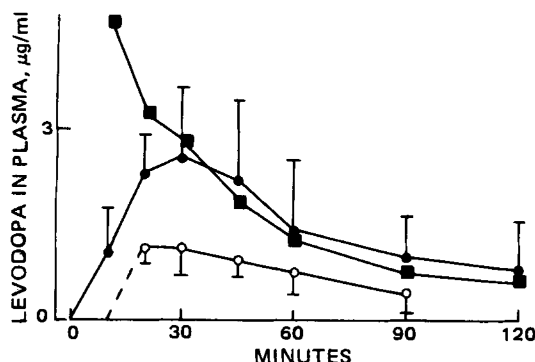

Figure 9—*Concentrations of levodopa in the plasma as a function of time following rectal administration of a microenema at pH 7.4 and a levodopa dose of 15 (● and ○), 7.5 (○), and 15 mg/kg (●) of sodium salicylate. Levodopa (10 mg/kg) was also given intravenously (■).*

rise in the plasma salicylate concentration after the simultaneous rectal administration of both salicylate and drug as shown in Fig. 10 for cefmetazole and levodopa. To examine the effect of salicylate levels independent of rectal absorption, the effect of salicylate given intravenously on the rectal absorption of cefmetazole and theophylline was studied. In this study, sodium salicylate was given by an intravenous infusion to maintain a relatively high plasma salicylate concentration of ~2 mg/ml. As shown in Table II and Fig. 10, salicylate in the plasma alone did not affect the loss of cefmetazole or theophylline from the solution perfusing the rat rectum. Furthermore, no salicylate was found in the perfusate, indicating that little if any salicylate was present in the rectal membranes after intraveous infusion of salicylate. Although salicylate is readily absorbed from the rectum to the plasma, the reverse does not occur under these conditions. It also appears that salicylate does not promote rectal drug absorption except when it is present in the rectal tissue. This is supported by the observation (2) that the enhancement of theophylline absorption from the rectum by salicylate was eliminated by washing the rectum with a buffer solution after pretreatment with salicylate. This is in contrast to the effect of sodium lauryl sulfate which continued after washing the rectum following pretreatment with sodium lauryl sulfate.

It appears that salicylate interacts with some feature of the rectal membrane facilitating the transport of drug substances from the rectum to the general circulation. Studies are continuing on the mechanism of this action.

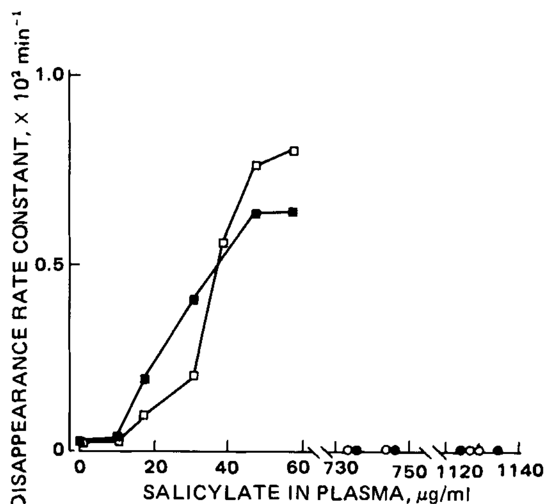

Figure 10—*Disappearance rate constant (× 10^2 min^{-1}) of cefmetazole (■) and levodopa (□) from perfusate as a function of salicylate concentration in the plasma following rectal administration. High plasma concentrations of salicylate following intravenous salicylate administration did not result in significant disappearance of cefmetazole (●) or levodopa (○).*

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Analysis of Iodochlorhydroxyquin in Cream Formulations and Bulk Drugs by High-Performance Liquid Chromatography

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Received June 6, 1981, from the *Quality Control Division, The Upjohn Company, Kalamazoo, MI 49001.* Accepted for publication October 23, 1981.

Abstract □ A high-performance liquid chromatographic method for the analysis of iodochlorhydroxyquin in creams and as bulk drugs has been developed. Iodochlorhydroxyquin was acetylated in the 8-position by reaction with acetic anhydride in pyridine. The resulting ester was mixed with the internal standard and chromatographed on a microparticulate silica column. Recovery was quantitative and the method was shown to be applicable to cream formulations from several manufacturers.

Keyphrases □ Iodochlorhydroxyquin—analysis in cream formulations and bulk drugs, high-performance liquid chromatography □ High-performance liquid chromatography—iodochlorhydroxyquin, analysis in cream formulations and bulk drugs □ Cream formulations—analysis of iodochlorhydroxyquin and bulk drugs by high-performance liquid chromatography

Iodochlorhydroxyquin (5-chloro-7-iodo-8-hydroxyquinoline) (I) has antifungal and antibacterial activities and is used in the treatment of inflamed skin conditions such as eczema, athlete's foot, and other fungal infections. Its use is generally limited to topical applications and is commercially available in lotion, cream, and ointment formulations. It is frequently formulated in combination with the corticosteroid, hydrocortisone. Monographs for

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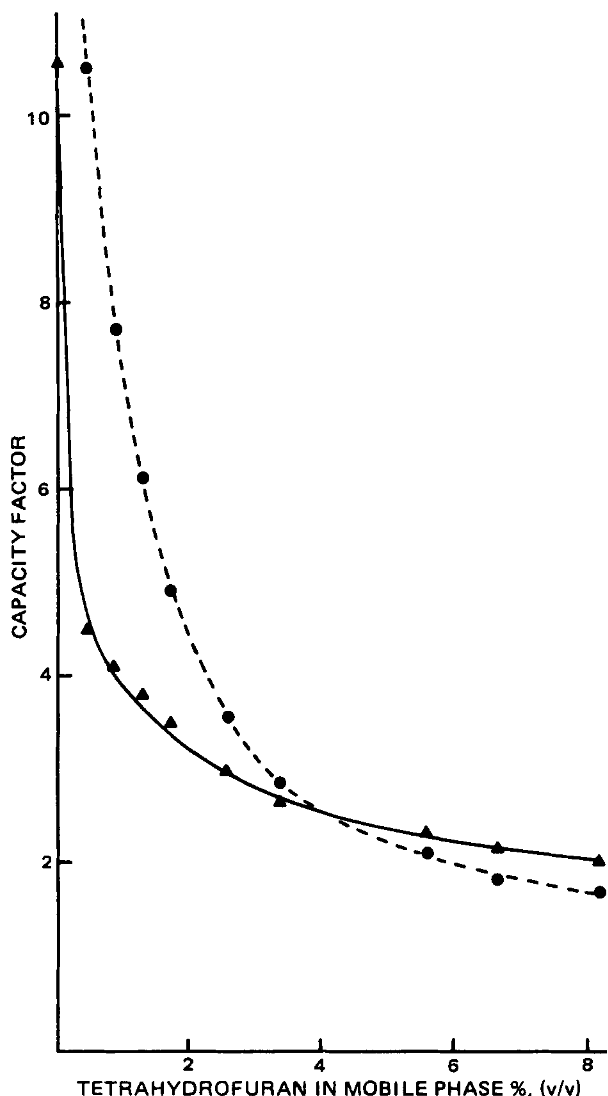


Figure 1—*Effect of tetrahydrofuran on the capacity factor of iodochlorhydroxyquin-8-acetate ester and the internal standard. Key: (●) testosterone acetate; (▲) iodochlorhydroxyquin acetate ester.*

I as bulk drug, cream, ointment, compound powder, and tablets appear in USP XX.

The USP XX assay for I in cream formulations involves extraction of the drug into carbon disulfide and quantitation by IR spectroscopy. The sample preparation in this assay is cumbersome in that it requires multiple extractions and one back-extraction step. Also, the use of quantitation by IR spectroscopy limits the extent to which the assay can be automated.

In this study a chromatographic method was developed with high specificity and an experimentally simple sample preparation. An approach utilizing acetylation of the 8-hydroxy group of I and normal-phase high-performance liquid chromatography (HPLC) was found to satisfy these conditions.

BACKGROUND

The analysis of I in dosage forms and biological samples has been accomplished by TLC, GC, and HPLC. Compound I has been analyzed using silica gel plates with a methanol–methoxyethanol–hydrochloric acid (88:10:2) developing solvent (1). Three unidimensional developments of the plate were required to completely isolate I from frequently encountered impurities. The following compounds were observed as impurities in samples of I: 5,7-dichloro-8-hydroxyquinoline (II); 5,7-diiodo-8-hydroxyquinoline (III); 5-chloro-8-hydroxyquinoline (IV); and 8-hydroxyquinoline (V). In a previous study (2), I was reacted with *N*-trimethylsilylimidazole to form the silyl ether in the 8-position. Silylated I and related compounds were separated and quantitated by GC using a methylsilicone column. The silyl ether was formed by reacting I with *N*-methyl-*N*-silyltrifluoroacetamide, and I was analyzed in several dosage forms by GC (3).

Table I—Relative Retention Times and Relative Responses for Related Compounds

Compound	Relative Retention[a]	Relative Response[b]
5-chloro-7-iodo-8-hydroxyquinoline (I)	1.00	1.00
5,7-dichloro-8-hydroxyquinoline (II)	1.08	0.07
5,7-diiodo-8-hydroxyquinoline (III)	0.94	0.64
5-chloro-8-hydroxyquinoline (IV)	1.45	0.04
8-hydroxyquinoline (V)	Not Detected	

[a] All compounds chromatographed as the 8-acetate ester. [b] Peak height response at 254 nm.

Extractive alkylation has also been used as a derivatizing procedure for I prior to GC analysis. Compound I is extracted into methylene chloride using tetraalkylammonium salts as ion pairing agents. Methyl iodide in the methylene chloride solution then reacts with I to form the 8-methyl ether which is then analyzed by GC. Tetrapentyl- and tetrahexylammonium hydroxide have been used as ion-pairing reagents (4). To avoid problems associated with the hydroxide portion of the ion-pairing reagent, tetrabutylammonium hydrogen sulfate has been employed as the pairing ion in the analysis of I in urine and plasma (5). Acetate ester formation has been used in the GC analysis of I in serum, urine, and milk (6). Compound I is extracted into pyridine–benzene (1:9) and then derivatized with acetic anhydride.

Iodochlorhydroxyquin has also been analyzed in urine by HPLC (7). This method used an anionic exchange resin and gradient elution to achieve satisfactory chromatography.

EXPERIMENTAL

Materials—Testosterone acetate[1], iodochlorhydroxyquin[2], 5,7-diiodo-8-hydroxyquinoline[3], 5,7-dichloro-8-hydroxyquinoline[3], 5-chloro-8-hydroxyquinoline[3], and 8-hydroxyquinoline[3] were used as received. Pyridine[4], glacial acetic acid[4], and acetic anhydride[4] were analytical reagent grade. Butyl chloride[5] and tetrahydrofuran[5] were distilled in glass. Water used in preparation of the mobile phase was deionized.

Mobile Phase—All mobile phases were prepared by mixing water-saturated butyl chloride with butyl chloride at a ratio of 1:1 and then adding the appropriate amounts of tetrahydrofuran and glacial acetic acid. The mobile phase used for analysis of I was butyl chloride–water-saturated butyl chloride–tetrahydrofuran–glacial acetic acid(55:55:3:2). All mobile phases were filtered before use.

Instrumentation—The HPLC system has been described previously (8). The column used contained microparticulate silica[6] (10 μm, 30 cm × 4 mm).

Quantitative Procedure for I in Creams—An accurately weighed quantity of cream equivalent to 30 mg of I was transferred to a 100-ml volumetric flask. Tetrahydrofuran (~70 ml) was added, shaken vigorously until the sample was completely dissolved, and tetrahydrofuran was added to volume. A standard preparation was prepared by dissolving an accurately weighed quantity of I (~30 mg) in 100.0 ml of tetrahydrofuran. The standard and sample preparations (5.0 ml) were transferred into suitable vials. Two milliliters of a pyridine–acetic anhydride mixture (1:1) were added to the sample and each vial was heated for 15 min at 60°. After the samples returned to ambient temperature, 15.0 ml of the internal standard solution, prepared by dissolving 450 mg of testosterone acetate in 60 ml of tetrahydrofuran and then diluting to 1000 ml with butyl chloride, was added and mixed thoroughly. A 3-ml portion of each sample

[1] Steraloids, Inc., Wilton, N.H.
[2] Ciba-Geigy, Summit, N.J.
[3] Aldrich Chemical Co., Milwaukee, Wis.
[4] Mallinckrodt, St. Louis, Mo.
[5] Burdick and Jackson Laboratories, Muskegon, Mich.
[6] μ-Porasil, Waters Associates, Milford, Mass.

Table II—Recovery of I Added to Placebo Formulation

Added, mg/g	Found, mg/g	Recovery, %
22.46	22.98	102.3
26.20	26.54	101.3
29.94	30.16	100.7
33.68	34.00	101.0
37.42	37.46	100.1

Table III—Analysis of I in Cream Formulations

Manufacturer	mg I/g[a]
A	30.6, 30.2
B	30.1, 30.1
	29.8, 29.7
	30.4, 30.3
C	30.5, 29.9
	29.9, 28.8
	30.0, 29.7
	30.2, 30.1
	30.0, 29.6
D	29.9, 30.0
	31.3, 30.7

[a] Duplicate assays. Each pair of results represents a different lot. Label quantity was 30 mg/g (3%).

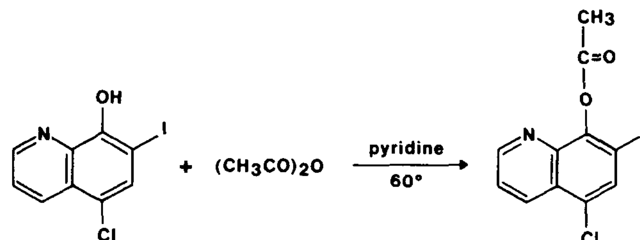
was transferred to a second vial and evaporated to dryness under a gentle nitrogen flow at 40°. Residue was reconstituted in ~15 ml of mobile phase. (Gentle warming and/or vigorous shaking may be necessary to ensure reconstitution.) Aliquots of the final solution were chromatographed and quantitated using peak height or peak area ratios.

Quantitative Procedure for I Bulk Drug—An accurately weighed quantity of I (~30 mg) was transferred to a 100-ml volumetric flask. The same procedure was followed as with cream.

RESULTS AND DISCUSSION

Initial attempts to develop a direct liquid chromatographic assay for I were unsuccessful. Using silica columns, the retention times were excessive. Using reversed-phase chromatography (octadecylsilane, 10 μm), reasonable elution times were obtained but the peaks exhibited unacceptable tailing in most cases. The observed behavior suggested that I was binding strongly to silanol groups on both types of column packings. To avoid this problem, the approach of acetylating the highly polar 8-hydroxy group was undertaken. The resulting 8-acetate ester was much less polar and could be chromatographed easily using a silica column and a modified butyl chloride mobile phase.

The derivatization reaction (Scheme I) is rapid under the chosen conditions. It was determined experimentally that one-half the amount of acetylating reagent (pyridine–acetic anhydride mixture) called for in the procedure would give quantitative conversion to the ester in 15 min at 60°. It was also found that the reaction gives essentially 100% recovery in 5 min at 60°. The amount of acetylating reagent and the length of reaction time used in the final analytical procedure, therefore, represent excess quantities.



Scheme I

The effects of tetrahydrofuran and acetic acid on the capacity factors for the 8-acetate ester of I and testosterone acetate were studied by varying the amounts added to the mobile phase. The amount of butyl chloride in the mobile phase was kept constant; however, the ratios changed slightly. The effects of varying the amount of acetic acid were minimal. However, small changes in the tetrahydrofuran concentrations caused dramatic shifts in the capacity factors for both compounds as shown in Fig. 1. Therefore, the concentration of tetrahydrofuran must be carefully controlled to obtain reproducible results.

To evaluate the specificity of the chromatographic system, several related compounds were derivatized by acetylation and examined for chromatographic retention and response. Of the compounds tested (Table I), 5,7-diiodo-8-hydroxyquinoline (III) showed the greatest potential for interference. Figure 2 showed the chromatographic tracings

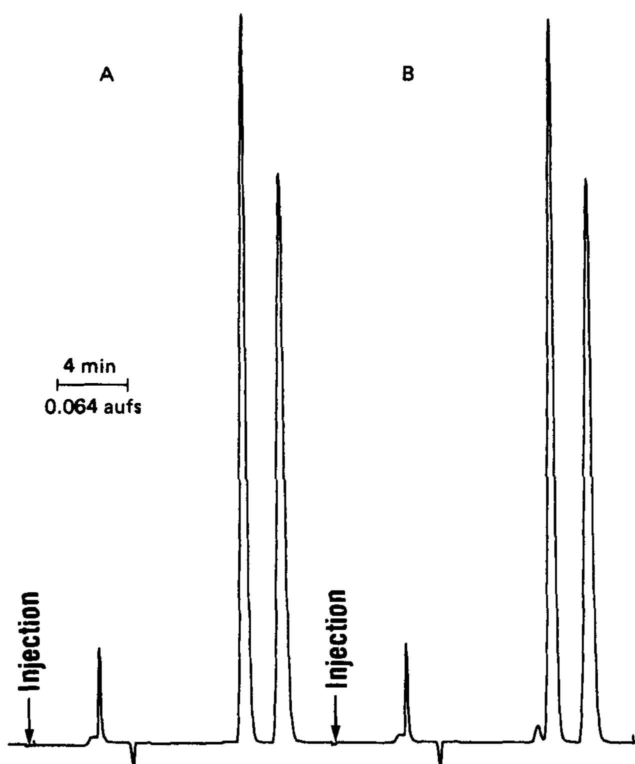


Figure 2—*Chromatographic tracings of a standard preparation before (A) and after (B) adding 3% 5,7-diiodo-8-hydroxyquinoline prior to derivatization step.*

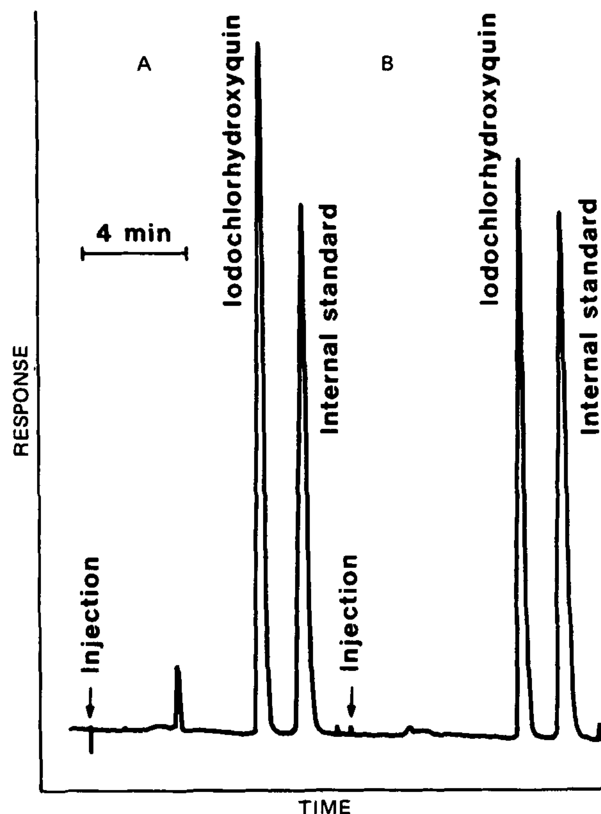


Figure 3—*Typical chromatographic tracings of sample preparation (A) and standard preparation (B). See text for details.*

Table IV—Analysis of Iodochlorhydroxyquin Bulk Drug

Lot	Purity, %	
	Peak Area Calc.	Peak Height Calc.
A	98.9, 98.3	99.4, 98.9
B	99.2, 99.9	99.5, 99.1
C	97.4, 98.7	98.9, 99.4
D	98.5, 98.4	98.3, 98.9
E	99.9, 99.4	99.2, 98.7
F	98.3, 98.9	98.2, 99.2

of a standard preparation before and after the addition of III in an amount equivalent to 3% of I. Baseline resolution was achieved and recovery of III was quantitative over the range of 1.5–5.0% expressed on the basis of I. While 5,7-dichloro-8-hydroxyquinoline elutes near the peak for I, its relative response at 254 nm is very low and should not interfere at low concentrations. Furthermore, no changes in assay results for I were observed with samples spiked with as much as 6% of III.

Typical chromatographic tracings for standard and sample preparations are shown in Fig. 3. No interferences were observed from formulation excipients, even though no sample clean-up steps were employed. No late eluting peaks were observed over an 8-hr period.

Placebo samples with added I were analyzed by this method to determine recovery efficiency. The recovery data (Table II) indicate that the procedure is quantitative for I over the range of 22–37 mg/g. This range corresponds roughly to 75–125% of label for the typical 3% cream formulations (30 mg/g). Replicate analysis of a single lot (n = 8) at 30 mg/g gave a 1.1% *RSD*. Results from the analyses of several lots of cream formulations from five manufacturers are shown in Table III. Good agreement with label content was observed in all cases.

A slightly modified procedure was used to analyze samples of bulk drug. Five samples of I (18–43 mg) were analyzed according to this procedure. The weight of I found was plotted against the weight of I added. The resulting linear regression equation had a slope of 1.00, an intercept of −0.02, and a correlation coefficient of 0.999. One lot of bulk drug was analyzed 10 times to determine the precision of the bulk drug assay. Using peak area ratios as the basis for calculation, the average value was 99.12% purity with a 1.1% *RSD*. Using peak height ratios, the mean was 99.21% purity with a 0.6% *RSD*. Six additional lots of iodochlorhydroxyquin bulk drug were analyzed in duplicate. The results of these assays are shown in Table IV.

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Distribution of Bile Salts Between 1-Octanol and Aqueous Buffer

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Received June 15, 1981, from the *Department of Pharmaceutical Chemistry, The University of Kansas, Lawrence, KS 66045.* Accepted for publication October 13, 1981.

Abstract □ The distribution of four bile salts: sodium cholate (I), sodium deoxycholate (II), sodium chenodeoxycholate (III), and sodium ursodeoxycholate (IV), between aqueous buffer and 1-octanol has been measured as a function of temperature between 25 and 55° and as a function of bile salt concentration at concentrations <0.1 mole/liter in the aqueous phase. The distribution isotherms obtained have been explained on the basis of reversible association in the aqueous phase. The treatment assumes that the bile acid exists as a monomer in the organic phase, which is verified by vapor pressure osmometry. A graphical method has been employed to estimate the association constants in the aqueous phase for the various equilibria encountered. An aggregation number of four for IV and 12 for I, II, and III has been estimated. From the results, thermodynamic functions associated with the transfer of each of the bile salts from water to octanol and those associated with association processes in the aqueous phase were calculated. These results are consistent with previous findings that the premicellar association of bile salts occurs by hydrophobic interaction. The thermodynamics of transfer of bile salts revealed an unfavorable enthalpic and favorable entropic contribution for all four bile salts. However, for IV, which is an epimer of III, both enthalpic and entropic contributions are reduced, compared to III, suggesting a pronounced effect of stereochemical orientation on hydrophobic interaction.

Keyphrases □ Partition coefficient—distribution of bile salts between 1-octanol and aqueous buffer □ Thermodynamics—distribution of bile salts between 1-octanol and aqueous buffer □ Surfactants—distribution of bile salts between 1-octanol and aqueous buffer

Bile salts are biological detergents which play an important role in the dissolution or dispersion of cholesterol and other lipids in the body (1, 2). The solubility of cholesterol in the sodium cholate–water and the sodium cholate–lecithin–water system was studied (3). It was shown (4) that bile salts were capable of solubilizing a large number of poorly water soluble organic and inorganic compounds. The solubilization of various steroidal hormones in bile salt solutions was investigated (5–7). It was demonstrated (8, 9) that the solubilities and dissolution rates of several unrelated poorly water-soluble drugs were

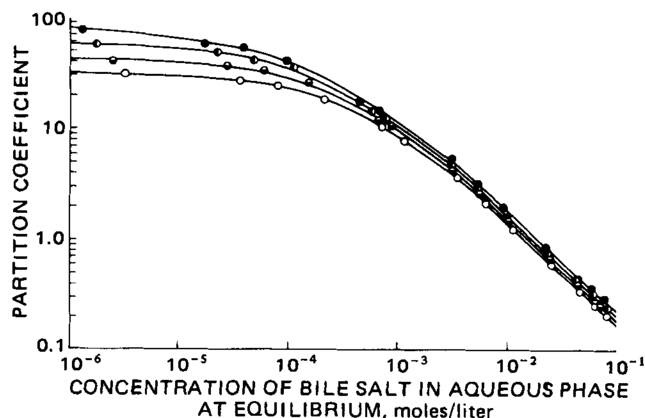

Figure 1—*Distribution isotherms for sodium deoxycholate at 25° (○), 35° (◒), 45° (◐), and 55° (●) between 1-octanol and 0.02* M *tromethamine buffer (pH 8). The solid line is calculated according to Eqs. 15*a *and* b.

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