Micellar Solubilization of Barbiturates I: Solubilities of Certain Barbiturates in Polysorbates of Varying Hydrophobic Chain Length

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Abstract □ The effect of the hydrophobic chain length of the nonionic surfactants, polysorbates, on the degree of solubilization of a series of 5,5-disubstituted barbituric acid derivatives was studied. The solubilities were found to increase as the hydrophobic chain length increases. A pseudo two-phase model, according to which the drug molecule is partitioned between an aqueous phase and a micellar phase, was selected to determine the effect of the chemical structure of the solubilizate on the degree of solubilization. The number of carbon atoms of the substituents on the 5-position, as well as their inductive effects, was found to determine the extent of solubilization.

Keyphrases □ Barbiturates—solubilization □ Micellar solubilization—5,5-disubstituted barbituric acid derivatives □ Polysorbates, barbiturate solubilization—hydrophobic chain length effect □ UV spectrophotometry—analysis

Molecules of materials classed as surface-active agents possess polar and nonpolar characteristics. They are able to remain in solution at higher concentrations by orienting themselves in aggregates known as micelles (1). Surface-active agents, at a concentration above the CMC, are widely used as a means of producing aqueous solutions of insoluble or poorly soluble drugs (2). This phenomenon is known as micellar solubilization. Nonionic agents are by far the most popular in pharmaceutical formulations. This is due mainly to their low toxicity combined with their good solvent action. Although a wide range of nonionic surfactants are available, those most frequently employed are the polyoxyethylene sorbitan fatty acid esters and the polyoxyethylene monoalkyl ethers. Numerous studies on the solubilities of various drugs in these nonionic surfactants were reported (2).

The barbiturates are among the most frequently employed hypnotic and sedative drugs. They are used both as the free acids and as salts (usually sodium or calcium). Because of the instabilities and incompatibilities of the salts, the free acids are recommended to be used with other soluble drugs. These acids are slightly soluble in cold water. This limited solubility represents a problem in formulating aqueous preparations and elixirs (3). Polysorbate 80 was used to prepare aqueous solutions of phenobarbital (4). Gusiakov *et al.* found that the solubility of barbital increases in 2% solutions of polysorbates 20, 40, 60, and 80[1] (5). Küttel (6, 7) reported the solubilities of phenobarbital, barbital, and butabarbital in solutions of polysorbates 20, 60, and 80 at room temperature (16–24°). None of these reports have presented quantitative information concerning the effect of the chemical structure of the solubilizer or solubilizate on the degree of solubilization. This paper deals with the study of the solubilization of certain 5,5-substituted barbituric acids, which differ from one another only in carbon content of their substituents, in aqueous solutions of polysorbates of varying hydrophobic chain lengths. The data presented might help in gaining some insight into the mechanism of solubilization of such semipolar pharmaceuticals.

EXPERIMENTAL

Materials—The following surfactants were used as received: polysorbate 20, polyoxyethylene 20 sorbitan monolaurate; polysorbate 40, polyoxyethylene 20 sorbitan monopalmitate; polysorbate 60, polyoxyethylene 20 sorbitan monostearate; polysorbate 80, polyoxyethylene 20 sorbitan monooleate.

The barbiturates used and their melting points[2] were: phenobarbital, 5-ethyl-5-phenylbarbituric acid, m.p. 173–174°; barbital, 5,5-diethylbarbituric acid, m.p. 185–187°; amobarbital, 5-ethyl-5-isoamylbarbituric acid, m.p. 153–155°; diallylbarbituric, 5,5-diallylbarbituric acid, m.p. 169–170°; cyclobarbital, 5-(1-cyclohexenyl)-5-ethylbarbituric acid, m.p. 170–171°; butethal, 5-ethyl-5-butylbarbituric acid, m.p. 120–122°; secobarbital, 5-allyl-5-(1-methylbutyl)barbituric acid, m.p. 89–91°.

Assay Procedure—All barbiturates were assayed by the differential UV spectrophotometric procedure of Walker *et al.* (8) using a Unicam SP 500 spectrophotometer. The presence of surfactants did not interfere with this method.

Solubility Determinations—Excess quantities of the solid barbiturate were placed in 50-ml. rubber-stoppered bottles together with varying concentrations of 25-ml. solutions of the polysorbates in 0.003 *N* sulfuric acid. The weakly acidic solution was employed as the solvent to suppress any dissociation of the barbituric acid derivatives. The bottles were rotated in a constant temperature water bath at 30 ± 0.2° for 24 hr. This time was found to be sufficient for equilibrium to be attained. After equilibrium, aliquot portions of the supernatant liquid were removed and assayed for their barbiturate contents.

RESULTS AND DISCUSSION

Solubilization of the Barbiturates—The solubility curves for the barbituric acid derivatives are shown in Figs. 1–7. The curves show the effect of concentrations of polysorbates, well beyond the reported CMC (9–11), on the apparent solubilities of the drugs. All barbiturates studied showed increased solubilities in the presence of the polysorbates. This increase in solubility is looked upon here as due to true or micellar solubilization. Other workers (12–14) have found increased solubilities of similar semipolar drugs in solutions of various nonionic surfactants due to the same phenomenon. The apparent solubility increased linearly with the concentration of the surfactant as would be expected of micellar solubilization of such polar solubilizates (11, 12, 15, 16, 18).

Effect of Hydrophobic Chain of Solubilizer—In comparing the solubilizing power of different homologs of solubilizers, the ratio of moles of solubilizate:moles of micelles is the sensible criterion for comparison (17). This comparison involves the assumption that all the surfactant in excess of the CMC is in the form of micelles and

[1] Marketed as Tween 20, 40, 60, and 80 by Atlas Chemical Industries, Inc., Wilmington, Del.

[2] Uncorrected melting points determined with a Thomas-Hoover Unimelt.

that the number of units in the micelle remains constant. Although this is an approximation, it still has practical merit (17). Polysorbates 20, 40, 60, and 80 have the same hydrophilic portion in their molecule but differ in the length of the carbon atom chain of their lipophilic portion. The solubilizing capacity of the different polysorbates could be compared by calculating the slopes of the solubilization isotherms beyond the CMC. These slopes were calculated using the method of least squares. Table I shows the solubilizing capacities expressed as milligram drug per gram polysorbate and as mole drug per mole micellar solubilizer. The weight of each barbiturate solubilized per gram of polysorbate has its practical usefulness. The molar concentrations of polysorbates were calculated from the following molecular weights: polysorbate 20, 1226; polysorbate 40, 1282; polysorbate 60, 1310; polysorbate 80, 1308. Since polysorbates are heterogeneous, the significance of the molar values has its limitation.

On examining the slopes of the isotherms expressed on molar basis, it is found that all barbiturates showed a slight but gradual increase in solubility as the hydrophobic chain length of the solubilizer is increased. This increase in solubility is considered to be due to increase in the volume of the hydrocarbon in the micelle interior. In an idealized picture of spherical micelles the alkyl portions may be visualized as being directed inward. An increase in their length would result in micelles of larger size, and larger micelles will accommodate more solubilizate (16). Similar results were obtained by other workers when examining the solubilities of other semipolar pharmaceuticals (11, 14).

Effect of Chemical Structure of Solubilizate—In order to compare the effect of the chemical structure of the solubilizate on the degree of solubilization, a pseudo two-phase model was selected. According to this model, the solubilizate molecule is partitioned between an aqueous phase and a micellar phase. McBain and Hutchinson (17) suggested that a truer view is seen if solubilization is regarded as a partition between the micelle and water. The formation of hydrocarbon regions in micelles is a good jusification for treating the micelles as a separate phase. The partition coefficient K, associated with this process, was determined according to the following equation:

$$K = \frac{[D_M]}{[D_{NM}]}$$

where $[D_M]$ and $[D_{NM}]$ are the concentrations of drug, expressed as moles per moles, in the micellar and nonmicellar phases, respectively. The slopes of the solubilization isotherms expressed on molar basis will give the values for $[D_M]$. The number of moles of drug solubilized per mole of acidified water at 30° will give the values for $[D_{NM}]$. Table II lists the values of K for the various barbiturates in the different polysorbates studied. In a series of compounds such as the 5,5-substituted barbituric acids, which differ from one another only in carbon content of their substituents, the distribution coefficient would be expected to change with the alteration of the lipophilic character of the substituents as well as the influence these groups exert on the rest of the molecule. This influence will be mainly in the form of polar or inductive effects (19). The partition coefficients of the barbiturates between the micellar pseudophase and the aqueous phase in decreasing order are: secobarbital > amobarbital > phenobarbital > cyclobarbital > butethal > diallylbarbituric acid > barbital. As seen, the value of K increased with increasing the

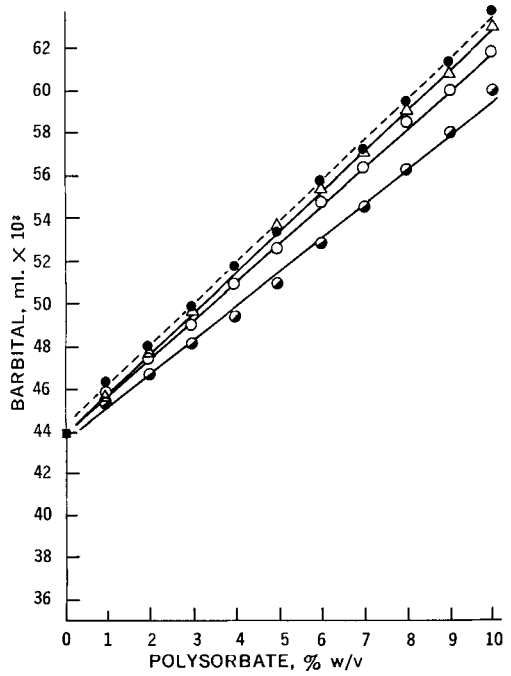

Figure 1—*Solubility of barbital in polysorbate solutions at 30°. Key: ■, solubility in water; ◒—, polysorbate 20; ○—, polysorbate 40; ●- - -, polysorbate 60; △—, polysorbate 80.*

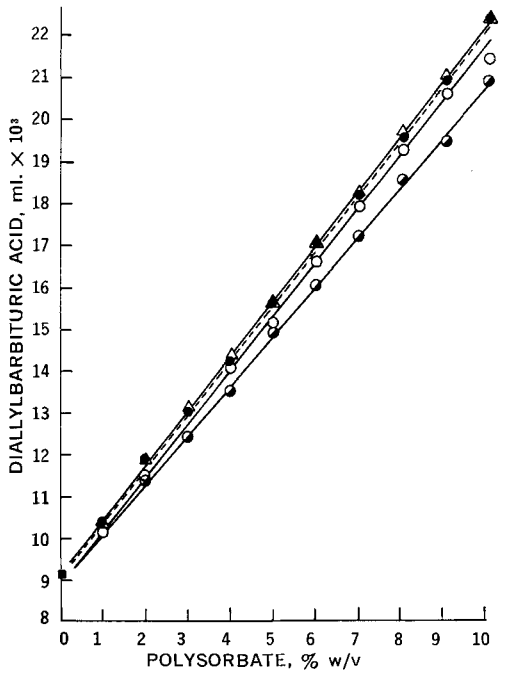

Figure 2—*Solubility of diallylbarbituric acid in polysorbate solutions at 30°. Key: ■, solubility in water; ◒—, polysorbate 20; ○—polysorbate 40; ●- - -; polysorbate 60; △—, polysorbate 80.*

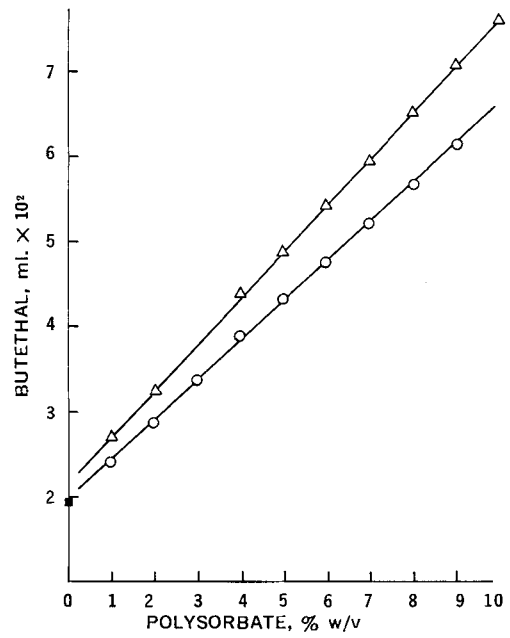

Figure 3—*Solubility of butethal in polysorbate solutions at 30°. Key: ■, solubility in water; ○—, polysorbate 20; △—, polysorbate 80.*

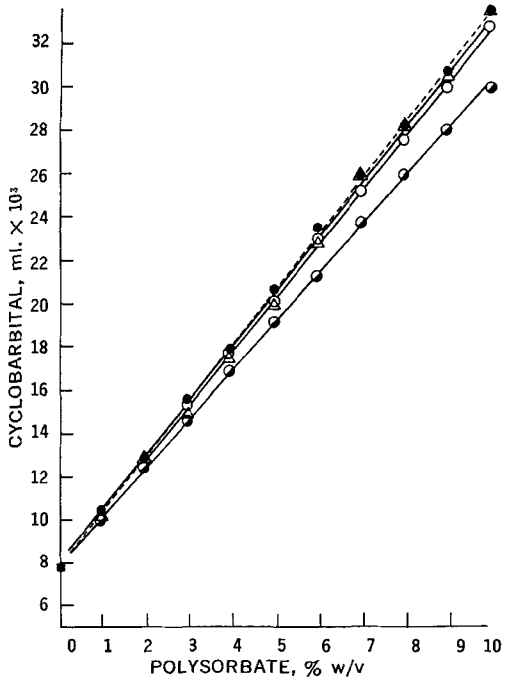

Figure 4—*Solubility of cyclobarbital in polysorbate solutons at 30°. Key:* ■, *solubiliy in water;* ◒—, *polysorbate 20;* ○—, *polysorbate 40;* ●- - -, *polysorbate 60;* △—, *polysorbate 80.*

number of carbon atoms in the substituents on the 5-position. These results compare favorably with the distribution coefficients of the same compounds between 1-octanol and water as determined by Hansch and Anderson (20). In their report the values of the distribution coefficients of the barbiturates between 1-octanol and water were in the following order: secobarbital > amobarbital > butethal > phenobarbital > cyclobarbital > diallylbarbituric acid > barbital. In comparing their results to the degree of solubilization of the barbiturates, as expressed by K, the authors found that phenobarbital and cyclobarbital show a higher degree of solubilization than what would be expected from their distribution between 1-octanol and water. As a result, phenobarbital and cyclobarbital showed higher K values than butethal which is reported to have a higher distribution coefficient. The increase in the extent of solubilization of these two barbiturates is attributed to the polar effect the phenyl and cyclohexenyl group exert on the hydrophilic portion of the barbiturate molecule. This polar effect will act in such a way as to increase the interaction between the hydrophilic portion of the barbiturate molecule and the hydrophilic portion of the polysorbate molecule. Such interaction may enhance the solubilization process. Further evidence for the effect of the phenyl group in enhancing the extent of interaction of the hydrophilic portion of the barbiturate molecule was obtained when the authors carried out an investigation of the solubilities of the same barbiturates in solutions of polyoxyethylene stearates of varying hydrophilic portion in their molecule. Phenobarbital was the

Table I—Solubilizing Capacity of Polysorbates for the Barbiturates at 30°

Drug	Surfactant	Solubility: mg. Drug/g. Surfactant	Solubility: mole Drug/mole Surfactant × 10²
Barbital	Polysorbate 20	30.0	19.9
	Polysorbate 40	33.0	23.0
	Polysorbate 60	35.3	25.1
	Polysorbate 80	35.0	24.6
Diallylbarbituric acid	Polysorbate 20	24.0	14.4
	Polysorbate 40	27.0	16.4
	Polysorbate 60	28.0	17.3
	Polysorbate 80	28.0	17.4
Butethal	Polysorbate 20	100.0	57.5
	Polysorbate 40[a]	—	—
	Polysorbate 60[a]	—	—
	Polysorbate 80	115.0	71.1
Cyclobarbital	Polysorbate 20	52.4	27.2
	Polysorbate 40	58.0	31.6
	Polysorbate 60	61.0	33.8
	Polysorbate 80	61.0	34.0
Phenobarbital	Polysorbate 20	55.1	29.1
	Polysorbate 40	61.0	33.7
	Polysorbate 60	63.0	35.5
	Polysorbate 80	66.0	37.2
Amobarbital	Polysorbate 20	32.0	17.2
	Polysorbate 40	38.0	21.7
	Polysorbate 60[a]	—	—
	Polysorbate 80	40.0	22.9
Secobarbital	Polysorbate 20	111.0	57.0
	Polysorbate 40[a]	—	—
	Polysorbate 60[a]	—	—
	Polysorbate 80	144.0	78.8

[a] Limited supply of the drug necessitated the use of a lesser number of surfactants.

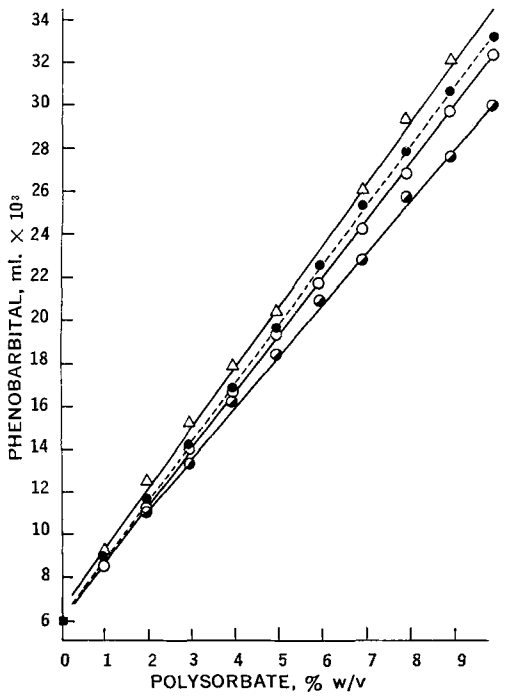

Figure 5—*Solubility of phenobarbital in polysorbate solutions at 30°. Key:* ■, *solubility in water;* ◒—, *polysorbate 20;* ○—, *polysorbate 40;* ●- - -, *polysorbate 60;* △—, *polysorbate 80.*

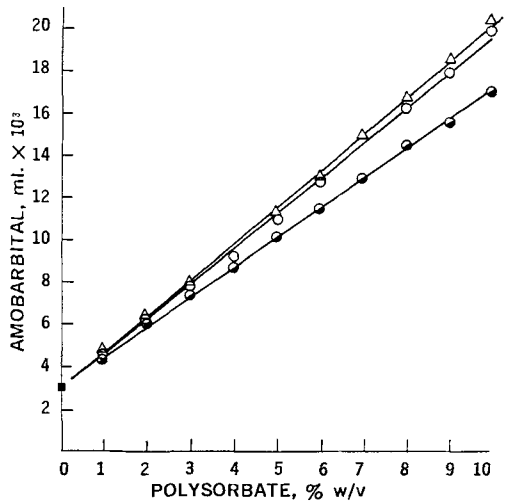

Figure 6—*Solubility of amobarbital in polysorbate solutions at 30°. Key:* ■, *solubility in water;* ◒—, *polysorbate 20;* ○—, *polysorbate 40;* △—, *polysorbate 80.*

Table II—Partition Coefficients for 5,5-Substituted Barbituric Acids Between the Micellar and Nonmicellar Phase at 30°

Drug	R_1	R_2	Surfactant	Partition Coefficient, $K \times 10^{-2}$
Barbital	C_2H_5	C_2H_5	Polysorbate 20	2.56
			Polysorbate 40	2.91
			Polysorbate 60	3.17
			Polysorbate 80	3.11
Diallylbarbituric	CH_2=CH—CH_2	CH_2=CH—CH_2	Polysorbate 20	8.73
			Polysorbate 40	9.94
			Polysorbate 60	10.48
			Polysorbate 80	10.55
Butethal	C_2H_5	n-C_4H_9	Polysorbate 20	16.43
			Polysorbate 40[a]	—
			Polysorbate 60[a]	—
			Polysorbate 80	20.31
Cyclobarbital	C_2H_5	1-cyclohexenyl	Polysorbate 20	19.29
			Polysorbate 40	22.41
			Polysorbate 60	23.97
			Polysorbate 80	24.11
Phenobarbital	C_2H_5	C_6H_5	Polysorbate 20	27.20
			Polysorbate 40	31.50
			Polysorbate 60	33.18
			Polysorbate 80	34.77
Amobarbital	C_2H_5	iso-C_5H_{11}	Polysorbate 20	31.79
			Polysorbate 40	40.11
			Polysorbate 60[a]	—
			Polysorbate 80	42.33
Secobarbital	CH_2=CH—CH_2	$C_3H_7(CH_3)_3OH$	Polysorbate 20	46.42
			Polysorbate 40[a]	—
			Polysorbate 60[a]	—
			Polysorbate 80	64.07

[a] Limited supply of the drug necessitated the use of a lesser number of surfactants.

only barbiturate to form an insoluble precipitate complex indicative of a greater extent of interaction. The results of this investigation will be the subject of a future communication.

Solubilization Type—Micellar solubilization was broadly classified into three types (16): first, adsorption on the surface of the micelle; second, incorporation in the hydrocarbon center of the micelle, a form of solution; and third, incorporation by penetration into the palisade layer of the micelle with the solubilizate oriented in approximately the same manner as is the surfactant molecule in the micelle. Mulley (1) suggests that solubilization even of polar materials in nonionic surfactants is probably due mainly to a solution process within the micelles rather than due to specific factors such as complex formation or adsorption on the surface of the micelles. He states that other factors obviously play a part but a solution process still predominates. The results obtained in this work support the assumption that the solubilization of the semipolar molecules, the barbiturates, in polysorbate solutions is essentially a micellar solubilization.

The order of the partition coefficient K, calculated from the solubility data of the various barbiturates studied, lends support to the suggestion of Mulley and leads the authors to believe that here also the type of solubilization is mainly a solution within the micelles together with some other factors playing a minor role. In the case of the barbiturates these factors are probably interactions between the hydrophilic portion of the solubilizer and that of the solubilizate.

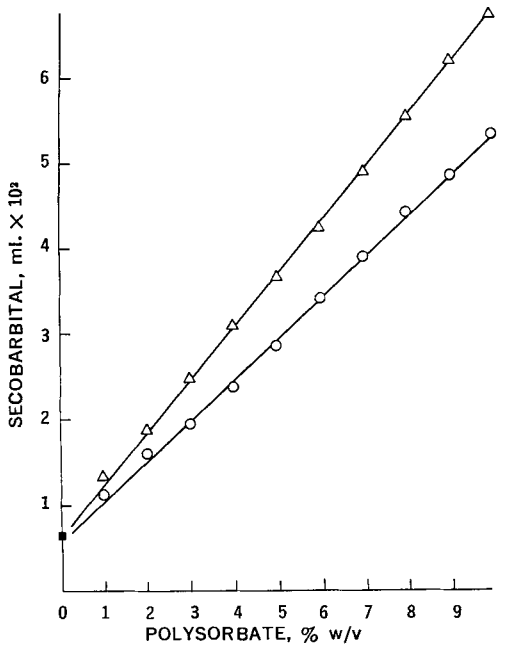

Figure 7—*Solubility of secobarbital in polysorbate solutions at 30°. Key: ■, solubility in water; O—, polysorbate 20; △—, polysorbate 80.*

SUMMARY

1. All barbiturates studied showed increased solubilities in the presence of the nonionic surfactants, polysorbates.

2. The amount of barbiturate solubilized was a linear function of the concentration of the polysorbates, characteristic of micellar solubilization of semipolar molecules.

3. The degree of solubilization increased with increase of the hydrophobic chain length of the solubilizer due to formation of larger micelles.

4. The increase in the number of carbon atoms in the substituents on the 5-position of the barbituric acid molecule, together with the polar or inductive effects of such substituents, determined the extent of solubilization.

5. The order of solubilization of the drugs compared favorably with the order of their distribution coefficients between 1-octanol and water.

6. Phenobarbital and cyclobarbital showed a greater extent of solubilization due to the inductive effect of the phenyl and cyclohexenyl groups resulting in a greater interaction between the hydrophilic portion of the solubilizer and that of the solubilizate.

7. The mechanism of solubilization is thought to be mainly a solution within the micelles together with other factors playing a role in the solubilization.

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Improved Thin-layer Chromatographic Identification of Tetracyclines and Their Degradation Products: Application to an Epimerization Study

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Abstract □ An improved thin-layer chromatographic method for the identification of pharmaceutically important tetracyclines and their degradation products is presented. A process is used which conditions the plates with optimum moisture content so that they may be stored without special precautions for as long as 4 weeks before use. Two solvent systems and four spray reagents for the separation and detection of tetracyclines and their degradation products are also presented. Tetracycline hydrochloride and chlortetracycline hydrochloride were allowed to epimerize in phosphate and acetate solutions, respectively. With the aid of UV spectroscopy and TLC analysis, it is demonstrated that epimerization is accompanied by extensive degradation.

Keyphrases □ Tetracyclines, degradation products—identification □ Epimerization, tetracyclines—reaction products separation, identification □ TLC—separation, identification □ UV light—TLC spot visualization

Thin-layer chromatography (TLC) has been used by a number of workers to separate and characterize certain tetracyclines of pharmaceutical importance (Table I) using adsorbent layers of silica gel (1, 2), kieselguhr (3–5), and microcrystalline cellulose (6). Difficulties encountered in these separations have been attributed to the property of tetracyclines to form chelate complexes with metallic ions and to lack of moisture in the support. Hence, sequestering agents (1–5) and glycerin (7) or mixtures of glycerin with polyethylene glycol 400 (PEG 400) (4) have been added to the support.

Of the various methods reported, two (3, 4) are most useful since they can resolve two or more tetracyclines in a single system on one chromatogram. Of these, the one employing the coating of kieselguhr containing EDTA and a developing solvent consisting of methyl ethyl ketone saturated with McIlvaine's buffer (pH 4.7) (3) was especially good for the separation of various degradation products of the tetracyclines. The other method (4), which employs acid-washed diatomaceous earth impregnated with EDTA at pH 7.0 and a glycerin–PEG 400 mixture, and which uses ethyl acetate as the developing solvent, was found satisfactory for the resolution of tetracyclines, though often it was necessary to chromatograph two to four times. In both cases, however, problems in their application were encountered. The most serious difficulty with the former method (3) was the frequent and erratic splitting and streaking of the tetracycline spots. The latter method (4) involves rigid adherance to a lengthy procedure in which the diatomaceous earth is repeatedly washed to remove binder and other acid-soluble materials. This was found not to be reproducible without considerable experience. Furthermore, the plates must be freshly prepared and used immediately. The method is, therefore, particularly inappropriate in situations where a large number of chromatograms must be run in a short time.

The tetracyclines have most commonly been detected on chromatograms by their fluorescence under long-wave UV light, either with or without exposure of the chromatograms to ammonia vapor (1, 3–5, 8). A few chromogenic spray reagents were described in the earlier literature (1) but have been largely abandoned in recent publications.

This communication describes a simple, rapid, and reproducible method for the separation of six tetracyclines presently marketed in numerous dosage forms.