I am grateful to V. ŠKRAMOVSKÝ of Charles' University for a gift of authentic 1:12-benzoperylene.

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An automatic stepwise elution dispenser for column chromatography*

Certain studies in our laboratory are concerned with the separation of proteins and nucleotides. In these studies stepwise elution column chromatography is used. Reasons for this type of elution are to isolate given activities and to provide enriched fractions thereof. Since the time required to elute a given column is often in excess of 12–14 hours, it became necessary to construct an apparatus, that was not time dependent, to automatically allot the various buffers used.

Apparatus

Fig. 1 shows the dispenser we built to solve the problem. Eleven buffers can be accommodated with the machine (including one buffer in the reservoir).

The automatic dispenser was constructed so that each buffer is contained in a separatory funnel (Fig. 1). A tygon tubing is used to connect the respective funnels to the reservoir. Each tube is closed with a hospital type clamp. A stainless steel wire is used to connect each clamp to a respective solenoid, which at the proper time opens the clamp and allows the buffer to drain into the reservoir. The reservoir (Figs. 1 and 2) has a capacity of 500 ml. It is equipped with a glass float (b) connected to a glass rod to which a counter-balance (c) is used. At such time as the reservoir becomes empty, the level (bearing the counter-weight) is so devised to close switch (d). The closed circuit moves the motor driven contact arm (e) to the successive contact point which activates a solenoid. The solenoid activation results in opening the hospital type clamp through which the tygon tubing was placed. This then allows the buffer to drain into the reservoir. As the reservoir fills, switch (d) opens. This results in disconnecting the line voltage from the motor that drives the contact arm until such time as the reservoir again becomes empty.

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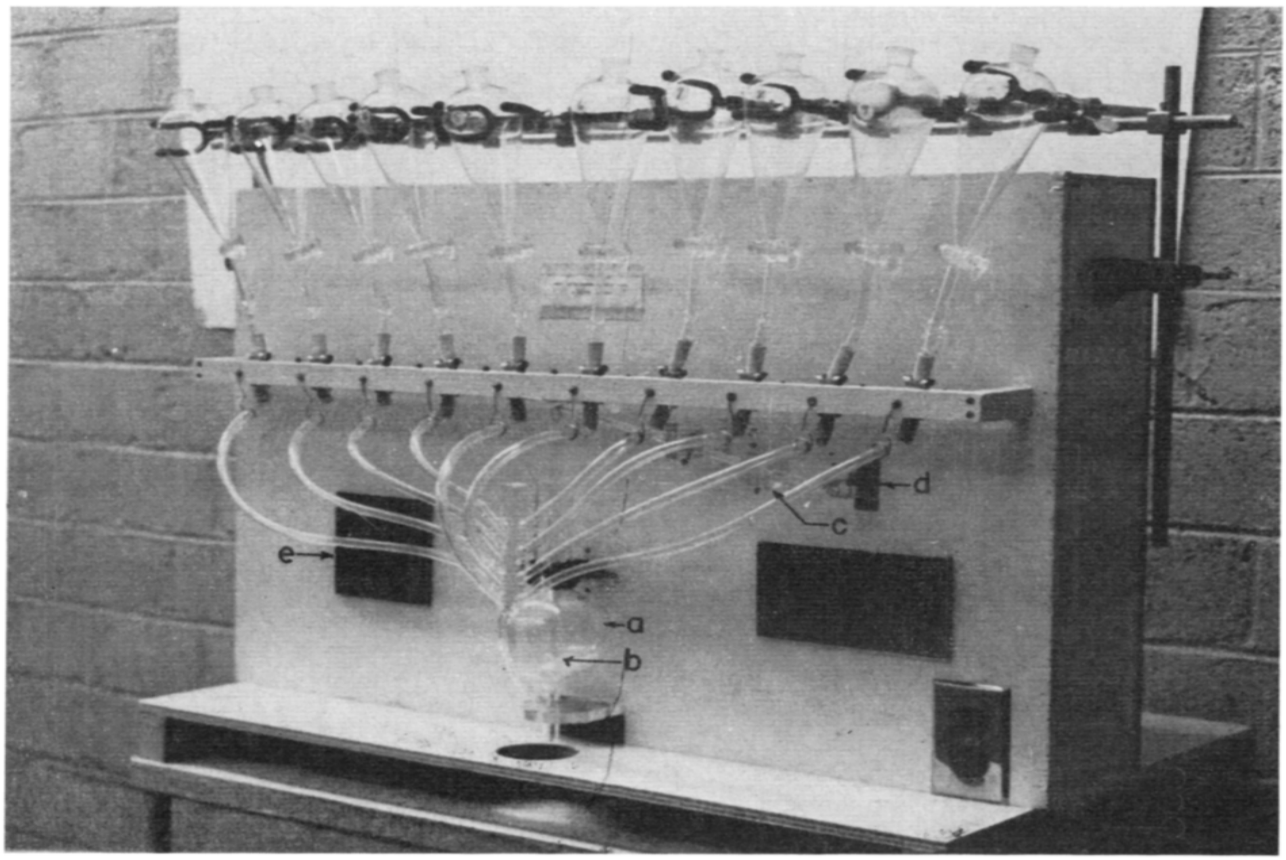

Fig. 1. Automatic stepwise elution dispenser for column chromatography. Dispenser can accommodate 11 buffers. With a slight modification it may be used for gradient elution.

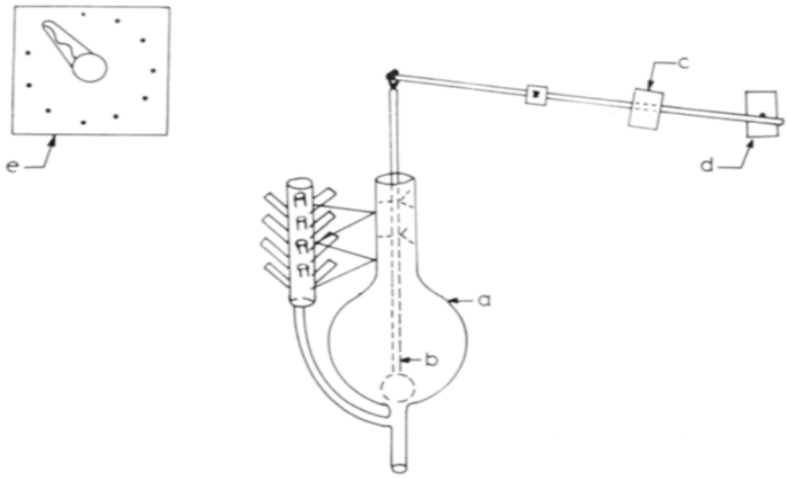

Fig. 2. Float valve and reservoir system used on dispenser.

The electrical diagram is shown in Fig. 3. Since the dispenser was used in the cold room (0–5°), we found it necessary to install a 100 W light bulb in the machine housing. This was done to furnish sufficient heat to prevent condensation of moisture on the electrical equipment.

Operation

Polyethylene tubing (i.d. 0.055 in.) was used to transport the buffers from the dispenser reservoir. A Mini Pump* was used to pump the buffers to the chromatography column. There is no reason that gravity flow would not work in the transporting of the buffers from the dispenser to the column; we used the mini-pump since in our situation it was more convenient to do so.

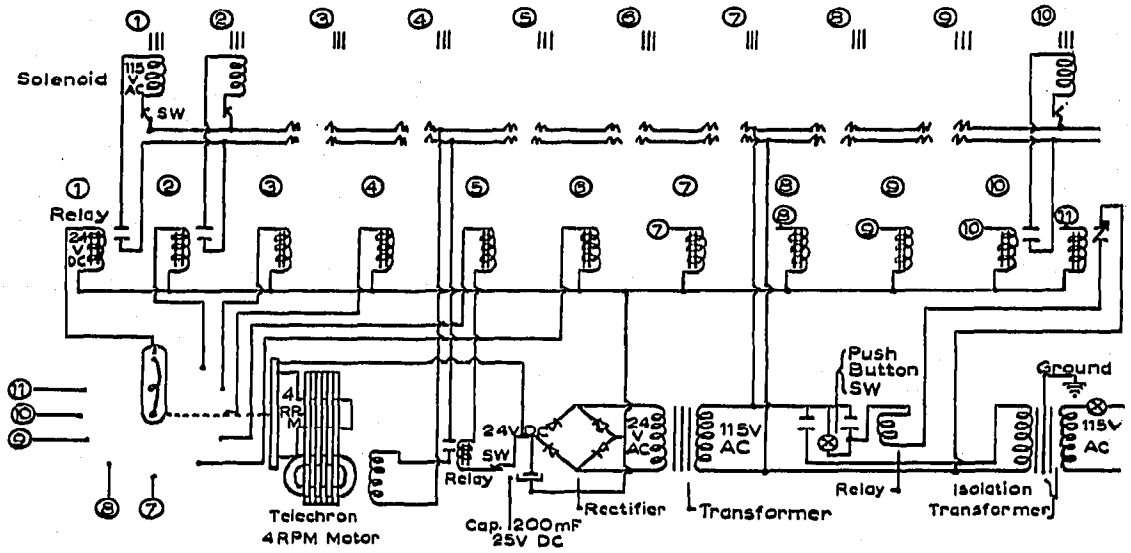

Fig. 3. Electronic diagram of the automatic dispenser.

Machine efficiency

In order to determine the amount of mixing between buffers, we decided to put liquid containing low levels of ^{32}P in separatory funnels numbered 1, 3, 5, 7, and 9. Liquid not containing radioactivity was put in funnels numbered 2, 4, 6, 8, and 10. Liquid

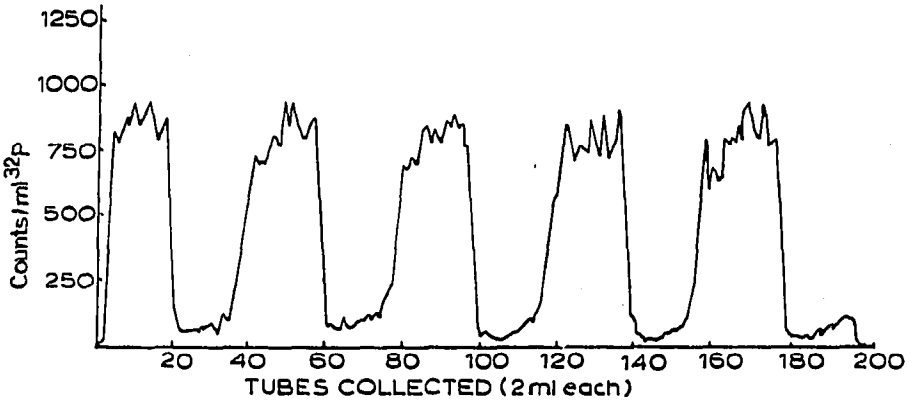

Fig. 4. Data obtained from collecting aliquots of liquid from dispenser. Alternate separatory funnels contained low levels of ^{32}P.

* Milton Roy Instruments System, Philadelphia, Pennsylvania.

was collected from the dispenser in 2-ml aliquots. The results are shown in Fig. 4. As may be seen from this graph, the mixing between liquids (corresponding to buffers) was negligible. Much more mixing would be expected within a column during the actual elution.

Comments

The machine is easily adaptable so that fewer or more buffers can be handled, as desired.

Another feature of the machine is that with one modification it could serve as a gradient type elution apparatus. Letting the reservoir (Fig. 2) be flask A and installing flask B equipped with a stirring bar below flask A, the result would then become an automatic gradient type elution system.

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Ion-exchange chromatography of some uncommon amino acids

During studies involving the isolation and identification of substances from the blood plasma and urine of patients with disease[1,2], the authors found it expedient to determine the chromatographic behavior of several compounds. The detailed reports by SPACKMAN, STEIN AND MOORE[3], and by HAMILTON AND ANDERSON[4], contain information relative to several "non-protein" amino acids. Such amino acids are not likely to be encountered in hydrolysis of natural proteins. However, the synthesis of peptides with uncommon amino acids leads to the possibility that their products of hydrolysis will contain unusual compounds. In addition, the list of compounds being identified in human blood plasma and urine is increasing. Previously unidentified or incompletely described compounds may appear in urine or blood plasma, especially in various disease states. Determination of the R_F of several compounds by ion-exchange column chromatography is expensive in time and materials, particularly if synthesis is involved. Accordingly, we wish to record our experiences.

Experimental

Column chromatography using sulfonated polystyrene cation-exchange resins[5], with the automatic recording method of SPACKMAN, STEIN AND MOORE[3]*, was used

* Phoenix Precision Instrument Co., Philadelphia, Pa., U.S.A.