Automated chromatography for stepwise elution of complex lipids

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SUMMARY An automated column-chromatographic system for stepwise elution of lipids is described. The automation is achieved with commercially available snap-on modules and Teflon valves.

SUPPLEMENTARY KEY WORDS column chromatography · modular instrumentation · photocells · solenoid-operated Teflon valves

Column chromatography with stepwise elution of complex mixtures of lipid by increasing proportions of polar in nonpolar solvent is widely used for isolation and analysis of polar lipids (1). The procedure is tedious and since the column cannot be allowed to run dry, the time of operation is limited to manual monitoring. Because of the time factor, one is often forced to compromise on optimal flow rate, sample loading, column dimensions, and solvent volume. Automation can free the chromatographic run from these restrictions. A flexible, relatively inexpensive method of automation is described.

The system uses snap-on modules, which can easily be modified for different experimental procedures. A 26 v DC power supply operates commercially available AND gates, relays, timers, photocells, and a stepper switch (Fig. 1). The eluting solvents and sample come only in contact with glass or Teflon. Since the Teflon valves are pneumatically operated through an air line indirectly controlled by solenoid valves, there is no heating of the solvent or sample. The entire system is run under nitrogen in order to reduce oxidation of lipid.

The following is an example of an automated chromatographic isolation of brain lipids by means of a Sephadex

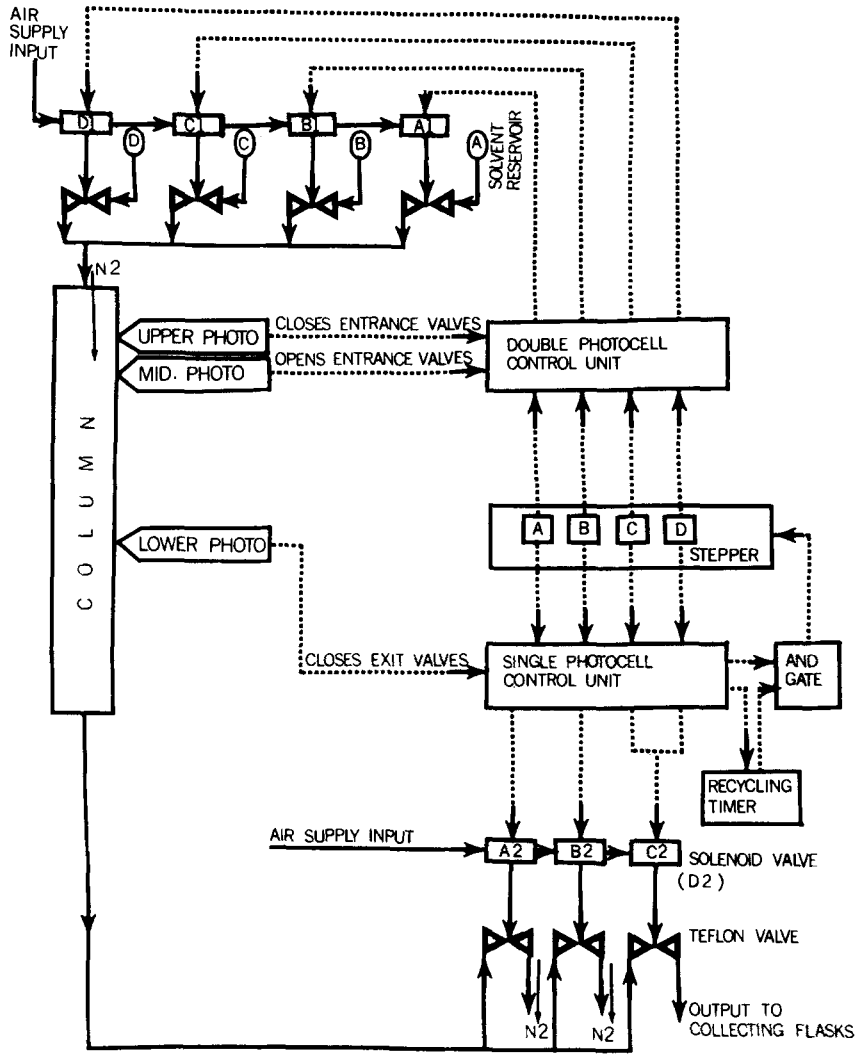

FIG. 1. Diagram of automated column for stepwise elution. Solid lines indicate Teflon tubing; broken lines are electrical connections; arrows depict direction of fluid flow. A_1, B_1, C_1, D_1 indicate the entrance solenoid valves, and A_2, B_2, C_2, D_2 refer to the exit solenoid valves. A, B, C, and D are the reservoirs of solvent entering the corresponding pneumatically operated Teflon valves. The exit Teflon valves empty into collecting flasks. N_2 refers to the nitrogen flow.

column using the elution system of Rouser, Kritchevsky, and Yamamoto (1). Reservoir A contains chloroform–methanol 19:1, saturated with water; reservoir B has chloroform–methanol–acetic acid 19:1:1.7, saturated with water; reservoir C contains chloroform–methanol 1:1; and reservoir D has chloroform–methanol 19:1. Receiving flask A collects all water-insoluble lipids. Receiving flask B collects ganglioside as well as some nonlipid contaminants. Receiving flask C is for waste solvent.

The sample is layered on to the column bed. The program is started by a starter button that moves the stepper switch from the hold to the A position. The stepper switch is used to select the proper solvent channel, which is then monitored by three photocells and their control units. The lower photocell is placed outside the column right above the absorbent bed. This photocell and its single control unit close and open the exit valve. When the run starts, the solvent level is above the lower photocell beam so the exit valve is open. Since the solvent level is below the middle photocell the A_1 entrance is opened and the solvent from A reservoir enters the column. The fluid level continues to rise until it reaches the upper photocell. This interrupts the upper photocell beam, which closes the A_1 entrance valve. The solvent level will then fall until it drops below the middle photocell beam. This then reopens the A_1 valve. Thus, the upper and middle photocell and the double control unit will continue to deliver solvent to the column. When the predetermined amount of solvent in reservoir A is ex-

hausted, the solvent level will continue to fall until it drops below the lower photocell beam, the single photocell control unit and a 30-sec recycling timer are activated. At the end of the 30 sec, the timer and the photocell control unit, operating through an *AND* gate, send a signal to the stepper, which moves the stepper switch to the *B* position. The use of a recycling timer operating through an *AND* gate prevents false signals from the lower photocell from prematurely activating the stepper switch.

When the stepper moves to position *B*, solenoids B_1 and B_2 are activated and open the corresponding valves. The apparatus then goes through a cycle similar to the above, but with the solvent in the *B* reservoir. When *B* is completed, the *C* cycle (which removes nonlipid contaminants and the acetic acid) is initiated. At the end of this cycle, the unit will either shut itself off, or if the long-cycle switch is set, goes through the *D* cycle, which will wash the column bed with chloroform–methanol 19:1 in preparation for a new sample run.

An elapsed time meter cumulatively measures the time for each solvent run, or by a switching panel records the total time of the four solvent runs. Since the Teflon valves require air pressure to remain open, and the solenoid valves require current to pass the air pressure to the pneumatically operated valves, any power interruption will automatically close all exit and entrance valves. The system gave a 96% recovery of a mixture of sphingomyelin and ganglioside.

The initial cost of parts for the equipment was approximately $3,000. Because of the modular design, a wide variety of applications can be obtained without additional major expense. For example, we have utilized the system to develop an automated two-dimensional chromatographic tank for thin-layer chromatography.

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