Mar 7th, 8:00 AM

Automatic Chemical Processing Systems for Extraterrestrial Biochemical Investigations

Donald C. Garwood
Philco Corporation, Aeronautic Division

Harry A. Taussig
Philco Corporation, Aeronautic Division

AUTOMATIC CHEMICAL PROCESSING SYSTEMS FOR EXTRATERRESTRIAL BIOCHEMICAL INVESTIGATIONS

Donald C. Garwood and Harry A. Taussig
Philco Corporation, Aeronutronic Division
Newport Beach, California

Summary

Implementing the concept of planetary exploration is a formidable task. Laboratory procedures and techniques that are performed routinely by scientists and technicians must be performed remotely, automatically, and reliably for long periods of time without direct human intervention. The subtle and sophisticated reasoning, planning, and execution that lies behind well-conducted research in terrestrial laboratories must be incorporated into the careful original design and mechanization of extraterrestrial experiments.

Mechanisms capable of automation for use in batch chemical processing of surface samples for scientific payloads have been devised and tested. Elemental processing operations common to a wide variety of possible life detection and biochemical characterization experiments were identified. It was determined that one device could perform a multiplicity of analyses if it could perform a limited number of elemental processing operations. Mechanisms were then designed for performing such elemental processing operations, and a breadboard processing device was fabricated incorporating the various processing operations, some completely automated (such as reagent metering) and others manually operated. The tremendous flexibility of the device to perform a wide variety of chemical procedures is evident. Tests of the breadboard in performing aqueous and organic extractions of soil samples indicate that completely automated, flight worthy chemical processing systems possessing great experimental flexibility are feasible and reliable.

Background Analysis

Analysis of Experiments

To accomplish the various experiments that will be required for recognition, identification and characterization of encountered life forms, it will be required that the experimental apparatus make intimate contact with an extraterrestrial environment. This interface is perhaps the most difficult to deal with since, at present, it contains the most unknowns. Many experiments require a portion of the environment to be brought within their analytic devices in order to complete various tests on the environment. This connection between the scientific payload and the environment being studied is established by the sample gathering and sample processing equipment. Therefore, an essential spacecraft subsystem of any payload for scientific surface exploration of the Moon or planets is a sample processing equipment.

Nearly all meaningful biological and biochemical experiments which have been suggested for detection and characterization of extraterrestrial life require a subsystem that would accomplish the chemical treatment of a sample. A very large number of biodetection techniques have been surveyed at Aeronutronic, from which it has been concluded that the chemical treatment of a sample is a broad and extensive experimental operation required by the majority of these experiments, and therefore worthwhile investigating.

Chemical processing is defined as the processing of a substance by effecting physical changes and phase changes which depend on the chemical properties of the sample, such as, dissolutions and extractions of samples with organic solvents and neutral, basic and acidic aqueous solvents. Separating phases (filtration), or performing chemical reactions (for example, carbonate fusions of minerals, pyrolysis of organic materials, etc.) in order to achieve separations of components of a mixture is also chemical processing. The eventual identification of the components of a complex mixture is substantially simplified by these separations because interference in the analysis for one substance by the presence of another substance is avoided. Finally, preparations of chemical derivatives are often necessary to render a sought substance suitable for specific analytic techniques. Derivative preparation is another function of chemical processing apparatus.

Chemical processing as considered here does not produce the primary scientific data but only prepares the sample for subsequent analysis which will be the main source of scientific information. The sample is presented to the analytic instrumentation where the scientific measurements are performed upon it subsequent to the chemical processing. The analytic instrumentation is not an integral part of the sample collection or the chemical processing subsystems. The position of chemical processing in a functional breakdown of a generalized experiment is shown in Figure 1.

Analysis of Processing Needs

To define the chemical processing needs of the various experiments that were considered, a more detailed study of the specific processing steps of each experiment was undertaken. Over one hundred biological detection and characterization experiments suggested for exobiological exploration of the planets were analyzed. Some of these experiments correspond in principle to those currently being developed by NASA and other scientists. Others are only conceptual at this time.

Individual chemical operations were identified by examining stepwise procedural flow diagrams

which were generated for each suggested experiment. In these diagrams, the individual steps taken to perform the experiment from beginning to the final analysis were documented. The individual steps were patterned after the methods used in biochemical analyses used in terrestrial laboratories.

Three examples of simplified procedural flow diagrams for typical experiments are given in Appendix A for illustrative purposes. From these, it is evident that a complex chemical procedure can indeed be expressed as sequences of elemental processing operations. Diagrams such as these were generated for over one hundred experiments and analysed as detailed below.

The experiments were studied to determine if they shared common chemical processing steps or operations and to identify these common operations. The three procedural flow diagrams presented are summarized in Table 1. Called out vertically are the experiments that were considered. Against these are presented the chemical processing steps required to accomplish the particular experiment. Below the three experiments is given the total number of times that particular chemical step was used to perform the three experiments. It can be seen that many steps are repeated in one experiment and that many steps are common to all three experiments.

Selection of Elemental Processing Operations

By performing this analysis on the large number of separate experiments considered, it becomes obvious that many experiments can be accomplished by a number of steps relatively small compared to the number of experiments accomplished. These steps are called elemental processing operations. In fact, almost all chemical and biochemical experiments can be performed with a very limited number of elemental processing operations. Table 2 lists 16 elemental processing operations with which almost all biochemical detection experiments can be performed.

Table 2. Elemental Processing Operations

1. Addition of a liquid to a solid.
2. Mixing liquid/solid phases (homoginization, etc.).
3. Separation of liquid/solid phases (filtration, centrifugation, decantation, floatation, etc.).
4. Concentration of liquid by evaporation.
5. Condensation of vapor (as in distillation).
6. Dialysis or ultrafiltration.
7. Addition of miscible liquid to another.
8. Addition of immiscible liquid to another.
9. Mixing miscible liquids.

Table 2 (contd.)

10. Mixing immiscible liquids.
11. Separation of liquid/liquid phases.
12. Addition of gas to liquid.
13. Separation of gas from liquid (porting of evolved gases).
14. Heating.
15. Cooling.
16. Timing (as timing the length of a reaction).

Types of Processing

The necessary elemental processing operations have now been defined that would be required to perform almost any type of biochemical processing. It must now be decided how these steps will be accomplished.

An investigation has shown that two general types of sample processing mechanisms are available, batch processing and continuous flow processing. Batch processing treats a limited amount of sample in discrete steps and is similar to the procedure used in the familiar chemistry laboratory experiment. Continuous flow processing, on the other hand, utilizes a series of pipes or tubes through which the sample and some type of supportive medium flow from station to station where operations are performed on the sample without interruption. Continuous flow processing is similar to that found in an oil refinery.

Continuous Flow Processing. Continuous flow processing was evaluated to determine its applicability to extraterrestrial biochemical exploration.

Continuous flow processing is characterized by interconnecting tubes and pumps that work continuously; these imply that it is best suited to perform a large number of identical analyses on a large number of samples. Technicon, Inc., has investigated the possibilities of continuous flow processing in a marketable device.[1] These devices have found application in places where large numbers of samples are put to the same test, for instance in a hospital where large numbers of blood and urine samples must undergo a routine analysis. Once an experiment or determination has been set up it is difficult to tear it down and construct another experiment.

The disadvantages of continous flow processing in its present stage are to be found in its relative inflexibility and the large amounts of fluid needed to transport the sample from station to station as well as the fluid needed to separate the samples from each other. The amounts of fluid needed are at least 10 times that of the sample. In an extraterrestrial laboratory where fluids are at a premium, this may be an important

consideration. This large fluid to sample ratio imposes serious limitations on the use of continuous flow processing for space exploration unless a very efficient solvent recovery device is found.

One possible method of increasing the flexibility of continuous flow processing is by the installation of a large number of valves. With the appropriate switching network of valves the number of experiments that could be performed with one set-up of the tubes could be increased. However, the chances the failure in one valve would prove catastrophic to the whole experimental system are seriously increased. Thus a malfunction in only one valve could prevent any experiment from being performed. For example, the whole of some critical solvent could be accidentally dumped if the solvent supply valve remains open. Redundancy in valve operation can be provided by replacing each key valve with a system of several valves.

Batch Processing. Batch processing, on the other hand, can work intermittently and on command. The batches which are processed can be rather small, thus being conservative of fluid. Since the batch must be transported from station to station, it makes little difference what the order of the station is. Batch processing is thus amenable to the philosophy of the elemental processing operations since these correspond to the stations in batch processing. In addition, batch processing corresponds closely to the usual activity found in the chemistry laboratory; this was also the approach taken in defining the procedural flow diagrams.

The disadvantage of batch processing lies in the transport of the batch from station to station or, conversely, the placement of the station relative to the batch being processed. It is of great advantage in batch processing to integrate many stations or elemental processing operations into one piece of equipment so that the transport mechanisms are at a minimum. Since batch processing uses the same station repeatedly, some consideration must be given to cleaning of the equipment after every processing operation.

In batch processing many of the elemental processing equipments are in the form of replaceable and disposable units. A failure in any one unit does not prevent the performance of subsequent experiments. Duplication of unit transfer and movement mechanisms can be provided to further insure reliable performance.

Design Considerations

Several additional considerations must be given to the design of a chemical processor. The two major considerations are of the size of the processor and of the restrictions imposed by sterilization.

It was found that the optimum size and volume of the processing apparatus was directly dependent on the amount of sample which the device would be required to handle. The size of the sample, in turn, is determined by (1) the sensitivity of each piece of analytic instrumentation to the particular substance being measured, (2) the efficiency with which the substance can be removed from the sample, and (3) the concentration of that substance in the sample or the sample richness. The greatest unknown is the last, sample richness. Taking into consideration the variations in sample richness in the terrestrial environment and then looking at a "bad" case, it was determined that a minimum sample size (assuming a gross heterogenious surface sample) is one gram. A nominal reagent volume for one treatment of this sample is ten cubic centimeters. As our knowledge of the environment to be sampled grows, these figures may well change.

Chemical processing subsystems intended for exobiological exploration must withstand dry heat sterilization at 125°C or greater for extended periods of several days. Dry heat sterilization combined with chemical interactions, aging effects, and mechanical properties necessary to satisfy functional requirements present problems in material compatibility. These compatibility requirements result in a somewhat restrictive list of fabrication materials. These are Teflon (tetrafluoroethylene), Viton (copolymer of hexafluoropropylene and vinylidene fluoride), stainless steel, commercially pure nickel, and Pyrex or fused silica glasses. Problems such as a sliding piston seal which are easily solved with conventional elastomer materials require the exploration of various design and fabrication techniques when using materials such as Teflon. Research in these areas is developing a new technology for the design and implementation of sterilizable chemical devices.

Conclusions

By the previous analyses, we have defined the requirements and criteria for the chemical processor to be used as a subsystem in supplying samples ready for analysis to the end instrumentation of biochemical experiments in extraterrestrial exploration. Each candidate experiment was analyzed for its elemental operations. It was found that almost all of the experiments could be performed with a very limited number of elemental processing operations.

Thus the chemical processor that has been suggested by this investigation has the following characteristics:

1. It is a batch processor.
2. It has the capability of performing a limited number of elemental

processing operations in any desired order.

3. The processor must be cleaned after each batch.

4. It must be compatible with the requirements for any space experiment, i.e., sterilizable, withstand launch.

5. It must be automatable.

The Processor

The breadboard device which will be described in the following section was intended for exploration and development of key or critical concepts of batch chemical processing. This breadboard is an experimental, initial device and not a prototype of an ultimate processor for biochemical processing. However, technologies and experience gained with this breadboard device are immediately applicable to prototypes for a variety of specific missions.

In the first iteration toward an automated chemical processor, a simplified model was undertaken which bears little resemblance to the ultimate flight hardware. No attempt was made to either miniaturize or lighten the present breadboard. The object of the first breadboard was to start with a soil sample, chemically treat the sample, by addition of liquid, filter the liquid from the solid so the liquid can be analyzed and clean the processor for the next sample. The guidelines for this breadboard were as follows:

1. The chemical reactions are to be contained in a cylinder called the reaction chamber and this cylinder is to be cleaned after every process.

2. The sample will be introduced into the processor by an outside device.

3. The extract is to be collected in a unit which can be removed to outside devices. This can be of a throw-away nature.

4. All reagents will be introduced from ampules with each ampule being used only once.

5. Wash water, cleaning solutions and gases can be contained in bulk supply and introduced by feedlines.

The elemental processing operations that were selected for this model were those that appear to be used the greatest number of times in the flow diagrams that were studied. The operations to be accomplished by this machine were:

1. Addition of a liquid.

2. Agitation of a liquid and a solid.

3. Agitation of a liquid and a liquid.

4. Addition of a gas to a liquid.

5. Separation of solid and liquid phases (filtration).

6. Separation of immiscible liquids.

It was found that these six operations could be conveniently combined into a workable and useful device. The individual parts of the machine are described in the paragraphs that follow.

The general arrangement of the breadboard chemical processor is explained graphically in the block flow diagram in Figure 2. The reaction and an overall view of an early model of the breadboard is shown in Fig. 10. The reaction chamber is the heart of the processor (see Fig. 7). In this chamber all the reactions take place as well as mixing and heating of the sample to be treated.

Below the reaction chamber is the filter unit (Fig. 7). This contains a filter or a set of filters as well as devices for agitation of the sample and devices for the unclogging of the filters should this be required. In the present breadboard model the test sample is introduced into the machine by placing the soil on top of the filter in the filter unit.

Below the filter unit is a combined suction and receptacle unit and a suction unit feed mechanism (Fig. 7). The suction unit consists of a piston and a cylinder (which attaches to the bottom of the filter unit). The piston can be moved downward applying suction to the filter unit or it can be moved upward forcing a liquid through the filter unit into the reaction chamber or back flushing the filter unit. The suction unit is removable and is one of the places in the processor where liquids may be removed for analysis. The suction unit feed mechanism contains the controls for moving the piston up and down, and a clamping device which secures both the suction unit and filter unit to the bottom of the reaction chamber.

Forming a ceiling on the reaction chamber is the upper piston assembly called the reaction chamber sealing piston. This is a stainless steel piston (Fig. 5) with a removable and replaceable teflon tip (Fig. 6). The piston may be moved up or down in coordination with the piston in the suction unit, thus applying pressure to aid filtration. At the end of an operation the piston may be moved down to help clean the walls of the reaction chamber by scraping off loosely adhering material. When the piston is moved all the way down through the reaction chamber into the filter unit, the piston tip expands into a recess in the filter unit where it is retained (Fig. 8). The piston is now withdrawn without the tip. The used and discarded tip is replaced with a clean tip before the next operation. The debris from the previous operation which has been removed from the chamber walls is now sealed in the

filter unit by the expanded piston tip and can be removed from the processor.

Provision has been made within the upper piston for utilizing an ampule (Fig. 3, 4, and 5). With these ampules chemical reagents are added to the sample. The ampule resembles a hypodermic syringe. It consists of an outer cylinder and a plunger. The reagent is hermetically sealed in the ampule and the assembly is sterilized. When needed it is brought into position on the inside of the chamber sealing piston. Upon seating, a double ended needle (Fig. 4) penetrates the seal in the face of the ampule and also provides a means of controlling the liquid flow as it enters the reaction chamber under the influence of the ampule plunger (Fig. 6). A mechanism is provided that controls the motion of the plunger inside the ampule and thereby meters the liquid.

A small port has been provided in the edge of the chamber sealing piston for porting of any gases that may be evolved during a reaction in the chamber. This port will be fitted with a pressure sensor and a means of collecting and storing the evolved gases.

In addition to ampules for placing liquids into the chamber, a special ampule can be used to remove liquids; this is called the transfer ampule. It is provided with a long needle which extends to the liquid level. By withdrawing the ampule plunger and simultaneously advancing the chamber sealing piston, the liquid is drawn into the ampule. This provides a second means by which liquids can be removed from the processor for analysis. The separation of two immiscible liquids can be accomplished with the transfer ampule provided the location of the boundary between the liquids is known.

Now that the individual components of the breadboard chemical processor have been described, a typical operation, an aqueous soil extraction, will be outlined.

A soil sample contained in a filter unit is brought into place, as is a clean suction unit, and both are clamped to the bottom of the reaction chamber. The ampule containing the appropriate solvent, water in this case, is brought into position. The reagent ampule feed mechanism then locks the ampule into a chuck, lowers it down the inside of the chamber sealing piston and seats it against the inside of the piston. A clean piston tip is brought into place and the chamber sealing piston is lowered into the chamber, picking up the piston tip in the process. When the chamber has been sealed, the seal on the ampule is broken by pushing down on the ampule so that the needle perforates the seal. A programmed amount of reagent is added to the soil sample. The soil and reagent mixture is then agitated for a predetermined time. Any gases that may be evolved are ported and stored for further analysis. The suction piston is lowered in coordination with the upper piston, forcing the fluid through the filter unit into the receptacle unit beneath the filter. When filtration is completed, the upper piston is in a position to release the piston tip which seals the soil remains into the filter unit. All the liquid is now in the receptacle unit, which is unclamped and removed for analysis. The filter unit containing the soil is discarded or used for further experimentation. The machine is cleaned and recycled to its original position and the various pieces that were used in the aqueous soil extraction are replaced.

A wash ampule has been constructed in addition to the reagent and the transfer ampules mentioned above. This ampule contains no liquid of its own but is fitted for connection to a bulk storage supply of liquids and gases. When extended into the reaction chamber a rod with radiating nozzles can be used to wash the walls of the reaction chamber with either water or a cleaning solution followed with a distilled water rinse. This wash ampule can also be used to fill the chamber with a cleaning solution should this become necessary.

A second method of cleaning the chamber is the piston tip, which has been previously discussed. The piston tip will scrape the walls of the chamber as it is lowered toward the filter unit where it will remain after the cleaning operation is completed.

Agitation or mixing can be accomplished in two ways. The first is by the wash ampule. The wash head containing the nozzles is lowered into the liquid to be agitated. Instead of using liquid from bulk storage, an inert gas is used for gas burst agitation.

A second means of agitation is provided in the filter unit. A ring of small nozzles surround the filter itself. These can be located above or below the filter, and in case a series of filters is used, between the filters. These can be actuated separately or together and provide gas burst agitation.

In addition to the aqueous soil extraction procedure described above, the machine is capable of many other biochemical procedures utilizing the selected elemental processing operations. Among those that can be performed are: precipitations, dissolutions, preparation of solutions, etc.

In order to utilize the inherent flexibility of such a machine a limited amount of other ancillary equipment has been considered. A heating coil to be placed near the filter unit would provide several functions. With moderate heat it could be used to speed chemical reactions and for distillations. The resulting vapor would be removed by means of the port in the upper piston wall. The concentrate or dry residue would remain in the filter unit. With greater heat

applied, fusions could be accomplished. With even greater heat, pyrolisis of organic materials prior to analysis with a gas chromatograph can be implemented.

As can be seen, with a few ancillary devices and the basic flexibility of the batch processing breadboard, a very large number of chemical analysis can be performed.

To explore the possibilities of automation it was decided that the most difficult or complex set of manual steps required to complete an elemental processing operation should be mechanized. This set exists in ampule handling, locking, seating, and reagent metering from the ampule and the reverse motions which return the ampule to storage. In its simplest form this involves ten separate motions. The relative complexity of the automated versus the manual ampule control and reagent feed mechanisms are evident from a comparison of Figures 9 and 10. Due to very successful operation of this mechanism, the authors are satisfied that the remaining operations are also amenable to reliable automation.

Conclusions

The performance and testing of the breadboard chemical processor are sufficiently conclusive to establish the feasibility of developing a prototype flight model. The ability to perform a group of elementary processing operations in any desired sequence provides flexibility of experimental procedure which is valuable in a thorough biological and geochemical exploration of the planets and the Moon.

In addition to exploring planets such as Mars and Venus that are now suspect of biological activity, geochemical exploration of the moon and other bodies can be accomplished. Many geochemical analysis procedures are adaptable to the concept of the chemical processor just as are the biological analysis that have been presented here. Chemical processing can be of substantial assistance in geochemical analysis of planetary surface materials by being employed to affect chemical separations into classes and purify an impure sample prior to instrumental analysis. With a combined geochemical and biological investigation package, utilizing as its central core an automated chemical processor, investigations of many of the planets and their moons could be accomplished.

In addition to extraterrestrial explorations, the chemical processor could be adapted to other environments which require the performance of chemical operations under circumstances which would not permit direct human intervention, such as a high pressure environment as exists on the ocean floor. The processor could also be used to perform chemical experiments in a high radiation environment. Furthermore, since the processing equipments will survive the thermal bake required for sterilization, applications can be envisaged in thermal environments which preclude human presence. In planetary explorations, the surface of Venus may provide such conditions.

The capabilities of extracting soluble materials from a sample of heterogeneous surface material, chemically modifying an intractable sample, and performing chemical separations would lead to an improvement in the quality of compositional analyses and further broaden the number and kind of analytic methods which may be employed in space exploration.

Acknowledgements

The authors gratefully acknowledge the assistance of Wilfred Bachle and Virgil Marple for the mechanical design and Joe Balough, Fred Cairncross, and Ken Crowder for the fabrication, assembly and testing. We would also like to thank Mr. Serge Golian for suggestions and invaluable discussions.

Appendix A

Below are presented three simplified procedural flow diagrams. The steps taken to perform the experiment are given in numerical order. Devices, such as escapes from loops, which were considered in the flow diagrams used for analysis have been omitted here in the interest of briefness and simplicity. The flow diagrams were derived from well known and documented biochemical procedures.

Detection of Optically Active, Water Soluble, Organic Macromolecules

The following procedure represents an experiment for the detection of optically active, water-soluble, organic macromolecules which are essential participants in the ordered and controlled performance of life processes.[2] A sample (assumed to be in a powdered form) is extracted with water. The water soluble materials are then treated to separate large molecules from the smaller molecules by dialysis. The presence of organic macromolecules is confirmed by the presence of organic carbon. The ability of the sample to rotate the plane of polarization of light is then determined.

1. Acquire sample.
2. Weigh sample.
3. Determine the amount of solvent (water) to be added to the sample (5 to 10 times the weight of the sample).
4. Acquire the solvent and add the appropriate amount to the sample as determined in Step 3.
5. Mix.
6. Filter.

7. Remove the residue.

8. Dialyze or ultrafilter the filtrate to remove small molecules and salts.

9. Determine organic carbon content in a portion (measured fraction of the total) of the dialyzed filtrate.

10. If the organic content of the non-dialyzable extract is high enough then proceed to remaining steps; if the organic carbon content is too low, acquire additional sample and go to Step 2.

11. Transfer dialyzed filtrate (which is now known to contain water soluble, organic macromolecules as the non-dialyzable extract) to the optical rotatory dispersion sample cell.

12. Measure the optical rotatory dispersion of the sample in a spectropolarimeter. Record data.

13. Remove sample; clean and recycle equipment to ready position.

Detection of Porphyrins

All terrestrial organisms are known to contain porphyrins (the porphyrin structure occurs in cholesterol, blood pigments, coenzymes, etc.). The conceptual scheme for detection of porphyrins involves extraction of a suitable sample with an organic solvent, treatment to prevent the interference of ions in the analysis, the conversion of porphyrinogens which may be present to porphyrins, and finally excitation and detection of the porphyrin by fluorescence.[3]

1. Check equipment (the equipment check should consist of checking out fluorometer and sample processing equipment).

2. Collect sample. (Surface soil sample preferred.) Sample size required is several grams.

3. Pulverize and grade sample by sieving through a coarse mesh screen. (The sample grading may be bypassed if the sample is collected in a manner which pulverizes large chunks or excludes such chunks as rocks. To assure that the chemical processing equipment is not damaged, large hard pieces should be removed. The sample should be in powder form to facilitate extraction.)

4. Weigh sample. The sample should weigh about one gram. (A rough weighing to about $\pm$ .05 grams is probably adequate. A volume measuring technique might also suffice.)

5. Acquire 3 ml of 4:1 ethyl acetate-acetic acid.

6. Transfer weighed sample to the chemical processor.

7. Add solvent to the sample.

8. Mix.

9. Filter.

10. Retain filtrate. (The filtrate will be an ethyl acetate-acetic acid solution containing porphyrins with their metal ions and porphyrinogens.)

11. Remove residue.

12. Transfer filtrate to the reaction chamber.

13. Acquire one ml of EDTA (ethylenediamine tetraacetic acid). (The EDTA is suggested to be a 0.01 M solution in 4:1 ethyl acetate-acetic acid. The EDTA serves the function of chelating metal ions, both those extracted from soil by the solvent and those associated with the porphyrins or porphyrinogens. These metal ions, unless chelated, may interfere in the fluorescence spectrum.)

14. Add EDTA to filtrate.

15. Mix.

16. Bubble oxygen through the solution. (The oxygen serves to oxidize the porphyrinogens to porphyrins.)

17. Fill fluorometer cell.

18. Transfer cell to fluorometer.

19. Excite fluorescence with source illumination having a wavelength of 400 mμ. The absorption band of the porphyrins at 400 mμ is used to absorb excitation energy. The porphyrin fluorescence is shifted considerably to the red region of the visible spectrum.

20. Record fluorescent spectrum between 600 and 750 mμ.

21. If the fluorescence intensity is so high that the detector is saturated, go to 22. If the fluorescence is detectable but does not saturate the detector over the wavelength region from 600 to 750 mμ, go to 30. If no fluorescence is detected, go to 24.

22. Reduce intensity of excitation source by one-half.

23. Go to 20.

24. Repeat 1 through 16 with a 10 gram soil sample, 30 ml of extraction solvent, 10 ml of EDTA solution.

25. Reduce to dryness and extract residue with two 1 ml portions of 4:1 ethyl acetate-acetic acid.

26. Filter.
27. Remove filtrate.
28. Do 17 through 20.
29. Remove cell from fluorometer.
30. Bubble through gaseous HCl to make 2M in HCl.
31. Return cell to fluorometer.
32. Scan spectral region from 600 to 750 mμ as before.
33. Remove cell.
34. Turn off fluorometer source.
35. Recycle fluorometer.
36. Remove sample from cell.
37. Clean cell and processing equipment.
38. Recycle all equipment to ready position.

Detection of Flavins

In the following procedure, flavins are extracted from a sample with water and sulfuric acid. Since flavins are practically insoluble in chloroform, the acidic extract is treated with chloroform to remove all chloroform soluble fluorescent materials which would later interfere in the detection of lumiflavin fluorescence.[4] The extract is next made basic and photolyzed which converts the flavins to lumiflavins. Since lumiflavins are chloroform soluble and since all other fluorescent, chloroform soluble compounds were previously removed, a chloroform extract of the photolysis mixture will contain lumiflavin free of interfering substances. Fluorescence of the chloroform extract indicates the presence of lumiflavins and, therefore, the presence of flavins in the original sample.

1. Check equipment. (A check for functioning of the processing equipment, the fluorometer, and the photolysis light source is required.)
2. Collect a sample of soil or suspected biological material. An excess of over five grams is desired if the sample is soil; over one gram if biological material.
3. Mechanically process the sample. (If the sample is soil, rough crushing and collection of particles less than a critical size is sufficient. If the sample is biological material it should be cut into small pieces or should be ground.)
4. Weigh the sample (1 to 5 grams). (A knowledge of the approximate sample weight (± 0.2 grams) is adequate. A knowledge of the gross sample density and its volume would also suffice.)
5. Transfer the sample to the chemical processor.
6. Acquire 10 ml of water.
7. Add to sample.
8. Mix.
9. Heat at 80°C for 15 min. while continuing to mix.
10. Acquire 3 ml of 1 N H_2SO_4.
11. Add to sample.
12. Heat at 80°C for 15 min. while continuing to mix.
13. Cool to ambient (25°C).
14. Filter.
15. Retain filtrate (~13 ml).
16. Remove residue. (The extraction of the flavins from the sample (if any) is now complete. The next sequence of steps is intended to remove interfering, chloroform soluble materials.)
17. Acquire 1 ml of chloroform.
18. Add to the filtrate from Step 15.
19. Mix.
20. Allow layers to separate.
21. Do liquid-liquid separation.
22. Retain top layer.
23. Fill fluorometer cell with chloroform (bottom) layer.
24. Transfer cell to fluorometer.
25. Activate fluorometer source (445 mμ).
26. Detect fluorescence (500 to 600 mμ).
27. Remove cell.
28. Remove chloroform from cell and excess chloroform solution.
29. If fluorescence is detected in Step 26, go to 17. If no significant fluorescence is detected in the chloroform extract, go to 30. (Extraction of interfering substances is now complete. The next sequence of steps is the alkaline photolysis of flavins to lumiflavins.)
30. Acquire 3 ml of 1 N NaOH.
31. Add to sample solution.
32. Mix.
33. Evaporate water under reduced pressure to a total solution volume of 2 ml.
34. Acquire 2 ml of 1 N NaOH.
35. Add to sample.

36. Mix.

37. Filter.

38. Retain filtrate.

39. Remove residue.

40. Transfer sample solution (filtrate) to photolysis apparatus.

41. Activate photolysis light source.

42. Illuminate the sample for a period of 30 minutes. (The period of photolysis is determined by the light source intensity and the maximum expected concentration of flavins.)

43. Remove the sample from photolysis apparatus.

44. Acquire 4 ml of chloroform.

45. Add to sample.

46. Mix.

47. Allow layers to separate.

48. Do liquid-liquid separation.

49. Remove aqueous (top) layer.

50. Retain chloroform (bottom layer).

51. Fill fluorometer cell with chloroform layer.

52. Transfer cell to fluorometer.

53. Excite fluorescence with source illumination having a wavelength of 445 mμ.

54. Record fluorescent spectrum between 500 and 600 mμ.

55. If the fluorescence intensity is so high that the detector is saturated, go to 56. If the fluorescence is detectable but does not saturate the detector over the wavelength region from 500 to 650 mμ, go to 58.

56. Reduce intensity of excitation source by one-half.

57. Go to 54.

58. Remove cell.

59. Shut off fluorometer source.

60. Recycle fluorometer.

61. Remove *sample from cell*.

62. Clean cell and processing equipment.

63. Recycle all equipment to ready position.

References

1. For example: Sturgeon, P., and D. T. McQuiston, A Fully Automated System for the Simultaneous Determination of Whole Blood Red Cell Count and Hemoglobin Content, Paper #35, Technicon International Symposium on Automated Analytical Chemistry, Sept. 16-18, 1964, N.Y.

2. Chargaff, E. and J. N. Davidson, The Nucleic Acids, Vol. I, Academic Press, Inc., New York, N.Y., page 336, (1955).

3. Schwartz, S., M. H. Berg, I. Bossenmaier, and H. Dinsmore, Determination of porphyrins in biological materials, Methods of Biochemical Analysis, Vol. VIII (ed. D. Glick), Interscience Publ., New York, N.Y. page 237, (1960).

4. Yagi, K., Chemical determination of flavins, Methods of Biochemical Analysis, Vol. X, (Ed. D. Glick), Interscience Publ., New York, N.Y., page 345, (1962).

EXPERIMENT	ADDITION	MIXING	FILTRATION	BUBBLING	EVAPORATION	HEATING	L/L SEPARATION	PHOTOLYSIS	COOLING	DIALYSIS
MACROMOLECULES	X	X	X							X
PORPHYRINS	X	X	X	X	X					
FLAVINS	X	X	X		X	X	X	X	X	
TOTAL NUMBER OF USES IN THREE EXPERIMENTS	11	10	6	3	2	2	2	1	1	1

TABLE 1. SUMMARY OF PROCEDURAL FLOW DIAGRAMS OF APPENDIX A.

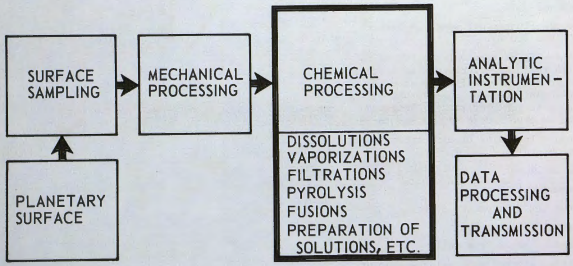

FIGURE 1. BLOCK DIAGRAM OF A GENERALIZED EXPERIMENT.

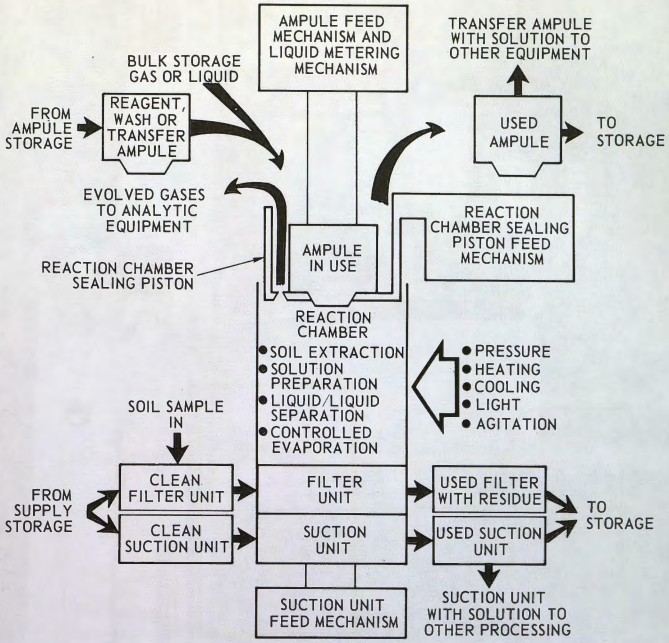

FIGURE 2. BLOCK FLOW DIAGRAM OF BREADBOARD CHEMICAL PROCESSOR.

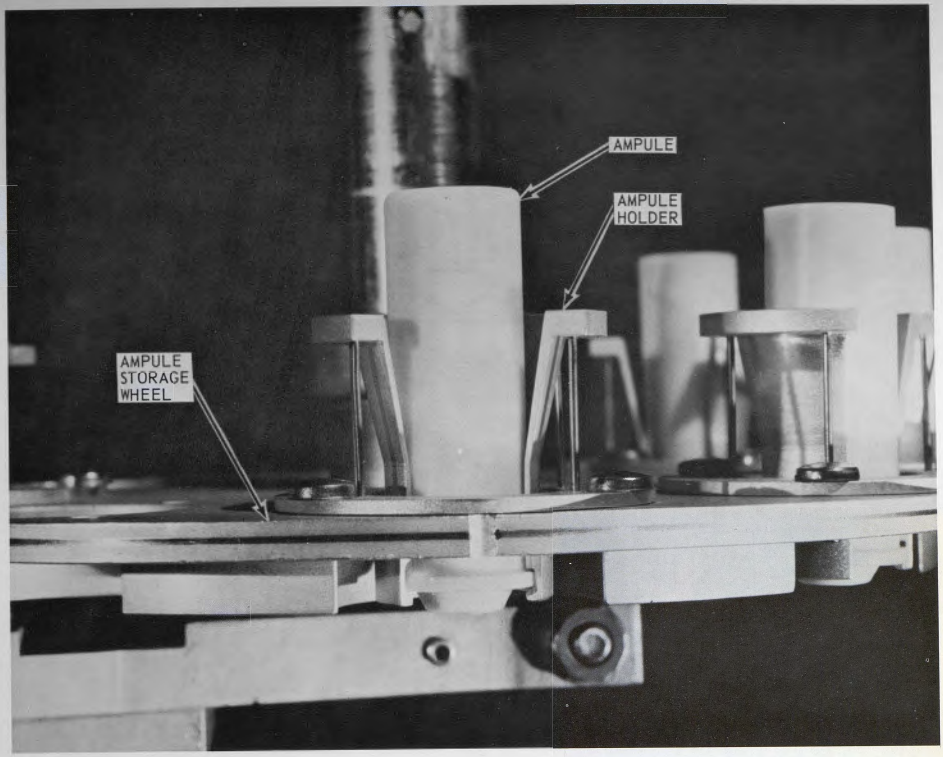

FIGURE 3. AMPULE IN STORAGE.

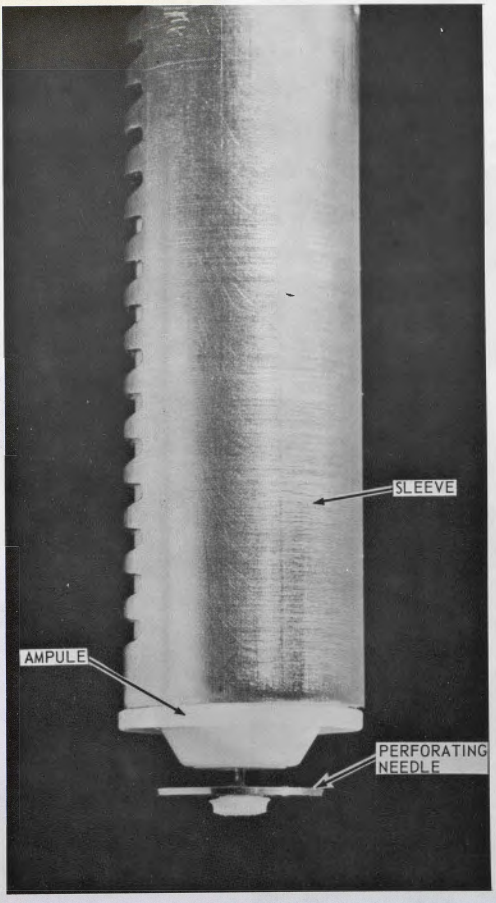

FIGURE 4. AMPULE REMOVED FROM HOLDER BY SLEEVE AND READY FOR INSERTION INTO PISTON.

FIGURE 5. AMPULE SEATED IN PISTON.

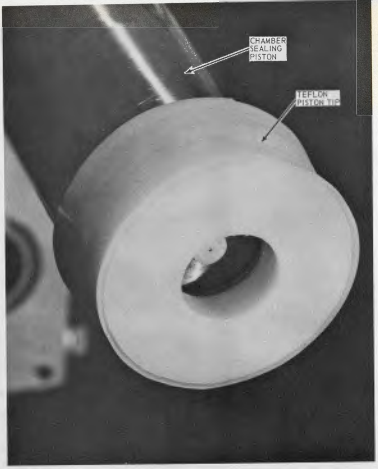

FIGURE 6. PISTON TIP IN PLACE ON PISTON.

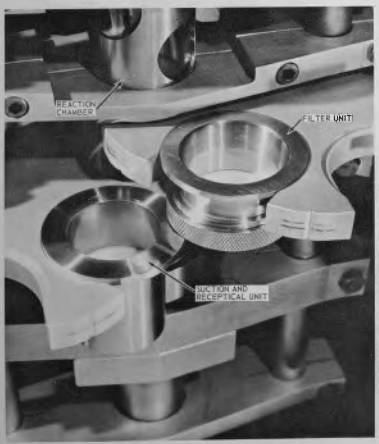

FIGURE 7. REACTION CHAMBER AND LOWER ASSEMBLY.

FIGURE 8. PISTON TIP RETAINED IN FILTER UNIT SUBSEQUENT TO FILTRATION.

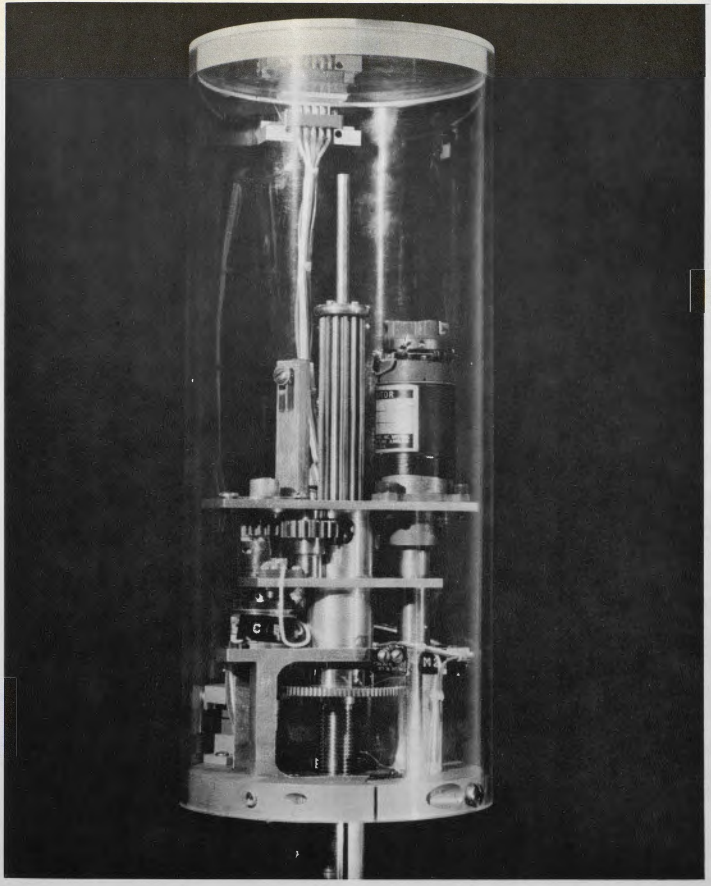

FIGURE 9. AUTOMATED AMPULE CONTROL AND REAGENT FEED MECHANISM WHICH REPLACED THAT SHOWN IN FIGURE 10.

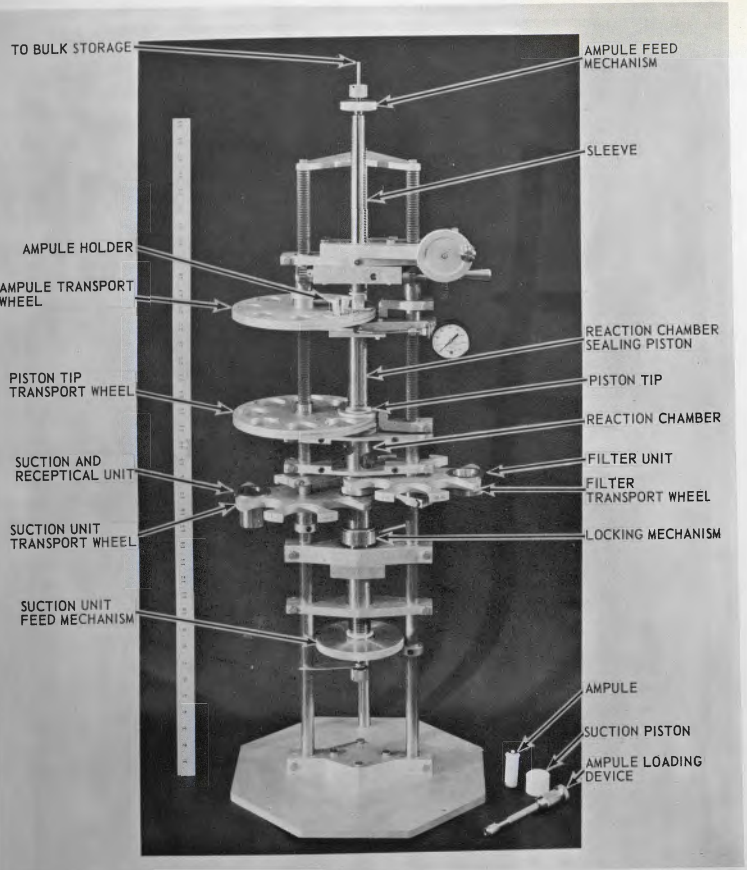

FIGURE 10. BREADBOARD CHEMICAL PROCESSOR.