CHROM. 4337

Pressure-elution pump for spacecraft chromatograph

The *in situ* analysis of extraterrestrial soil samples by means of soft-landed spacecrafts requires automatic chemical equipment which consumes minimum weight, volume, and electrical power.

During a feasibility study of a miniaturized amino acid analyzer[1] employing ion-exchange chromatography, the high-pressure metering pumps normally used in commercial instruments seemed prohibitive because of their weight and electrical power requirements.

An alternate way of forcing the eluent solution through the column is by means of pressure elution. The term 'pressure elution' implies that a gas under constant pressure is used to push the eluent liquid through the column.

The simple application of gas pressure to the eluent leads to several problems. For example, if one subjects the eluent solution to direct gas pressure any gas dissolved at the high-pressure column input will bubble out at the low pressure in the column exit causing the column to develop air pockets. Thus HARE[2], using nitrogen gas in direct contact with the eluent buffers, had to load the buffers into long coils to prevent bubble formation at the low-pressure column exit. In this way, presumably, the time required for the gas to diffuse through the buffer reservoir was too long to interfere. Another problem is that bubble formation would be troublesome with any flow-through detector cell.

The gas diffusion can be prevented by using a movable barrier of some sort to separate the driving gas and eluent liquid. JENTOFT AND GOUW[3] used a mercury barrier. A commercial pressure-elution pump (Waters Associates, Framingham, Mass.) using a polyethylene barrier is also available.

The approach in this paper is to use a movable metal barrier in the form of a metal bellows to separate the gas from the eluent solution.

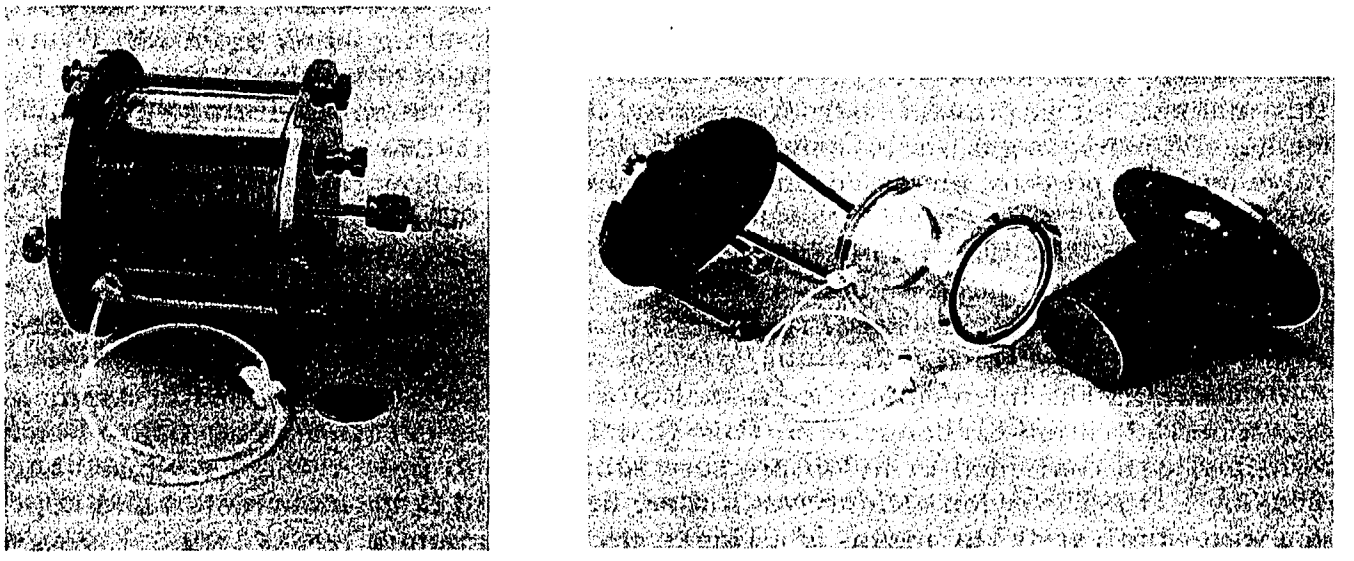

Fig. 1. Experimental pressure-elution pump.

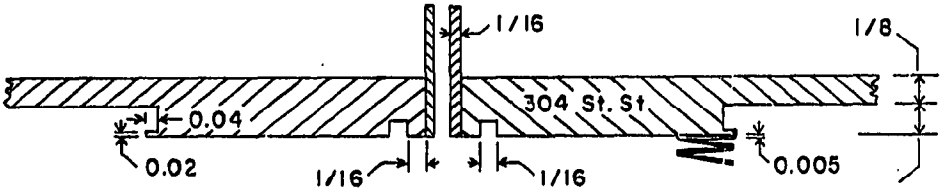

Fig. 2. Cross-section of top plate and gas inlet showing design necessary for arc welding. Dimensions are in inches.

Apparatus

Pump description. Photographs of the assembled and disassembled pump are shown in Fig. 1. It consists of a metal bellows operating inside a Plexiglass (Rohm and Haas acrylic plastic) cylinder. The cylinder is closed off at the ends by stainless steel end plates. The flange of the open end of the bellows is arc welded onto one of the end plates, the top plate, as shown in Fig. 2. A Teflon O-ring at each end provides a seal between the Plexiglass cylinder and the end plate. The O-rings are located in grooves in the cylinder as recommended by the manufacturer (Parker Seal Company, Culver City, Calif.).

In this experimental pump, the cylinder (length 2½ in., I.D. 2 in., wall 0.5 in.) was made of transparent Plexiglass to allow inspection for gas bubbles and bellows position during loading and elution.

The best commercial bellows for this pump was a so-called welded bellows (Model No. 61050, Metal Bellows Corporation, Chatsworth, Calif.). The bellows was so elastic that the pressure absorbed during one full stroke (1.25 in.) within the cylinder was only 1.8 p.s.i. Hence, at say 180 p.s.i., the flow rates at initial contraction and final complete expansion differ by only 1%.

Pump loading. The pump is loaded with eluent by applying partial vacuum to the gas port in the top plate. This causes the bellows to contract and produce suction in the liquid port in the Plexiglass cylinder.

The initial air in the liquid chamber is easily removed by a few filling cycles before connecting the pump to the column. After loading with eluent, the liquid port is connected to the center port of a 3-way valve, the loading valve, from which one exit leads to the column, and the other exit to the bottom of an eluent reservoir. In this manner, air is prevented from entering the liquid chamber during successive loadings. A nitrogen gas tank is connected to the gas port through a pressure-reducing valve and gas pressure increased until the desired liquid flow rate is obtained at the column exit.

Test solutions. The pump was tested by chromatographing a mixture of the principal bases from DNA and RNA.

Two test buffers were used: 1.6 *M* citrate adjusted to pH 6.5, and 0.05 *M* citrate adjusted to pH 5.58 with HCl.

Column. The microcolumn consisted of a 0.191 × 112 cm resin bed of Aminex A-4 spherical cation-exchange resin (Bio-Rad Laboratories, Richmond, Calif.). The resin bed was contained in nylon pressure tubing (1000 p.s.i. rating; 1/8 in. O.D.; 0.075 in. I.D.). The nylon column was formed into a loop (4 in. in diameter) and kept in a water bath at 55° by means of a Haake circulating pump.

Sampling valve. A sample injection valve was connected between the 3-way

loading valve previously mentioned and the column. This valve consisted of two 4-way valves connected in the standard manner recommended by the manufacturer for manual operation (Model CAV-4031 4-way Cheminert Valve, Chromatronix Inc., Berkeley, Calif.). The sample loop had a capacity of 150 μl.

Detection system. A UV monitor (Model UA-2, ISCO Inc., Lincoln, Nebr.), normally used for fraction collection, served as the detector. In this instrument a 100-μl flow-through cell with a 2-mm light path is illuminated with light at 254 mμ from a mercury lamp.

Results and discussion

The chromatograms (Fig. 3) show the results obtained when the pump is used

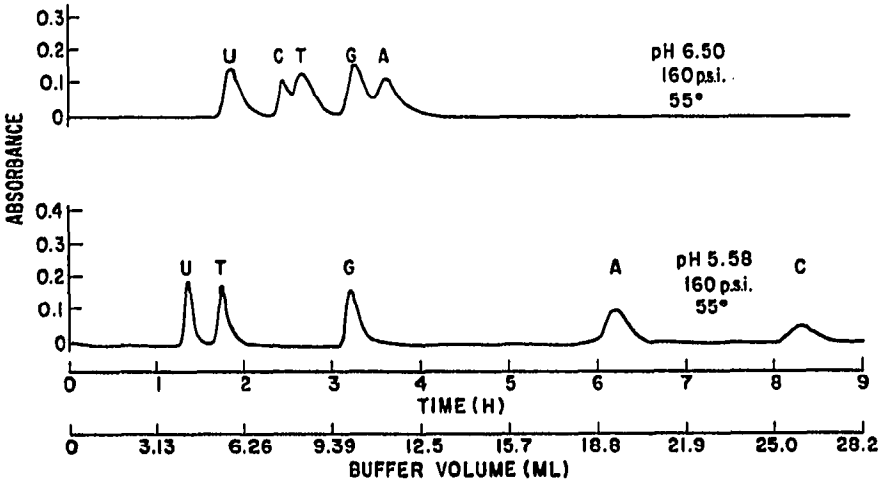

Fig. 3. Pressure-elution chromatograms. U, 43 nanomoles of uracil; T, 58 nanomoles of thymine; G, 54 nanomoles of guanine; A, 32 nanomoles of adenine; C, 41 nanomoles of cytosine; λ = 254 mμ.

on the microcolumn. The 'column gain', due to the small diameter of the column, is apparent since the compounds are detected at the nanomole level despite the rather simple detection system.

The pump was developed primarily for a spacecraft chromatograph employing single-buffer elution. For this purpose, the pump has the following advantages:

(1) It requires no electrical power. The driving energy is stored in the form of compressed gas.

(2) It is efficient. The slow isothermal expansion of the gas—an almost reversible process in the thermodynamic sense—results in nearly the maximum available PV-work.

(3) It is an 'integrated' device in the sense that the liquid reservoir is indistinguishable from the pump. Hence a separate liquid reservoir has been eliminated.

(4) The pump is compatible with microcolumns (I.D. $<$ 1 mm) which entail small volumes of eluent per run and low flow rates ($<$ 1 ml/h). The flow rate can be made as slow as one wishes. The upper pressure is limited only by the strength of the materials since no leakage is possible through the metal barrier. This particular pump was tested up to 200 p.s.i.

(5) The pump is non-pulsating.

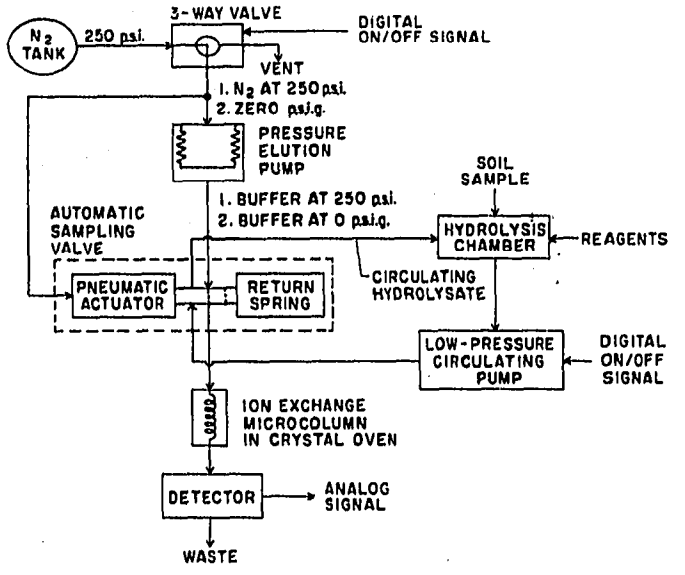

Fig. 4. Gross description of spacecraft chromatograph.

If pH-programming of the eluent is desired, several pumps could conceivably be operated in parallel. If several such pumps are operated in parallel at the same pressure, the pump has the disadvantage that the flow rates will differ if the column resistance is pH-dependent.

A possible system description for the analysis of soil hydrolysates is shown in Fig. 4.

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1 B. E. BONNELYCKE, K. DUS AND S. L. MILLER, *Anal. Biochem.*, 27 (1969) 262.
2 E. HARE, *Fed. Proc.*, 25 (1966) 709.
3 R. E. JENTOFT AND T. H. GOUW, *Anal. Chem.*, 38 (1966) 949.

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