Capillary teflon columns for adsorption and partition chromatography

We have previously[1] described U-shaped standard sized and capillary columns made of glass. In extension of work started with the U-shaped capillary columns we became interested in the possibility of constructing very long capillary columns.

Glass could not be used for these columns because of leakage through the junction of the swagelok connector and the glass tubing at very high pressures. There was also the danger of breakage of the glass at the high pressures necessary to force the fluid through very long capillary columns.

We turned in our search for a suitable material to teflon tubing because of its chemical inertness, semi-transparency and relatively high mechanical strength.

Construction of the capillary teflon columns

Teflon tubing. Unless special connectors are custom made in the machine shop for use in the column construction the commercial availability of the necessary connectors in increments of 1/16 in. only starting with a connector for 1/16 in. tubing limits the selection of tubing available for the construction of columns to sizes that are within the tolerances specified for the outside diameter of tubing to be used with the connectors.

We have in our work used two types of teflon tubing both with an outside diameter of between 0.121 and 0.126 in. to fit the tolerances of the 1/8 Gyrolok connector. One type has been the standard size flexible teflon tubing with an 1/16 in. (approximately 1.5 mm I.D.). The other is the thinner walled AWG size 10 with an outside diameter of 0.121–0.126 in. and an inside diameter of 0.103–0.107 in. (2.5 mm; both types of tubing available from Pennsylvania Fluorocarbon Company, Inc., Clifton Heights, Pa.).

Gyrolok connectors. We used in our work with the U-shaped glass columns swagelok (Crawford Fitting Company, Solon, Ohio) connectors. We found, however, that these connectors constricted the inside diameter of the capillary teflon columns somewhat and that this slowed the filling of the column. We changed therefore to a similar fitting the Gyrolok (Hoke, Inc., Cresskill, N. J.) tube fitting that does not constrict the inside of the tubing to a degree that slows the filling of the column.

Column construction. The columns were made simply by attaching gyrolok fittings to both ends of a piece of capillary teflon tubing of appropriate length. This is done by inserting the tubing through the fitting and turning the nut of the fitting 1¼ turn thereby swaging the fitting to the tubing. The tubing coming from the feed pump and going to the fraction collector is similarly equipped with gyrolok fittings.

Filling the capillary columns

Filling by gravity. It is possible with some column materials (alumina, silica gel) to fill the columns with the larger inside diameter by gravity alone. The technique for this is illustrated in Fig. 1 that shows to the left in the foreground a column ready to be filled to which a glass funnel has been attached at the top through a reducing union and a piece of thin walled teflon tubing. The columns are plugged at one end with glasswool or cotton. The columns are completely filled up, the funnels partially filled up with mobile phase. The column material is added in a slurry to the funnel and left to settle by gravity in the columns. The column to the right in the foreground of Fig. 1 is an almost filled column filled by this technique.

Filling by pump feeding. This technique is used for the capillary columns with small inside diameters and also for columns with larger inside diameter if the material used for the column preparation cannot be packed by gravity.

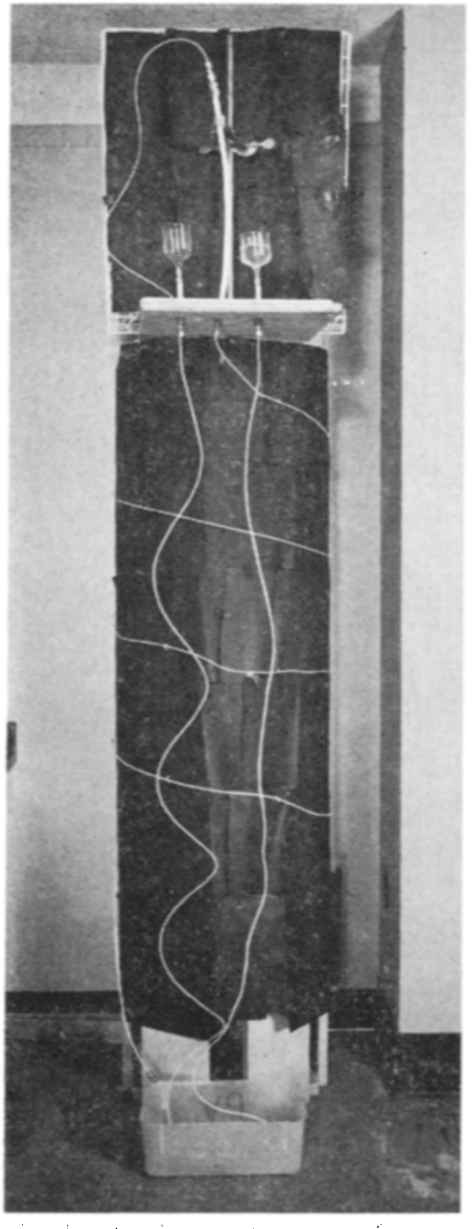

Fig. 1. Filling of capillary teflon columns. The columns with larger I.D. (2.5 mm) are filled by gravity in the foreground. In the background is an 8 m long capillary column with an I.D. of 1.5 mm being packed by pump feeding from the large diameter teflon tubing reservoir connected to the top of the capillary column through a Gyrolok union.

To fill the capillary column by pump action a piece of wide diameter (½ in. O.D.) teflon tubing is attached through a reducing union to the top of the capillary column (Fig. 1 background). This wider section of teflon tubing is as the capillary filled up with mobile phase. The column material is then poured into the wider teflon tubing through a glass funnel attached to the top of the tubing. The top end of the

teflon tubing is now connected through a reducing union to the chromatographic pump and the column material is then pumped into the capillary section when the pump is started up.

The pump feed technique can also be used to fill the previously described[1] U-shaped glass capillary tubing with material that could not without this technique be packed into that type of columns.

We have with the pump feed technique filled columns with a variety of hard to pack partition column materials including cellulose powder and celite.

Application of sample to columns

We have in our work transferred the sample in mobile phase to the columns using a tuberculine syringe equipped with a 4 in. long 22 gauge needle or with a needle to which very thin teflon tubing has been attached. The sample solutions have after introduction into the top part of the column been pushed into the column with compressed air or nitrogen applied through a reducing valve so that the pressure can be regulated at will. Syringe and sample container has been washed through with mobile phase a few times and the rinses washed down into the column with compressed air. The columns are now filled up with mobile phase and connected to the pump to start the chromatographic run.

Emptying the columns

The columns are very easy to empty. The glass wool or cotton plug at one end of the column is removed. The tubing leading to the pump is attached to the other end. The column material empties out of the column when the pump is started up.

Performance

The great savings in time obtainable through the use of the capillary teflon columns are demonstrated in Fig. 2. This figure compares a 36-h chromatogram of a steroid mixture performed on a conventional alumina column with the technique described previously[2,3,4] with 5- and 2.5-h capillary chromatograms of the same mixture on the same column material. All chromatograms were recorded with the automatic read-out system described earlier[4].

It can be seen that the 5-h capillary chromatogram is if anything slightly better in resolution than a 36-h chromatogram on a conventional column and that a 2.5-h capillary chromatogram although somewhat poorer in resolution than both a 5-h capillary chromatogram and a 36-h conventional chromatogram still would be acceptable for example for a clinical analysis for these steroids in biological fluids since the maximal overlap between individual steroids is of the order of 3% of the total amounts applied to the column.

The resolution of a complex steroid mixture of this nature in such a short time period makes column chromatography for such a mixture slower only than gas chromatography of the major chromatographic systems in analytical use. Separation of this steroid mixture by thin layer chromatography has in our hands been possible only by horizontal chromatography or special overrun techniques. Both these procedures take 4–6 h to perform. Fast paper chromatographic systems take 4–5 h for a steroid mixture of this nature.

Further development of the capillary technique might conceivably make capil-

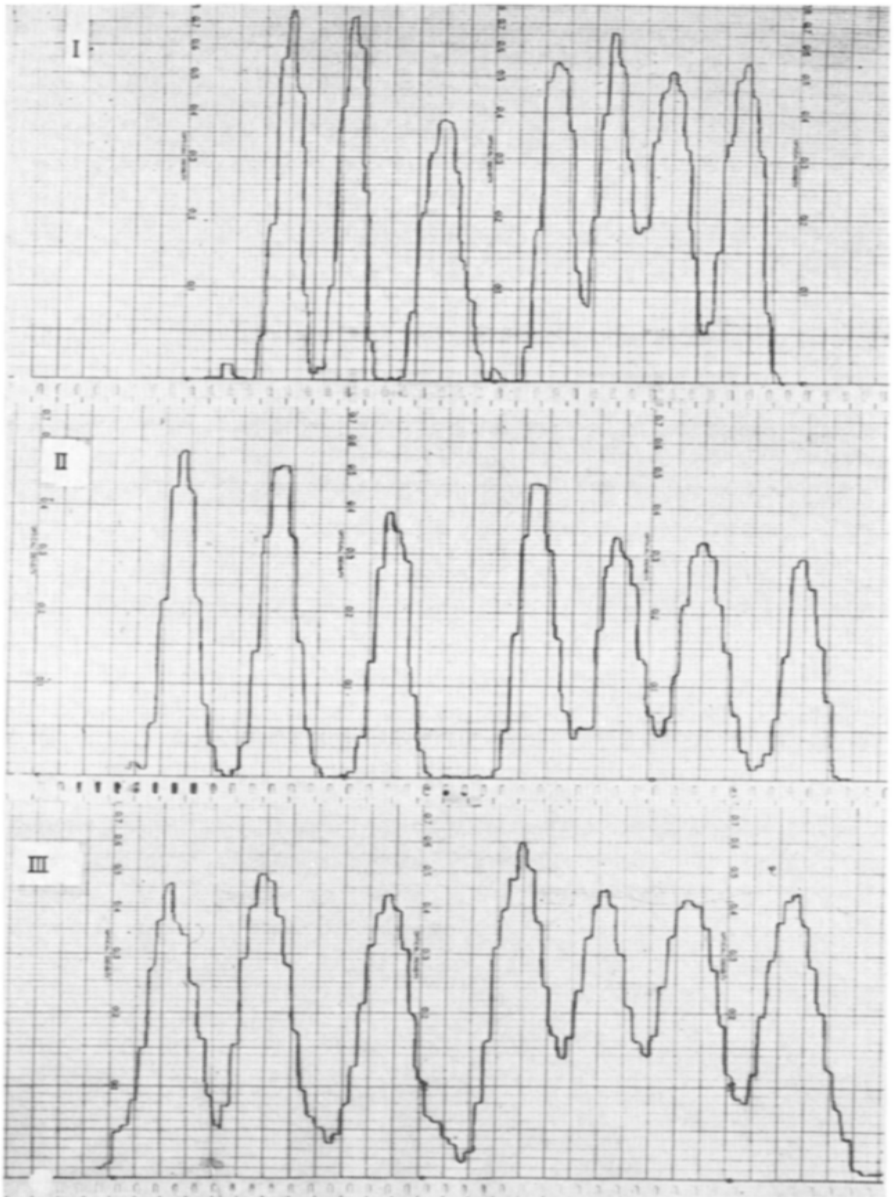

Fig. 2. Comparison of a conventional 36-h chromatogram (I) of the seven common urinary 17-ketosteroids obtained using a straight 40 cm long alumina column (I.D. 6 mm) with chromatograms of the same steroid mixture obtained using 8 m long capillary teflon columns (I.D. 1.5 mm). (II) shows a 5-h capillary chromatogram, (III) a 2.5-h capillary chromatogram. Gradient elution chromatography was used[2,3,4] and the chromatograms were recorded with our automatic read-out system[4]. The 17-ketosteroids separated are in order of elution: dehydroisoandrosterone, androsterone, etiocholanolone, 11-ketoandrosterone, 11-ketoetiocholanolone, 11-hydroxyandrosterone and 11-hydroxyetiocholanolone. Approximately 50 μg of each steroid was chromatographed.

lary column separations the fastest chromatographic method available for complex steroid mixtures.

Advantages of capillary teflon columns

The columns are non-breakable and will outlast glass columns by a wide margin in routine use. Making them involves only cutting off an appropriate length of teflon tubing and swaging on the Gyrolok fittings, and this is done in a few minutes. They

are very convenient to thermostat. We have for our 12 column multi-column runs arranged twelve such 8 ft. long columns in coils in a small waterbath. The columns are easy to fill and empty. They are easy to store and can as the U-shaped glass columns[1] be stored for later use once made up. They must, however, be kept in closed containers over mobile phase when stored since teflon is slightly porous to many organic solvents.

Advantages of the filling technique using pump feeding of column material

This technique is necessary for the filling of capillary columns, since it would be close to impossible to develop mechanical devices that could pack columns with such small inside diameters. The technique can however be used to advantage also for filling conventional sized chromatographic columns including the U-shaped glass columns described earlier[1]. The pump feeding technique is in our experience much faster than techniques using packing rods or other mechanical devices. A semi-automatic technique of this nature makes rapid batch filling of partition columns with material that is hard to pack with other methods possible.

Further developments of the capillary column technique

The 24 ft. long capillary columns we have used for the chromatograms in Fig. 1 has presented the limits of the technique for the capillary teflon columns in our experiments. The pressure obtained was 900 lb. per sq. in. The chromatographic minipumps used (Milton Roy Company, Philadelphia, Pa.) are rated for work to 1000 lb./sq. in. The bursting pressure of the narrow diameter teflon capillary tubing was found to be 1200 lb./sq. in. To get further with the development of the capillary column technique, it will therefore be necessary to turn to other pump types with higher pressure ratings and to metal tubing that can resist the high pressures involved.

Acknowledgement

The work was supported in part by Grants AM 04262 and GM 11940 and Institutional Grant MH 07292 from The National Institutes of Health, U. S. Public Health Service.

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Received May 12th, 1966