United States Patent [19]

Jones

[11] **3,926,809**

[45] **Dec. 16, 1975**

[54] **MULTI-COLUMN FRACTIONATOR**

[76] Inventor: **Roosevelt J. Jones,** 3854 Blodgett, Houston, Tex. 77004

[22] Filed: **Oct. 10, 1973**

[21] Appl. No.: **396,356**

[52] **U.S. Cl.** ..... **210/198 C;** 55/386

[51] **Int. Cl.²** **B01D 15/08**

[58] **Field of Search** ..... 55/386; 210/198 C, 31 C

[56] **References Cited**

UNITED STATES PATENTS

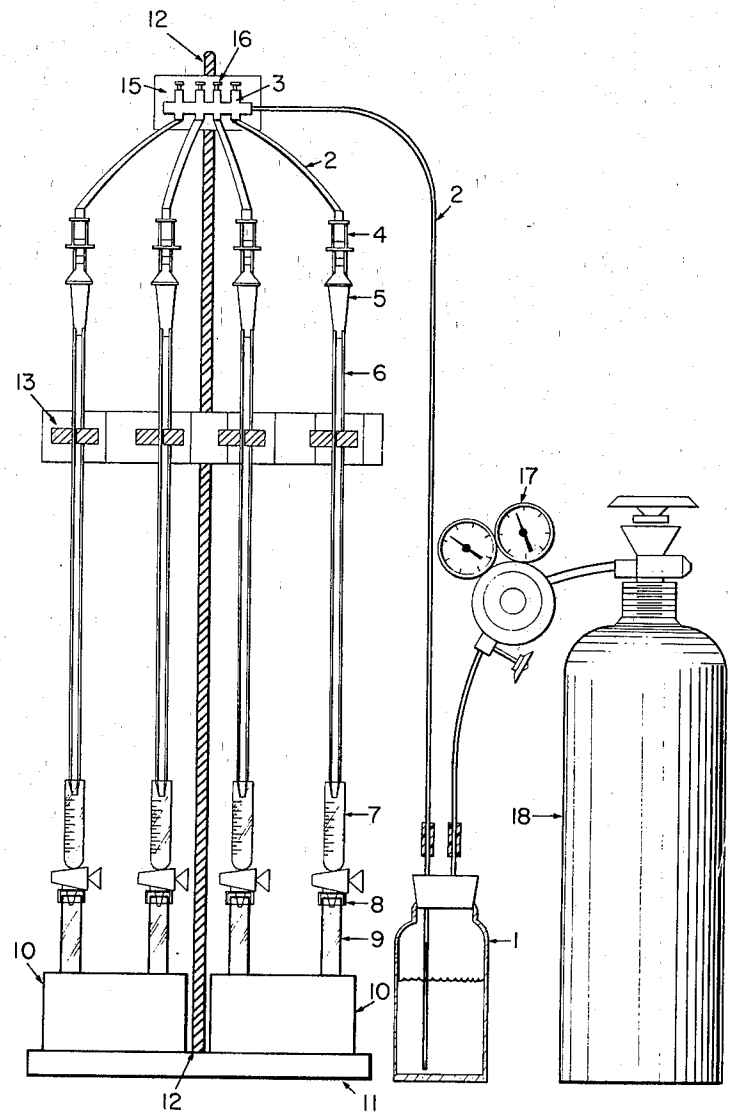
2,935,908	5/1960	Phillips, Jr.	210/198 C X
3,030,798	4/1962	Lichtenfels	55/386 X
3,150,517	9/1964	Kuffea et al.	55/386 X
3,156,548	11/1964	Perry	55/386 X
3,164,980	1/1965	Lord	55/386 X
3,169,389	2/1965	Green et al.	55/386 X
3,373,872	3/1968	Habina	210/198 C
3,504,799	4/1970	Ogle	210/198 C
3,518,874	7/1970	Habina	210/198 C X
3,653,840	4/1972	Silas	55/386 X

Primary Examiner—John Adee

[57] **ABSTRACT**

A chromatography apparatus for the simultaneous fractionation of two, three and four different mixtures of nucleotides, each on a separate ion-exchange column, using a stepwise elution procedure. Each elution solvent, used in a stepwise elution procedure, is forced by air pressure from a single interchangeable reservoir flask on to and through each of two, three or four chromatography columns at the same moment after having been dispersed equally by a multi-valve control.

3 Claims, 3 Drawing Figures



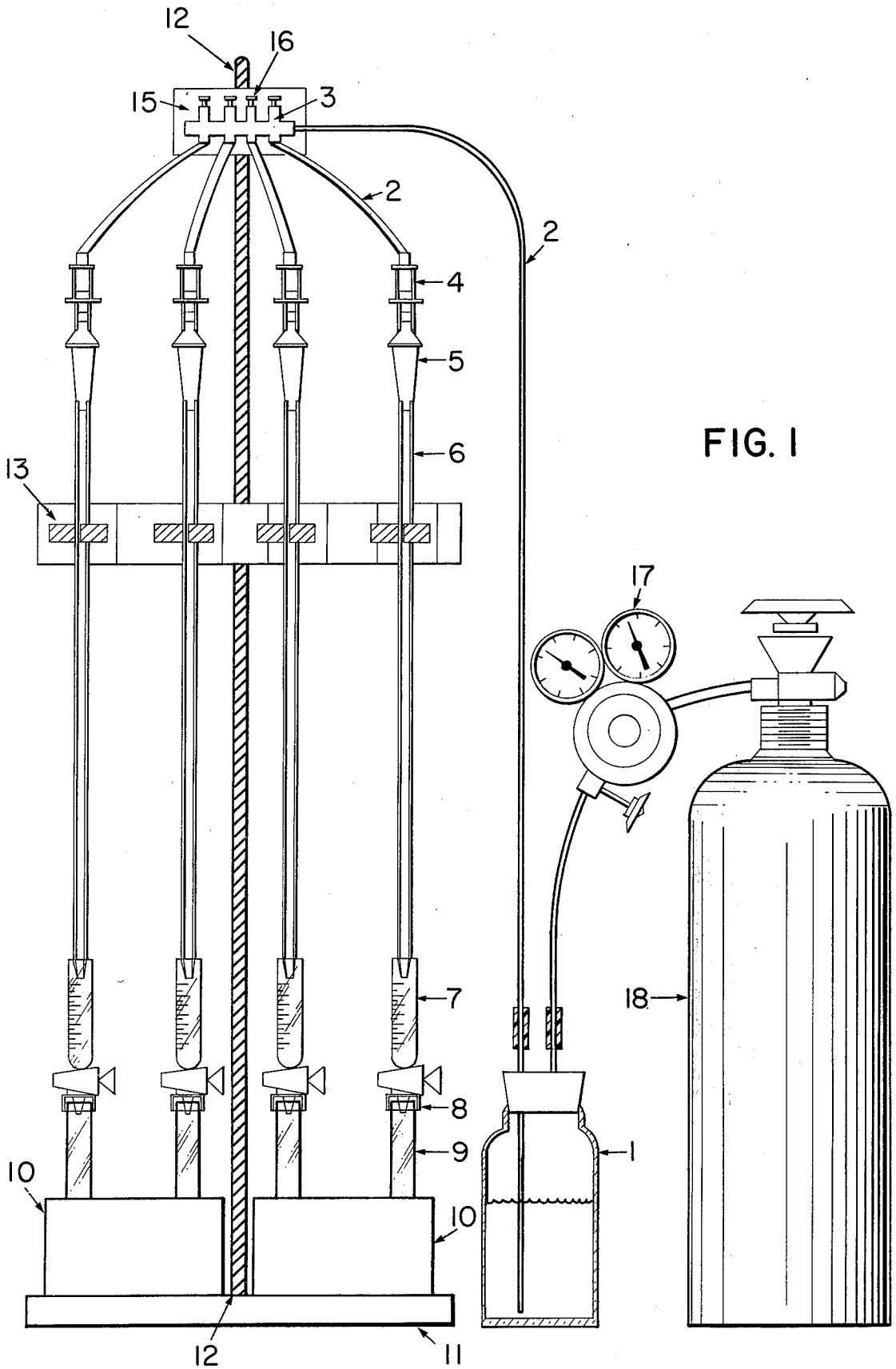
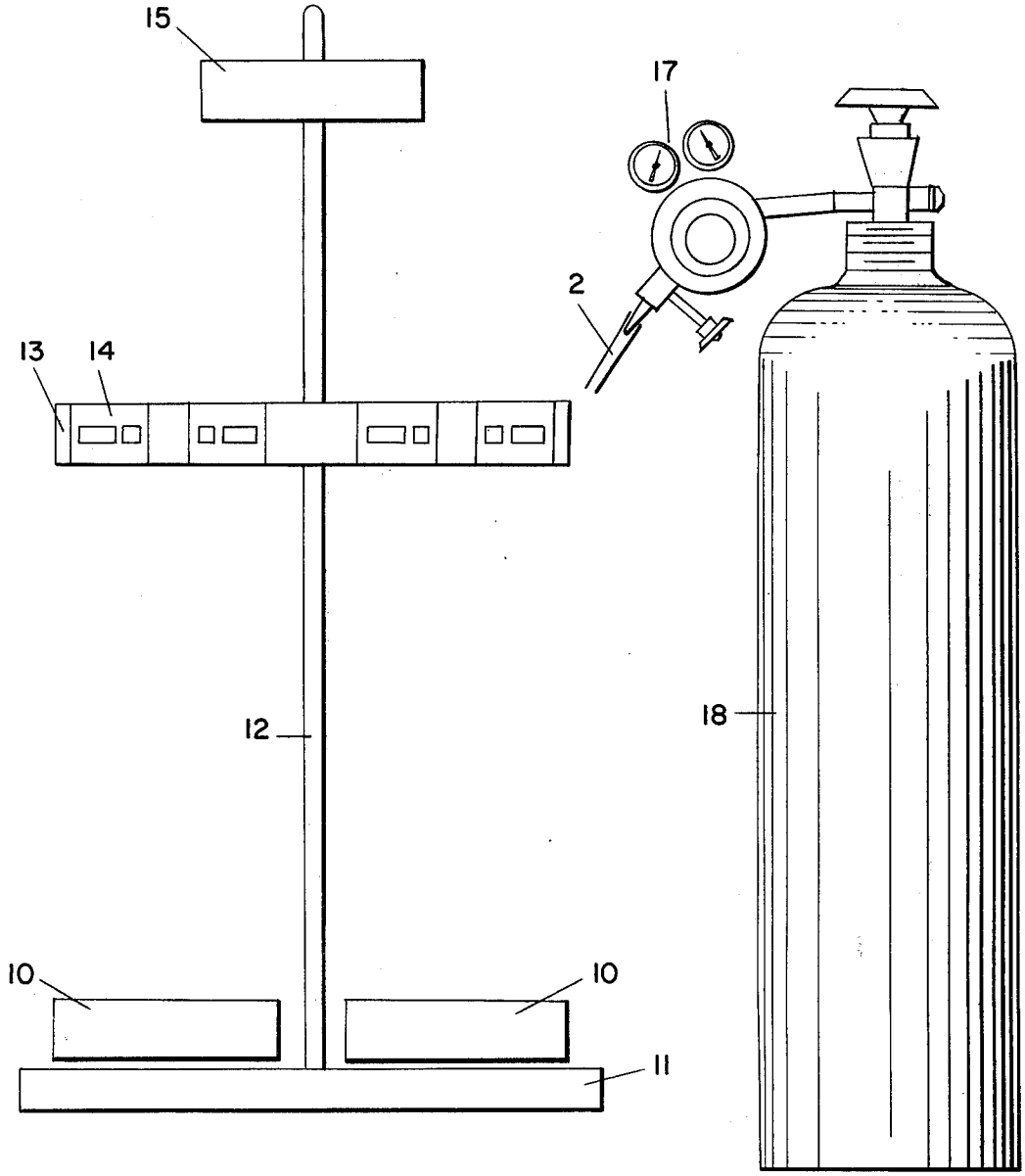


FIG. 1

FIG. 2



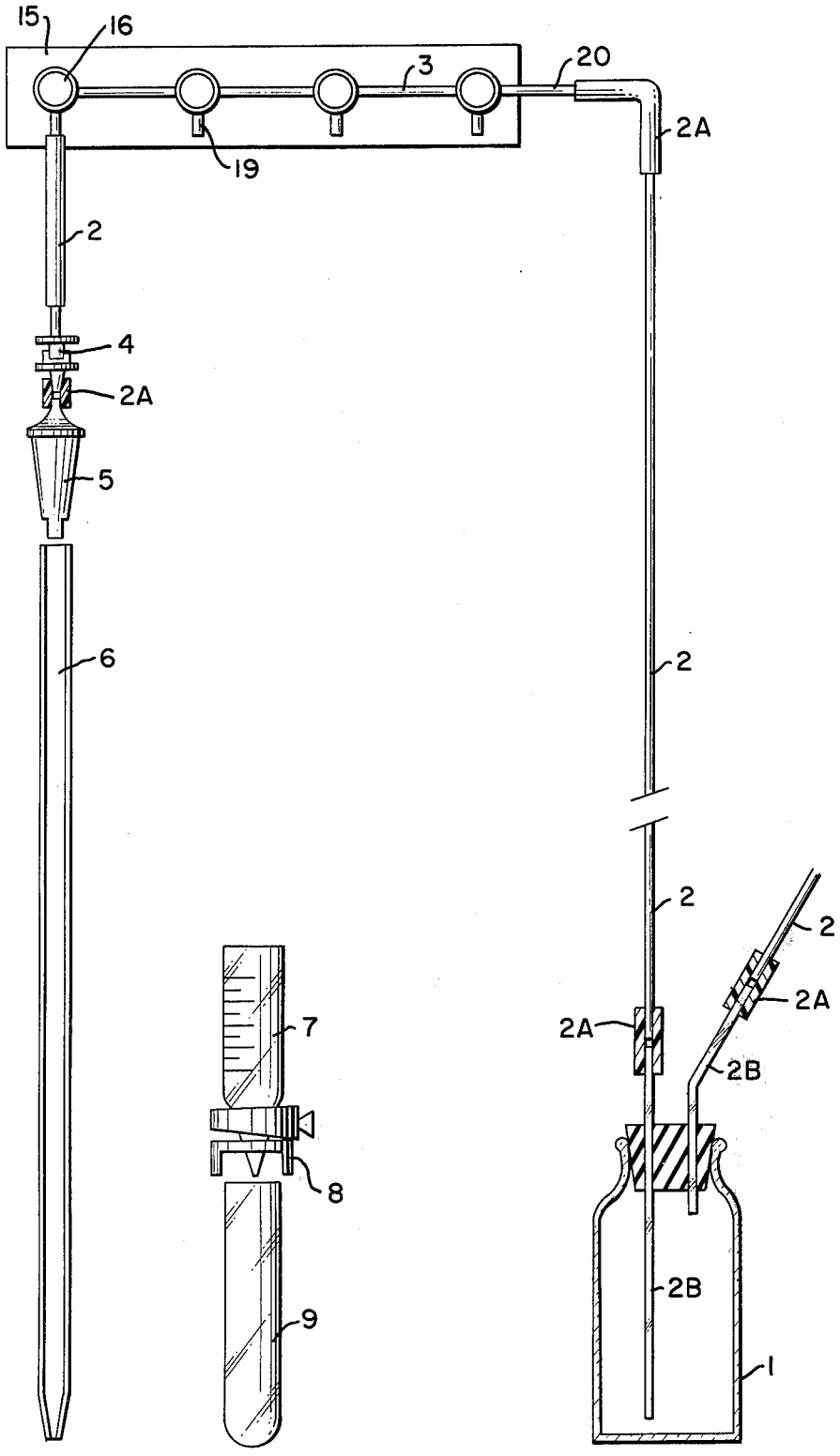


FIG. 3

MULTI-COLUMN FRACTIONATOR

BACKGROUND OF THE INVENTION

This invention relates in principle to known liquid ion-exchange chromatography apparatus (Cohn, 1950. Jour. of Amer. Chem. Soc. 72: 1471–1478) but differs in that four columns, rather than one, are employed to permit the fractionation of four separate and different mixtures of nucleotides during a single operation.

This apparatus requires a manual placing of each sample or mixture of nucleotides on to each of four ion-exchange resin columns prior to forcing the elution solvent of a given pH through the resin columns. The elution solvent of a given pH, when forced through the resin column, will selectively remove certain of the nucleotides of each nucleotide mixture. The apparatus was designed to permit a partial separation of the numerous (more than 20) nucleotides which may be present in two, three or four nucleotide mixtures in a rapid manner, so that individual nucleotide can be isolated from several nucleotide mixtures for subsequent quantitative and qualitative analysis using paper chromatography or thin-layer chromatography. The multi-column fractionator was designed primarily for the fractionation of ^{32}Phosphorus-labeled nucleotide mixtures of biological origin where small volumes of several nucleotide mixtures are to be analyzed. To study the synthesis of one or several different nucleotides, in vivo, as many as 12 or more biological samples labeled with ^{32}phosphorus must be quantitatively and qualitatively analyzed within a period of 4 to 6 weeks, since the half-life of ^{32}phosphorus is 14.5 days. An advantage of this apparatus is that it permits the simultaneous fractionation of two, three or four different nucleotide mixtures in less time than is required to fractionate two different nucleotide mixtures when two independent single column chromatography apparatus are employed successively.

SUMMARY

This invention is based on the novel concept that the elution of nucleotide mixtures from different chromatography columns in parallel would permit the quantitative elution of the same nucleotide when a given elution solvent and flow rate is employed. This invention was devised to permit the elution solvent of a given pH to be forced by air pressure from the reservoir flask through a multi-valve control on to and through each of two, three or four chromatography columns and into collecting tubes. The interchangeable reservoir flasks provide simplicity and save time during shifting from one elution solvent of one pH to another. A prescribed stepwise elution procedure requires eluting with several elution solvents of different pH during a single operation or complete fractionation. The multi-valve control makes it possible to disperse equally each elution solvent of a given pH on to and through each of two, three or four chromatography columns at the same moment. The multi-valve control permits the regulation of flow rate for each column. The eluent which flows from each chromatography column is collected and measured in burettes before transferring into collecting tubes. The collecting of eluent is done manually. The number of elution solvents of different pH and the specific pH of each elution solvent can be prescribed at the discretion of the operator. The size of the resin bed, type of ion-exchange resin used, and eluent flow rate are not limited to those described herein.

DESCRIPTION OF THE INVENTION

FIG. **1** is a schematic four-column liquid chromatography system in accordance with the present invention in one embodiment.

FIG. **2** shows the component parts of the multi-column support stand and the compressed air source as viewed from the front.

FIG. **3** is a schematic of the individual components and connections of the chromatography apparatus with one of its four chromatography columns illustrated.

MODE OF OPERATION OF THE INVENTION

By way of example and with reference to the accompanying drawings the embodiments of the present invention will now be described. As shown in FIG. **2** the multi-column support stand is assembled by fastening, with nut and bolt, the support rod **12** to the support stand base **11.** The multi-valve support **15** and the column clamp support **13** are clamped on to the support rod **12.** The multi-valve support **15** hold the multi-valve control **3** in place. The column clamp support **13,** with four spring clamps **14,** holds the chromatography glass columns **6** upright and in place. The ion-exchange resin (Dowex 1X8 formate) is prepared as described by Hurlbert, et al. 1957. Jour. of Biol. Chem. 209: 23–39. Each chromatography glass column (28 × 0.8 mm) **6** is partially filled with the Dowex-1 resin (bed size: 15 × 0.5 mm) and rinsed with deionized water prior to and after applying the nucleotide mixture. The nucleotide mixture. The nucleotide mixture can be a ^{32}phosphorus-labeled nucleotide extract, that contains 100,000 to 800,000 counts per minute, or anucleotide mixture, that contains 24 mM of nucleotides. Stepwise elution of nucleotides, as described by Hurlbert, et al. 1957. Jour. of Biol. Chem. 209: 23–39, involves making several graduated shifts in the concentration (from 0.01 M to 4 M formic acid; and 4 M formic acid plus varying concentrations, 0.1 to 1 M, of ammonium formate) of the elution solvent during a single operation. The various nucleotides (mono-, di- and triphosphates of the nucleosides of purines and pyrimidines) which posses different dissociation constants (pK values) are eluted individually as a function of the pH of the eluent (Cohn, 1950. Jour. of Amer. Chem. Soc. 72: 1471–1478). The elution volume for each concentration of the elution solvent varied from 5 to 20 ml between shifts, being collected in 5 ml fractions. To detect the presence of ^{32}phosphorus-labeled nucleotides in each eluent fraction a 1 ml aliquot is added to 15 ml of a scintillation fluid (POPOP-toluene and Trition X-100, 2:1) and analyzed with a Packard Tri-Carb Scintillation Counter for ^{32}phosphorus radioactivity. If the eluted nucleotides are unlabeled (containing no ^{32}phosphorus) their presence in each eluent fraction is determined using a Beckman Model DB Spectro-photometer, which detect ultraviolet-absorbing compounds. Final separation and identification of individual nucleotides, present in each eluent fraction or in pooled fractions, are accomplished by paper chromatography (Wyatt, 1955. *The Nucleic Acids of Chemistry and Biology*, Acad. Press, New York. Vol. 1: 243–265) or thin-layer chromatography (Randerath and Randerath, 1964. Jour. of Chromatography 16: 111–125.

To initiate the elution of nucleotides from the resin columns the reservoir jar **1** containing 400 ml of 0.01

M formic acid is connected to the compressed air tank **18** via the inlet glass tube **2B** and plastic tubing **2**, and to the multi-valve control **3** via the outlet glass tube **2B** and a plastic tubing **2**. The dripmeters **5** are disconnected from the chromatography glass columns **6** before air pressure is permitted to force the elution mixture from the reservoir jar **1**, through the multi-valve control **3** and into the dripmeters **5**. Adjustment of the gas regulator **17** and the valve control knobs **16** regulate the flow of the elution solvent. Using the dripmeters **5** the space above the resin bed of each chromatography glass column **6** is filled with the elution mixture before dripmeters **5** are fitted tightly into each chromatography glass column **6**. Final adjustment with the valve control knobs **16** is necessary to obtain the desired eluent flow rate of 0.75 ml per minute. Eluent volume is measured in a burette **7**, which fits into a burette plastic cap **8**, and collected in test tubes **9**, which are held upright in test tube holders **10**.

To shift from one elution solvent to the next elution solvent, whose pH concentration is higher, the following steps are followed. Air pressure is turned off with the gas regulator **17** before disconnecting the plastic tubing **2** that joins the gas regulator **17** to the reservoir jar **1**. The dripmeters **5** are disconnected from the chromatography glass columns **6** and held elevated above the multi-valve support **15** to permit the elution solvent to flow back into the reservoir jar **1** once the valve outlets **19** have been opened using the valve control knobs **16**. The previously used reservoir jar **1** is replaced with a reservoir jar **1** containing 400 ml of elution solvent of the next higher pH concentration. The elution solvent above the resin bed in the chromatography glass columns **6** is removed and replaced with the elution solvent of the next higher pH concentration.

The elution procedure is resumed after replacing each drip-meter **5** into their respective chromatography glass columns **6**. The presence of the plastic joint connector **4** which joins the connecting tube **2**, leading from the valve outlet **19**, and the dripmeter **5** permits disconnection of the apparatus at this point.

What is claimed is:

1. In an apparatus for analyzing samples of nucleotides using a plurality of chromatograpic columns the improvement comprising in combination:

interchangeable reservoir means, each said reservoir holding an elution solvent of a different ph concentration;

a set of four chromatographic columns; a four-way multi-valve control which disperses equally elution solvent of the same pH concentration on to each of said four chromatography columns at the same moment;

said columns containing ion-exchange resin capable of separating a micture of nucleotides; means connecting said valve between said reservoir and said columns;

a burette for measuring eluent volume collected from each of said chromatographic columns;

a set of collecting tubes held upright in test tube holder means;

pressure means connected to said reservoir means for forcing said elution solvent through said valve means and into each of said columns.

2. The apparatus of claim **1** wherein said reservoir means consist of wide mouth jar, clamp and rubber stopper.

3. The apparatus of claim **2** further including said columns, jar and tubes being constructed of glass.

* * * * *