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AN IMPROVED AUTOMATIC LIPID EXTRACTION AND THIN-LAYER SPOT APPLICATION SYSTEM*

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SUMMARY

The principle of operation was described in detail in a previous publication. The main features of the improved system are much greater speed of application, a simpler and more flexible programming system and simpler operation.

INTRODUCTION

An improved version of a previously described eight-channel automatic lipid extraction and thin-layer plate applicator system[1] has been developed. The basic operational principle of the original system is preserved in the new model. The key features of both systems are the use of programmed flow for automatic protein removal and lipid extraction directly from serum samples combined with thin-layer chromatographic (TLC) application of the lipid extracts.

Each channel has a container for lipid extraction connected by Acidflex tubing through an Auto-analyzer pump to a TLC applicator needle. Extraction containers are prepared from disposable Oxford sampler pipette tips by inserting a small cotton filter into their lower, narrower end, which is connected to the pump tubing. The applicator needles are supported vertically in a manifold, and their tips rest on a TLC plate placed on a hot plate.

Serum is added to isopropanol in each extraction container, and proteins are completely precipitated in 2 min and retained in the extraction chambers by the cotton filters; lipid extracts are then transferred onto the heated TLC plate by intermittent pumping at a rate allowing for continuous evaporation of isopropanol under streams of warmed air or nitrogen. The lipids accumulate on the plate in eight small spots, one for each channel. Solvent is added proportionally into the extraction chamber from a common reservoir[1].

Except for the temperature-controlled water-bath containing a coil for heating the drying gas and the programmer in the new system the various functional parts are integrated into a single housing measuring 25 × 34 × 75 cm (Fig. 1), containing only a single Auto-analyzer pump (B) as opposed to two such pumps in the original system. Since the Acidflex tubing used is quite small (0.02 in. I.D.) the sixteen pump

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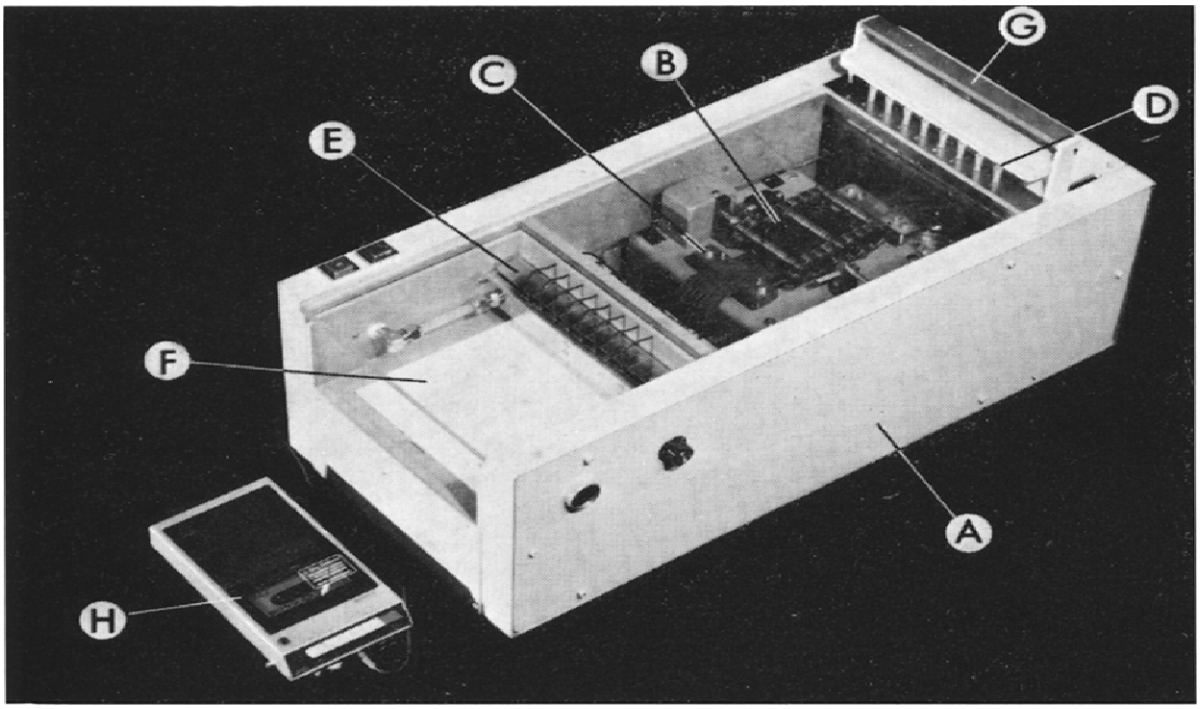


Fig. 1. The new automatic lipid extraction and thin-layer chromatography application system: A = Housing; B = peristaltic pump; C = end block; D = extraction containers; E = applicator manifold; F = thin-layer plate; G = sliding support for refill tubes; H = magnetic tape unit.

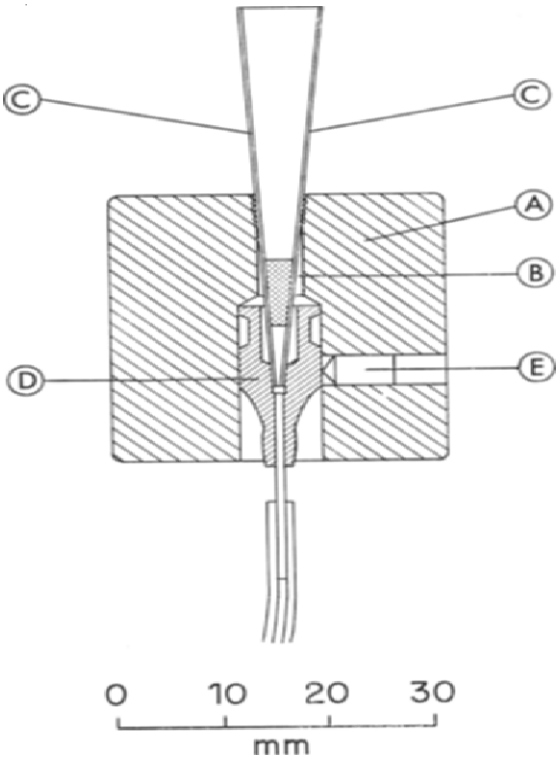
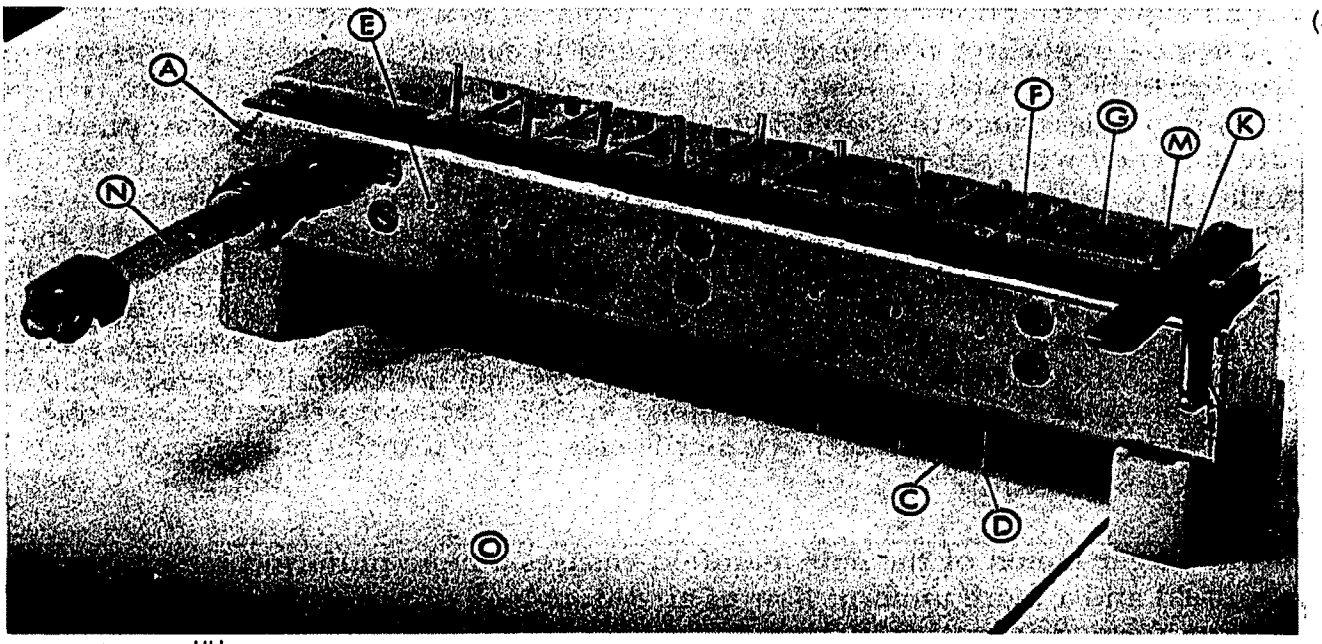


Fig. 2. Cross-section of extraction container support block. A = Support block; B = guide hole; C = extraction container; D = needle hub; E = set screw.

tubings needed are easily put on one pump using new end blocks (C). A new extraction container support block (D) as well as a new applicator manifold (E) have been developed. The TLC plate rests on a built-in temperature-controlled hot-plate. A simpler programming system using a Sony Cassette recorder (H) was also developed.

The extraction container support block consists of a 30 × 30 × 250 mm plated



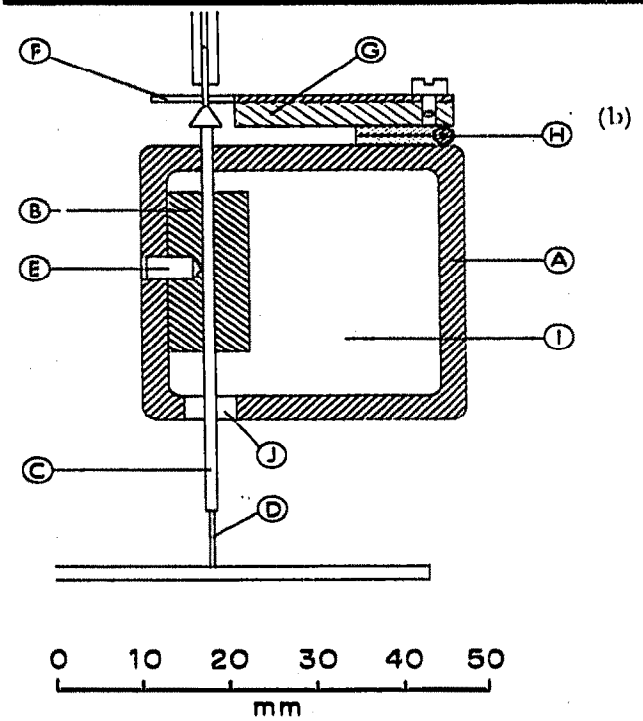


Fig. 3. New applicator manifold: (a) before insertion into applicator unit; (b) cross-section. A = Manifold tube; B = needle support plate; C = support tube; D = applicator needle; E = set screw; F = spring; G = leveler plate; H = hinge; I = manifold lumen; J = vent hole for drying gas; K = leveler bar; L = leveler spring; M = leveler adjustment screw; N = gas supply tube; O = thin-layer plate.

brass block (Fig. 2) (A) with a guide hole (B) for each of the extraction containers (C) the lower end of which fits directly into the hub of 22-gauge Luer hypodermic needles (D) fastened into the block by set screws (E). The tops of the guide holes (B) are threaded so that when the extraction containers are inserted into the manifold and twisted one-half turn, the lower tips of the extraction containers are pressed air-tight into the Luer hubs. One advantage of the new extraction container support block is that all eight extraction containers can be replaced in a matter of seconds.

The new applicator manifold (Figs. 3a and b) consists of a 40 × 40 × 250 mm square steel tube (A) with an inside 10 × 20 × 200 mm needle support plate (B), in which 55-mm long, gauge 18 stainless steel tubes (C) supporting the applicator needles (D) are fastened with set screws (E). All applicator needles are lifted vertically off from the TLC plate simultaneously by individual flat springs (F) fastened to the common leveller plate (G), which swings around the pivot point of the hinge (H) that is fastened to the top of the manifold. The drying gas is introduced into the manifold lumen (I) from which it escapes through the 6-mm holes (J) located over the sites of application. A sliding bar (Fig. 1, G) located above the extraction containers serves as a support for the refill tubes, which are moved 75 mm horizontally during replacement and filling of the extraction containers.

RESULTS AND DISCUSSION

The larger lumen of the new manifold ensures a more uniform pressure within the manifold and a more uniform flow of air through all of the eight vent holes, thus assuring uniform evaporation at all application sites. The large hole size also ensures greater flow of drying gas at reduced velocity resulting in faster evaporation and smaller spots. The reduction in gas velocity also reduces the chance of disturbance of the adsorbent layer at the site of application. The larger distance between spots (20 mm) in the new model prevents overlapping between adjacent spots when large quantities are applied for preparative chromatography. The fact that all applicator needles can be lifted off or lowered onto the plate simultaneously is not only convenient, but also reduces plugging of the needle tips since the spring-loaded applicator needles can be set so that their tips barely contact the plate surface.

As pointed out in the previous article[1], the system should be operated in the following sequence: (1) a 2-min delay for protein precipitation, (2) transfer time, and (3) intermittent pumping. Bursts of 1 Kc/s sinus waves recorded on the tape in the above sequence trigger a frequency sensitive relay which engages the pump motor.

Experiments revealed that elevating the needle tips from the plate surface between each stroke of the pump greatly increases the speed of solvent evaporation. If the applicator needles are lowered onto the plate only during every second pump stroke considerable preconcentration of the lipid extracts occurs at the hanging drops on the needle tips. Tests indicate that the rate of application may be doubled by implementing this principle into the automatic system. Using intermittent pumping for 0.2 sec every 3 sec a 20:1 isopropanol extraction and simultaneous TLC application of lipids from eight 10-μl serum samples in less than 10 min appears possible.

REFERENCES

1 E. FOSSLIEN AND F. MUSIL, *J. Lipid Res.*, 11 (1970) 605.