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Notes on Methodology

A calculation for locating the carrier gas front of a gas-liquid chromatogram

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► The linear relationship between the logarithm of the reduced retention volume (V_R') and the chain length (n) of a series of structural homologues is a feature of gas chromatography which facilitates the identification of components (1). To determine V_R' one must establish the time of appearance of the carrier gas front. In an ideal system this point is considered to be the moment when an infinitesimal amount of nonabsorbed gas introduced with the sample moves through the detector under over-all conditions of constant temperature and pressure gradient. James and Martin (1) designated the air peak as the carrier gas front, and the retention time of a component was obtained directly by measuring from this air peak to the peak of the component. When the chromatogram is recorded from a gas-density balance, the air peak is an obvious positive deflection and the retention measurements are easily made. However, with the use of an ionization chamber detector the air peak is a negative deflection and is frequently not seen (Fig. 1). In this case, the time of the appearance of the carrier gas front can be calculated in the manner described below, and the retention times of all components can be measured from this "mathematical air peak."

By definition, the volume of gas (uncorrected for compressibility) required to elute a component after the appearance of the gas front is given as

$$V_R' = tF_c = \frac{xF_c}{S} \qquad (1)$$

where t = retention time (minutes) which elapses from the appearance of the gas front to the appearance of the peak of a component, F_c = flow rate (cc. per min.) of the carrier gas measured at the outlet pressure and temperature of the column, x = distance (cm.) measured on the chart from the carrier gas front to the component peak and S = speed (cm. per min.) of the recorder chart.

From equation 1, the relationship between V_R' and the chain length,

$$\log V_R' \propto n \qquad (2)$$

can be expressed

$$\log x \propto n \qquad (3)$$

If the distance to a given peak is not measured from the true carrier gas front (x_0) but from some arbitrarily chosen point (y_0), this distance y will differ from x by δ, as shown in Figure 1. Thus,

$$x = y + \delta \qquad (4)$$

In turn, equation 3 can be expressed

$$\log (y + \delta) \propto n \qquad (5)$$

If three homologues in a chromatogram are chosen such that

$$n_2 - n_1 = n_3 - n_2 \qquad (6)$$

then it follows from equation 5 that

$$\frac{y_2 + \delta}{y_1 + \delta} = \frac{y_3 + \delta}{y_2 + \delta} \qquad (7)$$

or

$$\delta = \frac{y_2^2 - y_3 y_1}{y_3 + y_1 - 2y_2} \qquad (8)$$

Accordingly, one can measure from any arbitrary point (y_0) to the peaks of three homologues which obey equation 6, record these distances as y_1, y_2, y_3 respectively, solve for δ and measure from this arbitrary point to the point which should be the location of the carrier gas front. If δ is positive, x_0 precedes y_0; if δ is negative, x_0 follows y_0.

A simplification of this calculation is to designate the peak of homologue 2 as y_0. In this case y_2 is zero. Then the distance between the peaks of homologues 2 and 1 multiplied by that between homologues 2 and 3, divided by their sum (y_1 is a negative number in this formulation), is the distance from the peak of homologue 2 to x_0.

In Figure 2 is shown the plot of the relative retentions, r (relative to methyl stearate), of the n-saturated components of the chromatogram shown in Figure 1. As measured from y_0 there is an obvious deflection from linearity, but when measured from x_0 these values show a strictly linear relationship with chain length.

As mentioned above, it has been customary to designate the air peak as the carrier gas front (1). However, when chromatographic analyses are made by the

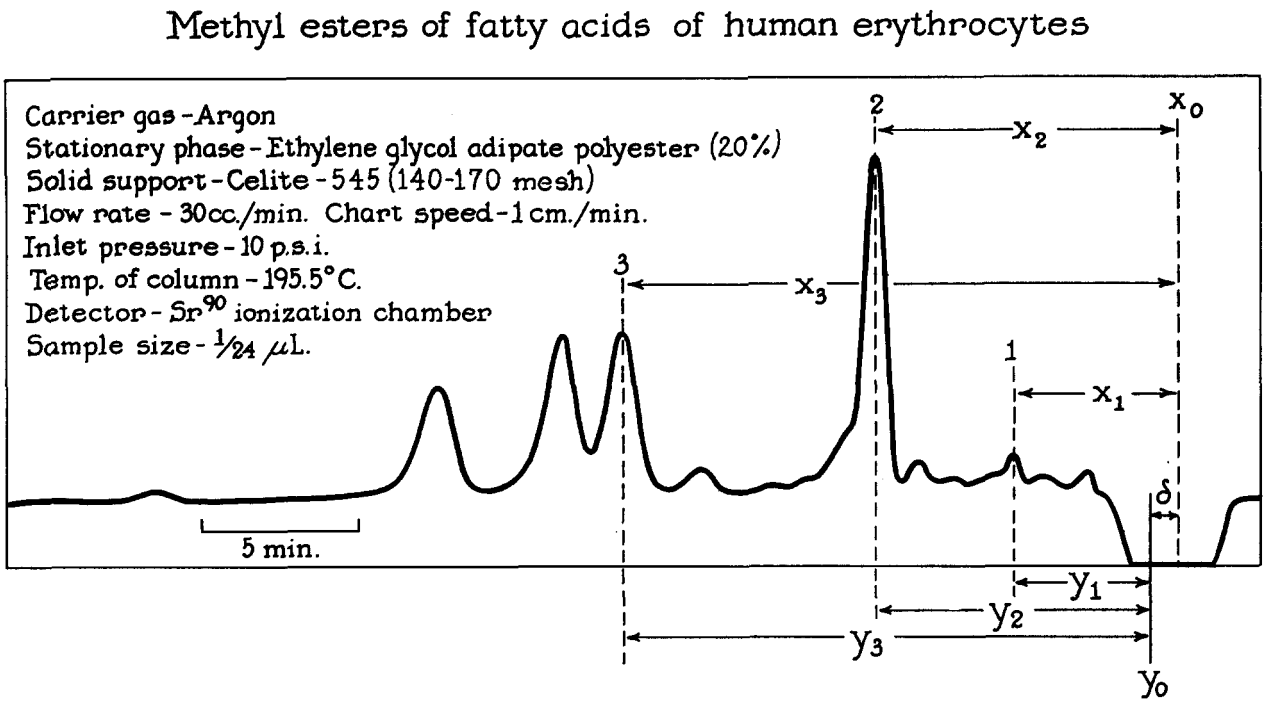

FIG. 1. Gas-liquid chromatogram of methyl esters of fatty acids of human erythrocytes. The homologues chosen for calculation of the location of the carrier gas front, x_0, are methyl myristate (1), methyl palmitate (2), and methyl stearate (3). The distances from an arbitrary point (y_0) to these homologues are designated y_1, y_2, y_3, respectively. For purposes of clarity the entire chromatogram is not shown, nor are all the components labeled. The chromatogram proceeds from right to left.

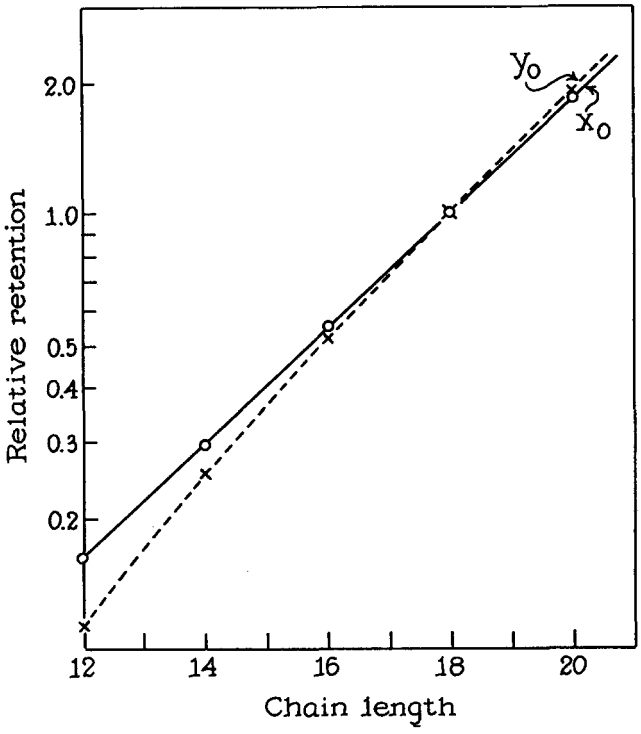

FIG. 2. Relative retentions of *n*-saturated components (relative to methyl stearate) as a function of the chain length. The broken line is the plot of the relative retentions of the components shown in Fig. 1, calculated from y_0. The solid line is the same plot for values calculated from x_0.

gas-flow interruption technique of sample loading, the carrier gas front calculated from equation 8 *precedes* the air deflection by a small but perceptible distance. This occurs in records made by the gas-density balance (Fig. 3) as well as by the ionization chamber detector (Fig. 4). This discrepancy is thought to be a function of the following considerations:

(*a*) The calculation presented above assumes a constant gradient between inlet and outlet pressures. This is not the case when the gas-flow interruption technique is used, since, when gas pressure is reapplied after loading the sample, the pressure gradient is not re-established instantaneously. Because the partition coefficient and hence the V_R' of every component is a function of gas pressure, any deviation from constancy of pressure invalidates the relationship expressed in equation 2. During this period of disequilibrium the components which partition most readily into the gas phase are subjected to the greatest distortion of V_R', whereas the components with the greater preference for stationary liquid phase (which appear last in the chromatographic run) are least affected. Figure 4 demonstrates that the calculated carrier gas front almost coincides with the air deflection when the calculation is made with three esters appearing late in the chromatographic analysis. How-

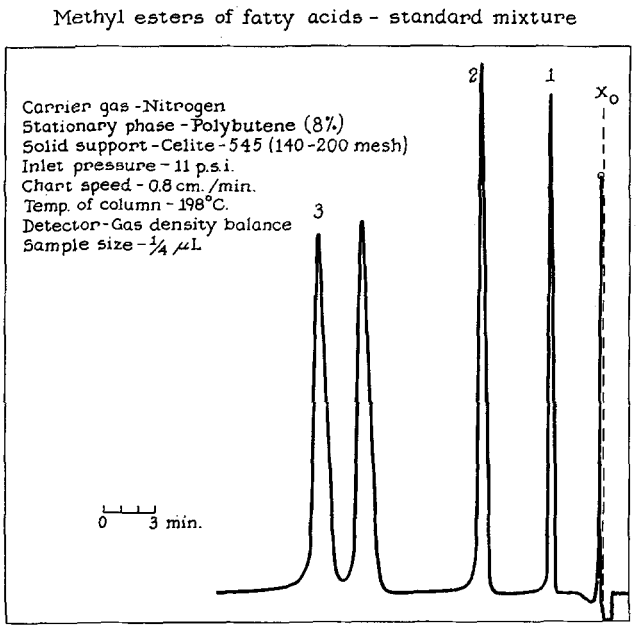

FIG. 3. Chromatographic analysis utilizing a gas-density balance. Methyl esters of myristic, palmitic, and stearic acids are designated *1*, *2*, and *3*, respectively. The unlabeled peak is methyl oleate. The calculated carrier gas front, x_0, precedes the air peak.

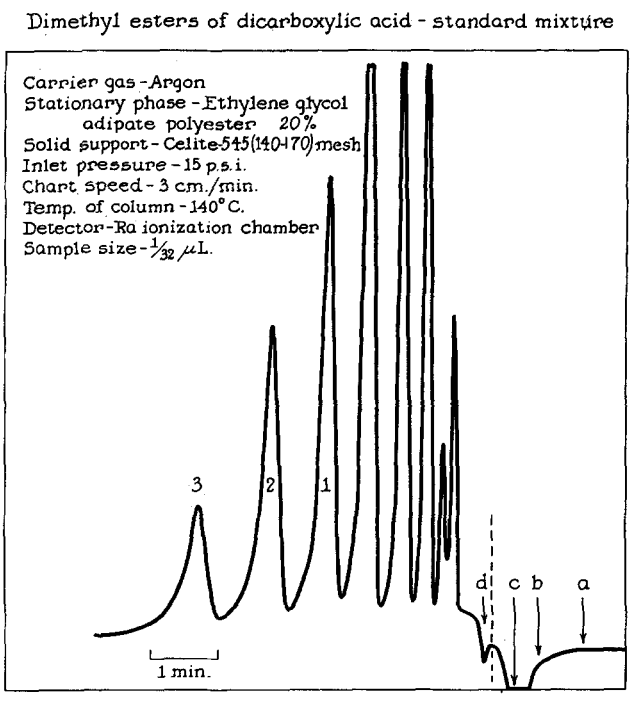

FIG. 4. Chromatographic analysis utilizing an ionization chamber detector. The gas-flow interruption technique of sample loading is used, illustrating the sequence of events at the start of the run. The carrier gas was obstructed (*a*), the inlet pressure fell to 5 pounds per square inch and the column was opened (*b*) to allow insertion of the sample. After the sample was applied, the carrier gas flow was re-established and the column closed (*c*). With this detection system the emergence of the air introduced with the sample appears as a negative deflection (*d*). The calculated carrier gas front (dotted line) precedes the air deflection. This calculation was made from the peaks designated *1*, *2*, and *3* (dimethyl esters of pimelic, suberic, and azelaic acids respectively).

ever, had the calculation been based on three early-appearing components, the discrepancy between the calculated carrier gas front and the air deflection would have been much larger. Thus the calculation of the location of the carrier gas front is most reliable when it is based on the three homologues with the longest retention times.

(*b*) The air deflection is created by components of the atmosphere which are introduced when the sample is placed on the column. If these distribute into the liquid phase, the air deflection is retarded.

When no air deflection is visible, the calculation of the location of the carrier gas front is made possible if familiar components are recognized which allow the application of equation 6. Then, from the retention volumes based on this calculated gas front, tentative identifications of unknown components can be made by reference to published tables of relative retentions.

REFERENCE

1. James, A. T., and A. J. P. Martin. *Biochem. J.* **50**:679, 1952.