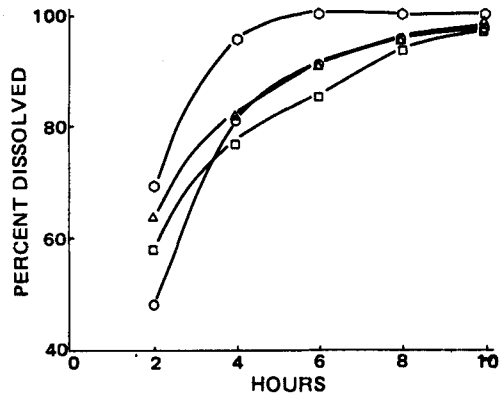

Figure 3—*Dissolution plots of all four batches after 60-min pretreatment. Key:* ○, *Batch A;* □, *Batch B;* ○, *Batch C; and* △, *Batch D.*

Table III shows that the number of tablets undissolved after 10 hr of dissolution with no pretreatment was greater for Product Y than for Product X. For Batches A and B with no pretreatment, all tablets tested were partially undissolved after 10 hr. Batch C similarly yielded four out of 10 undissolved, whereas the tablets in Batch D were totally dissolved. Pretreatment decreased both the number and size of undissolved tablet residues after 10 hr of dissolution. Overall, the 60-min pretreatment resulted in the fewest undissolved residues for all batches tested, suggesting it as the optimum pretreatment time. Furthermore, Product X appeared to perform *in vitro* better than Product Y with respect to the number of undissolved tablet fractions at all pretreatment times. This finding supports the F test result, which indicated that Brand X performed significantly better *in vitro* than Brand Y ($p = 0.05$).

SUMMARY

Pretreated tablets yielded higher dissolution profiles than nonpretreated tablets. The analysis of variance showed that pretreatment significantly increased the tablet dissolution profiles from all batches except Batch D at the $p = 0.0001$ level. Furthermore, Table III indicates that pretreatment in simulated gastric juice reduced both the number and the size of undissolved fractions. The 15-min pretreatment was adequate and was not statistically different from the other pretreatment periods. However, the 60-min pretreatment yielded higher dissolution profiles for all batches and fewer tablet residues. While the tendency for higher dissolution profiles cannot be supported statistically, this trend is consistent with the reduction in tablet residues after 10 hr of dissolution.

A relationship between the pretreatment time and the $t_{80\%}$ reduction appeared to exist, suggesting that a similar relationship may exist *in vivo* and should be studied. Product X performed better than Y, as indicated by higher dissolution profiles. The smaller $t_{80\%}$ values for Product X (Table II) support this argument. The F test using averages obtained after each time interval indicates that Product X was significantly better than Y at the $p = 0.05$ level. Furthermore, Table III shows that there were fewer undissolved tablet residues for Product X than for Product Y after 10 hr of dissolution in simulated intestinal fluid.

The batch ranking indicated that Batch C was the best batch irrespective of the pretreatment times, and it was followed by Batch D. Batches A and B were interchangeable, although Batch A appeared to be better than B for the 60-min pretreatment, as indicated by the lower $t_{80\%}$ value. Since the conclusions are based on *in vitro* data, any extrapolations to the biological system should be confirmed *in vivo*.

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Anomalous Solution Behavior of 2-Palmitate Esters of Lincomycin and Clindamycin

E. L. ROWE

Received June 22, 1978, from *Pharmacy Research, The Upjohn Company, Kalamazoo, MI 49001.* Accepted for publication April 18, 1979.

Abstract □ The aqueous solubilities of lincomycin and clindamycin 2-palmitate esters are compared. Clindamycin 2-palmitate hydrochloride has an unusually high solubility at 25°, which is due to micelle formation. Both compounds are surface active with relatively low critical micelle concentrations. However, since the Krafft point of lincomycin palmitate is ~43°, it does not form micelles below that temperature and appears to be quite insoluble until heated above 43°. The experimental monomeric solubilities of the two compounds agree with calculations based on group contributions to lipophilicity. Clindamycin 2-palmitate hydrochloride solutions are quite sensitive to ions, being salted out as unprotonated base in the form of oily droplets. Salting out correlates well with anionic strength, which is quite constant for the various salts studied. A viscosity maximum occurs with increasing salt addition, with the peaks of the different salts occurring at the same anionic strengths.

Keyphrases □ Lincomycin—palmitate ester, aqueous solubility, pH, temperature, micelle formation □ Clindamycin—palmitate ester, aqueous solubility, pH, temperature, micelle formation □ Antibacterial agents—lincomycin, palmitate ester, aqueous solubility, pH, temperature, micelle formation □ Antibacterial agents—clindamycin, palmitate ester, aqueous solubility, pH, temperature, micelle formation □ Aqueous solubility—lincomycin and clindamycin palmitate esters

Lincomycin and clindamycin are medium spectrum antibiotics whose hydrochloride salts are quite soluble in water. Both compounds have a bitter taste which is difficult to mask. Since clindamycin hydrochloride is considerably more bitter than lincomycin hydrochloride, chemical modification was required to make an acceptable liquid dosage form. A variety of prodrug esters were synthesized to reduce or eliminate the bitter taste of these anti-

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Table I—Aqueous Solubility of Lincomycin and Clindamycin 2-Palmitate Hydrochloride at 24.3°

I		II	
pH[a]	Solubility, mg/ml	pH[a]	Solubility, mg/ml
2.3	0.124	3.7	53.2
—	—	5.8	<0.001
7.4	0.0249	7.4	<0.0002

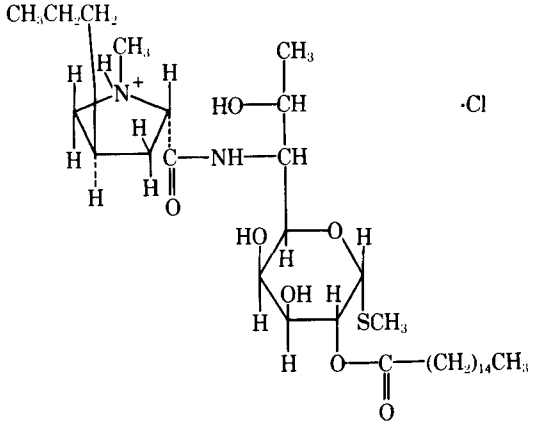
[a] Saturated solution pH at the end of the equilibration period.

biotics (1, 2). The esters are inactive until enzymatically hydrolyzed, which occurs readily *in vivo*, providing reasonably good blood levels of the parent compounds.

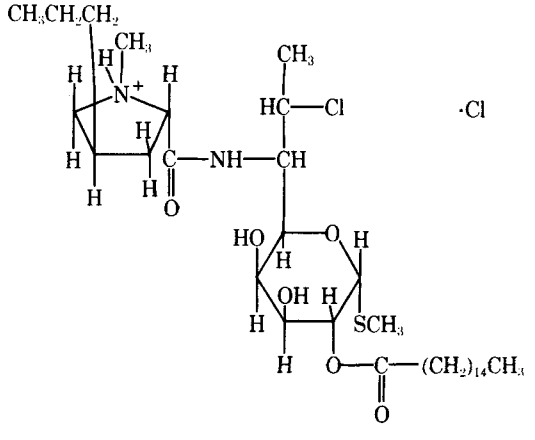
With respect to taste, stability, and bioavailability, lincomycin 2-palmitate hydrochloride (I) and clindamycin 2-palmitate hydrochloride (II) are the most useful of the prodrugs synthesized. This study explores some of the unusual solution properties of these esters, including surface activity, pH–solubility relationships, and sensitivity to ions.

EXPERIMENTAL

Materials—Compounds I and II were obtained from research stock and were used as received for the solubility studies. For surface activity measurement, II was purified by column chromatography. A concentrated aqueous II solution was eluted through a silica gel column with a solvent system of hexane–2-pentanone–pyridine (49:49:5). The filtrate was monitored by TLC, and a narrow fraction containing the main component was collected. The hydrochloride salt was formed by addition of chloroform saturated with hydrogen chloride gas. The product was precipitated by slow acetonitrile addition, filtered, washed with additional acetonitrile, and dried under vacuum at ambient temperatures.



I



II

Anal.—Calc. (when corrected for water): C, 58.35; H, 9.22; N, 4.00; Cl, 10.13; S, 4.58. Found: C, 57.92; H, 9.17; N, 3.91; Cl, 10.46; S, 4.42; H_2O, 2.25.

Flavored II granules were obtained from regular production stock. All electrolytes, solvents, and other chemicals were reagent grade.

Solubility Determination—An excess of the compounds was added to double-distilled water in tightly capped tubes and equilibrated for 2 or more days at 24.3 ± 0.3° using an oscillating shaker at 18 cpm.

A portion of the samples was filtered through medium glass frit funnels and again through 0.22-μm membrane filters[1]. The natural pH of the filtrates was measured with a pH meter[2]. The remaining unfiltered suspensions were each titrated with 1.0 *N* NaOH to ~pH 10, equilibrated for 2 or more days at 24.3°, and then filtered as before.

The solubility–temperature study on I was conducted in a constant-temperature bath[3] equipped with a sample holder that rotated at 6 rpm. The solutions were filtered through a preheated glass filtering apparatus[4]. Portions of the filtrate were transferred to vials with a preheated pipet. The solutions were either freeze dried and then redissolved in internal standard solution or extracted with chloroform after the aqueous solutions were made basic with sodium bicarbonate. The chloroform solutions were assayed by GLC using the conditions described previously (3, 4).

Surface Tension Measurement—A stock purified II solution was prepared in double-distilled water at ~10^{-2} *M*. Serial dilutions were made so that each successive solution had a concentration one-half that of the previous solution. The surface tension of each solution was measured at 25° in an automated drop-volume apparatus described earlier (5). The surface tension of the same solutions also was measured with a Wilhelmy plate apparatus[5].

Viscosity Measurement—Twenty-four grams of flavored granules containing II at a concentration of 37.5 mg of clindamycin activity per gram was added to 60-ml clear flint bottles. Stock electrolyte solutions were made from reagent grade salts and deionized water to give final concentrations of 6000 mg/liter.

These stock solutions were diluted to give a series of solutions with salt concentrations ranging from 100 to 6000 mg/liter. Forty-five milliliters of each salt solution was added to the bottled granules and shaken to obtain complete dissolution. Observations and measurements were made after standing at room temperature. Viscosity was measured using a viscometer[6] with a No. 2 spindle at 12 rpm.

RESULTS AND DISCUSSION

Comparative Solubilities of I and II—The solubilities obtained for I and II are shown in Table I. The solubility of II in the acid range was abnormally high (400-fold greater than I). It should actually be lower than I since the substitution of a chlorine group for a hydroxyl is expected to lower the aqueous solubility. At the very least, the solubilities should be about equal if the solvated protonated amine group dominates over lipophilic differences at C_7. This apparent anomaly was due to micelle formation by II, which occurs only in the protonated state. This phenomenon will be discussed more fully in the following section.

At pH 7.4 in the nonmicellar region, the solubilities of the two compounds differed in the "right" direction, *i.e.*, I was more soluble than II. The relative solubilities of I and II at this pH can be calculated from group contributions to lipophilicity (6). On this basis, one would expect I to be more water soluble (less lipophilic) than II. With the Hansch equation:

$$\log \frac{1}{S} = a \log P + b \qquad \text{(Eq. 1)}$$

where S is the molal solubility of the compound in water, P is the partition coefficient between 1-octanol and water, and a and b are constants, the solubility ratio of I and II can be obtained by combining their respective equations:

$$\log \frac{S_I}{S_{II}} = a(\log P_{II} - \log P_I) \qquad \text{(Eq. 2)}$$

The effect of group substitution on the partition coefficient can be evaluated using substituent constants:

$$\pi_x = \log P_x - \log P_H \qquad \text{(Eq. 3)}$$

[1] Type GS11, Millipore Corp., Bedford, Mass.
[2] Model NX, Sargent-Welch Scientific Co., Skokie, Ill.
[3] Constructed at The Upjohn Co., Kalamazoo, Mich.
[4] Medium glass frit funnel fitted with ground glass joints to a suction flask with an upward extended sidearm.
[5] Rosano surface tensiometer, Federal Pacific Electric Co., Newark, N.J.
[6] Model LVF, Brookfield Engineering Laboratories, Stoughton, Mass.

Table II—Critical Salting-Out Concentrations of Electrolytes for Clindamycin 2-Palmitate Hydrochloride

Salt	Molar Concentration	Ionic Strength	Anionic Strength
Na_2SO_4	0.014	0.042	0.056
$FeSO_4$	0.013	0.052	0.052
$MgSO_4$	0.017	0.068	0.068
$MgCl_2$	0.032	0.096	0.064
$CaCl_2$	0.027	0.081	0.054
NaCl	0.052	0.052	0.052
KCl	0.054	0.054	0.054
$NaHCO_3$	0.024	0.024	0.024

where P_x is the partition coefficient of a derivative and P_H is that of the parent molecule. When the appropriate constants are substituted into Eq. 2:

$$\log \frac{S_I}{S_{II}} = a(\pi_{Cl} - \pi_{OH}) \qquad \text{(Eq. 4)}$$

Taking established values for the constants (6) gives:

$$\log \frac{S_I}{S_{II}} = 1.339\ (0.39 + 1.16) = 2.075 \qquad \text{(Eq. 5)}$$

and:

$$\frac{S_I}{S_{II}} = 119 \qquad \text{(Eq. 6)}$$

Thus, I should be 119 times more soluble than II. The experimental solubility ratio in Table I at pH 7.4 is (0.0249/<0.0002) ≥ 125, which is a close approximation of the calculated value. The same semiquantitative solubility relationship holds true for the parent compounds, lincomycin and clindamycin. Their solubility ratio should also be 119. The respective experimental solubilities were determined previously (7) to be >400 and 4 mg/ml, giving a ratio of ≥100, again in close agreement with theory.

Surface Activity and Solubility—Clindamycin 2-palmitate hydrochloride (II) has an amphiphilic structure conducive to surface activity and micellar aggregation. The combination of a lipophilic palmitate side chain and a protonated head group is responsible for the enhanced solubility through association into micelles.

The compound is highly surface active, reducing the water surface tension from 72 to 30 dynes/cm. The data obtained from drop-volume measurements on the automated drop-volume apparatus (5) showed a distinct critical micelle concentration (CMC) at 3.40×10^{-4} mole/liter (Fig. 1). This finding was confirmed by Wilhelmy plate data, which gave a CMC of $\sim 3.5 \times 10^{-4}$ mole/liter. This value is about one order of magnitude lower than an estimate published earlier (8).

Above the CMC, the monomer concentration stays essentially constant, with the excess molecules forming micelles (9). Therefore, the monomer concentration in equilibrium with micelles is $\sim 3.4 \times 10^{-4}$ *M* or ~0.238 mg/ml.

As a first approximation, this value can be considered the monomer solubility of II. It is close to the experimental solubility of I (0.124 mg/ml, Table I). Apparently, lipophilicity differences due to the substitution at C_7 are overshadowed by the highly hydrophilic, protonated amine function.

Compound I has the same amphiphilic structure as II but does not appear to have sufficient surface activity to provide micellar solubilization

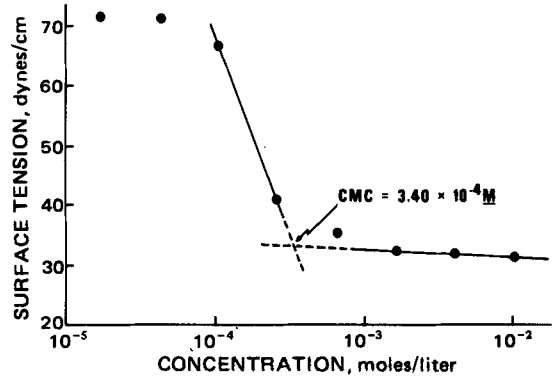


Figure 1—*Surface tension* versus *log concentration of aqueous clindamycin 2-palmitate hydrochloride solutions at 25°.*

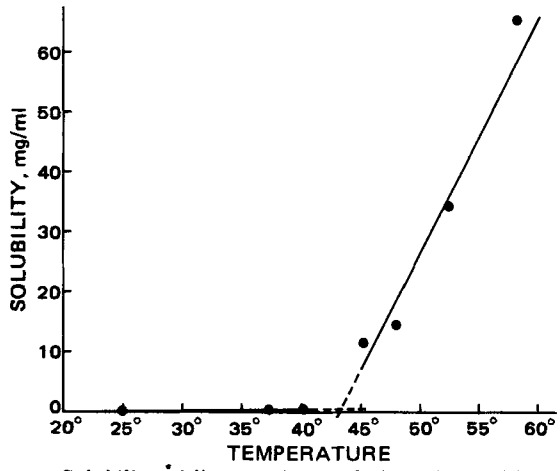


Figure 2—*Solubility of lincomycin 2-palmitate hydrochloride as a function of temperature.*

at 25°. However, at higher temperatures, the solubility increased sharply (Fig. 2). The solubility increased slowly with increasing temperature until ~43°, when a sudden change in slope occurred. Many surfactants exhibit similar solubility changes at a critical temperature known as the Krafft point (10). At this temperature, the monomer solubility has increased sufficiently to make micelle formation possible. Above the Krafft point, of course, the apparent solubility is due to micellar aggregation. The CMC at the Krafft point is $\sim 3.62 \times 10^{-4}$ *M* or ~0.247 mg/ml, which is a fair estimate of the monomer solubility at 43°. At 25°, the monomer solubility would be less and much closer to the experimental value of 0.124 mg/ml.

pH Dependence of Clindamycin Palmitate Hydrochloride Solubility—The solubility of II varied greatly with pH. In the free base form, II was extremely insoluble and difficult to measure quantitatively. Even at pH 7.4, which is slightly below the estimated pKa of 7.6[7], the observed solubility was below the detection limit (<0.0002 mg/ml). Below pH 4, the protonated drug had a monomeric solubility over 1000 times greater than the base solubility. Thus, the solubility–pH curve had a steep slope, and acidic II solutions appeared to precipitate out quantitatively as the pH increased. For example, if an acidic solution (pH 3) containing 15 mg of II/ml is titrated with base to pH 5.8 where the solubility of II is <0.001 mg/ml, >99.9% of the initial concentration would precipitate out. The pH–solubility profile shown in Fig. 3 was calculated from:

$$S_t = [CPH^+] + [CP] \qquad \text{(Eq. 7)}$$

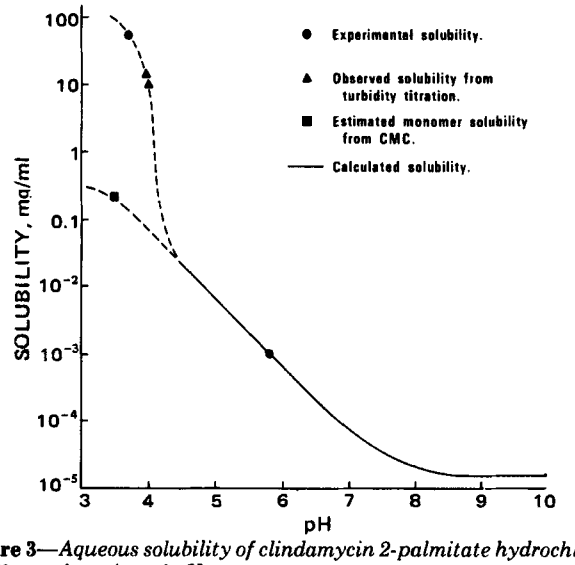


Figure 3—*Aqueous solubility of clindamycin 2-palmitate hydrochloride at 25° as a function of pH.*

[7] The pKa of II is difficult to determine because of the low solubility at high pH. However, the palmitate ester is too remote (both conformationally and along the chain) from the protonated amine function to have any noticeable effect on the pKa. Therefore, it is reasonable to assume that the pKa of the parent molecule, clindamycin, is unchanged by esterification at the 2-hydroxy position.

Table III—Effect of 2:1 Salt Mixtures on Salting Out of Clindamycin 2-Palmitate Hydrochloride from Syrup Solution

Mixture Number	Salt	Salt Concentration, mg/liter	Anionic Strength	Critical[a] Anionic Strength	Appearance of Reconstituted Solution
1a	$MgCl_2$	1333	0.028		Very slight turbidity
	Na_2SO_4	667	0.019		
	Total	2000	0.047		
				0.061	
1b	$MgCl_2$	2000	0.042		Very turbid with thick oil layer
	Na_2SO_4	1000	0.028		
	Total	3000	0.070		
2a	$CaCl_2$	1333	0.024		Very slight turbidity
	$NaHCO_3$	667	0.008		
	Total	2000	0.032		
				0.044	
2b	$CaCl_2$	2000	0.036		Turbid with oil layer
	$NaHCO_3$	1000	0.012		
	Total	3000	0.048		

[a] Calculated for the salt mixtures from the data in Table II, assuming that the individual values are additive.

where S_t is the total solubility, $[CPH^+]$ is the II concentration, and [CP] is the concentration of the unprotonated base form of II. The $[CPH^+]$ values at various pH's were obtained from the Henderson–Hasselbach equation:

$$\log [CPH^+] = pKa + \log [CP] - pH \qquad \text{(Eq. 8)}$$

where [CP] is assumed to be constant at pH 4–10 and was calculated to be $<1.56 \times 10^{-5}$ mg/ml using the experimental solubility value at pH 5.8 and the estimated pKa of 7.6. The solid line in Fig. 3, constructed from Eqs. 7 and 8, is a reasonably good estimate of the maximum solubility of II in the pH region shown.

As the pH decreases below the pKa, the concentration of protonated II molecules increases until the CMC is reached, at which time micelles form, causing a large jump in apparent solubility at ~pH 4. The experimentally observed CMC, which is a good estimate of the monomer concentration of II, was at 0.238 mg/ml. Without micelle formation, presumably the solubility would plateau at about this level. The triangular points (Fig. 3) are semiquantitative solubility values obtained from observations of the first appearance of turbidity when 1 *M* NaOH was added to syrup solutions of II.

The solubility curve in Fig. 3, while qualitative, does characterize the unusual pH–solubility relationship of II. The extremely low solubility of the base form kept the total solubility very low throughout most of the pH range. Only when the pH was 3 or 4 units below the pKa did it become significant. At pH 3.6, four units below the pKa, the protonated monomer concentration was 10^4 times the base solubility, which was at most 0.0001 mg/ml. Therefore, the monomer solubility at pH 3.6 was only ≤1 mg/ml. Of course, this result does not take into account the solubility due to micelle formation, which can amount to >50 mg/ml (Fig. 3).

Effect of Salts on Solubility—The solubility of II was greatly reduced by inorganic salts. When the salt concentration exceeded a certain critical value, the drug precipitated in the form of microscopic oil droplets, making the solution turbid. As the electrolyte concentration increased, the precipitation became more pronounced, leading to visible oil droplets and, finally, coalescence to an oily layer.

In a series of experiments, the reconstitution water for formulated flavored II[8] granules was spiked with salts normally found in drinking water. Observations were made on the first appearance of visible oil drops as the salt concentration was systematically increased. The critical oiling-out concentration of the different salts varied considerably (Table II), as did the ionic strength.

The individual contributions of cations and anions to ionic strength were calculated by:

$$\mu_\pm = mn_\pm Z_\pm^2 \qquad \text{(Eq. 9)}$$

where μ is the cationic or anionic strength, m is the salt molarity, n is the number of cations or anions per molecule, and Z is the charge on the ion. The cationic strength for oiling-out varied even more than ionic strength, but *anionic* strength proved to be fairly constant (Table II). The one exception, sodium bicarbonate, raised the pH as well as the ionic strength and thus had the additional effect of reducing solubility by pH change (Fig. 3). The pH effect of the other salts was minimal until the concentration approached 0.060 *M*, which was above the critical oiling-out concentration for all salts listed (Fig. 4). Thus, the pH of the sulfate and chloride solutions was not a significant factor in the "salting-out" phenomenon. The sharp increase in pH that occurred when sodium bicarbonate was added predominated over the ionic effect in reducing solubility.

The anion effect is additive, as shown by several qualitative experiments. Syrup solutions of II at ~25 mg/ml were made using water containing various salt concentrations (Table II). The pairs of solutions were made up so that one member of the pair had an additive anionic strength below the calculated critical value while the other member had an additive ionic strength above the critical value. Thus, the critical anionic strength of 2:1 magnesium chloride–sodium sulfate mixtures was calculated (assuming additivity) from the values in Table II to be 0.061. For 2:1 calcium chloride–sodium bicarbonate mixtures, the critical value was 0.044. Addition of the lower salt concentrations to clindamycin palmitate

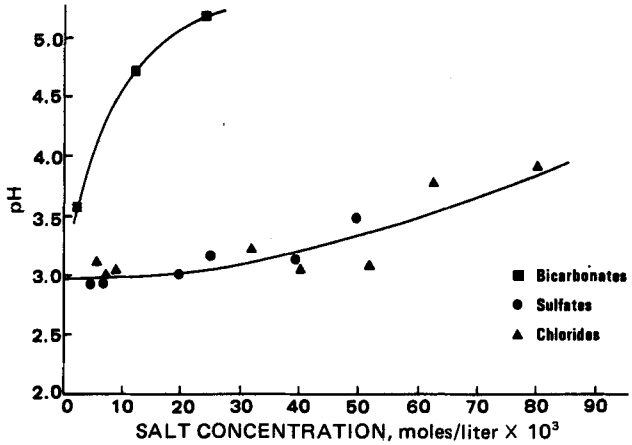


Figure 4—*Final pH of clindamycin 2-palmitate hydrochloride syrups reconstituted with various salt solutions.*

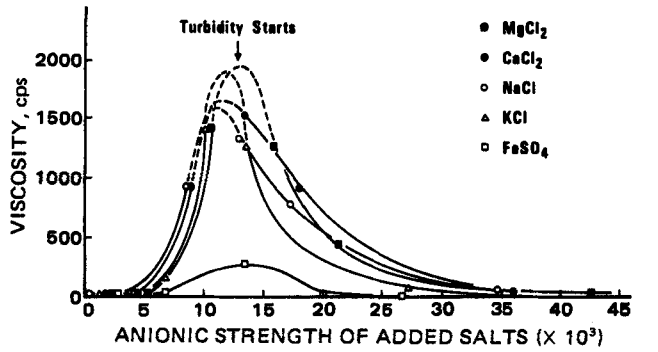


Figure 5—*Viscosity of clindamycin 2-palmitate hydrochloride solutions containing inorganic salts.*

[8] Cleocin Pediatric, 75 mg of clindamycin/5 ml.

syrup had very little effect while the higher salt concentrations caused II to "oil out" extensively (Table III). The oily precipitation was a salting-out phenomenon in which the monomeric solubility of II was reduced (11). Demicellization also may have occurred due to electrostatic suppression of the charge on the micelles by the increased ionic environment.

Effect of Salts on Viscosity—Inorganic salts also affect the viscosity of clindamycin palmitate hydrochloride solutions. Salt concentrations of ~400–800 mg/liter in syrup solutions of II caused the viscosity to increase with a fairly sharp peak. When concentrations were converted to anionic strength, as was done for the critical oiling-out point, a very close grouping of the peaks was obtained (Fig. 5). The anionic strengths of the peaks ranged from 0.011 to 0.013, the same range in which haziness or turbidity started in II solutions. At concentrations above this critical range, the viscosity was back to baseline levels well below the oiling-out point.

The chlorides were particularly effective in raising viscosity. Their peaks exceeded 1600 cps whereas the carbonate and sulfates produced more modest maxima. The common ion effect may have been dominant here, suppressing the dissociation of the II hydrochloride salt and thereby reducing the electrostatic repulsion forces between micelles (and also molecules). This can lead to increased intermicellar and intermolecular association forming flow-resistant aggregates or structures.

Following this hypothesis, as the salt concentrations increased, structure formation by II molecules increased until precipitation began, as evidenced by turbidity. The viscosity then peaked out and declined as the drug precipitated out in oily microdroplet form. Well beyond the peak viscosity, the oil droplets coalesced to form visible oil particles, at which point the precipitation was nearly complete.

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Reversed-Phase High-Performance Liquid Chromatographic Investigation of Levodopa Preparations I: Amino Acid Impurities

GARY W. SCHIEFFER

Received March 23, 1979, from the *Pharmaceutical Research Division, Norwich-Eaton Pharmaceuticals, Division of Morton-Norwich Products, Inc., Norwich, NY 13815.* Accepted for publication April 19, 1979.

Abstract □ A reversed-phase high-performance liquid chromatographic procedure with UV detection at 280 nm is presented for the determination of 6-hydroxydopa, tyrosine, 3-*O*-methyldopa, and other trace amino acid impurities in levodopa preparations. The method is fast and sufficiently sensitive to determine trace impurities at 0.1% of the levodopa concentration with a relative standard deviation of 4–6%. The trace impurities can be estimated at ≤0.01%.

Keyphrases □ Levodopa—analysis, high-performance liquid chromatography, amino acid impurities, commercial preparations □ Antiparkinsonian agents—levodopa, high-performance liquid chromatographic analysis, amino acid impurities, commercial preparations □ High-performance liquid chromatography—analysis, levodopa, amino acid impurities, commercial preparations

Current USP requirements (1) limit the impurities in levodopa [L-3-(3,4-dihydroxyphenyl)alanine] preparations to 0.1% of the levodopa concentration for 6-hydroxydopa [3-(2,4,5-trihydroxyphenyl)alanine] and to 0.5% for 3-*O*-methyldopa [3-methoxytyrosine or 3-(4-hydroxy-3-methoxyphenyl)alanine]. The designated USP method (1) for the estimation of these impurities is a TLC technique with a cellulose stationary phase and an acetic acid–butanol–methanol–water mobile phase. However, this laboratory found that the USP method suffers from a rather lengthy analysis time (>5 hr) and from the fact that the 6-hydroxydopa spot is not easily visualized. In addition, tyrosine, a frequently observed levodopa impurity, yields a spot with nearly the same R_f as 3-*O*-methyldopa. For these reasons, a quicker, simpler, and more universal method would be preferred.

BACKGROUND

A GLC procedure for the determination of levodopa purity was developed (2). However, this technique requires derivatization and was developed for impurities at a level of 1% with a stated detection limit for each component of ~0.1%. Thus, a more sensitive method is needed.

The standard amino acid analyzer procedure of separation on an ion-exchange column followed by detection of the ninhydrin color-forming reaction (3) was attempted but had long elution times (>80 min) and resulted in incomplete resolution of 3-*O*-methyldopa from tyrosine with the pH 3.4 citrate buffer employed[1]. Because of the greater speed and efficiency associated with microparticulate reversed-phase materials as opposed to ion-exchange materials (4) and because of the need to derivatize the amino acids (5) or to deposit a liquid phase on the solid support (6) when normal phase materials are used, a technique involving reversed-phase high-performance liquid chromatography (HPLC) was sought.

Knox and Jurand (7) resolved tyrosine from levodopa with reversed-phase HPLC by employing "soap" or ion-pair chromatography; the re-

[1] G. S. Denning, Jr., and G. Ginther, Norwich-Eaton Pharmaceuticals, Norwich, N.Y., unpublished results, 1970.

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