Paper Chromatographic Determination of Trimethyloctadecyl Quaternary Ammonium Chloride in Dimethyldioctadecyl Quaternary Ammonium Chloride[1]

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A quantitative paper chromatographic method was worked out for determining small amounts of trimethyloctadecyl quaternary ammonium chloride in dimethyldioctadecyl quaternary ammonium chloride. With a mixed solvent containing methanol, acetone, and water, the trimethyl-type quaternary travels just behind the solvent front, whereas the dimethyl-type quaternary and other components move very little. The trimethyl quaternary spot is developed with bromphenol blue and the resulting color is measured photometrically at 607 mμ. Standards are run on either side of the sample spot and are used for quantitative calibration. Accuracy and reproducibility of the method appears to be within 0.4% absolute.

COMMERCIAL dioctadecyldimethyl quaternary ammonium chloride contains a small and slightly variable amount of octadecyltrimethyl quaternary ammonium chloride. During application studies on this product, a need arose for a method to determine the amount of the trimethyl compound in the dimethyl quaternary. Since the chemical properties of these two quaternaries are so similar, the common analytical methods do not distinguish between them. A separation of the trimethyl quaternary chloride was therefore desirable prior to its determination. Paper chromatography has been applied to quaternary ammonium compounds (1,2). However these applications dealt neither with this type of mixture nor with the separation and determination of a minor constituent. Of the several solvent systems investigated for separating the trimethyl and the dimethyl quaternaries, a mixture of methanol, acetone, and water gave the most effective separation. With this solvent mixture the trimethyl quaternary traveled just behind the solvent front, whereas the dimethyl quaternary and other known impurities moved only very slowly or not at all.

Fumasoni *et al.* (3) indicated that electrochromatographically-separated quaternaries could be developed with iodine or Supranolrot BB and quantitatively determined. Of the several methods which were tried during the present studies, dipping in a bromophenol blue solution proved to be the most satisfactory method for developing the chromatograms. Use of this same reagent by Garcia *et al.* (4) for development with quaternaries came to our attention after this technique had been adopted. Light absorption by the developed chromatograms was found to be proportional to the concentration of trimethyl quaternary. A simple paper strip holder was adapted to a Coleman Senior spectrophotometer and used for quantitative determinations. Two standard solutions containing trimethyl quaternary concentrations straddling the unknown concentration were run on either side of the sample in order to provide a direct calibration for each chromatogram.

Experimental

Reagents and Apparatus

Standard Solutions. Two standard solutions of dimethyldioctadecyl quaternary ammonium chloride in chloroform (13 mg./ml.) were prepared to contain the upper and the lower limits of expected trimethyl quaternary ammonium chloride concentration. One contained 1 mg./ml. and the other 0.5 mg./ml. of trimethyloctadecyl quaternary ammonium chloride.

Developing Solvent. Mixture of 700 ml. of absolute methanol, 1,050 ml. of A.R. acetone, and 1,435 ml. of water.

Color Developer. Bromphenol blue, 80 mg., was dissolved in 20 ml. of water containing 4 ml. of 0.1N aqueous sodium hydroxide. After adding 10 g. of sodium carbonate the solution was diluted to 100 ml. with water.

Chromatographic Paper. Schleicher and Schuell filter paper, grade No. 589-blue, was cut with the grain into strips 4 in. x 18 in. Four strips, placed together, were coiled and soaked for 20 hours at room temperature in a 1-liter beaker containing absolute methanol. After pouring off the methanol, the strips were removed by grasping the ends on the inside of the coil and lifting up, allowing the strips to unwind. The strips were dried in an 80°C. forced-air oven for 10 minutes and stored in a closed container. Care was taken not to bend the strips during handling in order to prevent distortion of chromatograms.

Chromatographic Jar. A cylindrical glass battery jar, approximately 6 in. in diameter and 18 in. high, was charged with 300 ml. of solvent one to four hours before each run. The paper strips were suspended from a glass rod, wedged inside the top of the cylinder by means of a rubber policeman on either end. The top of the jar was closed with a piece of foam rubber covered with a sheet of rubber dam and held down with weights.

Color Measurement. Paper strips were placed in a specially constructed holder for absorbance measurements in a Coleman Senior spectrophotometer (see Figure 1).

Procedure. Samples were dissolved in chloroform, sample sizes being chosen to give between 0.5 and 1 mg./ml. of trimethyl quaternary ammonium chlo-

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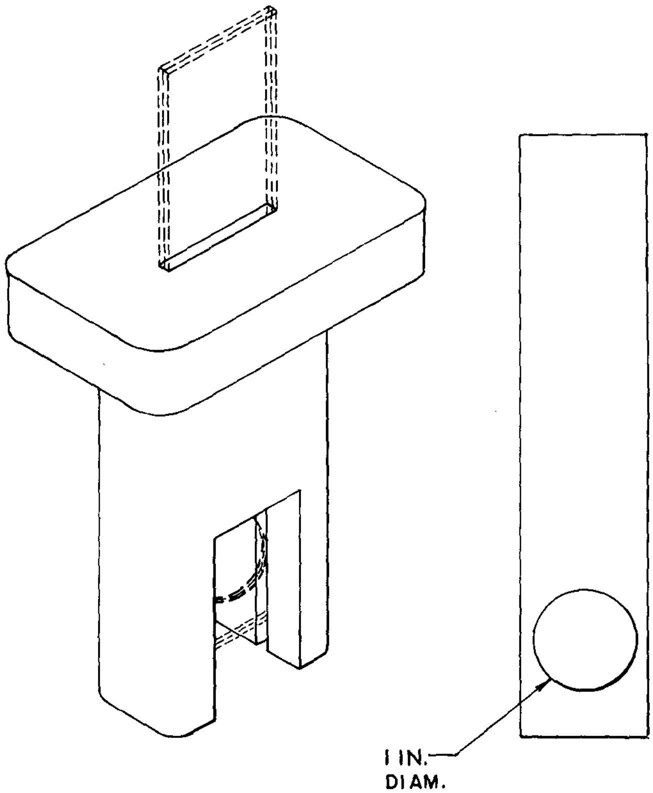

FIG. 1. Paper strip holder with metal plate.

ride, the concentrations of the two standard solutions. If the sample solution concentration was found to be outside this range, the concentration was adjusted and the run repeated.

A pencil line was drawn across the width of a paper strip (smooth side up) 1½ in. from one end. Three equidistant pencil dots were placed along this line starting ¾ in. from one edge of the paper. Another parallel line was drawn ½ in. from the same end, as a guide for depth of immersion in the solvent. A glass rod was laid under this end of the paper to keep it slightly elevated during the spotting operation. Solutions of 6 λ volume were spotted on the pencil dots from a 10 λ pipet graduated in 2 λ increments. Care was exercised to place the solutions on the paper in a reproducible manner, so that the wetted areas were approximately ½ in. in diameter. The center dot was used for the sample solution and the outer dots for the two standard solutions.

The strip was then carefully suspended in the chromatographic jar with ½ in. of the strip immersed in the solvent. The jar was covered and allowed to stand at a temperature of 23 to 27°C. After 16 hours the paper was removed, the location of the solvent front was marked, and the paper was allowed to dry by hanging for one hour at room temperature. The color was developed by holding the strip by its ends and slowly passing it back and forth three times through the bromphenol blue solution in a 6-in. Petri dish. The trimethyl quaternary appeared as a blue spot on a lavender background approximately ½ in. below the solvent front. Excess background color was immediately washed off both sides by holding the paper in a vertical position and directing a stream of water from a wash bottle along the top of the lavender color, allowing the water to flow down the paper over the spots. This washing, which required approximately 250 ml. of water, was continued until the background was white. See Figure 3 for final appearance.

The paper was allowed to air dry for one hour and then cut lengthwise into three strips, each containing a spot. Each of the three strips was placed in turn between the two metal plates, locating the spot in the center of the holes, and the plates were inserted into the plate holder. The plate holder was positioned in the spectrophotometer so that the light beam was centered on the middle of the spot. The absorbance of the spot was measured at 607 mμ, using a piece of unextracted paper as a blank. Absorbance *versus* milligrams of trimethyl quaternary was plotted for the two standard spots, and the concentration of trimethyl quaternary in the sample was calculated from this plot.

Discussion

In Table I are listed R_f values obtained on various high-molecular-weight fatty nitrogen derivatives. The R_f values indicate that the specified solvent system usually allows a separation of compounds having one long fatty chain from those having two. Commercial dimethyldioctadecyl quaternary ammonium chloride actually contains approximately a 3 to 1 ratio of octadecyl to hexadecyl alkyl groups. In the procedure, the trimethyloctadecyl and the trimethylhexadecyl quaternary ammonium chlorides travel together and are determined as one compound.

TABLE I

R_f Values with Methanol-Acetone-Water Solvent[a]

Compound	R_f
Trimethyloctadecyl quaternary ammonium chloride	0.92
Trimethyltallow quaternary ammonium chloride	0.93
Trimethyloctadecenyl quaternary ammonium chloride	0.93
Trimethylhexadecyl quaternary ammonium chloride	0.93
Trimethyldodecyl quaternary ammonium chloride	0.93
Trimethylcoco quaternary ammonium chloride	0.93
Dimethyloctadecylamine hydrochloride	0.92
Dimethyldioctadecyl quaternary ammonium chloride	......[b]
Dimethyldioctadecyl quaternary ammonium chloride[c]	......[b]
Methyldioctadecylamine hydrochloride	......[b]
Methyldioctadecylamine	......[b]
Dioctadecylamine hydrochloride	......[b]
Methyltrioctadecyl quaternary ammonium chloride	......[b]

[a] 8–12 Microgram quantities of indicated compound used in the described procedure.
[b] No significant movement.
[c] Commercial material purified by crystallization from acetone.

Besides trimethyloctadecyl quaternary ammonium chloride, the other minor constituents in commercial dimethyldioctadecyl quaternary ammonium chloride could include: methyltrioctadecyl quaternary ammonium chloride, methyldioctadecylamine, and methyldioctadecylamine hydrochloride. Unlike the trimethyloctadecyl quaternary ammonium chloride, these other components show no migration from the origin during chromatography.

Whatman No. 1 paper was tried in the initial work, but the developed spots were not as compact as those with the Schleicher and Schuell paper recommended in the procedure. Less compact spots also were noted upon using chromatographic jars with grossly different dimensions from those described in the procedure. It was found that the extraction of the chromatographic paper with methanol prior to use resulted in substantially increased absorbance readings for a given quantity of trimethyl quaternary chloride. Of several methods tried for extracting the paper, soaking several strips of paper in a beaker of methanol for 20 hours gave the best reproducibility in subsequent determinations. A clean working atmosphere is essential for reproducible results. Acid vapors cause fading of color in the spots. The color also fades slowly with time, being more noticeable with the highly colored spots as indicated in Table II.

Table III shows that recoveries of trimethyl quaternary chloride are essentially independent of the amount of dimethyl quaternary chloride over a wide range. This allows the adjustment of sample size in order to obtain optimum absorbance values.

TABLE II

Effect of Time on Color Intensity

Paper strip	Trimethyloctadecyl quaternary ammonium chloride	Absorbance	
		After 2 hours[a]	After 6 hours[a]
	mg./ml.		
1	0.5	0.17	0.17
1	1.5	0.64	0.59
2	0.5	0.13	0.15
2	1.5	0.70	0.65
3	0.5	0.15	0.14
3	1.5	0.63	0.57

[a] Time after developing color with bromphenol blue.

TABLE III

Effect of Sample Size on Color Intensity

Sample	Dimethyloctadecyl quaternary ammonium chloride	Trimethyloctadecyl quaternary ammonium chloride	Absorbance
	mg./ml.	*mg./ml.*	
1	67	1	.43
2	134	1	.44
3	268	1	.47

The linear relationship between the measured absorbance of the spots and the concentration of trimethyl-type quaternary is shown in Figure 2. The data in Figure 2 were obtained by running duplicate samples of synthetic mixtures containing the indicated percentages of trimethyloctadecyl quaternary ammonium chloride in dimethyldioctadecyl quater-

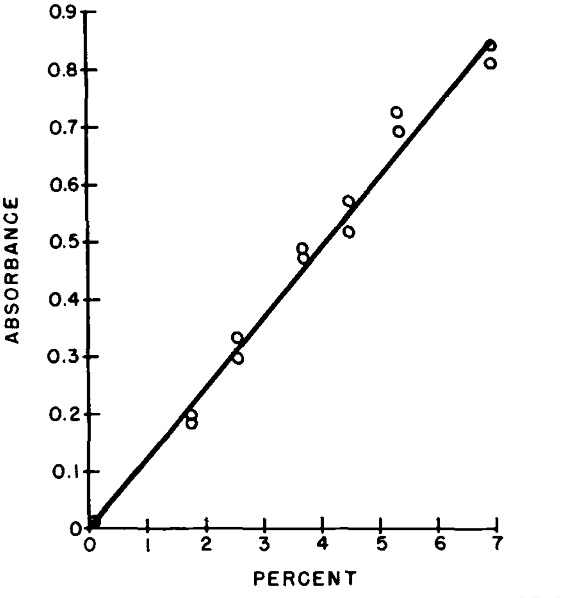

FIG. 2. Plot of absorbance *versus* percentage of trimethyl quaternary chloride for synthetic mixtures of trimethyloctadecyl and dimethyldioctadecyl quaternary ammonium chlorides.

TABLE IV

Analyses of Synthetic Mixtures and Commercial Samples

Quaternary ammonium chloride	Trimethylfatty quaternary ammonium chloride
	%
Synthetic dimethyldioctadecyl[a]	3.8, 4.3
Synthetic dimethyldioctadecyl[b]	7.2, 6.9
Synthetic dimethyldioctadecyl[c]	10.0
Synthetic dimethyldioctadecyl[d]	10.0
Commercial dimethyldioctadecyl	7.5, 7.0
Commercial dimethyldioctadecyl	5.9, 5.2
Commercial dimethyldioctadecyl	4.3, 4.1
Commercial dimethyldioctadecyl	6.8, 6.8, 6.5
Commercial dimethyldioctadecyl	4.0, 4.3, 3.8
Commercial dimethyldioctadecyl	4.9, 4.0
Commercial dimethyldioctadecyl	4.9, 4.4
Commercial dimethyldisoya	5.2, 5.0, 5.2

[a] Contains 3.6% trimethyloctadecyl quaternary ammonium chloride.

[b] Contains 7.2% trimethyloctadecyl quaternary ammonium chloride.

[c] Commercial material analyzing 4.7% trimethyloctadecyl quaternary to which has been added 4.8% trimethyloctadecyl quaternary ammonium chloride.

[d] Commercial material analyzing 5.6% trimethyloctadecyl quaternary to which has been added 4.7% trimethyloctadecyl quaternary ammonium chloride.

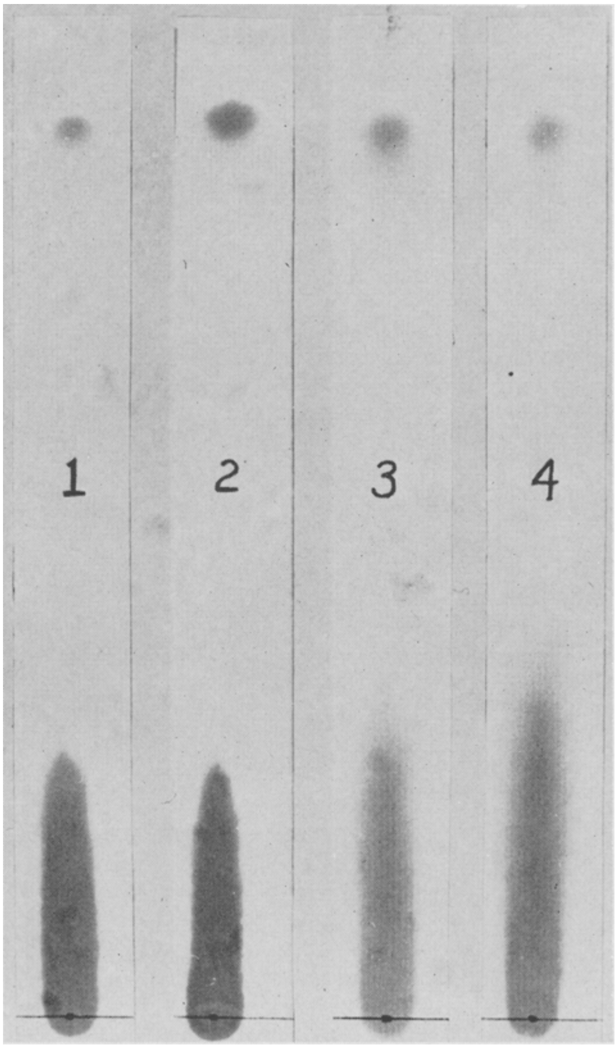

FIG. 3. Paper chromatograms of synthetic mixtures and commercial samples.

1. Synthetic mixture—1.8% trimethyloctadecyl quaternary ammonium chloride in dimethyldioctadecyl quaternary ammonium chloride.
2. Synthetic mixture—7.6% trimethyloctadecyl quaternary ammonium chloride in dimethyldioctadecyl quaternary ammonium chloride.
3. Commercial dimethyldioctadecyl quaternary ammonium chloride, analyzed 4.6% trimethyloctadecyl quaternary ammonium chloride.
4. Commercial dimethyldioctadecyl quaternary ammonium chloride, analyzed 3.9% trimethyloctadecyl quaternary ammonium chloride.

nary ammonium chloride. Although this linear relationship between concentration of trimethyl quaternary and absorbance was observed, there appeared to be some changes in the level of absorbance for a given range at different times. To overcome these variations and for best results during quantitative analysis, two standard mixtures, one containing less and the other more trimethyl quaternary than the sample, were run on either side of the sample on each paper. The absorbances of these two standards were then used as a calibration for calculating the amount of trimethyl quaternary in the sample.

Figure 3 is a photograph of several typical chromatograms of mixtures of purified compounds and of commercial materials.

Table IV lists results obtained on some synthetic mixtures and commercial samples.

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Fractionation of Castor Oil Methyl Esters by Liquid-Liquid Extraction

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LIQUID-LIQUID extraction technique has recently been applied to the fractionation of castor oil methyl esters obtained by the methylation of castor oil. A recent analysis of castor oil by Sreenivasan *et al.* (4) gives the following composition: ricinoleic acid 86.0%, dihydroxy stearic acid 1.9%, oleic acid 5.1%, linoleic acid 3.5%, and saturated acids 3.5%. McCormack and Bolley (3) indicate that while methyl esters other than methyl ricinoleate do not interfere with the ricinoleic acid reactions, it is nevertheless desirable to have the purest possible starting-material. They studied the separation of castor oil methyl esters by means of liquid-liquid extraction, using methyl alcohol and heptane at 8.4°C. and 24°C. It was observed that the hydroxyl value of esters in the methanol phase was greater than that of esters in the hydrocarbon phase. Kasturirangan and Laddha (2) studied the fractionation of castor oil methyl esters with furfural and cyclohexane at 30°C. The two-phase region for the system, castor oil methyl esters-furfural-cyclohexane, was much larger than for the system, castor oil methyl esters-methanol-heptane, at 24°C. and 8.4°C. indicating that furfural-cyclohexane gives a better performance. This fractionation of methyl esters of castor oil into fractions of preferably hydroxy and nonhydroxy esters is based on the observation that polar solvents will have more affinity for hydroxy esters. Further investigations indicated the possibility of the use of furfural-hexane and nitromethane-hexane as suitable solvents. In the present paper equilibrium and distribution data for the systems, castor oil-methyl esters-furfural-hexane and castor oil methyl esters-nitromethane-hexane, are presented. The effect of the change of concentration of the esters and solvents on fractionation of esters has also been studied.

Experimental

Materials used are given below.

Castor oil methyl esters were obtained by the methylation of castor oil, using HCl as catalyst. The mixed methyl esters thus obtained had the following physical and chemical constants: density $d^{30/4}$ 0.9250, refractive index 1.4602, specific rotation $[\alpha]^{30°/D}$ 5.48, saponification value 183.1, iodine value (Wij's, 30 min., 31°C.) 82.8, and hydroxyl value 165.8.

Furfural supplied by Allied Chemical and Dye Corporation, New York, was purified by vacuum distillation each time just before use. The specifications were density $d^{30/4}$ 1.124 and refractive index at 30° C. 1.5184.

Nitromethane from Fluka AG Chemische Fabrik Buschs SG. (Switzerland) was fractionally distilled, and the fraction between 100.5°–101.5°C. was collected and used. The specifications were density $d^{30/4}$ 1.1237 and refractive index at 30°C. 1.3770.

n-Hexane, as laboratory reagent-grade petroleum fraction supplied by B.D.H. Ltd., was rectified and the fraction collected between 67°–70°C. was used. The physical constants were density $d^{30/4}$ 0.6782 and refractive index at 30°C., 1.3761.

Experimental procedure. The equilibrium solubility data were determined by the synthetic method described by Treybal (5) at 30°C. For determining the phase distribution data, known mixtures of ternary constituents comprising castor oil methyl esters, polar and nonpolar solvents well within the heterogeneous region were placed in the constant temperature bath at 30 ± 0.1°C. after vigorous agitation and allowed to separate into two phases after attaining equilibrium. The individual phases were then separated and weighed. A weighed quantity of each phase was taken in a distilling flask, and the solvents were distilled in a water bath. Traces of solvents remaining were removed under vacuum by drying the esters on a water bath to constant weight under a stream of carbon dioxide. The total weight of the esters thus obtained from the two phases was checked against the weight of esters taken originally in the ternary mixture. The fractions of methyl esters thus obtained from the polar and nonpolar solvent phases were evaluated in terms of their hydroxyl values by following the pyridine-acetic anhydride method of Burton and Robert Shaw (1).