Reports—Current Topics—Queries

A Simple, Inexpensive Device For Column Chromatography

Biology often requires the use of column chromatography for the separation of components, such as proteins or pigments, from complex mixtures. The apparatus usually seems to fall into one of two categories: simple but inefficient or efficient but expensive. I have lately developed an inexpensive, versatile system that generally yields results comparable with those obtained by the use of commercial columns and accessories.

The chromatographic column itself consists of a piece of plain glass tubing of appropriate diameter. Soda glass is usually adequate and much more easily handled than Pyrex. In most cases, the diameter should be chosen such that the starting volume of the sample does not occupy more than 1 or 1.5 cm of column height. The length of the column depends largely on the nature of the proposed column-filling. Gel-filtration chromotography with a material like Sephadex tends to require a longer column than does ion-exchange chromatography with a substance like granular DEAE: a column 2.5 cm in diameter and 40 cm high is about right for the former, a column of the same diameter but only 25 cm long for the latter. The glass tubing should be cut cleanly to the desired length and not fire-polished. Fire-polishing, unless very carefully carried out, tends to produce a somewhat irregular bead, which may cause leaks.

The column bottom is made from a solid-rubber stopper that fits snugly; that is, it does not enter into the column more than about 5 mm. By means of a sharp knife or, preferably, a rotary file chucked into a small, hand-held power tool, a conical depression, roughly 2 mm deep, is made in the small end of the stopper. This will act as a collecting funnel. If the depression is too deep, remixing of components may occur. Next, the point and the hub are cut off from a hypodermic needle of appropriate diameter. For all but the biggest columns an 18-gauge needle is a good size. The remaining part of the needle is forced through the stopper until one end is centered in and exactly level with the bottom of the conical depression. Connection with external equipment can be made with polyvinylcholoride tubing. With most materials, *clear* tubing of the kind used in the electronics industry as insulating sleeving ("spaghetti") is, if well washed, quite satisfactory. 18-gauge tubing (0.042 inches internal diameter) will fit on an 18-gauge hypodermic needle.

Finally, a screen is provided to prevent the column-packing from coming through the needle. Thin, white, finely woven nylon works well. From the cloth is cut a disc having a diameter 4 to 6 mm greater than the small diameter of the stopper. This screen is placed over the small end of the stopper and, as the stopper is inserted into the tube, it becomes firmly and evenly wedged between the stopper and the column wall.

The top of the column may also be made of a stopper through which the shaft of a needle has been passed. The needle should protrude about 1 cm above and below the rubber. To facilitate addition of the sample without disturbance of the bed, a cup may be used. This is most conveniently made from a piece of plastic tubing (Lucite or Plexiglass) whose external diameter is 1 to 3 mm smaller than the internal diameter of the column. The cup should be 2 to 4 cm high. Both ends are sanded to a smooth surface. Then a piece of the same nylon material used previously is attached to one end. After a piece is cut somewhat larger than the external diameter of the cup, it is placed on a glass plate and the cup is placed on it. Then a very small amount of methylene chloride is applied at the junction of the cup and nylon along the perimeter. After a minute or two, the excess solvent will have evaporated and the nylon will be provisionally welded to the plastic. The cup is turned upside down and more solvent is applied sparingly to the nylon where it lies on the plastic. Use of an excess of solvent will cause occlusion of some interstices in the nylon due to flow of the dissolved plastic.

After the solvent has totally evaporated, the excess nylon can easily be removed by rubbing the edge of the cup on sandpaper or a file. After the column is loaded with the packing material, the well-washed cup is carefully lowered into contact with the top of the packing. The buffer level should be at the top of the cup. A piece of plastic tubing is then attached to the part of the hypodermic needle protruding from the small end of the upper stopper. The lower end of this tube should come within about 0.5 to 1 cm of the nylon screen.

To add a sample, it is only necessary to be sure that the sample material has a higher specific gravity than the buffer. This can be brought about by the addition of sufficient sucrose to bring the sample to about a 10% concentration. The sample can then be injected slowly by means of a hypodermic syringe and needle attached to the needle in the top stopper through a piece of plastic tubing. Similarly, the elution buffer can be added to the column through the needle in the upper stopper.

Werner G. Heim
Colorado College
Colorado Springs, Colo. 80903