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An Improved Density Gradient System for Forensic Science Soil Studies

The density of a homogenous material is one of the more useful of its properties. Being dependent on the nature and composition of the material, it can be used as both a point of identification of the substance and a comparison of its composition with similar substances; being constant, affected only by temperature, it is reproducible. Techniques and equipment exist to easily and precisely measure the density of most liquids and solids. The basic techniques and applications of density measurements in the field of forensic science are covered by Kirk [*1,2*].

One of the techniques described by Kirk is the use of the density gradient for the comparison of soil samples. This has become one of the classic methods for comparing soils to determine similarity of origin. If a set of burets are used as he describes, the technique is also useful for isolating and studying individual fractions of a sample. However, the tedium of precisely layering liquids in several tubes in a reproducible fashion detracts from the sensitive discrimination afforded by the technique. It is also time-consuming. This generates a high "preparation:interpretation" ratio. The agitation technique described by McCrone and Hudson [*3*] is much faster, but is limited for the comparison of soils from the standpoint of reproducibility of the tubes.

An apparatus has been designed to rapidly construct several identical gradient tubes simultaneously. The basic concept of the apparatus is a rectangular tank in which a gradient is prepared and into which a set of tubes, open at both ends, are simultaneously lowered. If the gradient in the tank is allowed to equilibrate and the tubes are lowered sufficiently slowly, identical gradients will exist in each of the tubes.

Equipment and Procedure

The basic density gradient apparatus is flexible as to size and number of tubes. These parameters determine the remainder of the dimensions of the apparatus. Throughout the following directions, the dimensions used in a typical model are given in parentheses (See Fig. 1).

1. Select the size of glass tubing desired (8-mm inside diameter) and cut the number of (45-cm) lengths desired (5).
2. Select a set of corks to fit the tubes (#1 regular).
3. Cut a rectangular piece of lead such that the corks will fit on it in a row with their

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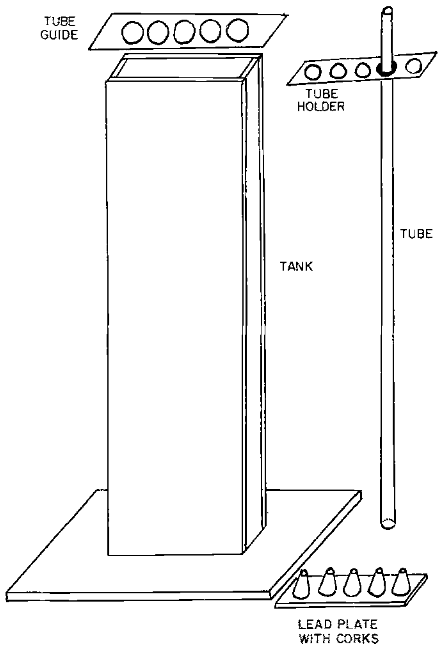

FIG. 1—*Basic density gradient apparatus.*

edges 2 mm apart (59 mm by 12 mm). Glue the corks to the lead with silicone rubber aquarium cement.

4. Measure the smallest rectangle which will fit around the lead plate loosely (60 mm by 13 mm). These are the dimensions of the inside of the tank. The height of the tank should be at least one inch shorter than the tubes (38 cm). The tank is constructed of ⅛-in.-thick glass. Allow for overlap on the two wider pieces of glass. (Two pieces of 7 cm by 38 cm by 3 mm and two pieces of 13 mm by 38 cm by 3 mm glass were required.)

5. Using silicone rubber cement, glue the tank together. Cut a piece of ¼-in. glass (15 by 15 cm) for the base. Glue the tank to the base using the silicone rubber cement.

A modification allows retrieval of individual fractions of a sample. This design is more difficult to construct, as the skills of a glassblower are required. The size and number of tubes are flexible and determine the remainder of the dimensions of the apparatus. Throughout the following directions, the dimensions used in a typical model are given in parentheses as a guide (see Figs. 2 and 3).

1. Select the size of glass tubing desired (7-mm inside diameter) and cut the number of (38-cm) lengths desired (5).

2. Select a set of male and female ground glass joints to fit the tubing (Ŧ 1030) and attach the upper ends of the male ground glass joints to each of the lengths of tubing.

3. Drill the desired number of holes (5) in a block of wood (20 by 10 by 2.5 cm) such that the female ground glass joints will fit snugly and with the edges almost touching. The holes must by perpendicular to the 20 by 20-cm plane and parallel. Check the alignment of the tubes when placed in the block before continuing.

4. Glue the female ground joints into place. Weldwood® epoxy is satisfactory. Apply a thin coat of glue over the top surface of the wood to facilitate gluing with silicone rubber later (see Direction 8 below).

5. Attach a Teflon® stopcock fitting (Ŧ 2) to the lower end of each female ground

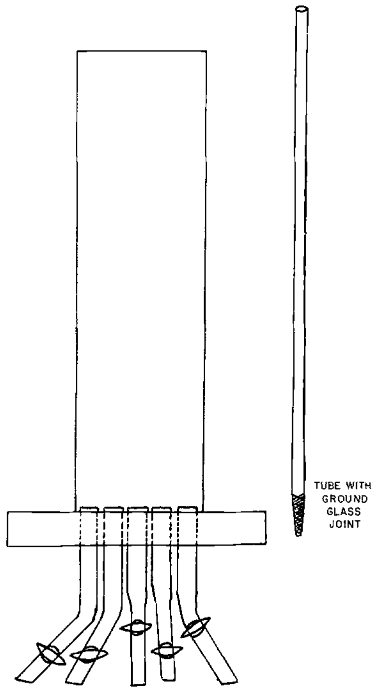

FIG. 2—*Modified density gradient apparatus, side view.*

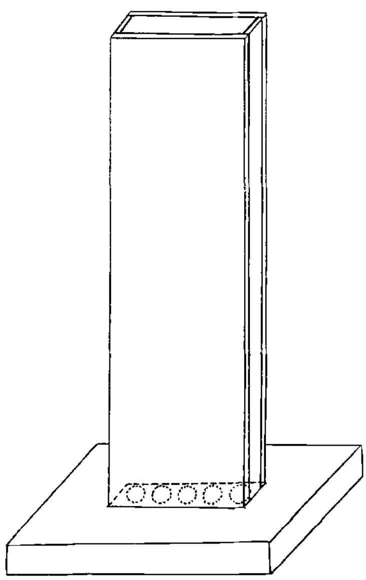

FIG. 3—*Modified density gradient apparatus, top-side view.*

glass joint. The stopcock must be arranged at varying angles so that they will all fit and be able to be turned.

6. Measure the smallest rectangle which will fit around the top of the female ground glass joints (20 by 92 mm). Add 3 mm to both directions and use these measurements as the inside dimensions of the tank. Allow for overlap (⅛ in.) on the edges of the wider pieces of glass. Construct the tank to the desired height (36 cm). (Two pieces of 36 cm by 11 cm by 3 mm and two pieces of 36 cm by 22 mm by 3 mm glass were required.)

7. Use silicone rubber aquarium cement to glue the tank together. Construct the tank before attaching it to the wood. Lay a thin bead of glue on the narrow edge of the narrow strip of glass and on the edge of the face of the wider strip of glass. Join the two pieces and hold perpendicular until the glue sets, about 15 minutes. Continue until the four sides are finished.

8. Because bromoform attacks most glues, lay a coat of silicone rubber over the area which will be exposed to the bromoform. (The silicone rubber cement is not attacked by the bromoform but will not bond to wood satisfactorily—See Direction 4 above). *Before this coat dries,* lay a bead of silicone rubber cement on the bottom edges of the tank, press the tank into place, and hold until the cement dries.

Procedure

The selection of the liquids for the gradient depends upon the density range desired. The heavier liquid is normally bromoform, which has a density of 2.89. The lighter liquid obviously must be miscible. Neither liquid should be miscible with the sample nor react with it. The liquids should not react with each other and should have similar vapor pressures if they are to be premixed and stored. If they are not to be stored, they can be slowly reactive (for example, bromoform and bromobenzene). If the liquids are nontoxic they can be used outside of a hood; however, the described apparatus can easily be used in a hood if the choice of liquids so dictates. An exhaust in the room is needed with bromoform. The amount of liquid required in the described apparatus is over twice that of the tubes alone. If the liquids are chosen so as to be separable, they can be reused to cut the cost. The liquids normally used are bromoform and 1,1,2,2-tetrachloroethane, which meet the above criteria.

The gradient in the tank can be prepared by several methods, depending upon the degree of linearity and the range of the gradient desired [*4,5*]. The technique of layering is most flexible but it requires preparing several sets of liquids and working in close proximity to the liquids for a longer period of time.

The technique of adding two liquids at controlled rates is less flexible but requires less contact with the liquids. It is also faster and does not require a previously prepared set of liquids. The apparatus shown in Fig. 4 is a simple method of producing a linear gradient.

The total volume of liquid required for the tank with the tubes inserted should be measured and then the amounts for each flask and for the reservoirs determined. The lighter liquid is placed in the first flask and the heavier in the second. A tube runs from the second flask to the top of the tank. A reservoir of the heavy liquid is poured into the tank to maintain the linearity of the gradient. The stirrer is turned on and the stopcock between the flasks is opened completely. The stopcock on the second flask is then par-

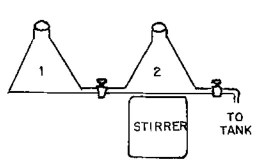

FIG. 4—*Assembly for preparing linear gradient.*

tially opened to provide the rate of flow desired. After the flasks are emptied, a reservoir of the lighter liquid is added to the top of the tank.

If desired, the order of the liquids can be reversed in the flasks and a tube run to the bottom of the tank. In this case a reservoir of the light liquid should be placed in the tank before starting.

In the event a nonlinear gradient is desired, a modification to achieve a two-step gradient is shown in Fig. 5. This modification is similar to the apparatus described by Lloyd [*6*]. The lighter liquid is placed in the first flask, the heavier in the second flask, and a mixture in the separatory funnel. The stopcock on the separatory funnel is opened completely and then the one on the second flask partially. As soon as the separatory funnel is emptied, the stopcock on the first flask is opened. This technique is useful for expanding the gradient around density 2.5.

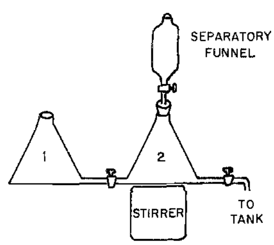

FIG. 5—*Modified assembly for preparing nonlinear gradient.*

After filling, the tank should be allowed to equilibrate for one to three hours.

A tube guide is fashioned from plastic and taped to the top of the tank. The holes in the guide should correspond in location to either the corks or the female ground glass joints in the bottom of the tank. A tube holder facilitates lowering the tubes simultaneously. It is fashioned from a pliable plastic, such as that used in sandwich boxes. Using a cork borer, holes are cut in the plastic just large enough to allow the tubes to be inserted but small enough to prevent them from slipping out. The tubes should be inserted such that the bottoms of the tubes are even.

The tubes must be lowered into the liquid slowly. A string can be tied to the tube holder and run through a pulley to provide better control on lowering the tubes. Upon reaching the bottom of the tank, they are gently maneuvered into place on the corks or into the female ground glass joints.

The samples are prepared and added to the tubes according to Kirk [*1,2*]. Acetone can be used to top off the tubes to observe the light components. A sheet of white paper can be placed behind the tank with a light behind it for photography if desired.

If, after observing the results, there is any question about the similarity of the gradients, the samples can be removed, filtered, and the procedure repeated in a relatively short time.

Individual fractions or layers of particles can be retrieved from the apparatus when modified with the stopcocks. The extreme density of the bromoform causes difficulty in controlling the flow of the liquid when collecting the lower layers. A slight amount of suction on the top of the tube helps to provide a more manageable flow.

Discussion

Because of the wide variety of complex mixtures encountered in soils, the density gradient tube has become one of the most widely used methods for forensic science soil comparisons. It is a sensitive, discriminating technique and relatively straightforward to interpret. Its major drawbacks have been the amount of time and effort required in relation to the amount of information obtained. The construction of the apparatus required and the increase in the amount of liquids necessary in the system described increases the cost of the analysis per run. But the amount of time required by the analyst is decreased drastically. In view of the complexity of soils, the time saved can be well spent on other complementary analyses which will increase the significance of the resulting conclusions.

Summary

The construction and use of the apparatus required for an improved density gradient system is described. The system is a combination of existing procedures, resulting in a system more suited for forensic science soil studies. The advantages consist of rapid, simultaneous filling of several tubes with identical density gradients and of the capability to remove individual fractions from the tubes for further study. Disadvantages are that an extra quantity of liquids is required and only a preset number of tubes can be filled at one time.

Acknowledgment

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