

## IMPROVED LIQUID ELUTION COLUMN SYSTEM

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## INTRODUCTION

Liquid column chromatography (LCC) is one of the methods of choice for the preparative separation of fragile biological materials and compounds of very high molecular weight. The method may never equal the remarkable performance of the rival gas-liquid chromatography (GLC) in respect to speed and packing stability, but would seem to be highly susceptible to improvement in other points of technique. In some cases the fault can be traced to some inadequacy in equipment design brought about by the use of glass. Yet, except in special applications requiring an all-glass apparatus, the use of glass can be limited to the column itself. Here visibility is helpful in guiding packing operations and in techniques where the movement of bands needs to be followed. Therefore, any non-contaminating material is justified which will help by introducing desirable features in other parts.

Sample loading, particularly in partition chromatography, is a delicate operation requiring skill and experience. While the purpose of this procedure is to minimize the volume of the starting band and to promote its even distribution, none of the several techniques proposed hitherto is infallibly successful in achieving this result or compares in speed, simplicity and reproducibility with the injection technique used in GLC. A successful adaptation of the latter to LCC as described below involves mechanical features only accessible through the use of metal.

Similarly, a removable column bottom fitting was designed to eliminate post-column dead volume effects as described by VANDENHEUVEL<sup>1</sup>, and other potential drawbacks of conventional systems. Use of easily replaceable filter paper disks in the packing support system, has made available a range of porosities and a uniformity in pore size not found in commonly used sintered glass, and has eliminated cleaning problems. Removability of the bottom fitting, which has also become a feature of commercial equipment, is helpful in techniques calling for the stepwise extrusion of the packing, and in general, in column unloading and cleaning.

The above, and other features described below, have required designs resulting in a system where complete tightness at glass to metal junctions is safely obtained with a little compression of standard "O" rings. This may be achieved through the use of precision bore glass tubing for the column.

VANDENHEUVEL AND HAYES<sup>2</sup>, BAULD AND GREENWAY<sup>3</sup>, BUSH<sup>4</sup>, and others, have insisted upon selecting for the column glass tubing of uniform bore, free of defects, in order to promote packing uniformity.

Precision bore tubing columns answer this description ideally and furthermore they permit the safe application of useful packing techniques having potential drawbacks in ordinary columns. Thus, when a filter paper disk is placed over the column packing to protect the top layer during sample loading operations, uneven contact along the disk periphery may promote formation of preferential flowpaths. Skewness in elution bands may also result from defects in the top layer structure when pressure is being applied with the packing piston, and the latter happens to deviate from the vertical even slightly. Both drawbacks are eliminated by the use of perfectly fitting packing tools in precision bore tubing columns as described by VANDENHEUVEL AND VATCHER<sup>5</sup>.

Temperature control of the column has long been established as an important factor contributing to the reproducibility of LCC results, particularly in partition chromatography. Jacketed columns are therefore used extensively. On the other hand, columns of various diameters and lengths are routinely used in many laboratories to cope with a variety of problems. The authors found it very convenient to group in a simple thermostated tank columns of various diameters. Fastened through "O" ring unions, the columns are effectively protected and remain completely visible at all times. Fittings for 1, 1/2 and 3/8 in. columns are described in the present article along with a thermostated tank system used by the authors. By reducing appreciably the time usually spent in erecting, disconnecting and cleaning the equivalent number of single column units, by eliminating the related paraphernalia, the breakage, and much of the bench space that would otherwise be occupied, compact and versatile systems of this sort improve the management of LCC problems.

#### DESCRIPTION OF EQUIPMENT

##### *Multicolumn tank system\**

Glass columns A, A... (Fig. 1) are fitted in tank B. Windows C, forming the front and back of the tank are made of 1/4 in. thick Lucite fastened to the tank frame by 1 in.-spaced, 1/16 in. diam. flat-head metal screws. Water tightness is obtained by a 1/16 in. thick, 1/4 in. wide Neoprene gasket cemented to the tank frame and lightly coated on the Lucite side with silicone grease. The frame itself is built by silver-soldering two vertical plates E to the top and bottom plates of the tank. Side plates E extend below the tank to base plate F. The tank frame and base plate are made of 1/4 in. thick plate. A series of fittings G and H\*\* are lead-soldered to the bottom and top plate respectively, permitting quick fastening of columns. These are ready for use when fitted with bottom fitting J.

Thermostated water, supplied from a bath not shown, is circulated through the tank (inlet K, outlet L). Alternately, the tank itself is fitted with a heating element and temperature regulator, and the water recirculated through an outside pump.

The rate of flow of the solvent being fed through M (elevated reservoir, or pump) is read on flow meter N and adjusted by needle valve O. The solvent is led through

\* Unless stated otherwise, all metal parts described under this heading are made of brass.

\*\* Details of parts G, H, J, R and S are given below.

the tank by stainless steel coil P. The supply of constant temperature solvent emerging at Q can be connected to any of the columns in use. It is shown connected to stainless steel injection device R and column piston S.

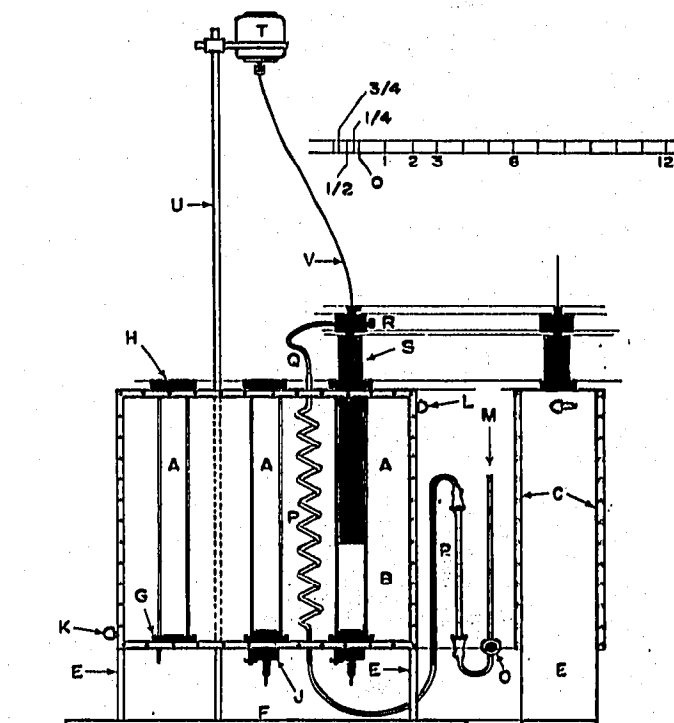


Fig. 1. Multicolumn tank system.

Stirrer motor T, supported by rod U is connected to R by a length of piano wire or a flexible shaft V.

In our present set up, 6 columns (two of each of the three diameter sizes described below) are fitted in a tank less than 11 in. wide. A total effective column length of 5.5 ft. is obtained thereby in a tank light and compact enough to be easily transported. The columns, the only glass parts in this equipment, are well protected from shock by the fastening system used and by the tank itself.

Columns are fastened in the tank through the arrangement shown in Fig. 2 where fittings G and H corresponding to 1 in. columns A, A... are described. Tightness is ensured by compression of "O" rings 3 and 6 obtained by screwing threaded collars 1 and 4 on corresponding collars soldered to bottom plate BP and top plate TP respectively. Neoprene gaskets 2 and 5 protect the column ends and prevent vertical motion. Only the diameters of parts just described need be adapted to fit columns of other sizes.

#### Column fittings\*

Bottom fittings J for the 1 (I, II, III), 1/2 (IV), and 3/8 in. (V) columns are shown in Fig. 3. All include three main parts, *i.e.*, threaded cap 7, central threaded spindle 8, and tightening sleeve 9. In the 1 in. column only, perforated cone 10 is included in

\* All metal parts described under this heading are made of type 316 stainless steel.

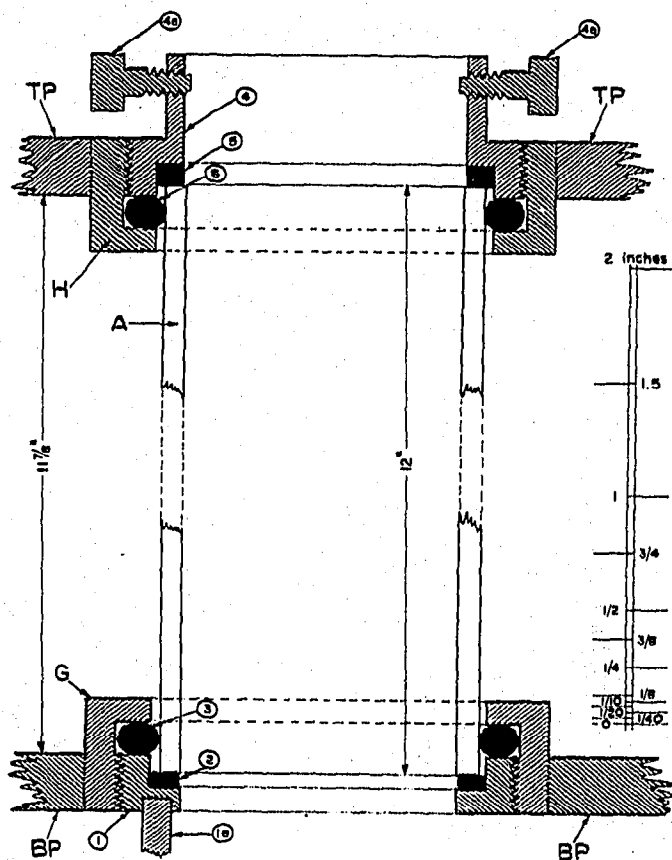


Fig. 2. Axial section showing tank fittings G and H for 1 in. column.

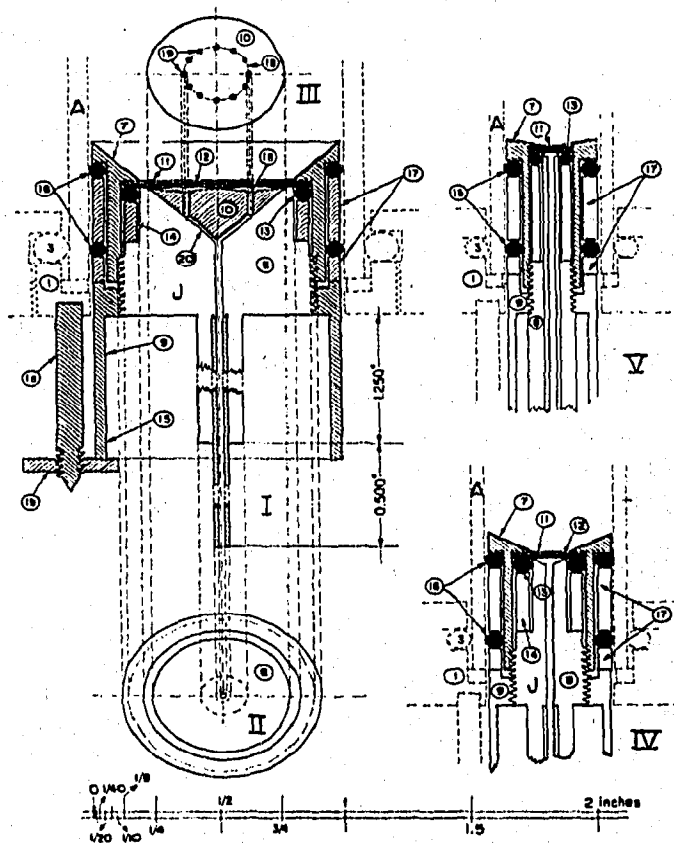


Fig. 3. Axial sections of bottom fittings for the 1 (I), 1/2 (IV) and 3/8 in. (V) columns. Bottom view of 1 in. fitting shown in (II); top view of perforated cone 10 shown in (III).



*Injection device R* shown in Fig. 5 can be used with all columns down to 3/8 in. diameter. It consists of two main threaded metal parts 29 (bottom) and 30 (top). Tightness at the junction of tube 28 and part 29 is ensured by Teflon washer 31. A larger Teflon insert 32 is located between 29 and 30. Leakage is prevented by gland 33 ("O" ring and spacer ring) and by "O" ring 34. Both "O" rings are compressed when 29 and 30 are screwed together. Insert 32 then forms the ceiling of injection

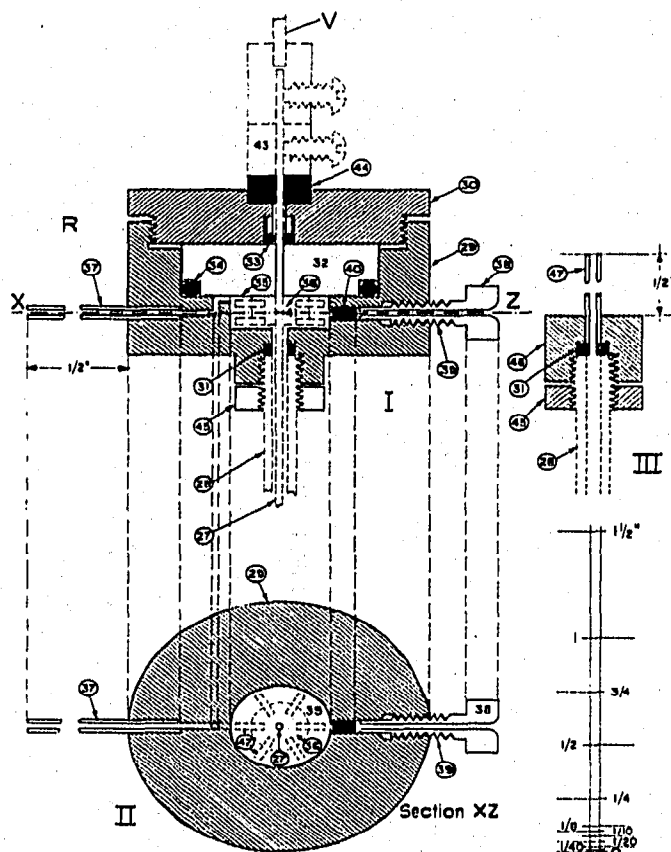


Fig. 5. Axial section of injection device (I) and adapter (III). Transverse section XZ (II).

chamber 35. The latter contains stirrer 36 fastened to stirrer shaft 27. Solvent proceeds to the injection chamber through inlet 37 while the sample is injected through port 38. The 22 to 24 gauge needle of the syringe containing the sample is inserted through screw 39 and pierces septum 40\*. The effective length of the 3/4 in. syringe needle is adjusted by a guard so that when the latter butts against 38, the needle will almost reach hub 41 of the stirrer as it emerges between the two rows of stirrer arms 42. Slowly incoming solvent will mix thoroughly with the injected sample. The resulting mixture will be carried down quickly and completely to the mixing chamber atop the column packing since the combined free volume of chamber 35 and tube 28 is only 0.15 ml.

Stirrer shaft 27 is connected to motor drive shaft V by the collar and screw arrangement shown. Adjustment of the pressure of hub 41 on Teflon insert 32 is obtained through collar and screw 43, while metal to metal friction is avoided by

\* 1/16 in. thick fluorocarbon rubber plug preceded by 0.005-0.010 in. thick Teflon disk.

Nylon washer 44. Connecting tube 28 is locked to part 29 by hexagonal nut 45. Fig. 5 (III) shows the connector replacing injection head R for intercolumn connection.

Table I shows the sizes and materials for all "O" rings used in the fitting for 1, 1/2 and 3/8 in. columns.

TABLE I  
INSIDE DIAMETER, I.D., CROSS-SECTIONAL DIAMETER, W, AND  
MATERIALS OF "O" RINGS FOR COLUMN FITTINGS OF VARIOUS SIZES

Related part	"O" ring*	Material**	Actual dimensions*** (in.) of standard "O" rings					
			1 in. column		1/2 in. column		3/8 in. column	
			I.D.	W	I.D.	W	I.D.	W
G, H (Fig. 2)	3 and 6	A	1.109	0.139	0.612	0.103	0.487	0.103
J (Fig. 3)	13	B	0.614	0.070	0.145	0.070	0.056	0.060
J (Fig. 3) and S (Fig. 4)	16 and 22	Back A Front B	0.864	0.070	0.364	0.070	0.239	0.070
R (Fig. 5)	33	A	I.D.	0.029				
			W	0.040				
	34	B	I.D.	0.614				
			W	0.070				

\* Number refers to number in figure quoted.

\*\* A = Silicone, fluorosilicone or fluorocarbon rubbers; B = Teflon for general application; in most applications, fluorocarbon rubber.

\*\*\*  $\pm$  tolerances (consult Catalog 5700, Parker Seal Co., Culver City, Calif.).

#### COLUMN OPERATION

##### *Packing and loading*

The middle column shown in Fig. 1 is fitted with bottom fitting J and ready to be packed. As a preliminary step, all air is expelled from the bottom fitting by connecting the solvent supply to the bottom outlet and allowing solvent to rise just above the filter paper while a slight vacuum (rubber bulb) is being applied from the top of the column. The column outlet is then closed and the packing slurry poured into the column.

Any packing mixture and any packing method described in the literature can be used. The packing procedure described by VANDENHEUVEL AND VATCHER<sup>5</sup> is particularly suitable to the present system since it takes advantage of precision bore columns by using precision packing tools.

Packing operations should be concluded by inserting a precisely cut, slightly oversized, thick filter paper disk which is pressed against the levelled packing with the packing piston. The necessary pressure is reproducibly obtained by placing the same weight on a plate fastened at the end of the packing piston shaft for the same length of time.

The column head is introduced after cautiously removing the packing piston and adding some solvent. Excess solvent will eventually enter the piston head and be discharged in a beaker held at the solvent inlet. Repeated suction applied to this inlet while the piston is being lowered will remove all air bubbles from the system.

The piston is finally locked in the final position already described. The solvent inlet is then connected to the solvent supply and solvent allowed to run from the column outlet into a beaker. Whatever conditioning is required by the LCC method used is carried out at this stage. In any case, solvent is allowed to percolate until the eluate is perfectly clear. The column outlet is then connected to whatever collecting or monitoring device is required.

Loading of the sample is carried out after arresting the flow of solvent and starting the stirrer motor (300-400 r.p.m.). While a liquid mixture can be injected as such, a solution or fine suspension of the liquid or solid sample in the minimum amount of solvent is generally used. A finely divided solid or liquid dispersion may result from mixing of the sample within the injection chamber. Solvent must be admitted very slowly at a rate and for a time predetermined experimentally. 2-3 ml in excess of the amount needed to obtain a clear solution in the mixing chamber above the packing is usually sufficient. The flow rate is then adjusted to the desired operational level and the stirrer motor switched off.

#### *Column combinations*

The smallest column length used with the present equipment is 2.5 in. for any of the column diameters. This can reach a maximum of 11.5 in. with one column and 23 in. with two. In the latter case the bottom outlet of the first column is connected to the inlet of the second with small gauge plastic tubing\*, the second column being fitted with the adapter shown in Fig. 5.

The interesting effects described by HAGDHAL<sup>6</sup> can be obtained by interconnecting columns of decreasing diameter. These have the advantage of visibility over the metal-clad column system proposed by this author.

When a monitoring instrument is used with solvent of constant composition, some of the solvent supply is diverted to the standard cell as described by VANDENHEUVEL AND SIPOS<sup>7</sup>. With linear gradient elution the same procedure is often sufficient. In non-linear situations, however, complete compensation for solvent variations can only be achieved with solvent percolated through a twin column, the solvent supply being equally divided between the two columns.

Any unused column fitted with J (Fig. 1) will function as a mixing device for continuous gradient solvent production. The required volume of starting solvent (*cf.* LEBRETON<sup>8</sup>) is placed in the column and the column head is lowered to the top of the liquid\*\*. Continuous mixing of diluting solvent flowing from the bottom produces the required graded solvent emerging from tube 37 (Fig. 5).

#### DISCUSSION

The present equipment resulted from the modification of an early model where 1/4 in. wall precision bore tubing was used. This type of tubing, a current production item in 1956\*\*\* is only available on special order today. Although the substitution of inexpensive 2 mm wall precision bore tubing necessitated a more elaborate design, the resulting equipment proved equally effective and simple to use.

\* Gauge 18 Teflon spaghetti, Fluorocarbon Products Inc., Camden 1, N. J.

\*\* The normal stirrer is replaced by a simple wire loop reaching the bottom of the column.

\*\*\* Precision Glass Products Company, Philadelphia 38, Pa.



In both column bottom fitting (Fig. 3) and column piston (Fig. 4) use is made of two external "O" rings. Soft fluorocarbon or silicone rubber is used for the backing "O" ring in order to obtain enough lateral expansion to ensure tightness under low compression. Made of tougher, non-contaminating Teflon, the "O" ring on the solvent side will expand only enough to prevent diffusion from either side.

Use of Teflon insert 32 (Fig. 5) avoids metal to metal friction of moving parts while stirrer 36 prevents segregation and immobilization of still undissolved sample particles. Both the rate of flow during the loading period and the dead volume constituted by the chamber above the packing should be kept as small as possible to ensure minimum initial band volume. Although the proposed loading procedure should be effectively equivalent to that usually practiced, the results are far less dependent on operator skill and experience in being determined by two easily reproducible conditions, *viz.*, the flow rate of solvent and the length of time assigned for this flow.

The example shown in Fig. 6 was chosen to permit comparison with GLC data obtained with the same mixture of methyl esters. It will be noted that a small negative peak appears at point F. This is an artifact resulting from a slight change in

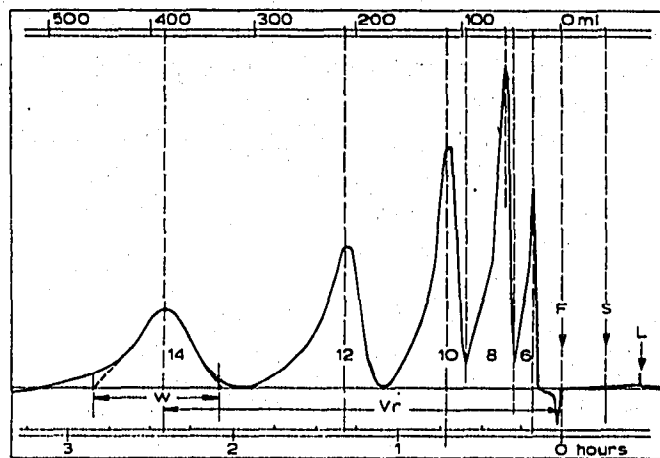


Fig. 6. Separation of methyl esters of the even numbered saturated fatty acids from  $C_6$  to  $C_{14}$  by partition chromatography. Sample,  $70 \mu\text{l}$  injected at L. From L to S, sample loading at reduced flowrate. Constant flowrate ( $2.62 \text{ ml/min}$ ) from S to end. Solvent front at F. In 1 in. column, 80 g siliconized firebrick holding 19 ml iso-octane. Developing solvent, 82% (v/v) ethanol- $\text{H}_2\text{O}$ . Chromatogram obtained with recording differential refractometer described by VANDENHEUVEL AND SIPOS<sup>7</sup>.

solvent composition following exchange with the injected sample. Point F corresponds to the solvent front and the small negative "ghost" peak is an enlarged reflection of the initial band. Indeed it is found that a plot of the logarithms of true emergence volumes  $V_r$  (counted from F to the peak maxima positions) against carbon numbers is rectilinear. The KEULEMANS<sup>9</sup> expression:  $\text{N.T.P.} = 16 (V_r/w)^2$ , yields 150 as the number of theoretical plates corresponding to  $n\text{-C}_{14}$  for this 8 in. column, or 225 theoretical plates per foot. This is comparable to 230 theoretical plates per foot ( $n\text{-C}_{14}$ ) found by HAWKE *et al.*<sup>10</sup> with a 6 mm polyethylene glycol succinate column in the GLC separation of the same esters. Separations obtained with other column packings, some resulting in even better resolution and reduced tailing, will be described in a future article.

## CONCLUSIONS

LCC equipment has not reached the degree of development and effectiveness found in its GLC counterpart. This is partly due to prejudice regarding design and materials from which the rival method appears to be free. Undoubtedly, successful work will still be carried out in simple LCC apparatus. It is even safer to predict, however, that without the limitations imposed by conventional equipment, LCC will make faster progress. The proposed sample injection device and column fittings unite several novel features with others already described in a combination eliminating many pitfalls in packing, loading and general procedure. The compact multicolumn arrangement, on the other hand, should improve sturdiness, management and versatility. Primarily designed for the comparative study of packing materials, this equipment is well suited to the standardization of conditions and the routine application of many forms of LCC, above all, partition chromatography. The high column resolution demonstrated by the example in Fig. 6 should encourage a search for further improvements.

## SUMMARY

A compact, sturdy and versatile thermostated multicolumn system is described. The design of the stainless steel fittings for the 1, 1/2, and 3/8 in. precision bore glass columns permits facile adaptation of the system to most liquid column chromatography problems while virtually eliminating many pitfalls involved in packing and sample loading.

## REFERENCES

- <sup>1</sup> F. A. VANDENHEUVEL, to be published.
- <sup>2</sup> F. A. VANDENHEUVEL AND E. R. HAYES, *Anal. Chem.*, 24 (1952) 960.
- <sup>3</sup> W. S. BAULD AND R. M. GREENWAY, in D. GLICK, *Methods of Biochemical Analysis*, Vol. 5, Interscience, New York, 1957, p. 966.
- <sup>4</sup> I. E. BUSH, *Chromatographic Separation of Steroids*, Pergamon, Oxford, 1961, p. 144 ff.
- <sup>5</sup> F. A. VANDENHEUVEL AND D. R. VATCHER, *Anal. Chem.*, 28 (1956) 838.
- <sup>6</sup> L. HAGDHAL, *Acta Chem. Scand.*, 2 (1948) 574.
- <sup>7</sup> F. A. VANDENHEUVEL AND J. C. SIPOS, *Anal. Chem.*, 33 (1961) 286.
- <sup>8</sup> P. LEBRETON, *Bull. Soc. Chim. France*, (1960) 2188.
- <sup>9</sup> A. I. M. KEULEMANS, *Gas Chromatography*, Reinhold, London, p. 112.
- <sup>10</sup> J. C. HAWKE, R. P. HANSEN AND F. B. SHORLAND, *J. Chromatog.*, 2 (1959) 547.