U-shaped columns for adsorption and partition chromatography

We have during the development of a multi-column chromatographic system (refs. 1–3) constructed U-shaped glass columns that were found for some applications to offer distinct advantages over conventional straight columns, also when they were used for single column chromatography.

Our interest in the U-shaped columns evolved from attemps to construct long, straight, capillary columns. Such columns were found impractical and very inconvenient in use since they approached or exceeded in length the height of the room we worked in. It was decided to bend the tubing into a U to make the columns easier to handle. The capillary columns of this shape were found so convenient in use that the design was adapted also for columns of more conventional length and diameters.

Construction of the U-columns

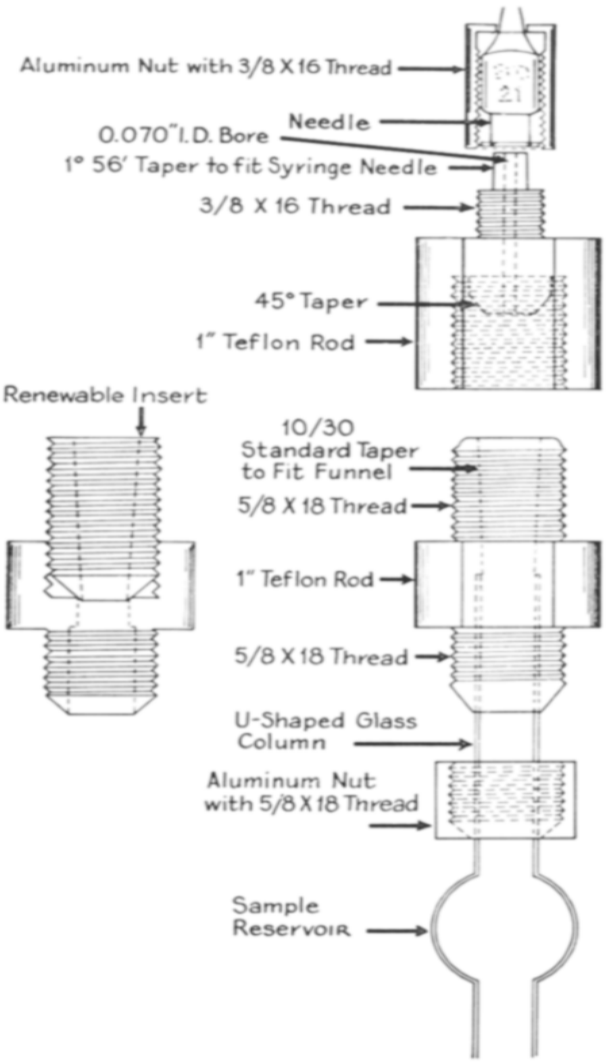
The columns have been made simply by bending a piece of glass tubing of appropriate diameter into a U-shape. A small sample reservoir can if needed be made with simple glass-blowing techniques at one end of the columns to facilitate the application of sample to the column. This has routinely been done in the case of the capillary columns, that otherwise may present difficulties in sample application. Heavy-walled glass tubing is preferable for column construction to give a column of greater mechanical strength and to make it possible to blow a sample reservoir in the column if needed.

The diameter of the glass tubing used in the construction can vary within rather wide limits if the teflon adapter is used on the columns. A set of, for example, three such adapters with increasing inside diameter will cover a wide range of outside diameters in glass tubing. However, tolerances are quite narrow for the swagelok adapter and tubing must either be selected to closely fit available swagelok fittings or a piece of glass tubing with the right O. D. must be annealed on each side to the top of the U-column, if swagelok connections are used for the columns.

Connections between the feed pump or eluant container and the U-column

Teflon adapter. This is the preferred adapter for columns exposed to moderate pressure only and for columns that are packed by a simple sedimentation technique. The bottom part of the adapter (Fig. 1) slides over the top of the chromatographic column. It is squeezed around the column by tightening the aluminum nut at the bottom to slightly more than finger tightness. This part of the adapter is left on permanently if the column is in continuous routine use. The top part of the adapter is screwed to the bottom part after the column has been filled. A removable insert (shown to the left on Fig. 1) has been made for the part of the adapter that is most exposed to wear. This modification is used if the adapter is in very frequent use. A needle is held by another aluminum nut to the top portion of the upper part of the adapter. Teflon tubing leads from here to pump or solvent container on the intake side and to the fraction collector on the outlet side.

Swagelok connection. If pressures are expected to be higher than 100 p.s.i. or if active packing of the column is necessary before chromatography a swagelok (Crawford Fitting Co., Solon, Ohio) connection is preferable. The swagelok fitting consists of a front and a rear ferrule that are squeezed around the tubing by a nut. The front



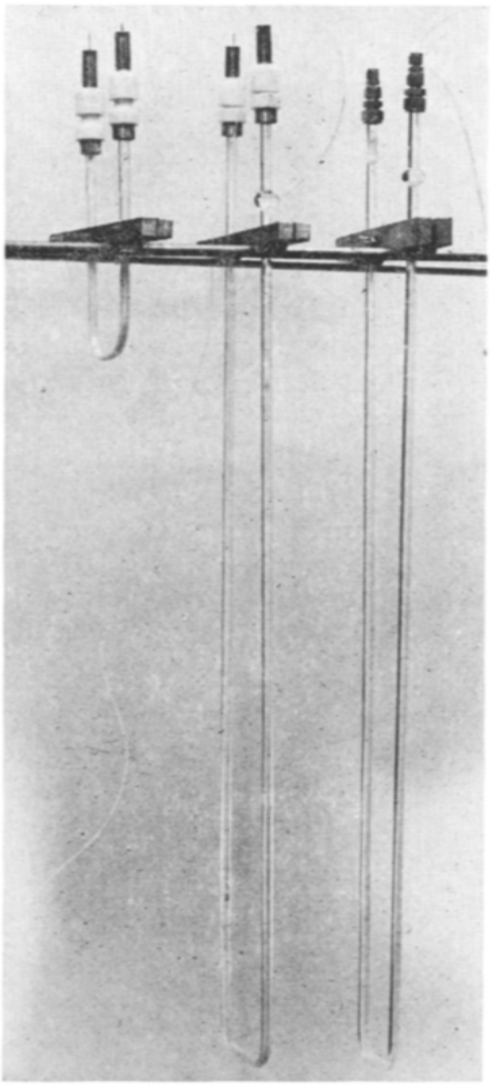


Fig. 1. Teflon adapter used to connect U-shaped column to eluant and fraction collector.

Fig. 2. A small 20-in. U-shaped column and two 8-ft. capillary U-shaped columns. The two columns to the left are equipped with teflon adapters. The column to the right with swagelok fittings.

must be teflon to reduce breakage on tightening the swagelok connection. A swagelok reducing unit is used to make the connection from the glass tubing on the column to the teflon tubing (Fig. 2, right).

Filling the U-columns

Using the teflon adapter. The bottom part of this adapter has an inside female 10/30 standard tapered joint. Glass funnels with corresponding male 10/30 joints are inserted into these joints on both sides of the column. Half the amount of column material to be used is after filling of the columns and partially filling of the funnels with solvent added in suspension to each of the two funnels. The columns are allowed to form by sedimentation. A glass fiber or cotton plug is put on top of the column on the low-pressure side and the column material is compressed and pushed against the

plug by applying appropriate pressure on the high-pressure side. The column is now ready for application of the sample. This can be done with a small syringe equipped with a long needle. After the sample has been washed down on the column with a few portions of eluant under appropriate pressure the adapter is filled up with eluant and connected to pump or container and the column is ready for chromatography.

Using the swagelok connection. Gravity filling of the columns is in this case performed by connecting the swagelok on the column through a straight union of the same diameter to a small piece of wide diameter teflon tubing swaged to the other end of this union. A glass funnel with a stem of appropriate diameter is inserted into the teflon tubing and the columns are filled as above. With this type of connection a perforated disk packer can also be used for partition column materials like Celite where active packing is necessary. The rod in the packer has to be flexible in the small section going into the horizontal part of the U. A stainless steel spring inside teflon tubing performs well for this purpose. The column is stamped from both sides of the U's after filling the horizontal part first. We have not been able to pack capillary columns adequately with hard to pack material like cellulose powder but larger sized diameter tubing packs with little more difficulty than straight columns. After packing the sample is applied to the top of the column with a syringe equipped with a long needle as above.

Emptying the columns

The columns empty easily after use if they are washed out with tap water under pressure. Thin polyethylene tubing connected through an adapter to a cold water faucet is pushed inside the column after removal of the fiberglass plug. The column material is rapidly washed away as the polyethylene tubing is gradually pushed through the column.

Performance

The types of columns and connections used in our work are shown in Fig. 2. The smaller 20-in. U-column with teflon adapters shown to the left in the figure is now used in routine gradient elution chromatography of steroids[1–3] instead of the water-jacketed straight columns of similar length used previously. The 8-ft. long capillary columns with teflon adapters or swagelok connections (Fig. 2, middle and right) have been used to speed up the chromatography of the steroids.

To illustrate the performance of the U-shaped columns some chromatograms of seven 17-ketosteroids separated by gradient elution chromatography on alumina columns with the technique described previously[1–3] are shown in Fig. 3. The chromatograms were recorded with our automatic read out system[3]. The top chromatogram was obtained using a straight column, the middle chromatogram by using a U-shaped column of similar length. The differences in resolution is not greater than is observed between individual chromatograms in a multi-column run using conventional straight columns. These two chromatograms were from 36-h runs. The bottom chromatogram shows a 12-h run on an 8-ft. long capillary column. A resolution essentially similar to that obtained in the three times as slow short column run is obtained on these columns in the shorter time.

Both the capillary and ordinary U-shaped column have besides for adsorption chromatography on alumina also been used with good results for partition chromato-

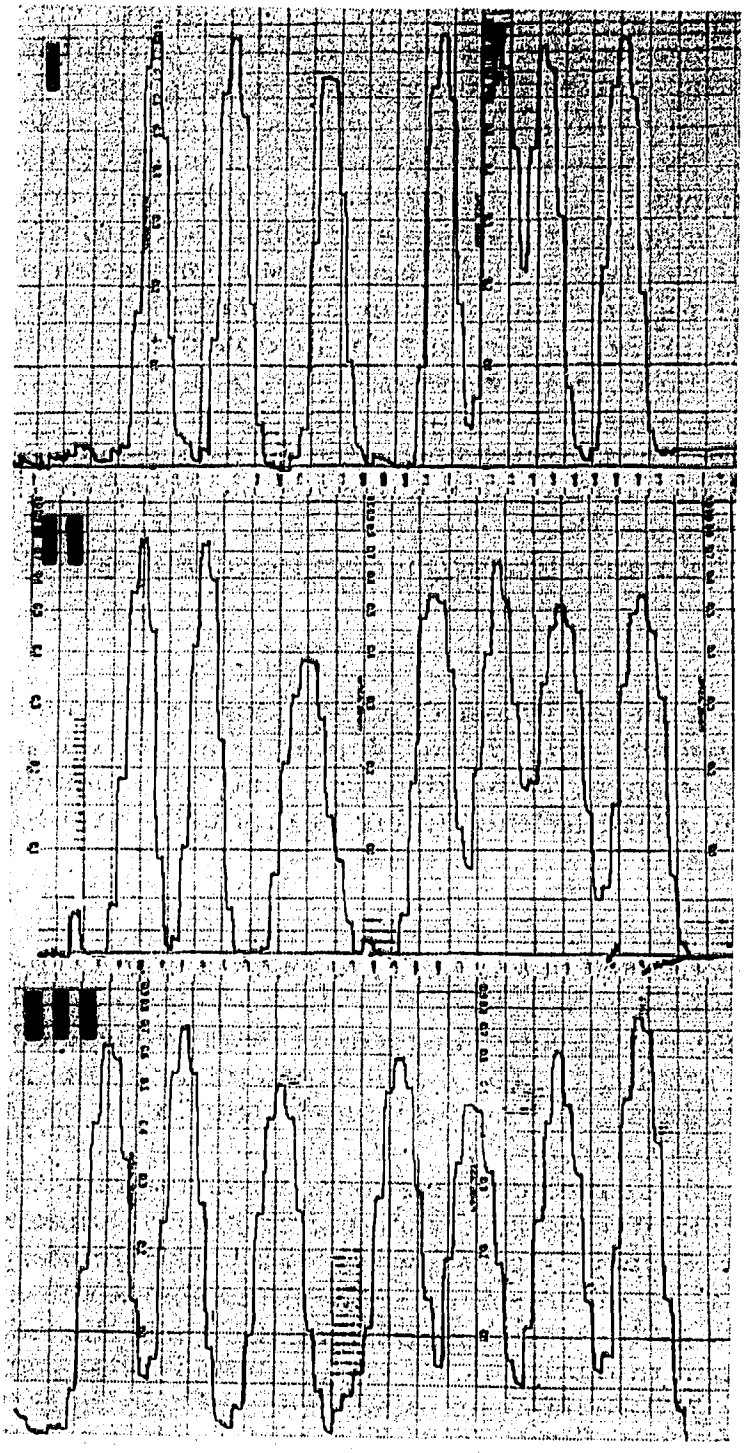


Fig. 3. Chromatograms of the seven major 17-ketosteroids present in human urine obtained and recorded with the technique described previously[3]. The top chromatogram is from a straight column run, the middle chromatogram was done on a U-shaped column of similar length. Both these chromatograms were run for 36 h. The bottom chromatogram is from a 12 h run of the same substances on an 8-ft. capillary column.

graphy of steroids on silica gel with water as the stationary phase. Columns of this material can as the alumina columns be packed by gravity. Partition chromatography on cellulose powder with propanediol as the stationary phase has been performed on U-shaped columns of standard dimensions only.

Advantages of the U-shaped columns

Conventional sized columns. These columns are less expensive and simpler to construct than the usual straight columns. No special glass-blowing skills are needed for the construction since the column is made simply by bending a piece of glass tubing into a U. No glass disk or other column support is needed in the columns. They are much easier to thermostat than conventional columns since the column can be simply dipped into a waterbath for thermostatting and no elaborate system with water-jackets connected to a system carrying circulating water is necessary. An advantage deriving from the low cost of the columns and the U-shape is that it is easy to prepare the columns in quantity before use and store them under solvent, packed and ready for chromatography at a later time. We have stored alumina columns this way for over a month under benzene and found them fully effective at the end of this period. The columns are more compact and take up less space. This is particularly valuable in multi-column systems where thermostating is needed. A small waterbath can easily accommodate 10–20 U-shaped columns.

Capillary columns. These columns are not as convenient in use as the smaller U-columns. They are, however, necessary to perform capillary column chromatography. This type of chromatography will in many applications give better resolution than shorter columns with larger diameter. The capillary columns may also be used to give a considerable reduction in the time necessary to run a chromatogram. Very long capillary columns may bring the running time in column chromatography down drastically if the technical problems involved can be solved.

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Research Center, Rockland State Hospital, Orangeburg, N.Y. (U.S.A.)

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JOSEPH F. SAYEGH
CLINTON S. WITHERELL

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