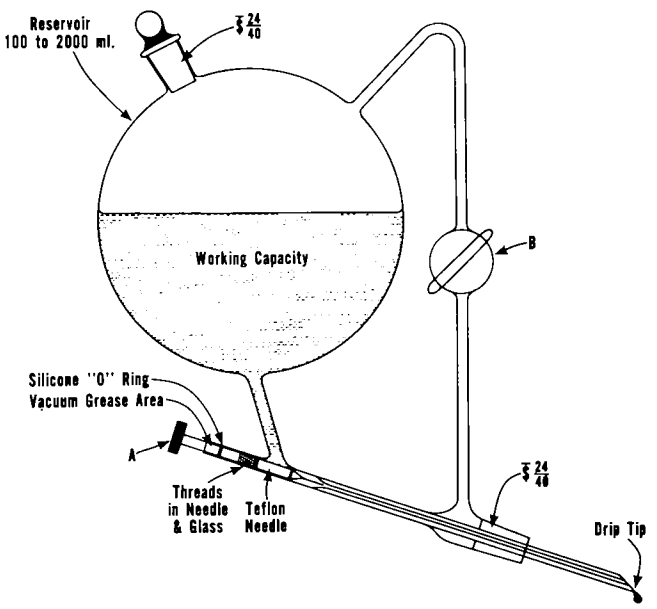

FIG. 1. Diagram of reservoir showing important features (Parts not drawn to uniform scale).

offers a distinct advantage over conventional stopcocks which invariably contaminate the sample by allowing it to flow over the lubricated area.

Samples may be degassed before distillation through vacuum stopcock B. When extremely high vacuum is not required, traces of solvent may be degassed from the sample during distillation. The completely accurate and fine flow control of the needle valve makes this operation possible. With conventional stopcocks it is often impossible to adjust flow if degassing is not carried out before distillation.

Reservoirs based on this design can save much time and frustration during molecular distillations. These savings, combined with low cost and ease of fabrication, make them particularly attractive for either small samples or large quantities sufficient for a full day's operation.

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Modified Silver Ion Thin-Layer Chromatography

SINCE THE INCEPTION of the technique, many papers have appeared in the lipid field citing the use of the silver ion in thin-layer chromatography (TLC), countercurrent distribution, and column chromatography. The results presented here demonstrate that *ammoniacal* silver ion plates have advantages over those prepared from aqueous solutions.

Commercially available Silica Gel G (30 g) was slurried with 60 ml of 12.5% silver nitrate in 28–30% ammonium hydroxide. The procedure of Barrett et al. (1) was used for preparing aqueous silver nitrate layers. Two parts of 12.5% aqueous silver nitrate solution were mixed with one part silica gel (wt/wt). Uniform 0.25-mm layers were then spread on 2 × 20-cm and 20 × 20-cm glass plates with a Colab No. 2810 applicator (Colabs Lab., Inc., Chicago Heights, Ill.) modified in this laboratory (2). After the chromatoplates had air dried for 30 min, they were activated in an oven for 30 min at 110C. Approximately 40 μg of the fatty acid methyl esters (obtained from the Hormel Foundation, Austin, Minn.) in 5 μl of chloroform were applied approximately 1 cm from the lower edge of the plate. Development of the chromatoplates was carried out in a chamber saturated with chloroform-ethanol 99:1 (v/v). The separations were visualized either by spraying with a 0.2% solution of 2′, 7′-dichlorofluorescein in ethanol, or by charring according to the procedure of Privett and Blank (3) or Barrett et al. (4). The charring procedure was used when the results were to be documented by photography. Because of uncontrollable environmental factors, such as relative humidity, the ammonium hydroxide and aqueous prepared plates were always handled simultaneously from preparation through development to insure the validity of the comparison. Solvents and other reagents were reagent grade and used without further purification.

Methyl stearate, methyl oleate, and methyl linoleate were used for comparison of the resolution obtainable on each of the different plate preparations. Approximately 40 μg of each methyl ester was spotted on the plates and developed by the ascending technique. Figure 1 shows the resolution of the esters on Silica Gel G plates impregnated with the ammonium hydroxide solution of silver nitrate (right) and with the aqueous silver nitrate solution (left). Resolution on the ammonium hydroxide silver ion plates was always superior to that of the aqueous prepared silver ion plates; in addition, the resolving power of the plates was maintained for a longer period. Silver ion plates prepared with aqueous solution lost their resolving power, presumably through the absorption of water, approximately twice as fast as the ammoniacal plates under the same conditions. On the silver ion plates, without ammonia, the distance of separation between the saturates and the monoenes was much smaller than the distance between the monoenes and the dienes (Fig. 1). With the ammoniacal silver ion plates, these distances were more nearly equal. No hydrolysis on the plate was observed in either case, as judged by the absence of polar hydrolytic products appearing as spots near the origin. Plates prepared by both methods appeared to have equal loading capacity as determined with methyl linoleate (10–100 μg). An increase in concentration was accompanied by an increase in R_f value.

Special precautions should be taken to protect silver ion TLC plates from moisture, air, organic matter, and light (causing them to darken). After exposure to direct sunlight for 1 hr, no noticeable darkening of the ammoniacal silver ion plate was observed; however, plates prepared without ammonia turned brownish black during the same period. Although the rate of discoloration is greatly reduced in the ammoniacal silver ion plates, it is not prevented: after two or three days unprotected plates on a laboratory bench

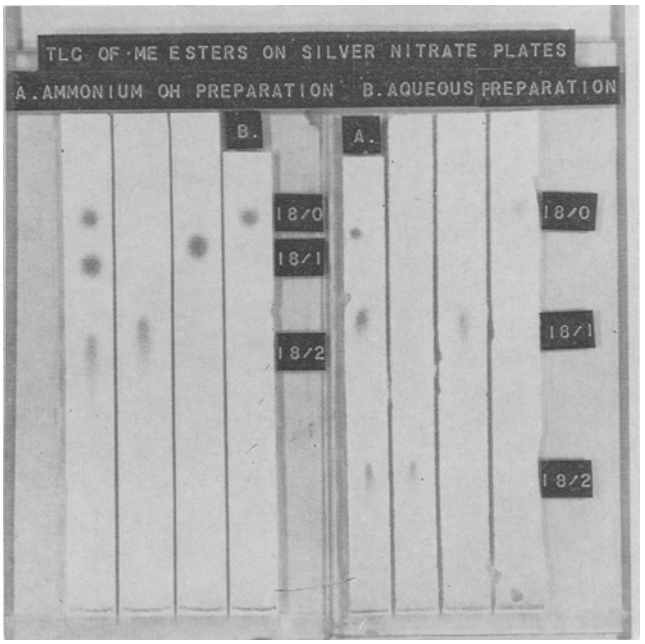

FIG. 1. Comparison of the resolution of methyl stearate, methyl oleate, and methyl linoleate on TLC plates impregnated with silver nitrate. *A*, plate prepared with an ammonium hydroxide solution. *B*, plate prepared with an aqueous solution.

do become darkened. Plates stored in a desiccator protected from light do not show discoloration even after four days.

In a comparison between the ammoniacal silver ion and the aqueous silver ion solutions for corrosiveness, the former was found to be less corrosive toward most metals used in the construction of TLC applicators and spreaders than the latter.

Kolthoff and Sandell (5) have reported that slightly soluble metal hydroxides dissolve in ammonia with the formation of complex ammino ions. From this supposition, the following set of equations was formulated:

1. $AgNO_3 + NH_4OH \leftrightarrows AgOH\downarrow + NH_4NO_3$
2. $NH_4OH \rightleftarrows NH_3 + H_2O$
3. $AgOH + 2NH_3 \rightleftarrows Ag(NH_3)_{2+} + OH^-$

When a stoichiometric amount of ammonium hydroxide was added to the silver nitrate, a precipitate was formed, in agreement with Equation 1. Upon the addition of more ammonium hydroxide, the precipitate dissolved, and is in agreement with Equation 3. The electrophilic ammino ion (Equation 3) apparently forms a stronger coordination complex with the nucleophilic π bonds than does the silver ion alone. This assumption is supported by the fact that improved resolution was obtained in the separation of the fatty acid methyl esters using TLC plates impregnated with the ammoniacal silver ion.

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On the Origin of the More Saturated Hydrocarbons of Skin Surface Lipid

HYDROCARBONS OTHER THAN SQUALENE or a hydrocarbon that gives the Liebermann Burchard test, have been consistently reported in studies of surface lipids of man and animals (1). Although a part of this material undoubtedly represents external contamination (1) (since the fraction resembles petroleum hydrocarbons), and although biosynthesis is unlikely since incubation of 1-C^{14} acetate with human skin slices failed to incorporate label into hydrocarbons under conditions where fatty acids, squalene and cholesterol became labelled (2), such material could still enter the body through the diet and be excreted intact through the skin. In support of this idea, cats fed hexadecane deposited it in the skin and in other tissue (3). Furthermore, rats fed a diet containing 50% mineral oil became greasy and appeared to excrete oil through the skin (4).

To determine whether the more saturated hydrocarbons of skin surface lipid could originate from excretion of dietary hydrocarbon, normally present at low levels, rats were fed 1-C^{14} octadecane, their skin surface lipid was wiped off at intervals, and the hydrocarbon fraction was separated and assayed for radioactivity as follows: Five male Sprague Dawley rats (about 290 g each) were individually stomach fed 1.8 mg 1-C^{14} octadecane (5.0×10^7 counts/min/g, New England Nuclear Corp., Boston, Mass.) in 0.50 ml corn oil. Paired control rats were stomach fed 0.50 ml corn oil only. Food was withheld on the day before stomach feeding but was available subsequently *ad libitum*. Several days before stomach feeding each rat was shaved from the neck to the base of the tail and from flank to flank (with clippers previously soaked in $CHCl_3$ to remove lubricating oil), then fitted with a large conical plastic collar which extended beyond the tip of the nose thus preventing oral contact with the body, feet and anus. Each rat was housed in a clean cage equipped with a wide net wire screen to minimize contamination from excrement.

At intervals as indicated in Figure 1, their backs were wiped with hexane soaked fat-free cotton pledgets. After each wiping the rat fed labelled hydrocarbon was moved to a new cage and his paired control was moved into the cage he vacated. If the animals could contaminate their backs from excrement on the cage, then the surface lipid of the paired control rats should have become radioactive. The surface lipid of the control rats had the same activity as background. This showed that the animals were not being contaminated with their excrement.