the measurements.

A viscosity bath[2] (Fig. 9) was used in these studies. The bath temperature was controlled to within ±0.005° by a thermotrol temperature controller. This controller uses a resistance thermometer as the temperature sensing element. When the temperature was changed, the new temperature was approached in such a fashion that cyclic variations did not exceed the final selected temperature by 0.002°. The bath was constructed with an outside jacket heated by a separate mechanism, thus providing an effective insulation against room temperature variations.

The bath medium used was a mixture of 50% propylene glycol and water. The medium was agitated (using a Hallikanien Jet-Stir Impeller) which caused the bath medium to flow radially outward at a high velocity through the hollow blades as well as in directions normal and tangential to the blade surfaces. This provided an effective and patternless agitation of the bath medium and enabled quick and even distribution of heat. The bath medium was cooled with an auxiliary portable cooling unit[3] using a 70% propylene glycol–water mixture.

[2] Kinematic model 1115, Hallikanien Instruments, Berkeley, Calif.
[3] Blue M Electric Co., Blue Island, Ill., model PCC-13A.

For measurements of mercury height in the capillary, a micrometer slide cathetometer[4] was used. With this instrument mercury heights could be read directly to 0.01 mm. and estimated to 0.001 mm.

The temperature was measured using a platinum resistance thermometer[5] and a Mueller resistance bridge.[6] A milli-microvoltmeter[7] was used as the null-point detector. The resistance on the Mueller bridge was read to 0.0001 ohms and the temperature could then be computed to 0.001°. For convenience of interpolation, the resistance temperature relationship was programmed on a computer (IBM-1401) using the Calendar and Van Dusen formulas (16, 17), and the measured resistance could be converted readily to temperature in degrees centigrade.

METHODOLOGY

Preparation of Samples—The samples were melted on a hot metal plate, kept as a melt for 15 min., and then poured into a heated casting block. To obtain Polymorph *A* the block was placed in a vacuum desiccator and allowed to solidify slowly at room temperature while the desiccator was being evacuated using a high-vacuum pump. To prepare Polymorph *B*, ice was placed in the bottom of the desiccator and a metal plate on top of the ice, and the block containing the melt was then placed on the metal plate. The sample was evacuated as before.

The samples were analyzed by X-ray diffraction and IR procedures to determine the polymorphic type. Due to the great influence any Polymorph *A* seed might have on the rate, the Polymorph *B* samples used for transformation studies were also analyzed using a microscope with a hot-stage. The sample was melted and examined for Polymorph *A* seeds, and only Polymorph *B* which contained no seed after melting was used in the transformation studies.

Dilatometric Procedure—For studies dealing with melting behavior, exactly 1 g. of the compound in the form of ribbons was weighed into the sample tube. The 2-mm. bore dilatometer was assembled with the sample tube and attached to a vacuum pump. The sample was degassed overnight using a vacuum pump.[8] The next morning, residual gases were removed with the aid of an oil diffusion pump by evacuating for three additional hours at a vacuum of 1×10^{-6} mm. of mercury pressure. After the degassing operation, the outlet leading to a mercury reservoir was opened, allowing the mercury to enter the dilatometer. When the dilatometer was completely filled, the stopcock was closed and the pressure was allowed to rise slowly until normal atmospheric pressure was reached.

An identical procedure was followed for the transformation studies except in this case 0.3 g. of the sample was used and a 1-mm. bore capillary dilatometer was employed. By using ribbons rather than powder, the sample could be degassed efficiently and errors due to the presence of voids (air pockets) were effectively eliminated.

For the melting behavior studies, the temperature of the bath was raised slowly at a rate of 3°/hr. and the dilatometric height readings were taken at intervals of 0.5° using the optic micrometer. Readings were taken over a temperature range from 30° to a few degrees beyond the melting point of the sample being studied. For the reversion studies, the bath was preset at 80° and the assembled dilatometer was placed in the bath. The time was recorded and readings were taken at appropriate intervals of time.

MATERIALS USED

The chloramphenicol esters used in the study were specially synthesized for the dilatometric studies and are not representative of chloramphenicol palmitate in commercial products. Both the 94 and 99.9% pure chloramphenicol palmitate were synthesized from palmitoyl chloride prepared from 94 and 99.9% pure palmitic acid,[9] respectively. The chloramphenicol stearate was synthesized from 99.14% pure stearic acid.[10] The chloramphenicol heptadecanoate and nonadecanoate were synthesized from the respective acid

[4] The Gaertner Scientific Corp., Chicago, Ill., model M-349.
[5] Rosemount Corp., Minneapolis, Minn., model 162C.
[6] James G. Biddle Co., Plymouth Meeting, Pa., model 601003.
[7] Keithley Instruments, Inc., Cleveland, Ohio, model 149.
[8] Duo-Seal, The Welch Scientific Co., Chicago 10, Ill.
[9] Applied Science Labs. Inc., P.O. Box 440, State College, Pa.
[10] C. P. Hall Co., Chicago, Ill.

chlorides[11] having a purity of 99.5%. The palmitic acid[12] used was recrystallized twice from chloroform and had a melting point of 63.2°. The stearic acid was also recrystallized twice from chloroform and had a melting point of 70°.

The purity of all the samples was determined by vapor phase chromatography.

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[11] Lachet Chem., Inc., Chicago, Ill.
[12] Eastman Organic Chemicals, Rochester, N. Y.

Physiologic Surface-Active Agents and Drug Absorption II: Comparison of the Effect of Sodium Taurodeoxycholate and Ethylenediaminetetraacetic Acid on Salicylamide and Salicylate Transfer Across the Everted Rat Small Intestine

STUART FELDMAN* and MILO GIBALDI

Abstract □ The effect of sodium taurodeoxycholate (STDC) and ethylenediaminetetraacetic acid (EDTA) on the transfer of salicylamide and salicylate across the everted rat intestine was studied. STDC produces a small but statistically significant increase (11–21%) in the intestinal transfer rate of salicylamide over a concentration range of 5–100 m*M* but has no measurable effect at a 1 m*M* concentration. There is a small but statistically significant increase (about 20%) in the intestinal transfer rate of salicylate after the intestine has been exposed to 25 m*M* EDTA but the chelating agent has no significant effect on salicylamide transfer. The results indicate that both EDTA and STDC alter membrane structure and thereby affect permeability but by two different mechanisms.

Keyphrases □ Surfactants—drug absorption □ Salicylate, salicylamide transfer—EDTA, sodium taurodeoxycholate effect □ Everted intestine—transfer rates □ Colorimetric analysis—spectrophotometer

The previous report in this series concerned the effects of sodium taurodeoxycholate (STDC) on the transfer rate of salicylate ion across the everted rat intestine (1). The bile salt was found to produce a change in the permeability of the intestine to salicylate; a small increase in transfer rate was noted at concentrations of STDC near or about the CMC and a much larger increase in membrane permeability was observed at STDC concentrations above the CMC. These findings were consistent with other reports on the effects of bile salts on the canine gastric mucosa (2) and goldfish membranes (3).

The purpose of the present study was to contrast the effects of STDC on the membrane transfer of unionized and ionized molecules and to explore the possible commonality of effects of disodium ethylenediaminetetraacetic acid (EDTA) and bile salts on drug transfer across the everted rat small intestine. Salicylate and salicylamide served as model drugs for this purpose.

EXPERIMENTAL

Intestinal Transfer Rate Measurements—The cannulated everted intestine method developed by Crane and Wilson (4) was used with the modifications described previously (1). Male Sprague-Dawley rats (Blue Spruce Farms, Altamont, N.Y) weighing 200–265 g. were fasted 20–24 hr. prior to each experiment. Water was allowed *ad libitum*. Each everted intestinal segment, 10 cm. in length, was suspended in 80 ml. of mucosal solution at 37° and oxygenated with a mixture of 95%:5% oxygen-carbon dioxide. The mucosal solution was Krebs-phosphate buffer (KPB) without calcium or magnesium, at either pH 6.0 or 7.4, and contained varying concentrations of sodium taurodeoxycholate (STDC) or disodium ethyl-