notes on methodology

Automated tank for one- or two-dimensional thin-layer chromatography

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SUMMARY An automated one- or two-dimensional TLC tank is described. The solvent front is monitored by a photocell or recycling timers.

SUPPLEMENTARY KEY WORDS modular instrumentation · lipids · gangliosides

Chromatographic analysis of complex mixtures of lipids are carried out by various combinations of column and thin-layer chromatography (TLC). The latter is often preferred for rapid quantitative and (or) qualitative analysis. In a previous publication we described a method for automating the stepwise eluion of complex brain lipids on a Sephadex column by means of an all glass-Teflon system (1). The system has been expanded so that it can now also automatically perform one- or two-dimensional thin-layer chromatographic analysis.

The system, using snap-on-modules,[1] includes a 26 DC power supply[2] that operates commercially available recycling timers,[3] relays,[4] a photocell,[5] and solenoid valves[6] controlling pneumatically operated Teflon valves.[7] The tank is cut out of a single block of 10 × 10 inch Teflon and is grooved in two of its inner sides, *A* and *B*, in order to accommodate the thin-layer plate and the appropriate solvent (Fig. 1). It is attached to a 1 rpm motor[8] with a torque of 600 in oz (538 cm/g) by means of a clamp-type frame. The frame also holds the glass plates tightly against the Teflon block. The frame is made of two 15 × 1 inch aluminum bars connected at each end with a machine screw and wing-nut. The motor is connected via its shaft to one of the aluminum bars by a flange and reducing bushing. The photocell and its light source are attached by means of clamps to the tank frame and can be set at any predetermined height or position.

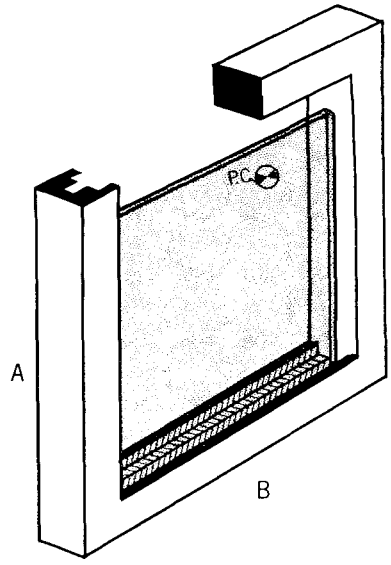

Fig. 1. Teflon tank with internal grooves on sides *A* and *B*. The stippled area represents the TLC plate, and *P.C.* represents the photocell. The tank has rotated 90° and is in position for second solvent run.

Fig. 2 shows the block-logic diagram of the TLC system. TLC for the major lipid extract (major brain lipids minus ganglioside) was performed according to the method of Rouser, Kritchevsky, and Yamamoto (2). The plate is placed into the tank, and 45 ml of the first solvent system is introduced. Glass plates are then placed against the Teflon tank and clamped firmly by means of the clamp frame. For the two-dimensional analysis of the major lipid extract the mode switch is placed in the photocell position. In this position the recycling timers Nos. 1 and 2 are cut out of the system, and the photocell is connected directly into timer No. 3 (drain-dry timer). When the system is activated the first solvent starts to rise. The thin-layer plate which is relatively opaque becomes translucent as the solvent front rises. When the solvent front reaches the prepositioned photocell which is arbitrarily set at a height of 15 cm from the origin, the translucence of the thin-layer plate at this point will permit activation of the photocell. The photocell sends a pulse that starts timer No. 3 (drain-dry timer). This timer, which has been preset for 30 min, opens Teflon valves *A* and *C*. Valve *A* allows nitrogen to flow into the tank and to exit with the first solvent through valve *C*.

Patent application submitted.

Abbreviations: TLC, thin-layer chromatography.

[1] Snap-on-modules Nos. SS4047J, 4066J, 4069J, and 4012J; Scientific Prototype Mfg. Corp., New York.

[2] Power supply No. 4027J; Scientific Prototype Mfg. Corp.

[3] Recycling timer No. 1419; Lehigh Valley Electronics Engineering & Mfg. Co., Inc., Fogelsville, Pa.

[4] Relays No. 1360; Lehigh Valley Electronics Engineering & Mfg. Co., Inc.

[5] Sigma Model 8A photocell unit; Allied Electronics Corp., Chicago, Ill.

[6] Solenoid valves, Sol-3-24VDC; Chromatronix Inc., Berkeley, Calif.

[7] Teflon valves, type CAV; Chromatronix Inc.

[8] RPM motor Allied No. 41F7159C; Allied Electronics Corp.

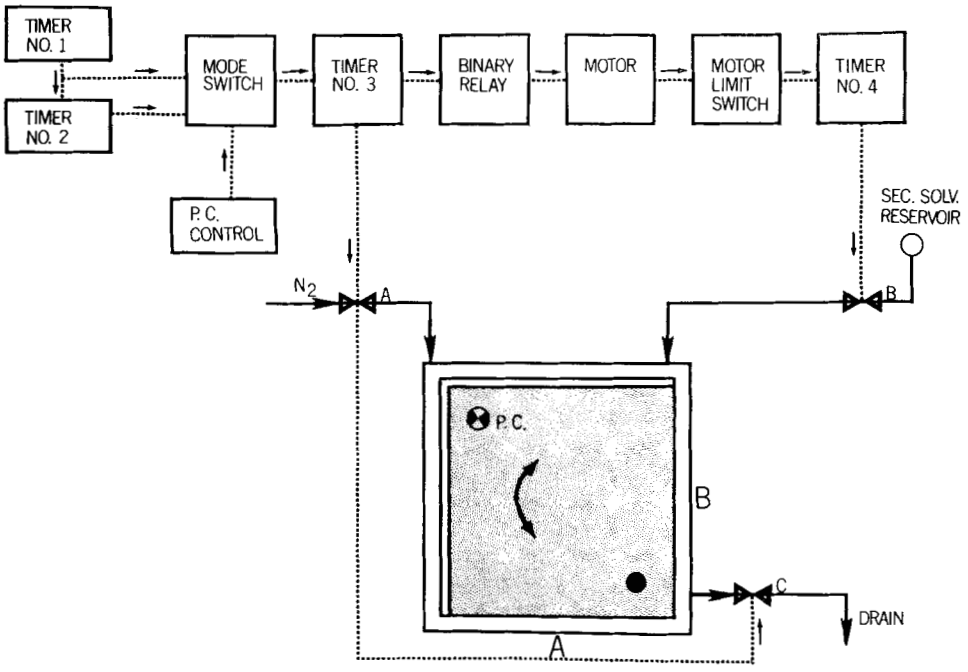

FIG. 2. Block logic diagram. Solid lines represent fluid or nitrogen (N_2); dotted line represents electrical current. Stippled area and *P.C.*, as in Fig. 1. The black circle is the point of application of the sample. The bidirectional arrow indicates the 90° rotation of the tank from position *A* to *B* and its reversal. The Teflon valves, *A*, *B*, *C*, are shown as double triangles.

At the end of the 30 min, which is sufficient time to allow the tank to be drained and dried, the timer closes valves *A* and *C* and switches the binary relay to its second state, in which position it starts the motor. The motor turns the tank 90° and stops when the motor limit switch is actuated by contact with the Teflon tank. At this point the second solvent timer (No. 4) starts and opens valve *B* for a period of 1 min. This allows 45 ml of the second solvent to enter the tank. The solvent front rises in the second dimension to the level of the photocell, and the drain-dry cycle is repeated. At the end of this time period the entire cycle ends because the binary relay is in its second state, and the motor limit switch is actuated. This combination of signals ends the run.

For ganglioside analysis a one-dimensional, two-solvent ascending system is used according to the method of Rouser et al. (2). The mode switch is moved manually to the time position. This brings timers Nos. 1 and 2 into the system, bypasses the motor and the motor limit switch, and removes the photocell control unit from the circuit. It had been determined that ascending chromatography with the first solvent for 5 hr and with the second solvent for 2 hr gave optimal separation of gangliosides. In order to start the run, a manual push button is activated. (In the two-dimensional system, the photocell takes the place of this manual start button.) At the end of 5 hr the output of timer No. 1 starts timer No. 2 which has been set for 2.5 hr. Timer No. 1 also activates timer No. 3 (the drain-dry timer) that has been set for 30 min. Timer No. 3 opens valves *A* and *C* and repeats the drain-dry cycle as previously described. At the end of the 30 min period, timer No. 3 closes valves *A* and *C* and sets the binary relay to the second state. This in turn starts the second solvent timer (No. 4) as previously described. At the end of this period, timer No. 4 shuts off, but timer No. 2 which is set for 2.5 hr will continue to run for the remaining 2 hr, after which time its output will once again activate timer No. 3 and repeat the drain-dry cycle. At the end of the run, the entire system is left in a hold position.

The automated system permits analysis in either one or two dimensions and can be easily modified to include three or more solvent sequences. The automated column and tank system reduces the probability of human error, markedly decreases the time necessary for routine analysis, and releases personnel for other duties.

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