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A Feasibility Study on Miniaturizing an Automatic Amino Acid Analyzer for use on Apollo Mission and Mars Voyager Mission.

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Introduction.

An amino acid analysis of the surface of the Moon and Mars would be of great scientific interest and importance. This analysis on the Moon will be indicative of its early history. The presence of amino acids on the surface of Mars will be strong evidence for the presence of life, provided it can be shown that these amino acids are not of non-biological origin. Since it does not seem likely that amino acids could have survived on the surface of Mars for several billion years, a highly sensitive amino acid analyzer constitutes a life detection system of moderate reliability.

The most widely used commercial amino acid analyzer at the present time, the Beckman-Spinco Model 120, has a volume of approximately 73 ft.<sup>3</sup>. In order to meet the requirements for the Apollo and Voyager mission, this instrument has to be reduced from its present size by a factor of 150. Several automatic reaction steps have to be added to make this instrument suitable for space missions. These include acid hydrolysis of the sample and the separation of the resulting amino acids from contaminating metal compounds. In addition the sensitivity of these instruments will be substantially higher than the present commercial model.

In order to achieve the large reduction in size and weight of the commercial instrument, we are conducting this feasibility study by miniaturization in two stages. The first stage is the construction of a semi-micro amino acid analyzer, and the second stage is the construction of the micro model suitable for space flight use.

The semi-micro model is being constructed essentially from commercially available components except for the miniature rotary valves, the hydrolysis

chamber, the reaction coil heating chamber, and flow cell with a long light path. Special attention is being given to the selection and arrangement of components for the spectrophotometric unit in order to achieve the greatest compactness and sensitivity.

During the present period of this feasibility study, primary attention has been given to the construction of the semi-micro model. We feel that this model will be a valuable contribution to instrumentation for biochemical research as a replacement for the over-sized and already obsolete instrument. The design of the micro model is in progress, making extensive use of our experience in the construction of the semi-micro model. Although the construction of this instrument is just beginning, we feel that this instrument can be successfully completed.

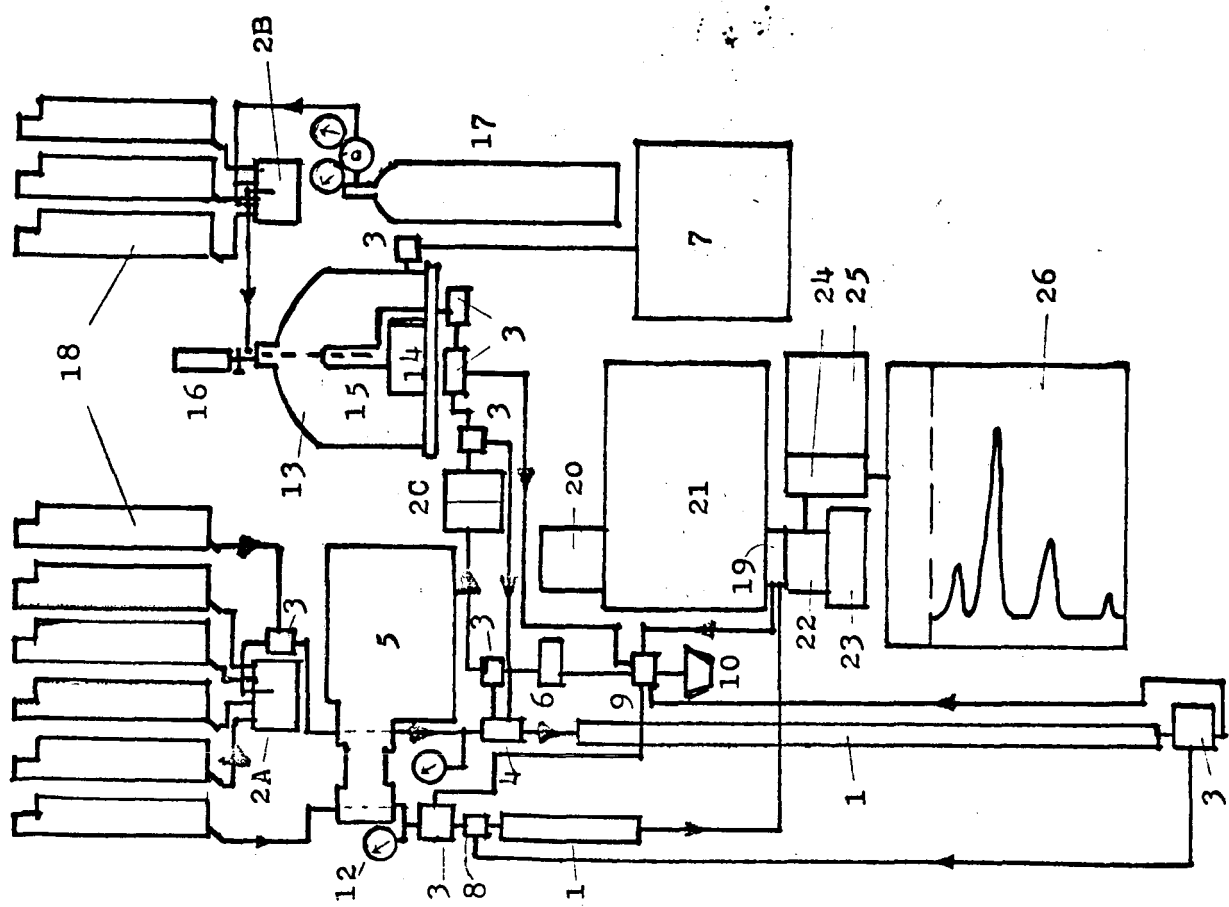
1. Construction of component parts

In order to reduce the size and weight of the commercial instrument and to make the system suitable for a fully automated analysis of Lunar and Martian soil, it is necessary to redesign and develop a number of essential component parts. The flow diagram is shown in figure 1.

a) Miniature rotary valves. We have chosen rotary valves instead of solenoid valves whenever possible because they can be made absolutely pressure tight. Also they have a very small hold up volume and permit very simple programming of multistep operations. The original device, designed in our laboratory several years ago, was too large for use in the semi-micro or micro model. This rotary valve was redesigned and reduced to 1/6 of its original size. This change involves mainly the use of a U-shaped channel through the rotating core of the valve instead of the slanted channel used previously. A more compact and more reliable electronic control circuit for the rotary valve was constructed and

# Flow Diagram of the Semi-Micro Amino Acid Analyzer

1. Ion exchange column, 60 x 0.9 cm, electrically thermostated,
2. Miniature rotary valves :  
 2A four point valve  
 2B six point valve  
 2C eight point double valve,
3. Solenoid valves: 2-way and 3-way,
4. Electrically controlled 4-way valve for alternate pathways,
5. Metering pump with two pumping heads,
6. Miniature diaphragm pump,
7. Vacuum pump,
8. Mixing manifold,
9. Drain manifold,
10. Drain bucket,
11. Reaction coil in electrically controlled heating chamber,
12. Pressure gauges,
13. Hydrolysis chamber,
14. Heater-stirrer,
15. Hydrolysis vessel,
16. Sample funnel,
17. Nitrogen cylinder,
18. Reservoirs for buffers, ninhydrin reagent, HCl, NaOH and water,
19. Flow-through cuvette,
20. Lamps,
21. Monochromator,
22. Photomultiplier,
23. Power supply for photomultiplier,
24. Stabilized operational amplifier,
25. Power supply for operational amplifier,
26. Recorder.



successfully tested in our laboratory.

b) Automated acid hydrolysis unit. Experiments with desert sand from the vicinity of Yuma, Arizona, have shown that the level of free amino acids after hydrolysis is about 20 times higher than the level before hydrolysis. Alkaline hydrolysis is not as efficient in releasing amino acids. If we assume that the composition of extra-terrestrial samples will be similar to desert sand samples available on earth, then acid hydrolysis represents a necessary step in the amino acid analysis of Lunar and Martian soil samples.

The design of a fully automated unit for acid hydrolysis has been completed, and the component parts of this unit are presently under construction in our laboratory. The automatic hydrolysis unit is designed to fit the ion exchange analysis system, but this unit may also prove useful for systems using other analytical procedures.

c) Compact pumping arrangement. Accurate metering of all liquid flow is essential for the reproducibility of mobilities on ion exchange columns and consequently for the reliability of the analysis. However, most accurate metering pumps are very bulky and contain only a single pumping head. The recently introduced DCL-Parvalux pump can be operated with up to 6 pumping heads. This reduces the weight and space requirements for this operation. We have obtained this pump and tested its performance for metering the buffer as well as the ninhydrin lines. A pressure ceiling of 250 psi was the only obstacle against using this arrangement in automatic amino acid analysis because the resins usually employed in these systems will produce back pressures up to 500 psi. This back pressure problem was overcome by using two new types of ion exchange resins (UR-30 of Beckman-Spinco and Aminex A-4 of Bio-Rad).

These resins give excellent resolution of all amino acids at greatly reduced back pressures (160-200 psi). Our tests indicated that the multihead pump can be used to advantage in combination with low pressure resins.

d) Electrical heating systems. We have replaced the bulky water baths and water jackets currently used to thermostat the chromatographic column and the ninhydrin reaction coil by compact electric heating devices. This development permits improved control and flexibility.

e) Detection system. In order to assure sufficient sensitivity of this miniature amino acid analyzer, it is important to improve all components of the detection system. We therefore have designed a special flow cell with a 30 mm light path. This component is presently under construction. Our semi-micro design will combine a very compact monochromator system and lamp arrangement with a photomultiplier tube for both visible and ultraviolet. A stabilized operational amplifier is provided for electronic amplification of the photomultiplier signal. This signal will be recorded on a small single trace recorder which will replace the bulky multipoint recorder of the commercial instrument. This detection system is designed to increase the sensitivity of the commercial amino acid analyzer by a factor of 100, and it may prove very useful in current biochemical equipment. The detection unit for the micro model will be constructed using a light source, filter and photomultiplier. This system is currently being designed.

## 2. Chemical Investigations

In addition to equipment design and construction we have carried out investigations on the chemical aspects of the project.

The detection of the amino acids is currently based on their reaction with the ninhydrin reagent. We have conducted a preliminary search for reagents which might prove superior to ninhydrin either because of higher sensitivity or because of greater selectivity. Two such reactions, trinitrophenylation and dansylation, seemed very promising and were studied in detail.

a) Trinitrophenylation. The reaction of trinitrobenzene sulfonic acid is highly selective for primary amino groups. However, its sensitivity is only 2/3 of the ninhydrin reagent, and it is very sensitive to light. The reagent in solution deteriorates even on prolonged standing at room temperature in the dark. However, reaction with suitable groups is very rapid at room temperature and almost quantitative in contrast to ninhydrin. The free amino acids can be regenerated from these complexes by treatment with  $NH_4OH$ . This fact would prove advantageous in peptide separation and purification since the ninhydrin reaction with peptides is sluggish and also destroys their amino terminal residues.

b) Dansylation. The reaction of 5-dimethylaminonaphthalene-1-sulfonyl chloride with amino groups gives a product that absorbs 50 times as strongly as the ninhydrin product. However, the absorption is in the ultraviolet where the buffer ions and organic solvents contribute a high background absorption. This problem may be overcome by measuring the fluorescence of the dansyl- derivatives. The present method of choice is ninhydrin, in our opinion, but these other possibilities are still being investigated in our laboratory.

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