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One-stage solvent system and one-dimensional thin-layer chromatographic separation of milk simple lipid classes on 20 × 20 cm plates

MANNERS *et al.*[1] reported that no one-stage TLC solvent system was availabl for separating the main simple lipid classes. But STORRY AND TUCKLEY[2] used a one stage, one-dimensional system on 20 × 34 cm plates for separating blood plasm simple lipids of a lactating cow. Several two-stage, one-dimensional systems hav been used with 20 × 20 cm[1,3] and 20 × 34 cm[4,5] plates. These techniques were use to separate simple lipid classes of gut contents[1], serum enzyme digest[3], pig intestine and plasma[4] and rat liver[5]. It is well known that the amount of each lipid class fron any biological extraction will vary with the source, and efficient separation by TL will require a particular solvent system. Because the simple lipids in cow's milk con tain *ca.* 98% triglyceride, it is difficult to separate free fatty acids and cholesterc esters. This was found when the techniques of KELLEY[3] and STORRY AND TUCKLEY were studied in our laboratory.

We believed that a one-stage solvent system for separating the main simpl lipid classes with standard TLC equipment would be helpful to other investigators Such a system was developed for the simple, rapid and efficient separation of simpl lipid classes in milk.

Methods

All solvents were ACS grade and used as obtained from the manufacturer Silica Gel G (according to Stahl) was purchased from Brinkman Instruments, Inc. N.Y. Plates were activated for 1 h at 120° and stored in a desiccative cabinet unti used. Approximately 1 h before use, a developing chamber was saturated with vapor of its solvent. The solvent systems moved 15 cm from the point of spotting. The spot were observed by spraying the plates with H_2SO_4–H_2O (1:1) and heating in an ove at 120° until the organic material charred.

The solvent system of VOGEL *et al.*[6], utilizing petroleum ether–diethyl ether formic acid (180:20:2) with plates 0.25 mm thick (Fig. 1A), was compared with a nev solvent system of hexane–ethyl acetate–formic acid (175:25:2) with plates 0.50 mn thick (Fig. 1B). Plates were prepared with a Desaga adjustable applicator using suspension of Silica Gel G in 0.01 *M* sodium carbonate[7].

The reference mixture of simple lipid classes (Fig. 1A and B, No. 1) containe *ca.* 10 μg of each of the following compounds: monoolein, 1,2- and 1,3-dioleins cholesterol, free fatty acid (C_4–C_{18}, C_{18-}, $C_{18=}$), triglycerides (trilaurin, tripalmitin tristearin, tripalmitolein and triolein) and cholesterol acetate. The monoglyceride diglycerides and unsaturated triglycerides were purchased from Applied Scienc Laboratories, Inc., State College, Pa.; the others from Fisher Scientific Co., Medford Mass. They were dissolved in chloroform–methanol (2:1) and the mixture applied t the plates with a disposable micropipet.

An application of the technique was witnessed in a lipolysis study of cow's mill obtained from the University Dairy Plant. Lipid hydrolysis was induced by mixin equal portions of raw and homogenized milks, heating the mixture to 30°, and placin it in a refrigerator overnight at 4°. Free fatty acid values (FFA) were determined b

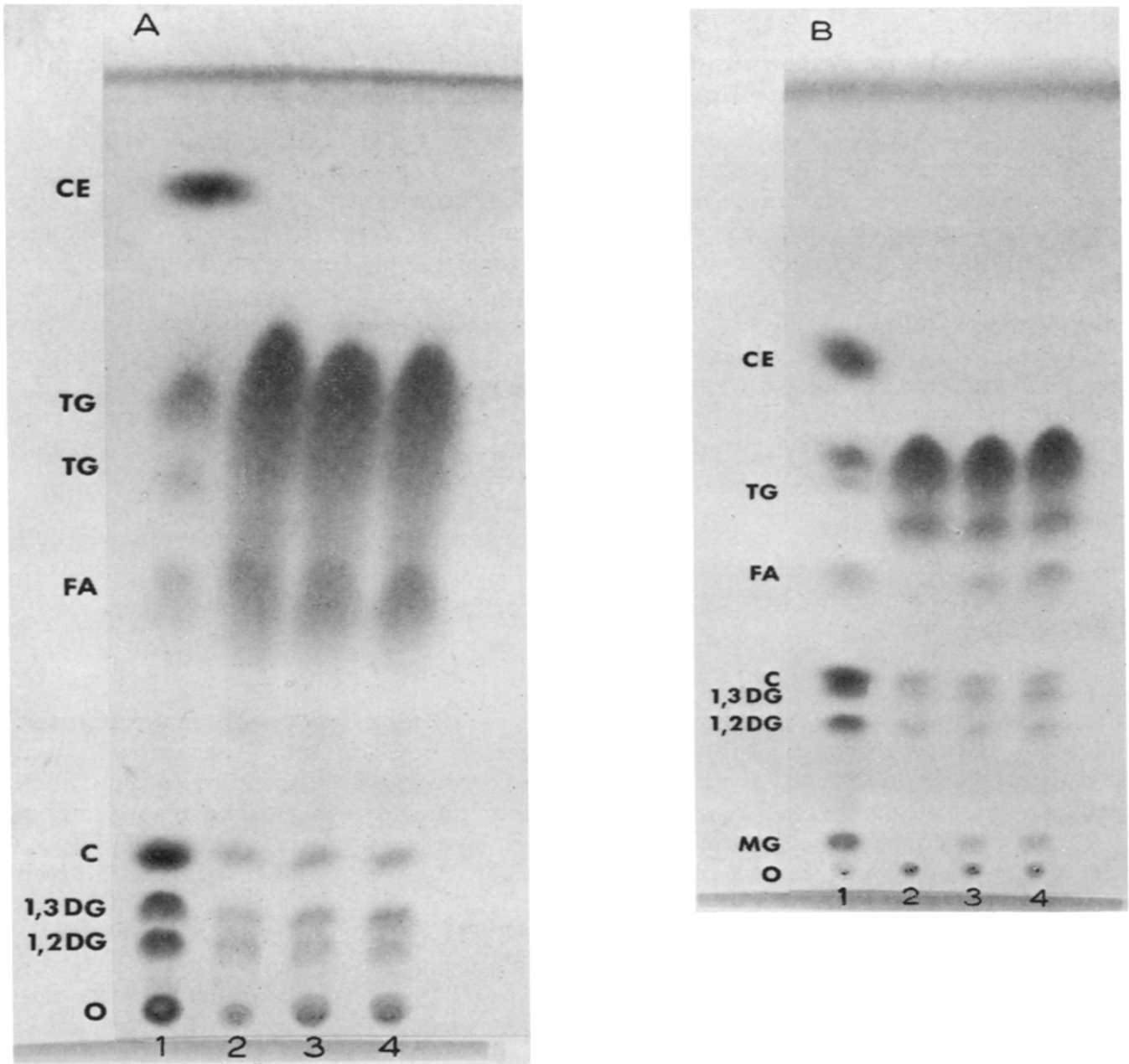

Fig. 1. Separation of simple lipid classes in two solvent systems. A, VOGEL *et al.*[6] solvent system of petroleum ether–diethyl ether–formic acid, 180: 20: 2, and B, our solvent system of hexane–ethyl acetate–formic acid, 175: 25: 5. Abbreviations: O = origin; MG = monoglyceride; 1,2DG = 1,2-diglyceride; 1,3DG = 1,3-diglyceride; C = cholesterol; FA = free fatty acid; TG = triglyceride; CE = cholesterol ester. Samples: (1) reference mixture of simple lipid classes; (2) total milk lipid extract, FFA 0.58; (3) total milk lipid extract, FFA 7.86; (4) total milk lipid extract, FFA 18.50.

the method of THOMAS *et al.*[8], as modified in our laboratory[9]. Three samples with specific FFA were prepared by mixing appropriate volumes of pasteurized hydrolyzed milk with pasteurized milk. Milk lipid (Fig. 1A and B, No. 2–4) was extracted by the method of MOJONNIER AND TROY[10], dissolved in chloroform–methanol (2:1), and spotted on the plates at a concentration of *ca.* 175 μg with disposable micropipets.

Results and discussion

VOGEL *et al.*[6] developed a one-stage TLC solvent system for rapid separation of most simple lipid classes on 20 × 20 cm plates. It has been the common solvent system used in our laboratory. But when reference simple lipid classes are analyzed (Fig. 1A, No. 1), the monoglycerides remain at the origin. And when milk lipid extracts are analyzed (Fig. 1A, No. 2–4), the free fatty acids trail with the triglycerides and the monoglycerides remain at the origin with complex lipids. The influence of lipid class concentration on separation ability is seen on this plate.

Our new solvent system overcomes these problems (Fig. 1B). With reference simple lipids (No. 1), the monoglycerides move off the origin. And the separation of free fatty acids from milk lipid triglycerides is distinct (No. 2–4). Excellent separation of the main simple lipid classes except 1,3-diglyceride and cholesterol is seen in Fig. 1B.

An application of the differences in solvent systems is witnessed in Fig. 1A and B, No. 2–4. As lipolysis proceeds, the amount of monoglyceride and free fatty acids increases. This is clearly seen only with the new solvent system (Fig. 1B).

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