April 29, 1969 H. K. BLUME 3,440,864

LIQUID CHROMATOGRAPHIC COLUMN

Filed Oct. 3, 1966 Sheet 1 of 3

Fig. 2.

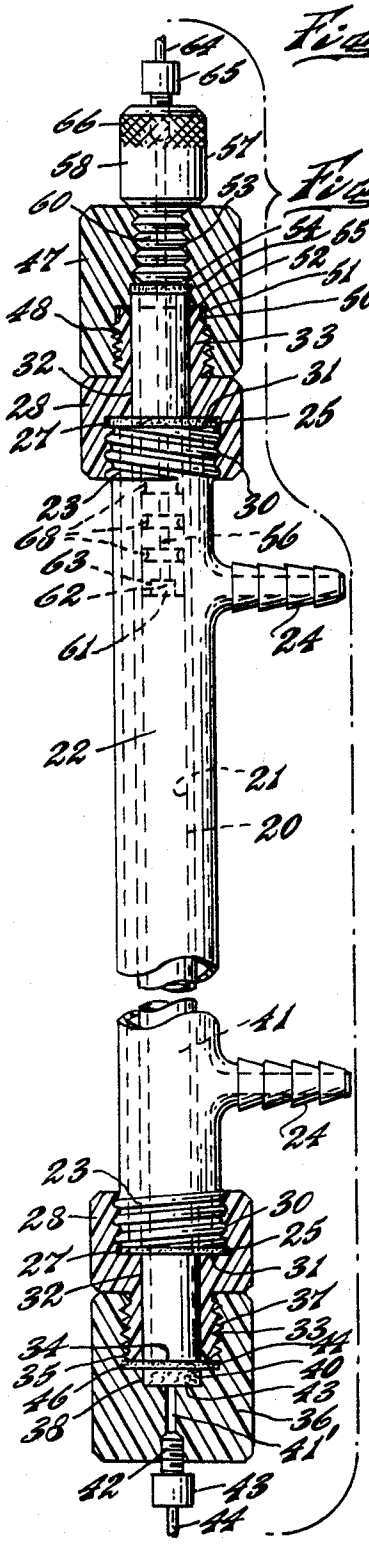
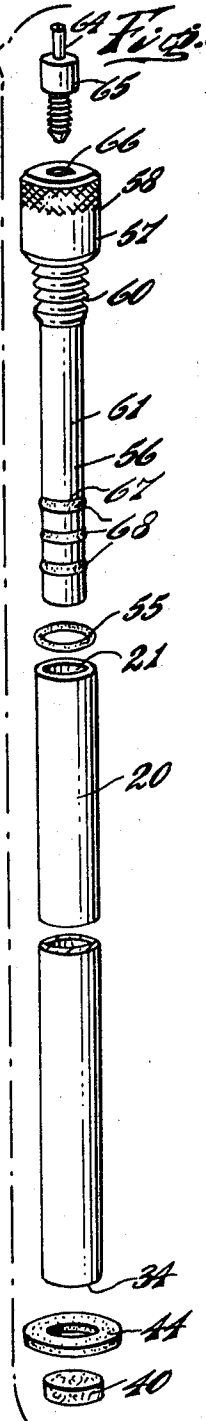
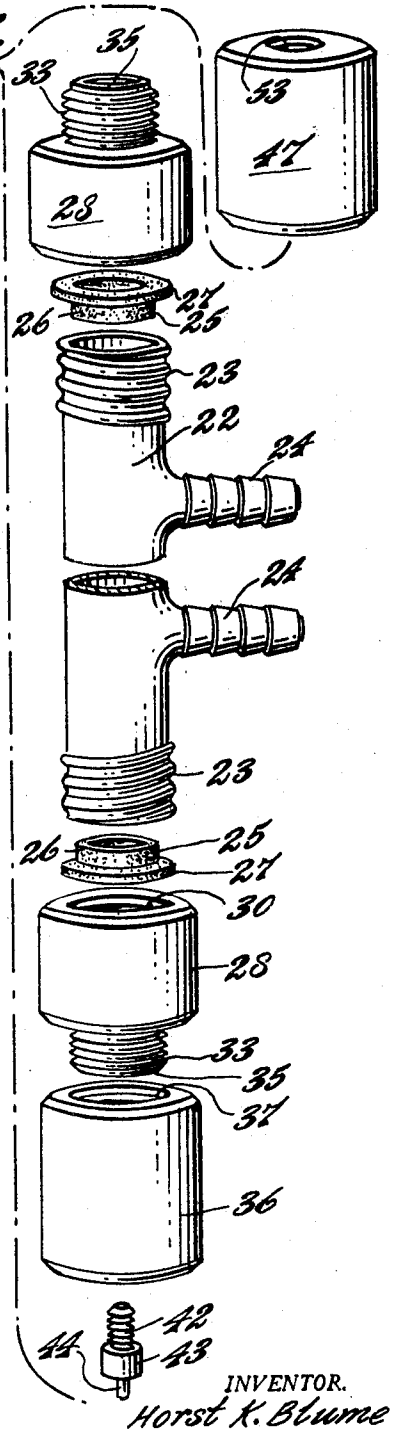


Fig. 1.

Fig. 3.





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LIQUID CHROMATOGRAPHIC COLUMN

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Fig. 3a.

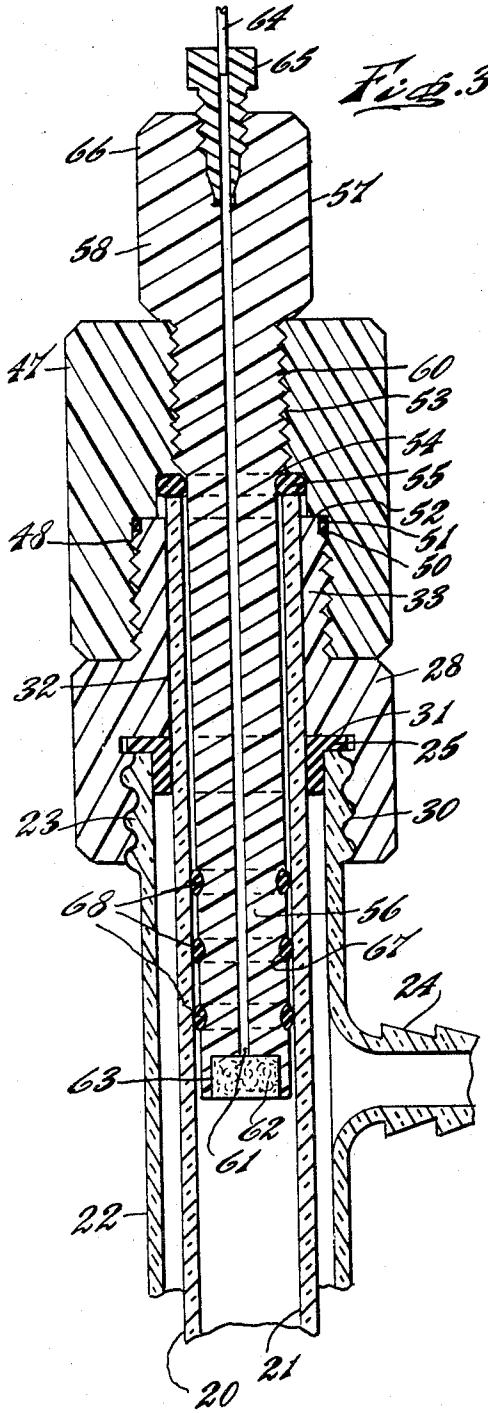


Fig. 4.

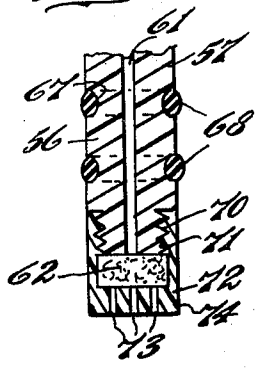
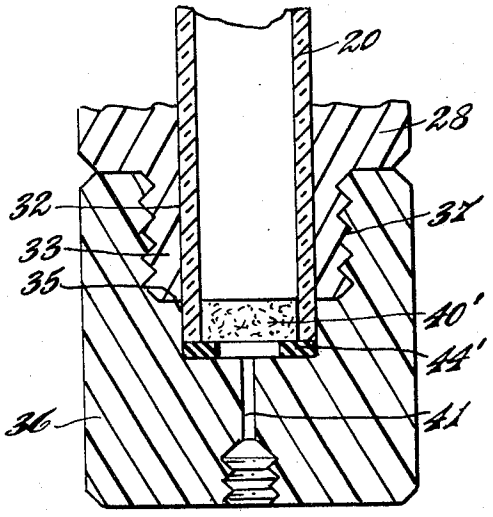


Fig. 5.



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LIQUID CHROMATOGRAPHIC COLUMN

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Fig. 6.

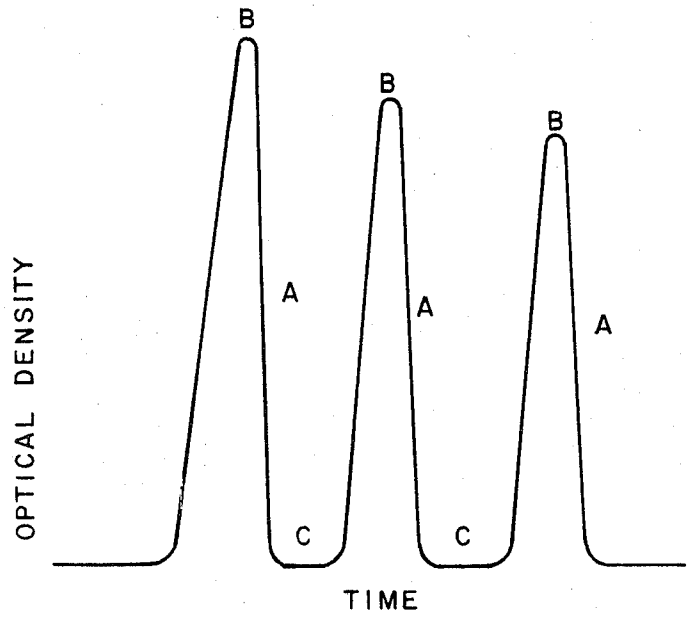
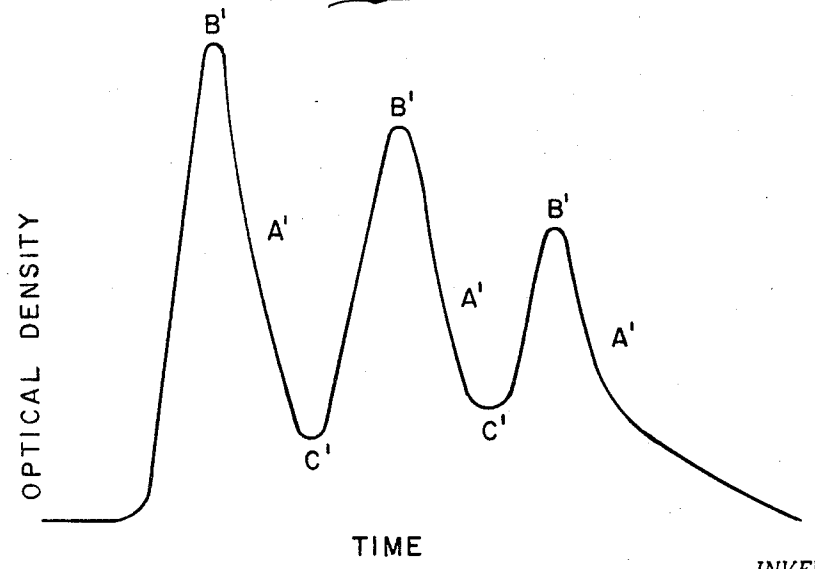


Fig. 7.



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United States Patent Office 3,440,864 Patented Apr. 29, 1969

3,440,864

LIQUID CHROMATOGRAPHIC COLUMN

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Int. Cl. G01n 11/08
U.S. Cl. 73—61.1 14 Claims

ABSTRACT OF THE DISCLOSURE

A chromatographic column having an absorbent bed and an adjustable plunger for bringing pressure on the bed and eliminating the void space between the inner end of the plunger and the bed. A porous cushioning pad may be provided on the end of the plunger to additionally take up the void space between the plunger and the bed.

The present invention relates to liquid chromatographic columns containing adsorbents.

In summary, the invention is concerned with an improved liquid chromatographic column capable of withstanding elevated pressures. One of the important features of the invention is that any void space which may exist or may later occur between the end of the bed and the adjoining end structure can be taken up by movement of a plunger in the axial direction under axially-applied force from a screw. One or preferably both ends of the bed are preferably in contact with porous cushioning material, so that if desired the column can be placed under a resilient pressure which will constantly take up any void space. Furthermore, in the present invention it is no longer necessary to tool the ends of the tube which contains the adsorbent bed itself, so that less danger of cracking occurs. Furthermore, the column of the invention is protected against contamination as by material which is crystalized on the connectors.

A purpose of the invention is to permit a chromatographic column to operate under relatively high internal pressure which may be incident to pumping, injecting of samples or the like.

A further purpose is to eliminate any void space which initially exists or may occur due to packing down of a chromatrographic column by permitting adjustment of the distance between one end of the column and a plunger under the action of an axial screw, with or without exerting pressure on the adsorbent bed.

A further purpose is to interpose a porous cushion at one or both ends of an adsorbent column between the column and the connectors and to permit application of resilient pressure through a plunger to the column against the cushion, so that as the column compacts, the cushion will tend to take up any available space.

A further purpose is to avoid tailing off of the chromatographic curve on the descending side due to the presence of void space in the column.

A further purpose is to avoid the necessity for opening the column and thus possibly contaminating it with solid materials which tend to form by evaporation at the ends of the column, by making the column in a self-contained form which does not require frequent opening, permitting injection of the sample at some other point in the system.

A further purpose is to surround the tube containing the adsorbent bed with a jacket having threaded ends and to obtain the endwise force for tightening gaskets on the tube by placing the jacket longitudinally in tension, thus permitting tightening of gaskets against the end of the tube, tightening of gaskets against the end of the jacket and also between the jacket and the tube.

Further purposes appear in the specification and in the claims.

In the drawings I have chosen to illustrate one only of the numerous embodiments in which the invention may appear, selecting the form shown from the standpoints of convenience in illustration, satisfactory operation and clear demonstration of the principles involved.

FIGURE 1 is an axial section of the chromatographic column of the invention.

FIGURES 2 and 3 are an exploded perspective of the chromatographic column of FIGURE 1.

FIGURE 3*a* is a fragmentary axial section of the device of the invention at the plunger end or optionally at both ends.

FIGURE 4 is a fragmentary axial section of the device of the invention showing a modification of the construction at the inner end of the plunger.

FIGURE 5 is a fragmentary axial section of the device of the invention showing a modification of the construction at the bottom of the column.

FIGURE 6 is a chromatographic curve plotted using the improved column of the invention, the ordinate being optical density or concentration and the abscissa being time. The curve shown in FIGURE 6 is merely diagrammatic and illustrative.

FIGURE 7 shows a curve similar to FIGURE 6 which illustrates in a diagrammatic and illustrative manner differences in the chromatographic curve which may be obtained if there is void space at the top of the adsorbent bed.

The present invention relates to an improvement in liquid chromatographic columns which contain an adsorbent bed which may be an ion exchange resin, an adsorbent gel or another suitable chromatographic column-filling material.

Prior liquid chromatographic columns were not completely satisfactory. Even though the column is completely filled initially with the adsorbent bed, there is a tendency after three or four uses of the column for the adsorbent particles to pack, thus, reducing the volume and tending to create a void space, usually between the top of the column and the top connector. This is seriously objectionable because a sample introduced into the column enters this void space and tends to diffuse or mix, for example with elutrient, before it comes in contact with the adsorbent bed. This tends to produce a tailing off of the chromatographic curve plotted by the mechanism on the descending side, rather than obtaining a sharp descending line from the hump.

Liquid chromatographs which plot curves in response to electrical impulses are well known in the art, and are described for example in Phoenix Precision Instrument Company Catalogue No. K–8000–CVG–6000–E (1965).

The presence of void space also slows down the response.

If you are operating with a gradient produced, for example, by a suitable pump, the effectiveness of the chromatograph is reduced by diffusion between one incoming sample and a residue of a former sample due to the presence of the void space. Also, a secondary mixing effect occurs, and the amount of secondary mixing is not predictable and may differ from one sample to another.

Another difficulty which has been present in older chromatographic column designs is that they were not capable of withstanding pressure.

In the older columns it was commonly necessary to open the column frequently, and slight leakage and evaporation tended to deposit crystals on the connector at the top of the column. When the column was opened, these crystals would in some cases drop into the top of the column and produce a false response.

Furthermore, in prior art columns in many cases the column tube itself was tooled at the ends, and the machining necessary to tool caused stresses which would often cause breakage and require early replacement.

The present invention contemplates an improved chromatographic column which eliminates these difficulties. The column tube itself has a precision bore, and a straight plunger fills this bore and seals to it. The plunger is capable of being advanced as the top of the column tends to settle under axial screw action, so that it can remain in firm engagement with the top of the adsorbent bed.

Furthermore, the structure in contact with the column at one or both ends is desirably provided with porous cushioning material which is capable of slight compression under pressure and can be compressed by the plunger pressure and will apply follow-up pressure to the column as it tends to shrink.

FIGURES 6 and 7 plot for comparison two chromatographic curves of optical density against time. In FIGURE 6 there is good resolution using the column of the invention, and in FIGURE 7 there is poor resolution using a prior art column which provides void space at the top of the bed. Thus, in FIGURE 6 there are steep descents A, and they are close to Gaussian distributions whereas in FIGURE 7 the descents A' are gradual because one component has tended to mix with another or with the elutrient. Furthermore, in FIGURE 6 all peaks B are higher than corresponding peaks B' in FIGURE 7. Also, in FIGURE 6 the valleys C descend to the origin whereas in FIGURE 7 the valleys C' do not descend to the origin.

Thus, it will be evident that not only does the invention prevent tailing off on the descending part of the curve, but it also permits operation under high pressure, of the order of 1000 p.s.i. or even higher.

The column in the present invention is intended to remain closed until it is necessary to replace the adsorbent bed, thus avoiding contamination as from crystals which may accumulate on the end connector. The introduction of a sample is accomplished at some other point in the system as by a sample injector which can be of prior art type.

In the column of the invention it is no longer necessary to tool the ends of the column tube, and therefore this source of breakage is avoided.

As shown in the drawings, the chromatographic column of the invention comprises a tube **20** which has straight side walls free from any flanging or threading, and is interiorly precision bored at **21** to receive a plunger as later described. The tube **20** is desirably made of glass, although it can, where desired, be formed of a suitable noncorrosive metal such as stainless steel or of a suitable plastic.

Surrounding tube **20** and in spaced relation to it is jacket **22** provided with male threads **23** at the opposite ends and somewhat shorter than the tube **20** to fit the end connection to be described. The jacket has inlet and outlet hose connections **24** for introduction and removal of a temperature regulating medium, such as hot or cold water, brine, glycerine, steam or other heat transfer medium.

The jacket may be suitably made of a heat resisting plastic, such as polytetrafluoroethylene (Teflon TFE), or the copolymer of tetrafluoroethylene and 5 to 35% of hexafluoropropylene (Teflon FEP), or glass.

The space between the jacket **22** and the tube **20** is closed at the top and bottom by an annular gasket **25** which is L-shaped in cross section and has a tubular portion **26** extending and sealing between the jacket **22** on the outside and the tube **20** on the inside, and a flange portion **27** extending radially outward from the end of the tubular portion **26**. The gasket **25** is preferably made of a synthetic rubber, such as neoprene or silicone rubber.

The gasket **25** is forced into the space between the jacket and the tube and also compressed at the flange portion **27** by jacket connectors **28** at the top and bottom

having female threads **30** cooperating with the male threads **23** on the top and bottom of the jacket and having a radial and annular seating portion **31** which is capable of compressing the flange portion **27** on the gasket **25** against the end of the jacket **22**, and also forcing the tubular portion **26** of the gasket **25** into the space between jacket **22** and the tube **20**.

The jacket connector at the opposite end from the jacket has a bore **32** which closely conforms to the exterior of the tube **20**, and the jacket connector extends away from the jacket, terminating in a male threaded nipple or extension **33** through which the bore **32** extends.

The jacket connector may be made of polyvinylchloride, polyfluoroethylene (Teflon TFE), a copolymer of tetrafluoroethylene and 5 to 35% of hexafluoropropylene (Teflon FEP) or polytrifluorochloroethylene (Kel-F). In case, however, it is desired to employ organic solvents in the column, it is preferable to produce the connector of Teflon TFE or FEP or Kel-F.

With the bottom wall of the tube **20** annular and radial as at **34** and flush with the bottom of the nipple **33** which also has a square end **35**, a tube connector **36** having a recess provided with female threads **37** cooperating with the male threads **33** on the nipple is tightened against the jacket connector. The tube connector **36** has a central socket **38** of about the same diameter as the outside of the tube and in this is inserted a porous disc **40** and sometimes called a cushion, preferably having cushion properties, such as porous Teflon TFE or FEP, porous polyethylene, porous stainless steel or porous fiber glass. When the cushion is described as porous it will be understood that the pores are interconnecting. The porous disc or cushion **40** occupying the recess **38** prevents the entrance of particles from the adsorption bed **41** which fills the interior of the tube **20** into a capillary bore **41'** extending through at the axis of the tube connector **36** from a threaded socket **42** at the bottom which receives a threaded connector **43** from a capillary tube **44** connecting to another part of the chromatograph, to the porous filter cushion **40**.

Above the cushion **40** is placed a ringlike gasket **44** which can be compressed between the tube **20** and the end of the nipple **33** at the top and between the cushion **40** and a straight annular portion **45** on the tube connector **36**. The gasket **44** occupies a recess **46** in the tube connector which is preferably slightly larger than the interior of the threads and is capable of holding the gasket **44** in place.

The gasket **44** is preferably of a synthetic rubber such as silicone rubber.

The tube connector itself is desirably made of polyvinylchloride or Teflon TFE or FEP or Kel-F. There is an advantage however in using one of the polyfluoroethylenes where an organic solvent will be present because of the resistance to attack by organic solvents.

At the top a tube connector **47** has at the bottom a recess provided with female threads **48** which engage with the male threads on the nipple **33**.

Near the outer end and at the interior the nipple **33** has an inner conical recess which is wider at the top and which receives optionally an auxiliary annular gasket or ferrule **51** of conical form composed of Teflon TFE or FEP or Kel-F or polyethylene and being compressed between the conical wall **50** of the recess, the outside of the tube and a square end portion **52** on the recess in the tube connector **47**.

The tube connector **47** has above the end of the tube a plunger bore **53** which has female threads on it, and near the bottom just above the square end of the tube **20** there is a gasket recess **54** which receives an O-ring **55** suitably of synthetic rubber such as silicone rubber, and which is compressed between the wall of tube connector **47** on the outside and the top, against the end of tube **20** at the bottom, and by the plunger to be described at the inside.

Extending down and filling the precision bore inside **21** of the tube **20** is a forward cylindrical portion **56** of

a plunger **57** which has a head **58** at the top and a male threaded portion **60** intermediate between the head and the cylindrical forward portion which cooperates with the threads **53** on the tube connector **47**.

Extending from end to end through the plunger is a capillary bore **61** which communicates with a porous cushion **62** in a recess **63** on the forward end and communicates with the rest of the chromatograph by a capillary tube **64** which is connected by a threaded connector **65** in a threaded bore **66** communicating with the capillary bore **61**. The porous cushion **62** on the forward end of the plunger is desirably of porous Teflon TFE, or FEP, or Kel-F, porous polyethylene, porous strainless steel or porous fiber glass. The pores are interconnecting.

Along the length of the cylindrical portion **56** of the plunger there are a series of annular gasket recesses **67** which receive O-rings **68**, the O-rings being compressed between the bore **21** of the tube **20** and the recesses **67** of the plunger. The O-rings are preferably made of a synthetic rubber such as silicone rubber with Teflon coating.

In initially filling the interior of the tube with the adsorbent bend with the jacket connectors in place and the tube connectors in place but the plunger withdrawn, the filling of the bed with ion exchange resin or gel adsorbent is full enough so that when the plunger is inserted it will not be possible to screw it all the way down, but not full enough to prevent engagement of the plunger threads. Thus the plunger is capable of intermediate takeup.

The plunger is preferably made of a suitable plastic such as polyvinylchloride, Teflon TFE or FEP or Kel-F and if organic solvents are to be used is preferably made of a Teflon or Kel-F.

In operation, once the proper quantity of adsorbent is placed in the tube and the plunger is inserted until it firmly engages the top of the bed, it will be evident that if the plunger is tightened with reasonable tightness and cushions are provided in the form of the porous material at the top and bottom, the cushions are capable of undergoing slight compression, and this will provide a slight takeup when the bed reduces its overall volume as it usually does after three or four utilizations. If, however, there is still some void space above the bed, the plunger can be screwed down until it again firmly engages the top of the bed, thus eliminating the void spaced. This advance of the plunger can be accomplished without opening the column, and furthermore it does not interfere with operating of the column under pressure. Avoidance of opening the column prevents the possibility that crystals of chemicals which may have formed by evaporation near the top around the fittings because of slight leakage can contaminate the next or subsequent runs.

Once the void space is eliminated, the tendency to reduce tailing of the chromatograph curve on the descending side due to admixture between one sample and the residue of a former sample or with the elutrient is eliminated, and sharper chromatographic indications are obtained. The fact that the bores in the tube connectors are of capillary dimensions and that capillary tubing brings the sample to the column from an outside sample injector tends to prevent the mixing of the sample with the elutrient.

The fact that the ends of the tube itself are free from threads prevents the likelihood of early tube failure, which has formerly been a feature in chromatographic columns.

The use of a jacket of an elastic plastic along with jacket connectors and tube connectors of a similar plastic tends to reduce the danger of breakage of the tube during storage, shipment or use.

If desired, the lower part of the device can be made identical with the upper portion by providing identical connectors and a plunger at both the bottom and the top so that the plungers can oppose one another, as suggested by FIGURE 3.

In many cases it is preferable to modify the lower end of the plunger as shown in FIGURE 4 by threading it at **70** and threading on to the external thread **70** internal threads **71** of a cap **72** which compresses a cushion **62** of porous material as above mentioned at the lower end of the plunger. Distribution of the sample from the capillary bore **61** is accomplished through capillary longitudinally extending bores **73** in the cap. The cap is desirably of a plastic such as Teflon, and its outside circumference **74** tightly conforms to the interior of the tube **20**.

In FIGURE 5 I show a preferred modification of the construction of the bottom of the column in which the tube **20** seats a modified gasket **44'** against the interior bottom of the jacket connector **36**. In this case a porous disc or cushion **40'** is placed inside the tube **20** at the bottom of the bed of adsorbent, and it will prevent particles of the bed from entering the capillary bore **41** and also will permit the resilient compression against the column provided by the bed.

In view of my invention and disclosure, variations and modifications to meet individual whim or particular need will doubtless become evident to others skilled in the art.

Having thus described my invention, what I claim as new and desire to secure by Letters Patent is:

1. In a chromatographic column, a tube defining the column wall and adapted to contain an adsorbent bed, said tube having a uniform bore, a first tube connector secured to one end of the tube in liquid tight engagement therewith, said first connector having a bore for communication with the bed, a second tube connector secured to the other end of the tube in liquid-tight engagement therewith, said second tube connector having an axial threaded bore, and a plunger extending through the threaded bore of the second tube connector and in liquid-tight contact with the interior of the bore, the plunger having threads thereon which engage the threads in the threaded bore and permit advance of the plunger to eliminate void space between the bed and the plunger, the plunger having a longitudinal bore extending therethrough and communicating with the bed.

2. A column of claim **1**, in combination with a jacket surrounding the tube and making liquid-tight joints with the first and second tube connectors at the opposite ends.

3. A column of claim **2**, in combination with gasket means interposed at the opposite ends between the first and second tube connectors and the ends of the jacket and also radially between the jacket and the tube.

4. A column of claim **1**, in combination with porous cushioning means at the inside of the first tube connector in the path of flow through the bed and permitting cushioning of axial pressure applied by the plunger on the bed.

5. A column of claim **4**, in combination with porous cushioning means also on the inner end of the plunger in the path of the flow through the bore in the plunger.

6. A column of claim **1**, in combination with porous cushioning means on the inner end of the plunger.

7. A column of claim **1**, in combination with a porous body on the inner end of the plunger, and a cap secured to the inner end of the plunger, holding the porous body in place, having a longitudinal distributing passage extending to the bed and in tight engagement with the interior wall of the tube.

8. A column of claim **1**, in combination with gasket means interposed at the opposite ends between the ends of the tube and the first and second tube connectors.

9. In a chromatographic column, a tube having a uniform internal bore and adapted to contain an adsorbent bed, a jacket surrounding the tube in spaced relation and relatively shorter than the tube, the jacket having threads at the opposite ends, jacket connectors threading on the jacket at the opposite ends and surrounding the tube in engagement therewith, jacket gaskets compressed between the jacket, the jacket connectors and the tube at the oppo-

site ends, the jacket connectors having threaded extensions around the tube, a first tube connector threaded on one of the jacket connectors, extending endwise beyond the tube at that end and having a bore extending therethrough, a tube end gasket compressed between the first tube connector, the end of the tube, and the corresponding jacket connector, a second tube connector threaded on the extension of the jacket connector at the opposite end, a porous cushion at the bottom of the tube adjacent the bed, and end gasket compressed between the second tube connector and the end of the tube, there being an axial threaded bore through the second tube connector, a plunger extending through the axial threaded bore in threaded engagement with the threads in the bore of the second tube connector and through the interior of the tube at the adjoining end, there being an axial bore through the plunger, gasket means sealing between the plunger and the bore of the tube, there being communication through the first tube connector.

10. A column of claim **9**, in which there is a recess at the inner end of the plunger, in combination with a second porous cushion in the recess at the inner end of the plunger.

11. A chromatographic column comprising a tube, connectors at opposite ends in sealed engagement with the tube, there being a threaded bore through each of the connectors and there being a bed of adsorbent in the tube, and plungers threaded through the theaded bores of the connectors and having cylindrical portions extending into the tube and making sealed engagement with its walls, each of the plungers having a longitudinal passage therethrough permitting communication with the bed, and the plungers being relatively adjustable to eliminate void space between the inner ends of the plungers and the bed.

12. In a chromatographic column, a tube defining the column wall, said tube being straight from end to end inside and outside and having a uniform bore, the tube being adapted to contain an absorbent bed, a jacket surrounding the tube in spaced relation, a first connector making a liquid-tight joint to one end of the jacket, extending inwardly to engage the tube and having a threaded portion surrounding the tube, a second connector surrounding and sealing to the tube at said one end, making threaded engagement to the first connector and having a passage for communication with the interior of the tube, a third connector making a liquid-tight joint to the other end of the jacket, extending inwardly and engaging the tube and having a threaded portion surrounding the tube, a fourth connector making a liquid-tight joint to the end of the tube, making threaded engagement with the third connector surrounding the tube and having a threaded bore in line with the interior of the tube, and a plunger having an opening therethrough adapted to engage one end of the bed and threading in the bore of the fourth connector, the plunger being adjustable with respect to the fourth connector.

13. A chromatographic column according to claim **12**, in combination with porous cushioning means in the end of the plunger.

14. A chromatographic column according to claim **13**, in combination with porous cushioning means between the second connector and the column and extending across the passage in the second connector.

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J. W. ROSKOS, *Assistant Examiner.*

U.S. Cl. X.R.

73—23.1; 210—31, 263