J Forensic Sci, Apr. 1979, Vol. 24, No. 2

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A Liquid Gradient Density Screening System for Soil Sample Identification Studies Using a Minicomputer

The use of density gradient systems for the identification and comparison of soil samples is a well-recognized procedure [*1,2*]. In the usual manual method, liquid from a series of mixtures consisting of various ratios of high and low density liquids, such as 1,1,2,2-tetrabromoethane and bromobenzene, is added to a glass tube positioned vertically in a stand having a ruled background. The liquid is added up to each line and two or more identical tubes are prepared for comparison of the unknown against the reference material. A simple 8- to 10-step gradient is usually sufficient to obtain a usable resolution for most soil samples.

Different approaches have been developed to overcome the time-consuming, tedious process of precisely layering liquids in tubes in a reproducible fashion to float and separate the soil samples to gain the sensitive discrimination afforded by this technique. McCrone and Hudson [*3*] developed an agitation technique for the preparation of density gradients that is much faster than the classical method [*1,2*] but does have limited reproducibility for the comparison of soils.

Nute [*4*] developed a flow system that prepares a series of identical gradients in one run but suffers from the disadvantages that the system requires skill to construct and can lead to difficulty in the preparation of an identical gradient at a later date.

To overcome these problems a simple system was developed to automate the classical manual procedure to prepare a variable step gradient (either small or large [41 step]) reproducibly and automatically with solenoid burets. The gradient so prepared can then either serve as the whole density gradient for the separation of the sample or as a method of selecting the appropriate gradient for the manual procedure. In the device the solenoid-operated burets are opened in sequence for various controlled time intervals. Although the controlling device is a programmed calculator with a laboratory interface that provides multiple options, identical operation can be obtained by using any dedicated computer microprocessor system that controls electrical relays. Equivalent operation can be had by cutting three appropriate cams that are fixed onto a motor shaft. The cams would operate microswitches that would, in turn, operate the buret and mix solenoids for the desired time. The idea of the operation is that initially the buret containing the denser liquid (tetrabromoethane) is opened for any desired time interval (such as 8 s) and this liquid is allowed to flow down into the mixing chamber. Then the bromobenzene-containing buret is opened for the desired time, allowing this liquid to flow to the mixing chamber where the bromobenzene-tetrabromoethane mixture is stirred for 20 s and then drained

Received for publication 5 July 1978; revised manuscript received 18 Sept. 1978; accepted for publication 20 Sept. 1978.

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down the side of the gradient tube for 7 s. No bromobenzene is added in the first cycle (0 s). The next cycle could consist of a 7.8-s operation of the first buret and a 0.2-s operation of the second buret, followed by the same mixing and drainage cycle. The other cycles could then follow the same pattern: 7.6, 0.4; 7.4, 0.6; 7.2, 0.8; and so on. After the density gradient has been prepared in the tube, the sample is added in the normal manner and the next tube is positioned under the delivery tube to prepare the next gradient column. Samples should all be added at the same time to insure the reproducibility of the columns and to allow observation of each sample as it enters the column.

Equipment and Procedure

The following equipment is necessary for the procedure: a rack to support glass tubes that are 1 m long and 4 mm inside diameter; solenoid-operated burets or valves (available from Laboratory Supplies Co., Inc., Hicksville, N. Y.); a laboratory interface with an internal clock (Midwest Scientific Instruments) for operation with a Wang Model 700 calculator; a nitrogen gas tank and pressure regulator; and 1,1,2,2-tetrabromoethane and bromobenzene as reagents.

Figure 1 illustrates the mixing head and the two solenoid-operated devices, which can be either a buret or an aspirator bottle with the flow controlled by the solenoid. The aspirator bottle, because of its larger volume, by itself acts essentially as a constant pressure head so that the flow of liquid remains constant throughout a run. Hypodermic needles (20 g) are connected to the exit flow from the solenoid to act as a flow restrictor. A glass capillary tube (3-cm length of a broken thermometer) is connected

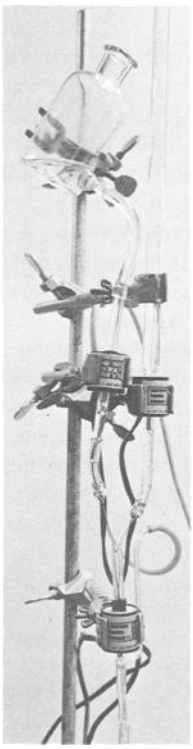

FIG. 1—*Apparatus for adding reagents.*

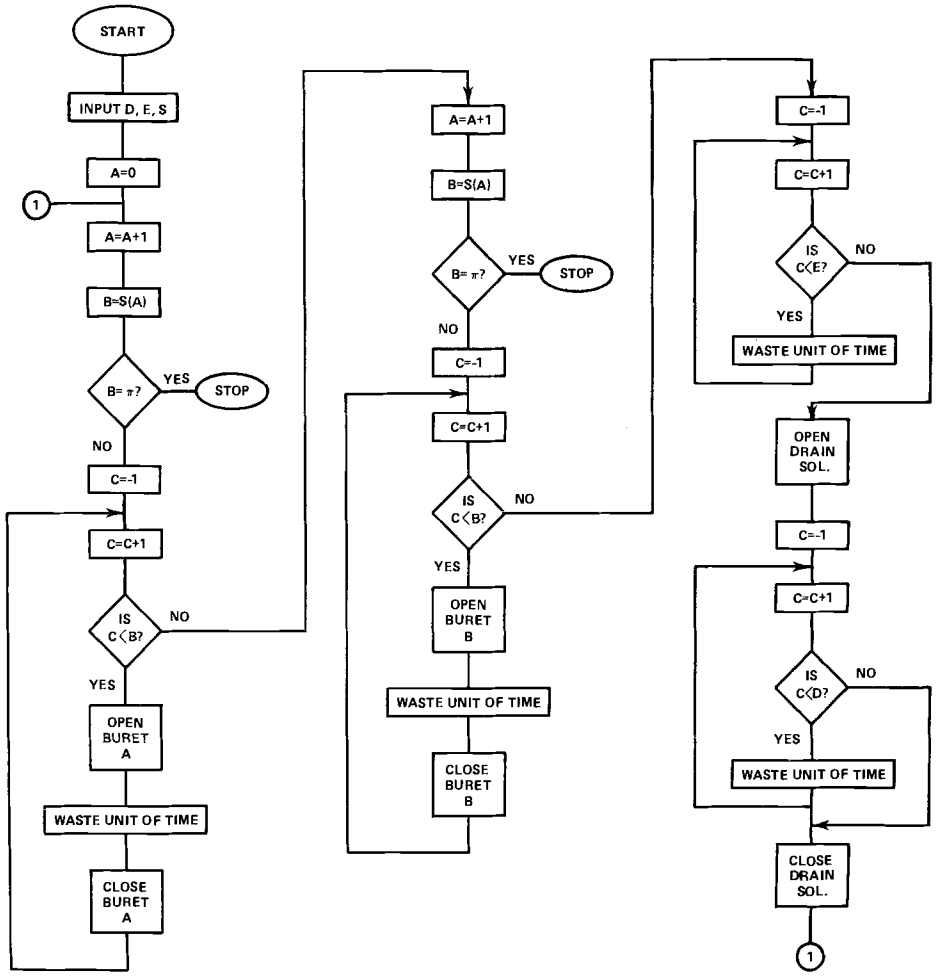

FIG. 2—*Program flow chart, where* D *is the number of drain cycles,* E *is the number of wait cycles before drain starts, and* S *is a vector containing wait cycles for Buret A and Buret B in chronological order and ending with* π *as an entry (for example, 3, 0, 2, 1, 1, 2, 0, 3,* π*). Other variables are internal.*

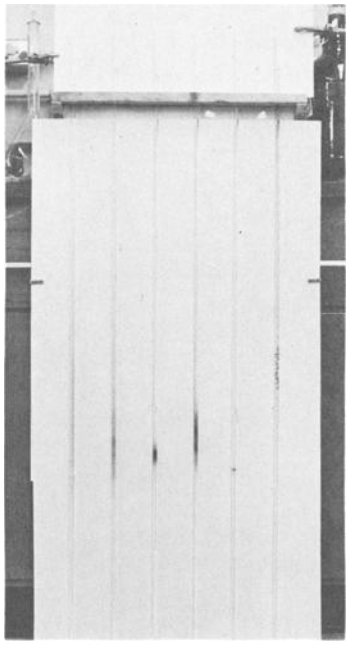

FIG. 3—*Soil separation of a 41-step gradient.*

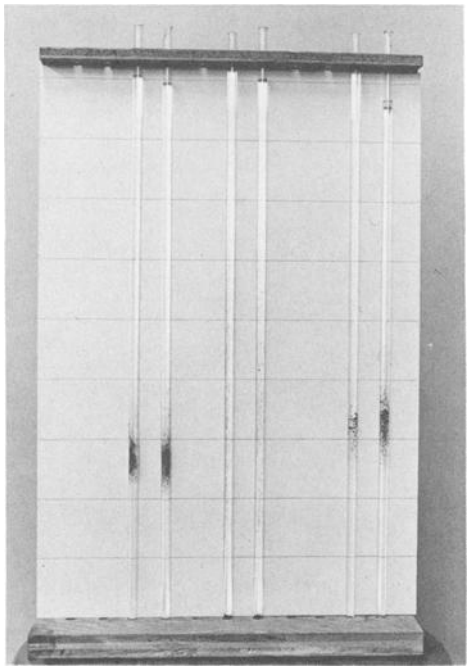

FIG. 4—*Soil separation of a 9-step gradient selected from the 41-step gradient.*

in the nitrogen flow line to act as a restrictor and to allow an easier adjustment of the slow stream of gas bubbles that mixes the liquid from the bottom of the mixing chamber.

The flow chart for the program is shown in Fig. 2. The time for each addition cycle (8, 0; 7.8, 0.2; 7.6, 0.4; and so on) is entered into storage. The pattern can be in any desired sequence and of indefinite length and stops when the appropriate command π is entered. The times for the mixing and drainage cycles are also entered individually (once in the beginning) and remain constant for the series. In practice a time interval of 0.2 s is entered on the interface clock. Then an entry of 40 time cycles in the storage register is equivalent to 8 s of addition, 39 cycles would be 7.8 seconds, and so forth.

Figure 3 shows the separation of samples consisting of sea sand (silicon dioxide), soil, and peat moss with a 41-step gradient. The total volume of liquid in the tubes is approximately 32 ml and the liquid height is about 90 cm. Because the tubing is not matched, a 3% variation in volume causes a 2.7-cm variation in liquid level height and could cause difficulty in interpretation. The system reproducibly adds liquid volume. The usual manual procedure of adding to a fixed, ruled line adjusts the liquid height, not volume. This difference should be considered when liquid gradients are prepared and, if desirable, the gradient system can be used for the rapid preparation of a multistep gradient that serves for selection of the appropriate gradient to be employed by the conventional procedure. Figure 4 shows a 9-step gradient selected from the 41-step gradient and prepared by the system to match a soil sample.

Summary

The construction and use of an automated, computer-controlled system for the preparation of a density gradient is described. The advantages consist of an automated, reproducible preparation of a density gradient having a large number of steps with an efficient use of small liquid volumes. The disadvantage is that a small variation in volume (approximately 3%) is reflected by an appropriate change in column height.

References

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