JOURNAL OF LIPID RESEARCH
ASBMB
Downloaded from www.jlr.org by guest, on June 19, 2012

Separation of 1- and 2-monoglycerides by thin-layer adsorption chromatography on hydroxyl-apatite

ALAN F. HOFMANN

Department of Physiological Chemistry, University of Lund, Sweden

[Received for publication March 26, 1962]

» Hydroxyl-apatite was introduced by Tiselius (1) in 1954 as an adsorbent for chromatography of proteins.

It has subsequently proved to be of value for the separation of plasma proteins (2), especially lipoproteins (3). We have recently used this adsorbent for the thin-layer chromatography of proteins (4), preparing suitable layers by means of a polymeric binding agent (Zytel 61, an alcohol-soluble polyamide[1]).

It was learned later that quite satisfactory layers could be prepared without a binding agent and that such layers showed many interesting properties when applied to the chromatography of lipids. The adsorbent is considerably weaker than (activated) silicic acid and has a lower capacity, but it seems useful for the separation of certain relatively polar lipid classes. The definition of the spots is improved by adding calcium sulfate as a binding agent but still remains slightly inferior for polar lipids to that obtained on layers of Silica Gel G,[2] the adsorbent most commonly employed for lipid separations (5). Nonpolar lipids show considerably inferior definition.

We report here preparation of the plates and conditions for the separation of 1- and 2-monoglycerides. In agreement with Privett and Blank (6), we have been unable to separate these isomers on thin layers of Silica Gel G by either adsorption or partition chromatography.

Hydroxyl-apatite was prepared as described by Anacker and Stoy (7), and passed through a 150-mesh sieve. For the preparation of the plates, a commercial apparatus was used.[3] Ten grams of hydroxyl-apatite was stirred with 800 mg of analytical-grade calcium sulfate, homogenized in 37–39 ml of distilled water, and spread. The plates were allowed to dry at room temperature; activating the plates by heating at 150° for 60 min greatly improved their chromatographic properties for low-temperature work. Plates prepared in this way were superior with respect to resolution to plates prepared without binder; with 1%, 4%, or 15% calcium sulfate; or 0.5%, 1%, or 4% Zytel 61 as binder. For preliminary experiments, microscope slides were coated as described previously (8).

1-Monoolein was a generous gift of Distillation Products, Rochester, N.Y., and contained about 5% 2-isomer, as assayed by periodate oxidation (9, 10) before and after perchloric acid-induced isomerization (11). 2-Monoolein (containing 5% 1-isomer) was synthesized by Drs. Lennart Krabisch and Bengt Borgström in this laboratory; details of this synthesis are to be published.

When chromatography was attempted at +10°, the isomerization seemed pleasingly less, but the resolution became strikingly worse. Separation could only be obtained with aliphatic ketones as developing solvent. The most satisfactory system was methyl isobutyl ketone alone. A mixture of methyl propyl ketone and methyl *n*-amyl ketone 1:1 (v/v) was less satisfactory but gave moderate definition and clean separation. With methyl isobutyl ketone as developing solvent,

[1] E. I. DuPont de Nemours Co., Inc., Wilmington, Delaware.
[2] E. Merck AG, Darmstadt, Germany.
[3] C. Desaga, GmbH, Heidelberg, Germany.

Separation of 1- and 2-monoglycerides occurred readily with many solvent combinations and with a few single solvents at room temperature. The best single solvent was methyl propyl ketone. Satisfactory solvent combinations were dioxane and iso-amyl acetate 1:1 (v/v) or dioxane and di-*n*-butyl ether 1:1 (v/v). However, considerable isomerization took place with these and every solvent system used, as evidenced by three spots that appeared when two-dimensional chromatography of the 2-monoglyceride was performed; 1-monoglyceride being formed during chromatography in each direction.

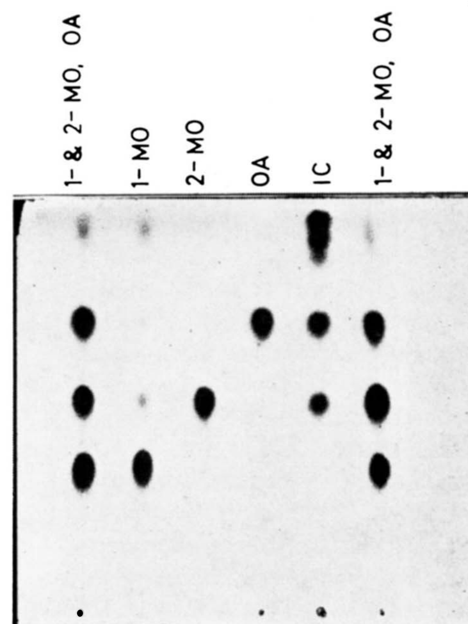

FIG. 1. Photograph of a typical chromatogram. From left to right: a mixture of 1- and 2-monoolein and oleic acid (1- & 2-MO, OA); 1-monoolein (1-MO); 2-monoolein (2-MO); oleic acid (OA); lipids from fresh, human-intestinal content obtained by intubation during absorption of a meal rich in lipid (IC) (2 ml of intestinal content was collected in 6 ml of heptane–petroleum ether–ethanol 1:1:1 (v/v), and a 5-μl aliquot of the upper phase applied to the plate); a mixture of 1- and 2-monoolein and oleic acid (1- & 2-MO, OA). About 10 μg of each model compound was applied. Adsorbent: hydroxyl-apatite with calcium sulfate as binder, prepared as described. Developing solvent: methyl isobutyl ketone at +10°. Detection with 10% ethanolic phosphomolybdic acid. The plate was predeveloped with acetone to remove substances that might react with phosphomolybdic acid and that travel with the front.

Downloaded from www.jlr.org by guest, on June 19, 2012

typical R_f values were: 1-monoglycerides, 0.42; 2-monoglycerides, 0.59; and fatty acids, 0.78 (see Fig. 1). All phospholipids remained at the origin; diglycerides, cholesterol, and triglycerides moved close to the front. The R_f values of the respective monoolein and monopalmitin isomers were identical. Two-dimensional chromatography of 100 μg of 2-monoolein showed very little apparent isomerization, the degree of isomerization being estimated as less than a few per cent.

Both the plate and the chromatography chamber containing the developing solvent were left at +10° for several hours to equilibrate before the samples were applied. The samples should be applied shortly before the chromatogram is run, as irreversible adsorption as well as some isomerization may occur if the sample is applied to the plate several hours before. After completion of the chromatographic run, the solvent was evaporated from the plate in a well-ventilated oven at 150°. The plates were then sprayed with a solution of phosphomolybdic acid in ethanol, 10 g/100 ml.

Although separation of isomeric monoglycerides is thus easily obtained, 1,2- and 1,3-diglycerides generally move together on hydroxyl-apatite. They can be separated by using chloroform–heptane 3:7 (v/v), but this separation is less satisfactory than that observed on Silica Gel G by using diethyl ether–petroleum ether mixtures (6, 12) or acetone–cyclohexane 15:85 (v/v).

It might be noted that the reactivity of phosphomolybdic acid, 10 g/100 ml absolute ethanol, which is an excellent spraying reagent for detecting lipids after separation by thin-layer chromatography on many adsorbents, is strongly influenced by temperature. Certain compounds (e.g., monopalmitin and 3α-hydroxy-12-keto-cholanic acid) do not react at 100° but react vigorously when the plate is heated to 160–180°. Furthermore, even with highly reactive lipids such as cholesterol, free or conjugated bile acids, and unsaturated glycerides or fatty acids, the contrast between spot and background is much greater when the plate is dried at 160–180° after chromatography and then sprayed immediately upon removal from the oven while still hot. Intense blue spots appear instantaneously against a pale-yellow background; no reheating is necessary.

Hydroxyl-apatite therefore appears to be an adsorbent that possesses valuable characteristics for the chromatographic resolution of mixtures of polar lipid classes. Recent experiments in our laboratory have also shown good resolution of choline phospholipids and bile acid derivatives. Hydroxyl-apatite may well prove to be a valuable alternative to silicic acid for column chromatography of lipids including steroids.

The author expresses his gratitude to Dr. Bengt Borgström who pointed out the value of hydroxyl-apatite as an adsorbent and made many helpful suggestions. This project was made possible by the generous gift of pure 2-monoglycerides prepared by Dr. Lennart Krabisch in this laboratory.

This work was done during the tenure of a postdoctoral fellowship of the National Heart Institute, U.S. Public Health Service. Additional support from the U.S. Public Health Service (Grant H-5302, Metabolism) is gratefully acknowledged. Miss Bodil Åkesson gave excellent technical assistance.

REFERENCES

1. Tiselius, A. *Arkiv. Kemi* **7**: 443, 1954.
2. Hjertén, S. *Biochim. et Biophys. Acta* **31**: 216, 1959.
3. Borgström, B., C. Naito, and P. Wlodawer. *Acta Physiol. Scand.* **54**: 359, 1962.
4. Hofmann, A. F. Protides of the Biological Fluids (Proc. 10th Colloq.) Hub. Peeters, Ed., Amsterdam, Elsevier Publishing Co., 1962 (in press).
5. Mangold, H. K. *J. Am. Oil Chemists' Soc.* **38**: 708, 1961.
6. Privett, O. S., and M. L. Blank. *J. Lipid Research* **2**: 37, 1961.
7. Anacker, W. F., and V. Stoy. *Biochem. Z.* **330**: 141, 1958.
8. Hofmann, A. F. *Anal. Biochem.* **3**: 145, 1962.
9. Desnuelle, P., and M. J. Constantin. *Biochim. et Biophys. Acta* **9**: 531, 1952.
10. Pohle, W. D., and V. C. Mehlenbacher. *J. Am. Oil Chemists' Soc.* **27**: 54, 1950.
11. Hartman, L. *J. Sci. Food Agri.* **11**: 191, 1960.
12. Hofmann, A. F., and B. Borgström. *Federation Proc.* **21**: 43, 1962.

ASBMB
JOURNAL OF LIPID RESEARCH
Downloaded from www.jlr.org by guest, on June 19, 2012