strates that the β-glucosides predominate over the α-glucosides. As opposed to D-galactose no trace of aldehydes or septanosides occurs. The mixture obtained from direct methylation in DMF however contained 0.3% of the aldehyde with a relative retention of 2.32 (Table IV). In addition, traces of the sugar anhydride, 2,3,5-trimethyl - D - glucosane - α - [1,4] - β - [1,6], and incompletely methylated compounds were present in these mixtures. It has not been resolved whether this anhydride takes part in the glucose equilibrium in solution or is an artificial product formed by water elimination from incompletely methylated glucose.

Arabinose. This case is analogous to glucose in that the methyl ethers formed from the methylglycoside contained only the furanosides and pyranosides. The mixture obtained by direct methylation was found to exhibit a peak with a relative retention of 2.50 indicating the presence of the aldo form (0.4%).

CONCLUSION

It is possible to determine the amounts of various ring forms and carbonyl forms of equilibrium mixtures of sugars in solution. The proportion of isomers changes under varying conditions. Investigations of this nature are very important in studies concerning the mechanism of mutarotation. Not only should α-β-isomerization and transitions of furanoses and pyranoses be considered, but, as in the case of D-galactose, the presence of septanosides must be taken into account.

Table V. Content of Different Ethers in Methylated D-Arabinose

2-m. column (0.4 mm.) with 20% polyethylene glycol on kieselguhr (0.2–0.3 mm.). T = 150° C. Retention relative to succinic acid ester

Substance	Relative retention	Glyc. 0.013% HCl Ag_2O/CH_3I	Dir. perm. BaO/CH_3I
2,3,5-Trimethyl-α-methyl-D-arabinoside	1.60	61.1%	3.3%
2,3,5-Trimethyl-β-methyl-D-arabinoside	2.04	26.5%	34.1%
2,3,4,5-Tetramethyl-al-D-arabinose	2.50	...	0.4%
2,3,4-Trimethyl-β-methyl-D-arabinoside	3.05	6.3%	26.0%
2,3,4-Trimethyl-α-methyl-D-arabinoside	3.20	5.9%	36.6%

Gas chromatography is the first reliable method that demonstrates the existence of carbonyl forms of sugars in solution which, according to the above scheme, are important intermediates in transitions of the ring forms.

By using the results of analytical investigations it is possible to devise simple syntheses of pure isomeric methyl ethers (*3*). Tables II to V indicate the feasibility of obtaining very high yields of any desired component merely by varying the conditions under which the methylglycosides and permethyl ethers are formed. In the past the synthesis of pure isomers was always a troublesome task which included many steps and, as in the case of carbonyl and septanose forms, was not always successful. Direct methylation and subsequent separation by preparative gas chromatography now produces the desired substances in a very short time.

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Pressure Changes during Passage of a Solute through a Theoretical Plate in Gas Liquid Chromatography

R. P. W. SCOTT

Unilever Research Laboratory, Sharanbrook, Bedford, England

▶ Peak distortion owing to solute partial pressure is considered theoretically and the elution curve equation, derived from the plate theory, is modified to account for the effect. A pressure curve, coincident with the elution curve, is confirmed experimentally using a novel pressure transducer. The effect of solute partial pressure is negligible on analytical packed columns for charges of less than 1 mg., but significantly distorts early peaks on preparative scale columns. The increase in exit flow from a column during the elution of a peak is also considered and a simple method given for molecular weight determinations using an anemometer detector.

DURING THE PASSAGE of a solute through a theoretical plate the column pressure will increase owing to the partial pressure of the solute. This change in column pressure will cause transfusion of the solute band away from the normal peak maximum due to the difference in volume flow of carrier gas on either side of the peak. To assess the significance of this pressure effect on peak shape, column efficiency; and retention volume, the plate theory has to be modified and another elution curve equation derived. Such an equation would indicate conditions where this effect would cause errors in results or reduce column performance.

To confirm the existence of a pressure pulse coincident with the elution curve, it was necessary to employ a suitable pressure transducer that would continuously monitor the pressure at a point in the column as a solute band passes (*1*) and it will be seen that this transducer acts as a detector with a sensitiv-

ity commensurate with that of the katharometer.

The possibility of determining molecular weights by volume measurements taken during the elution of a peak has also been considered theoretically and verified experimentally.

THEORY

The mass of gas passing any point in a column is assumed to be constant for a given flow. This assumption is, at present, controversial but as the theory derived from this premise is in accord with experimental data the assumption is taken as correct. It follows that as solute vapor enters a theoretical plate the number of molecules in the gas phase will rise and the pressure increase. The increase in pressure is equal to the partial pressure of the solute vapor and in the p'th plate is given by the expression:

$$\Delta P_p = \frac{X_{g_p}}{M} V_g \times \frac{2.24 \times 10^4}{V_g} \times \frac{T_p}{273} \times 760$$

i.e.,

$$\Delta P_p = \frac{X_{g_p}}{M} \times \frac{2.24 \times 10^4}{273} \times 760 \times T_p = \gamma X_{g_p} \quad (1)$$

where

$$\gamma = \frac{2.24 \times 10^4}{M} \times \frac{T_p}{273} \times 760$$

The pressure flow curve for a theoretical plate will, therefore, be of the same form as the elution curve.

Due to the partial pressure of the solute, the volume of carrier gas passing through the column at a given point will not be constant, and will change when the solute band is present. Thus, the elution curve derived from the plate theory will be only approximate if charges of significant sizes are employed.

Consider three consecutive plates in a gas liquid chromatographic column and make the usual assumptions pertinent to the plate theory (*3*). Assume also, that the inlet/outlet pressure ratio (*8*) is small such that the pressure falls linearly along the column length.

$p-1$	p	$p+1$
$X_{g_{p-1}}$ V_g	X_{g_p} V_g	$X_{g_{p-1}}$ V_g
$X_{L_{p-1}}$ V_L	X_{L_p} V_L	$X_{L_{p+1}}$ V_L

In time δt let the gas pass from plate $p-1$ to plate p and from plate p to plate $p+1$. Now the volume of gas passing between adjacent plates in unit time will be equal to the ratio of the pressure difference between the plates and plate impedance.

Thus, volume of gas passing from plate $p-1$ to plate p in time

$$\delta t = [\omega + (\Delta P_{p-1} - \Delta P_p)] \frac{\delta t}{e} = \left[1 + \frac{\gamma}{\omega}(X_{g_{p-1}} - X_{g_p})\right] \frac{\omega}{e} \delta t = \left[1 + \frac{\gamma}{\omega}(X_{g_{p-1}} - X_{g_p})\right] \delta V$$

$$\text{As } \frac{\omega}{e} \delta t = \delta V$$

In a similar manner the volume of gas passing from plate p to $p+1$ is

$$\left[1 + \frac{\gamma}{\omega}(X_{g_p} - X_{g_{p+1}})\right] \delta V$$

There will be a resultant mass change in plate (p) of dm where:

$$dm = X_{g_{p-1}}\left[1 + \frac{\gamma}{\omega}(X_{g_{p-1}} - X_{g_p})\right]\delta V - X_{g_p}\left[1 + \frac{\gamma}{\omega}(X_{g_p} - X_{g_{p+1}})\right]\delta v = \left\{X_{g_{p-1}} - X_{g_p} + \frac{\gamma}{\omega}\left[X_{g_{p-1}}(X_{g_{p-1}} - X_{g_p}) - X_{g_p}(X_{g_p} - X_{g_{p-1}})\right]\right\}\delta V \quad (2)$$

As

$$dm = V_g \delta X_{g_p} + V_L \delta X_{L_p}$$

and

$$\delta X_{L_p} = K \delta X_{g_p}$$

then

$$dm = V_g \delta X_{g_p} + K V_L \delta X_{g_p} = (V_g + K V_L)\delta X_{g_p} \quad (3)$$

Equating 2 and 3

$$\frac{\delta X_{g_p}}{\delta V} = \left\{X_{g_{p-1}} - X_{g-p} + \frac{\gamma}{\omega}\left[X_{g_{p-1}}(X_{g_{p-1}} - X_{g_p}) - X_{g_p}(X_{g_p} - X_{g_{p-1}})\right]\right\} \frac{1}{V_g + K V_L} \quad (4)$$

As the solute band is essentially distributed over $6\sqrt{n}$ plates and n is large

$$X_{g_{p-1}} - X_{g_p} \geq (X_{g_{p-2}} - X_{g_{p-1}})$$

and

$$X_{g_p} - X_{g_{p+1}} \geq (X_{g_{p-1}} - X_{g_p})$$

Thus Equation 4 may be rewritten in the form

$$\frac{\delta X_{g_p}}{\delta v} = \left\{X_{g_{p-1}} - X_{g_p} + \frac{\gamma}{\omega}\left[X_{g_{p-1}}(X_{g_{p-2}} - X_{g_{p-1}}) - X_{g_p}(X_{g_{p-1}} - X_{g_p})\right]\right\} \quad (5)$$

where the flow of gas is measured in plate volumes and the variable V is changed to v where

$$v = \frac{V}{V_g + K V_L}$$

The justification for making the necessary assumptions to transform Equation 4 to Equation 5 is given in the Appendix.

Let

$$X_{g_p} = Y_{g_p} + f_p(v)$$

where

$$f_p(v) << Y_{g_p}$$

and

$$Y_{g_p} = \frac{X_o e^{-v} v^n}{n!}$$

i.e., $f_p(v)$ may be considered as a perturbation of the normal error function curve. Then $f_{p-1}(v) - f_{(p)}(v)$ and $f_{p-2}(v) - f_{p-1}(v)$ may be ignored and Equation 5 becomes:

$$\frac{\delta X_{g_p}}{\delta v} = Y_{g_{p-1}} - Y_{g_p} + \frac{\gamma}{\omega}[Y_{g_{p-1}}(Y_{g_{p-2}} - Y_{g_{p-1}}) - Y_{g_p}(Y_{g_{p-1}} - Y_{g_p})] + \frac{\gamma}{\omega}[f_{p-1}(v)(Y_{g_{p-2}} - Y_{g_{p-1}}) - f_p(v)(Y_{g_{p-1}} - Y_{g_p}]$$

Now as

$$Y_{g_{p-1}} >> (Y_{g_{p-2}} - Y_{g_{p-1}})$$

and $Y_{g_p} >> Y_{g_{p-1}} - Y_{g_p}$

then

$$\frac{\gamma}{\omega}[f_{(p-1)}(v)(Y_{g_{p-2}} - Y_{g_{p-1}}) - f_p(v)(Y_{g_{p-1}} - Y_{g_p})]$$

is of lower order magnitude. Thus,

$$\frac{\delta X_{g_p}}{\delta v} = X_{g_{p-1}} - Y_{g_p} + \frac{\gamma}{\omega}[Y_{g_{p-1}}(Y_{g_{p-2}} - Y_{g_{p-1}}) - Y_{g_p}(Y_{g_{p-1}} - Y_{g_p})]$$

The general solution to this equation may be seen on differentiation to be

$$X_{g_n} = X_o e^{-v} \frac{v^n}{n!} \times \left[1 + \frac{\gamma}{2\omega} X_o e^{-v} \frac{v^n}{n!}\left(\frac{n^2}{v^2} - 1\right)\right] \quad (6)$$

Replacing v by $n + w$, noting n is large and applying Stirlings theorem,

$e^{-v}\frac{v^n}{n!}$ may be reduced to the error function $\frac{e^{-w^2/2n}}{\sqrt{2\pi n}}$ thus Equation 6 becomes

$$X_{on} = X_o \frac{e^{-w^2/2n}}{\sqrt{2\pi n}} \times \left[1 + \frac{\gamma}{2}\frac{X_o e^{-w^2/2n}}{\omega\sqrt{2\pi n}}\left(\frac{n^2}{(w+n)^2} - 1\right)\right] \quad (7)$$

It should be emphasized that this solution only holds for moderate charges (see Appendix), that is, for small values of $\gamma X_o/2\omega$. The value of $\gamma X_o/2\omega$ can also be increased by reduction of ω, the pressure drop per plate, and the equation will therefore only hold for columns with a realistic value for ω.

Equation 7 shows that the partial pressure of the solute distorts the normal error function curve and the amount of distortion varies directly with the charge size and inversely with the molecular weight of the solute and the column impedance.

From Equation 7 the elution curves for different charges and for the same charge, but taken at different points in the column, are shown in Figures 1 and 2, respectively.

The curves were calculated for the following column solute systems: column length, 120 cm.; column diameter, 6 mm.; liquid phase, squalane; liquid phase on support, 15%; efficiency, 2500 theoretical plates; volume of gas/plate, 0.010 ml.; volume of liquid phase/plate, 0.00109 ml.; temperature, 75° C.; solute, *n*-heptane; molecular weight of solute, 100; partition coefficient of solute at 75° C., 150; sample distributed initially over 10 theoretical plates, 0.5 cm.; pressure drop across column, 7 p.s.i.; column flow, 60 ml. per minute; Pressure drop/plate at 60 ml. per minute, 0.15 mm. of mercury. Finally, $\gamma/2\omega = 7.2 \times 10^5$.

It is emphasized that the curves in Figures 1 and 2 do not account for the initial band width of 10 theoretical plates due to the distribution of the charge over 0.5 cm. of column length. The initial band width of the charge will broaden the elution curve in the manner described by Klinkenberg (*4*) and may be estimated by summing the variances of the initial and final bands. It should be noted that the curves obtained from the 10-mg. charge should be considered very approximate as Equation 7 is not applicable to large charges (see Appendix). For this reason Equation 7 was not applied to charges greater than 10 mg.; furthermore, they could not be accommodated initially by 10 theoretical plates. Excessive charges produce high partial pressures of solute resulting in the rapid transfusion of the

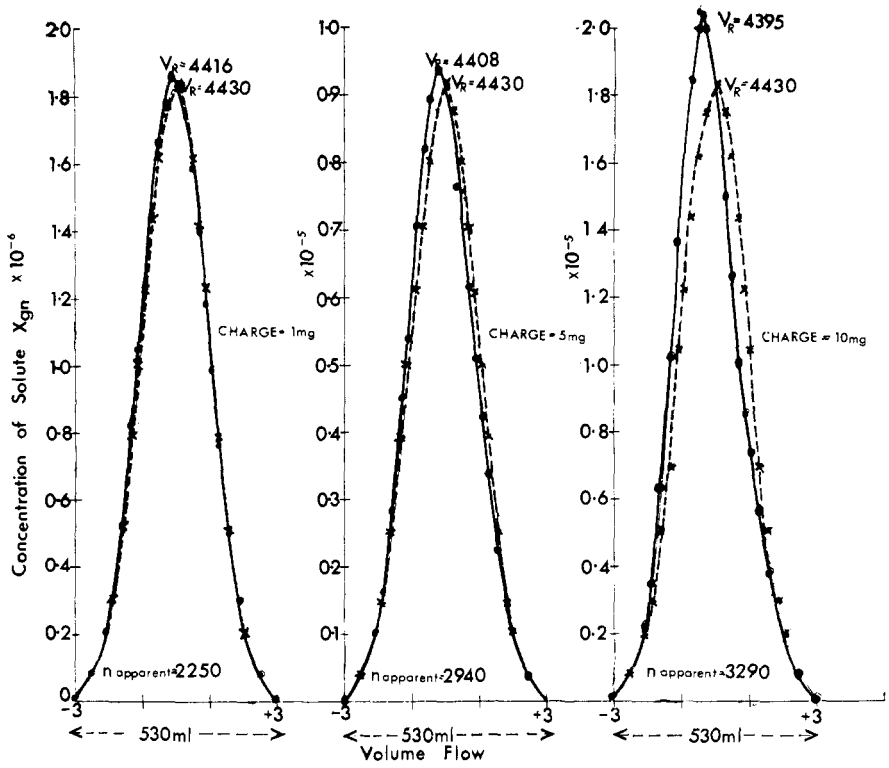

Figure 1. Theoretical elution curves for different charges of *n*-heptane

——— $X_n = \frac{X_0}{\sqrt{2\pi n}} e^{-w^2/2n}\left[1 + \frac{X_o}{\sqrt{\pi n}}\frac{\alpha}{2\omega} e^{-w^2/2n}\left(\frac{n}{(n+w)^2} - 1\right)\right]$

- - - - $X_n = \frac{X_o}{\sqrt{2\pi n}} e^{-w^2/2n}$

solute to neighboring plates. This transfusion continues until the partial pressure of the solute is reduced to a level where the normal elution process takes place.

Resulting from the pressure pulse that accompanies the solute band down the column, an increase in carrier gas flow must occur as the solute is eluted from the end of the column into the detector. This effect has been noted by van der Craats (*2*).

The increment of gas volume ΔQ occupied by M grams of the solute vapor is given by the following equation assuming the fugacity of the vapor to be unity.

$$\Delta Q = m\frac{K}{K+1}\frac{2.24 \times 10^4}{M}\frac{Tc}{273} \quad (8)$$

For two solutes A and B

$$\frac{\Delta Q_A}{\Delta Q_B} = \frac{K_A}{K_A+1}\cdot\frac{K_B+1}{K_B}\cdot\frac{M_B}{M_A}\cdot\frac{m_A}{m_B}$$

and if K is large

$$\frac{\Delta Q_A}{\Delta Q_B} = \frac{M_B}{M_A}\frac{m_A}{m_B} \quad (9)$$

Thus if a sensitive anemometer is employed as a detector in such a manner that no properties of the solute can affect the detecting system, then Equation 9 affords a simple method of measuring molecular weight.

If m_A, m_B, and M_A are known, and ΔQ_A and ΔQ_B measured, then

$$M_B = \frac{\Delta Q_A m_B M_A}{\Delta Q_B m_A} \quad (10)$$

DISCUSSION OF THEORY

During elution, the partial pressure of the solute sharpens the leading edge of a peak and slopes the trailing edge. This phenomena has been discussed by Littlewood (*5*) and has been demonstrated experimentally by Pollard and Hardy (*6*). These workers' results, however, include the effect of thermal changes in a column (*7*) which cannot be differentiated from the peak distortions owing to solute partial pressure alone. The explanation given by Littlewood is not exact as he assumes that the high concentrations in the elutions curve move more rapidly through the column than the lower concentrations. In fact, because of a pressure gradient along the column, it is the peak front that moves more quickly through the column than the peak tail. On a packed column the effect is small for charges of less than 1 mg. The peak asymmetry caused by the pressure gradient is similar to that produced by thermal effects in a column (*7*). The relative proportions of the peak asymmetry due to the thermal and pressure effects are difficult to assess, as the

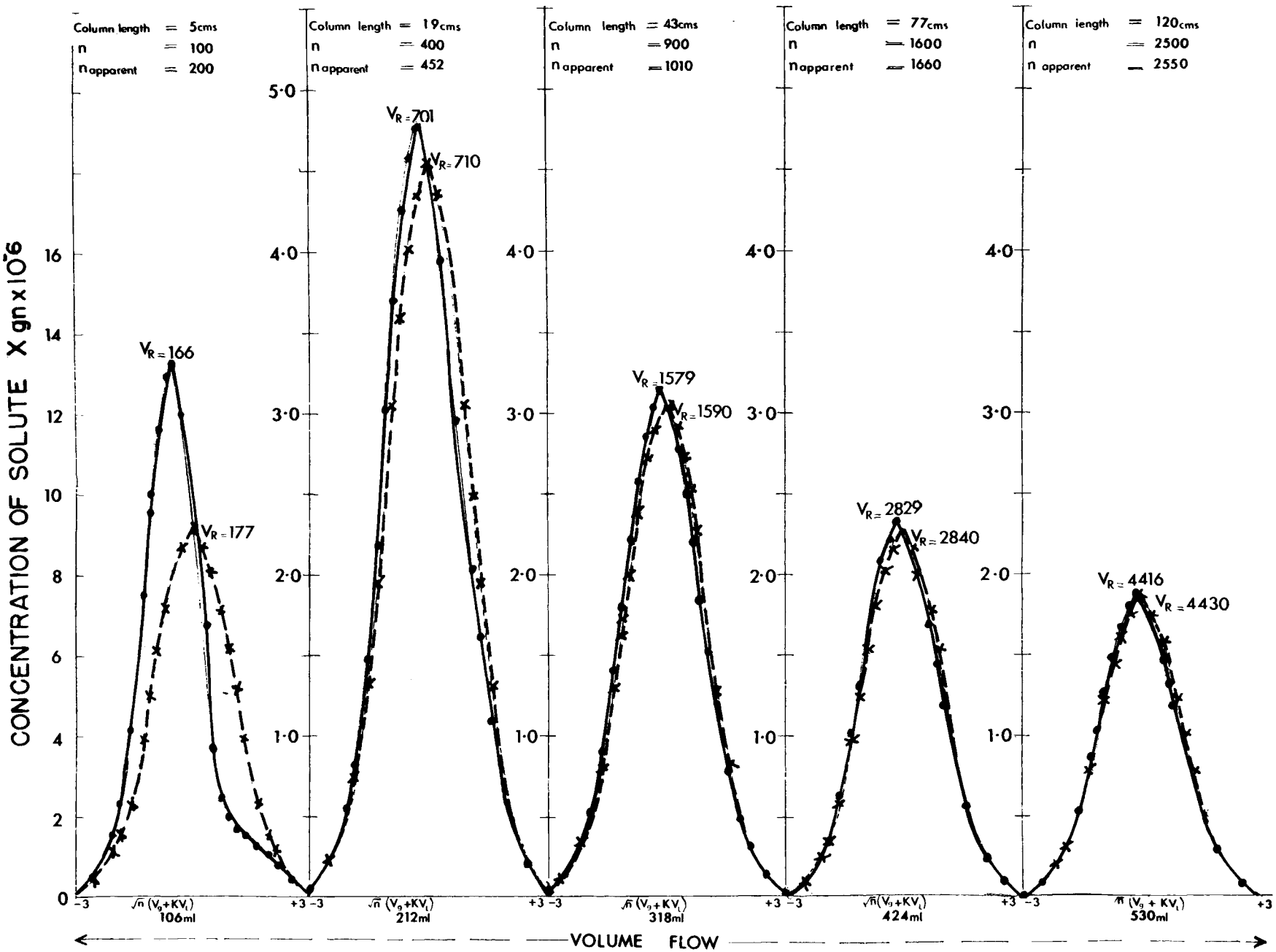

Figure 2. Elution curves for 1-mg. charge of *n*-heptane taken at different points in column

Solid and dotted lines have same meaning as in Figure 1.

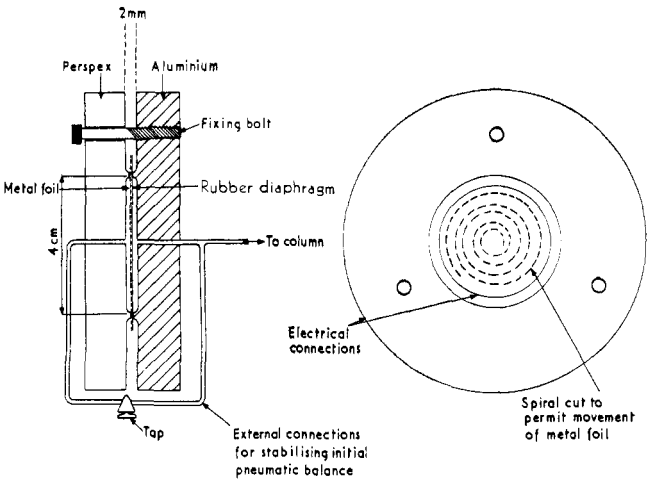

Figure 3. Diagram of pressure transducer

thermal changes that occur will depend on the heat of solution of the solute–solvent system concerned. Generally, the thermal effect will be the major factor contributing to peak asymmetry.

The magnitude of the peak asymmetry due to the solute partial pressure depends on three factors, γ, ω, and X_o. For a given charge, X_o varies inversely as the plate capacity and thus is also inversely proportional to the partition coefficient of the solute and the percentage liquid phase on the support: γ varies inversely as the molecular weight and ω directly with packing density. The greatest peak asymmetry will therefore occur on columns with low packing densities, carrying a small percentage of liquid phase on the support and for substances of low molecular weight that are eluted early in chromatogram.

Although the resulting asymmetry is small for charges of less than 1 mg., on preparative columns the pressure effect will make a significant contribution to the asymmetry of the early peaks.

For charges in excess of 1 mg. on packed columns the simple plate theory may not be applicable and efficiencies measured in the usual way (*1*) will be in excess of their true value (Figures 1 and 2). It should be stressed, however, that these apparent high efficiency values will only result from the pressure alone. If the pressure effect is accompanied by thermal effects and column 'over-load,' these latter will predominate and the measured efficiency will be lower than the true value. The effect of solute partial pressure on retention volume measurements is also shown in Figure 1 and 2. It is seen that values for retention volumes can be significantly smaller than those measured in an ideal system.

Measurement of the increase in column flow during the elution of the solute from the column affords a simple method of molecular weight determination. Equation 8 shows that the volume occupied by the solute vapor is inversely proportional to the molecular weight. Thus by using an anemometer detector, the peak areas produced by given masses of solute can be used to calculate their molecular weights. It may be that the GowMac Gas Density Bridge, which is known to be flow sensitive, is in fact partly acting as an anemometer detector. Flow changes during peak elution must effect the response of all flow sensitive detectors, particularly the flame thermocouple detector and katharometer. If a very accurate anemometer were used, it might be possible to determine the fugacity of the vapor for a substance of known molecular weight.

EXPERIMENTAL

It was required to establish that a pressure pulse did, indeed, accompany the solute band through the column and to verify the method of molecular weight determination using an anemometer detector.

Measurement of Pressure Change. The column pressure was measured by means of the transducer shown in Figure 3. It consists essentially of a plastic diaphragm with a metalized surface which acts as one plate of a parallel plate condenser. The diaphragm was made of thin latex rubber sheet to which a thin sheet of aluminum foil was attached by contact adhesive. The disk of foil carried a spiral cut to permit deformation without crinkling. The transducer was situated in one arm of a capacity bridge (Marconi Universal Bridge Type T.F. 2700) the output of which was fed to a 1-mv. potentiometric recorder.

The transducer was connected to the column by a tube sealed into a small hole in the column wall by an epoxy resin. By opening the tap, (Figure 3) both sides of the diaphragm were maintained at the same pressure while the column operating conditions were being established. Prior to charging the columns the metalized side of the diaphragm was isolated by closing the tap.

The transducer was calibrated against static water pressure and the calibration curves obtained were linear. The maximum sensitivity realized was 8 mm. of water at a signal-to-noise ratio of 2. This corresponded to a capacity change of 0.014×10^{-12} farads and a plate deformation of approximately 10^{-5} cm. It would appear that the sensitivity of the transducer could be further increased by the use of more sophisticated bridge unit operating at a higher frequency. As a detecting system the transducer had sensitivities commensurate with that of the katharometer detector.

Charges of 0.5 to 10 μl. of diethyl ether were placed on the column and the resulting pressure pulses recorded. The curve relating charge size with peak height in mm. of water is shown in Figure 4, together with the trace of a pressure pulse derived from a 5-mg. charge.

Determination of Molecular Weight. The anemometer detector used was the flame thermocouple detector (*9*) chosen because of its high sensitivity to changes in flow rate. This detector, however, responds to both changes in column flow and to changes in calorific value of the carrier gas owing to the presence of the eluted solute. It was necessary, therefore, to operate the detector in such a way that it responded explicitly to changes in column flow and to ensure that the eluted solute never entered the detector. This was achieved by the following column systems. The column, 30 cm. long, 4 mm. in diameter, packed with 100–120 B.S. mesh firebrick carrying 15% squalane as a liquid phase, was connected to a 20-foot length of 5-mm. i.d. copper tubing, which in turn, was connected to a second column, 20 cm. long, 4 mm. in diameter packed with 30–60 B.S. mesh active charcoal. A single substance injected onto the partition column resulted in two peaks appearing on the chromatogram. The first, a positive peak, occurred as the solute was desorbed from the partition column; the second, a negative peak, occurred as the solute was irreversibly absorbed on the absorption column. The copper tube introduced a time lag into the system and served to separate the desorption and adsorption peaks.

Eight-microliter charges of a number of volatile solutes were injected separately onto the column and a measure of the area of each adsorption curve was obtained by cutting the peak out and weighing it. The adsorption curve was used to circumvent the assumption that $K \gg 1$ in Equations 9 and 10. Each peak mass was corrected by dividing by the specific gravity of the respective solute to account for the varying densities of the substances used. The corrected weight of each peak, which is proportional to the same mass of solute is shown plotted against the reciprocal of the respective molecular weight in Figure 5, together with the desorption and adsorption curves for diethyl ether.

DISCUSSION OF RESULTS

The calibration curves obtained indicate the linear response of the transducer to pressure change. The

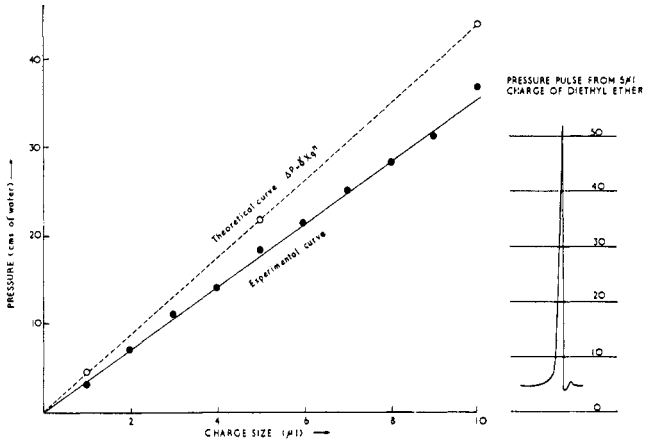

Figure 4. Theoretical and experimental curves relating pressure at peak maxima to charge size

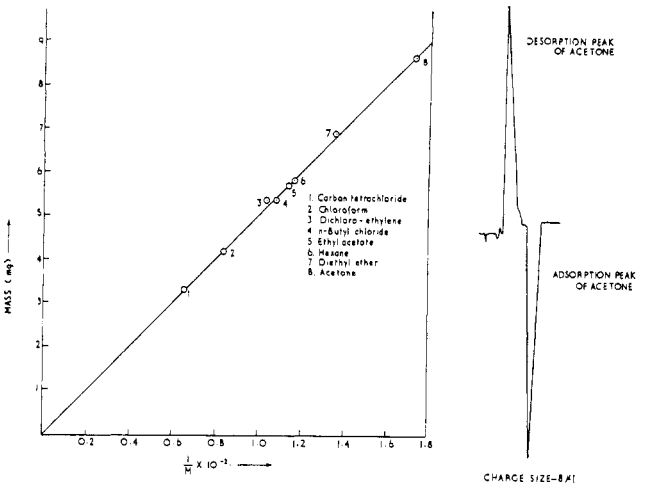

Figure 5. Graph of peak mass *vs.* molecular weight

curve relating peak height in cm. of water to charge size supports Equation 1 and substantiates the existence of a pressure pulse coincident with the solute band as it passes down the column. The pressure transducer affords yet another detecting system for use in gas chromatography although it does not offer any obvious advantage over those already in use. It should be noted, however, that for a linear transducer, predictable response will be inversely proportional to the molecular weight of the vapor detected.

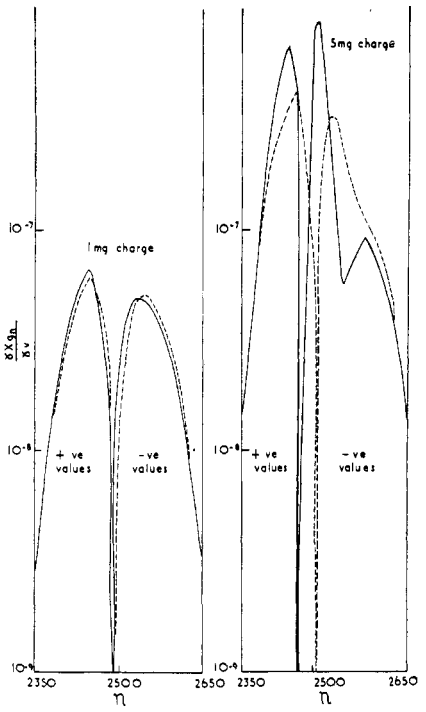

Figure 6. Computed values of $\frac{\gamma X g_n}{\gamma v}$ from true and approximate forms of differential equation

——— $\frac{\partial X g_n}{\partial v}$ from Eq. 4

- - - - $\frac{\partial X g_n}{\partial v}$ from Eq. 5

The results in Figure 5 show how a gas chromatograph employing an anemometer detector may be used indirectly for the determination of molecular weights. A more precise control of the operating conditions and the use of an integrator could give measurements with a high degree of accuracy.

The increase in column flow as a solute is eluted will affect all flow sensitive detectors and may account for some of the conflicting reports on the performance of such detectors. The increase in gas volume during the elution of a solute will contribute to the measured retention volume if liquid samples are employed but not if a gas sampling procedure is used. The effect of vapor volume on retention volume measurements using liquid samples will be small for charges of less than 1-μl., but if precise measurements are required a correction can be applied using Equation 8.

APPENDIX

In the development of the equations of the elution curve, certain simplifying assumptions were made to transform Equation 4 to Equation 5, and furthermore, the solution of Equation 5 was assumed to take a specific form. To test the validity of these assumptions the solution of Equation 5 was substituted in Equations 4 and 5 and the two respective values of $\frac{dXg_p}{dV}$ were calculated by means of a digital computor. The conditions used were those given for the determination of the curves in Figures 1 and 2 and as $\frac{dXg_p}{dv}$ was a difference function, the results were calculated to 16 significant figures to eliminate 'rounding off' errors. Values of $\frac{dXg_p}{dV}$ for charges of 1 and 5 mg. are shown plotted against plate number in Figure 6. It is seen that for 1-mg. charges, close agreement between the curves is obtained. For a 5-mg. charge the deviation is greater but the solution given by Equation 6 still gives a fairly good representation of the shape of the elution curve. The approximation used to transform Equation 4 to Equation 5 tends to smooth out the function. The elution curve maxima are shown in Figure 6 as a sudden change in the sign of $\frac{dXg_p}{dv}$ which thus locates the position of the maxima. It is seen that the displacement of the peak maximum is even greater where no approximation is made and thus the distortion of the 5-mg. and 10-mg. peaks shown in Figure 1 is less than the true value. The deviations of the values of $\frac{dXg_p}{dV}$ for the two equations is very significant for a 10-mg. charge and the curve for this charge size shown in Figure 1 can only be considered as a rough impression of the shape to be obtained.

LIST OF SYMBOLS

P_p = Column pressure in the p'th plate, mm. of Hg
ΔP_p = Small change in column pressure on the p'th plate
T_p = Temperature of p'th plate = column temperature
M = Molecular weight of solute
K = Partition coefficient of solute with respect to the liquid phase
X_{g_p} = Concentration of solute in the gas phase in the p'th plate, grams per ml.
X_{l_p} = Concentration of solute in the liquid phase in the p'th plate, grams per ml.
X_o = Initial concentrations of solute in gas phase on the first plate, grams per ml.
V_g = Volume of gas/plate, ml.
V_L = Volume of liquid phase/plate, ml.
ω = Pressure drop/plate, mm. of Hg
e = Plate impedance, mm. of Hg
n = Column efficiency, total number of theoretical plates
V = Volume of gas, ml.

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Determination of Carbohydrates in Glycolipides and Gangliosides by Gas Chromatography

CHARLES C. SWEELEY and BARBARA WALKER

Department of Biochemistry and Nutrition, Graduate School of Public Health, University of Pittsburgh, Pittsburgh, Pa.

▶ **Simultaneous gas chromatographic determinations were made of glucose, galactose, galactosamine, and sialic acid in neutral glycolipides and gangliosides. Methanolysis in dry, dilute methanolic HCl was used to convert the oligosaccharide portion of the glycolipides to monomeric carbohydrates. Hexoses were converted to methyl glycosides, *N*-acetylhexosamine was partially converted to hexosamine hydrochloride, and sialic acid gave the 2-O-methyl ketal of methyl neuraminate. Separate procedures were used to convert these products of methanolysis to trimethylsilyl derivatives for gas chromatography. Samples of sialolactose and ceramidetrihexoside were used to validate the procedures for determination of hexose and neuraminic acid. Reproducible determinations of these components were made with a relative error of ±5%. Problems were encountered in the quantitative determination of hexosamine by gas chromatography. Several alternative methods are given, and possible explanations for the difficulties are discussed.**

RECENT progress has been made in the application of gas chromatography for the separation of carbohydrates and related polyhydroxy compounds. Since these substances are not sufficiently volatile for gas chromatography, many studies have been directed to preparing suitably volatile derivatives by a method which is simple, quantitative, and applicable to a wide variety of compounds. Determinations which have been reported up to the present time have been made mainly on three types of derivatives: polymethyl ethers, polyacetyl esters, and polytrimethylsilyl ethers.

Since 1958, when McInnes *et al.* (*11*) reported a classic study on the separation of tri-*O*-methyl derivatives of methyl pentopyranosides and tetra-*O*-methyl derivatives of hexopyranosides by gas chromatography, a number of reports have been made of the determination of various sugars in the form of polymethyl ethers. Generally adequate separations have been achieved on both polar and nonpolar columns, but anomeric pairs are sometimes difficult to resolve. Probably the most serious problem in using the methyl ethers as general derivatives for routine analyses results from the long time required for their preparation and the possibility of variable yields. For some purposes, however, especially in structural studies on the location of glycosidic bonds in oligosaccharides, the use of these derivatives has proved to be of considerable value.

The separation of polyacetyl derivatives of carbohydrates by gas chromatography was first investigated by Bishop and Cooper (*3*) in 1960; determinations of a variety of sugars, as acetates, have since been recorded. The volatility of *O*-acetyl esters of carbohydrates is generally sufficient for separations on both polar and nonpolar columns. With substances having relatively high molecular weight, such as disaccharides, Jones and Perry (*9*) report that glass beads are the best inert support for liquid phase. Recently, Bishop, Cooper, and Murray (*4*) have investigated several types of thermally catalyzed changes of acetyl derivatives during gas chromatography. They noted that deamidation, changes in ring size, and rearrangements of acetal, ketal, and *N*-acetyl groups are likely to occur with these derivatives. For this reason, the polyacetyl derivativess hould probably be avoided as a general derivative for routine analyses.

Hedgley and Overend (*7*), Witsch (*20*), and Ferrier and Singleton (*5*, *6*) described conditions for the preparation and determination of *O*-trimethylsilyl derivatives of a number of carbohydrates. A general method was subsequently described for the preparation of these remarkably volatile compounds (*1*, *15*). The reaction proceeds to completion in a very short time, and seems to be limited only by the solubility of the carbohydrate in pyridine. A distinct advantage of the method is that injections may be made directly from the reaction mixture into the gas chromatograph. Relative retention times of more than 100 carbohydrates, as trimethylsilyl derivatives, have been recorded (*1*, *15*). Our conclusion, from these studies, has been that this derivative is ideally suited for routine determinations.

Extensive reviews by Bishop (*2*), Kircher (*10*), and Wells, Bentley, and Sweeley (*17*) compare separations achieved with the various derivatives, and describe useful conditions for the determination of these compounds.

An important area of application of these techniques, in the biomedical field, is for the determination of various carbohydrates which occur in complex glycolipides. The glycolipides are a family of compounds which contain a lipoidal group attached to an oligosaccharide composed of one or more different carbohydrate monomeric units, among which hexoses, hexuronic acids, inositol, hexosamines, and *N*-acylneuraminic acids have been found. A difficult analytical problem arises from the fact that a number of closely related glycolipides often occur together in relatively low concentration. They all