AIDS FOR ANALYTICAL CHEMISTS

Use of a Density Gradient Column to Measure the Density of Microspheres

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THE OAK RIDGE National Laboratory is engaged in the development of fuel elements for nuclear reactors. The Advanced Gas Cooled reactor will contain pyrolytic-carbon coated microspheres in its fuel elements. The core of the microsphere contains UO_2 and/or ThO_2. Each particle weighs about 100 μg, has a diameter varying from 250 to 450 microns and a volume varying from 0.10 to 0.01 μl. Refinements in the fabrication of these fuel elements necessitated the determination of density on individual microspheres as well as the determination of density distributions for a particular microsphere coating run.

The density gradient technique offers unique advantages for routinely determining densities on many small microspheres, from the standpoint of time of analysis, simplicity of equipment, and accuracy of measuring very small differences in particle densities. Perhaps the most important analytical feature of a gradient column is that many particles can be analyzed in one column. As many as 200 microspheres have been individually analyzed for density in a single column.

A density gradient is formed by establishing a concentration gradient between two solutions of differing density.

For all practical purposes, the process of diffusion in a density gradient column is too slow for the routine formation of a concentration gradient. Some form of partial mixing is necessary. Oster and Yamamoto (*1*) describe several methods of mixing. The best method consists of introducing the first liquid at a constant flow rate into a mixing chamber containing the second liquid and letting the mixture flow into the gradient tube. The gradient will be linear if the flow rate into the gradient tube is twice the flow rate of the first liquid. This condition may be approximated if the original densities of the two liquids are very close. Another way to approximate this condition is to require the volumes in the mixing chamber to be large with respect to the volume of the gradient column. Equilibrium is established in the gradient tube soon after mixing.

Because the gradient is not exactly linear over a large density range, standard particles are required to calibrate the column. These standards must be small in size to avoid disturbing the gradient when they are dropped into the column and to make it easier to measure their exact position. All standards were less than 2 mm in diameter. Standards greater than the density of borosilicate glass were made from lead-composition glasses. Standards less than the density of borosilicate glass were made by enclosing a pocket of air in capillary tubing.

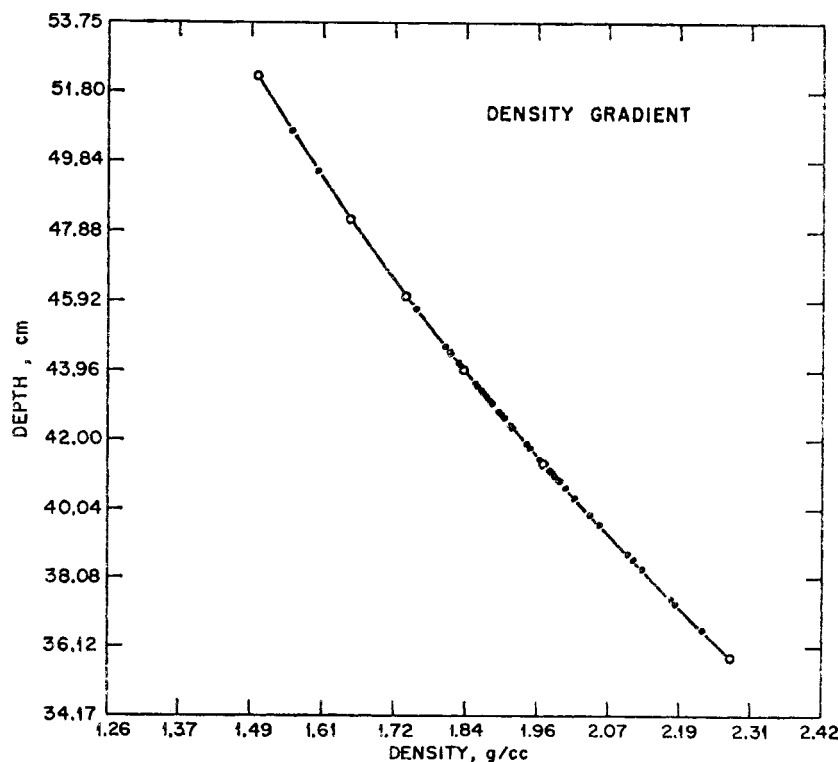(1) G. Oster and M. Yamamoto, *Chem. Rev.*, **63**, 257–68 (1963).

Figure 1. Density *vs.* cathetometer position

A primary density standardization of each glass standard is necessary. The calibration consists of a modified pycnometric sink-float procedure (2). With proper precautions the absolute density of an individual bead may be measured to 1 part in 10,000.

The gradient tube used is similar to that described by Oster and Yamamoto (1) except that the dimensions are reduced considerably. The gradient tube is 10 mm i.d. borosilicate glass tubing and is 40 cm in length. The reservoir volumes are 50 ml each.

Each column covered a relatively wide density range of 2 grams/cc. Organic and inorganic columns worked equally well for the carbon-coated microsphere. High densities approaching 5 gram/cc are obtained by saturating H_2O with a thallous formate–malonate double salt and maintaining the column at about 85°C.

A cathetometer is generally used to obtain position measurements on the standards and samples. The cathetometer used in these experiments measured to within $\pm 5 \times 10^{-3}$ cm. No movement could be detected with the cathetometer on the 100-μg carbon-coated microspheres after a 5-minute period in the column. Neither could the effects of slight temperature variations along the column length be detected with the cathetometer.

The accuracy of this method of measurement was tested by measuring the density of small high-purity KCl crystals in an organic gradient column containing benzene and tetrabromoethane. The density agreed to within 2 parts per thousand of the value given in the International Critical Tables.

Because the plot of position *vs.* density is not linear for any one gradient column, curve fitting is required. This method is subject to some error because of graphical misinterpretations as well as being time-consuming when many samples are being determined. Therefore, a computer program was written to analyze, tabulate, and plot the data.

A typical plot returned from the C.D.C. 1604A computer is shown in Figure 1. The open circles represent standard particles while the smaller black dots represent sample particles. The computer fits the best third-order polynomial to these standard points by a least squares method and then plots the sample particles on the curve. This plot refers to a 50-particle batch of microspheres representing the density range of a single coating run.

Other useful output information which accompanies this computer plot is the calculated value of the standard data points from the "best fit" third-order polynomial equation, the variance of each standard point as calculated from all of the standard data points, sample density, mean sample density, sample standard deviation, and the standard error of fit. The standard error of fit tells quantitatively how well the standard data fits the calculated equation. Mathematically, it is analogous to the standard deviation of the height.

(2) M. Gordon and I. A. Macnab, *Trans. Faraday Soc.*, **49**, 31–9 (1953).

Micropipet Encapsulation for Gas Chromatography Samples

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THE INTRODUCTION of sub-microliter quantities of volatile liquids into a gas chromatograph with a syringe is complicated by the variable amount of material which may be boiled out of the needle (1). In work with radioactive compounds this source of error is troublesome, since normalizing curves are not generally used in the quantitative analysis of radioactivity. Although it is possible to produce consistent peak heights for given sample volume, an analysis of contained radioactivity indicated that with variable volumes, inconsistent results are obtained.

Methods of introducing sealed samples into gas chromatography columns have been previously described (1, 2). The relatively elaborate equipment and technique required to measure the quantity of encapsulated material make these methods time consuming and expensive. The use of precise bore capillary tubing in the form of disposable micropipets (Figure 1) simplifies the measurement and encapsulation of sub-microliter samples. These pipets used in conjunction with a heated crushing device makes precision of a few per cent readily obtainable over a range of volumes less than one microliter. A machinist's rule is used to make the measurement of the contained volume.
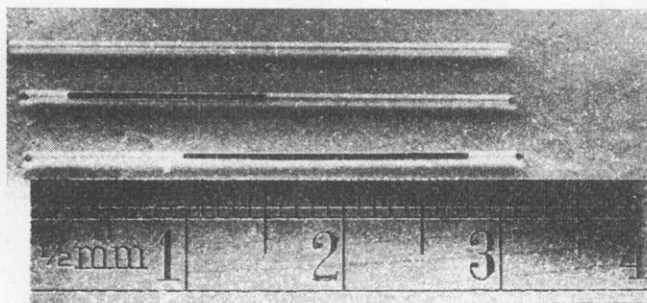
(1) A. G. Nerheim, ANAL. CHEM., **36**, 1686 (1964).
(2) R. L. Bowman and A. Karmen, *Nature*, **182**, 1233 (1958).

Figure 1.   One-microliter micropipets in position for measurement

EXPERIMENTAL

Drummond Scientific Co., Microcaps, disposable micropipets were used to encapsulate and measure the samples. Two separate lots of one-microliter tubes were used for this work, having overall lengths of 33.8 mm and 31.8 mm, respectively. Accurately standardized ^{14}C-tagged toluene was used as the test material. An ampoule crusher (Figure 2) similar to that described by Bowman and Karmen (2) was fitted in the place of the standard injection block of a Wilkens Model 1520 gas chromatograph. Radioactivity measurements were made with a Nuclear-Chicago Model 4998 gas chromatography proportional detector system and with a Nuclear-Chicago Model 6725 liquid scintillation spectrometer.