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Thermodynamic Functions in High Performance Liquid Chromatography of Saccharides

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High performance liquid chromatography of aldohexoses, the corresponding 6-deoxy derivatives, and several malto-oligosaccharides has been examined with a Hitachi #3013-N column, and with aqueous CH_3CN eluents, at various temperatures. It has been shown that when the concentration of CH_3CN in the eluent is higher than 30%, the system works as a partition chromatography, whereas with pure phosphate buffer eluent (H_2O) it works as a gel filtration column. Some of the values of the free energy $\Delta G°$ and enthalpy $\Delta H°$, assignable to the glucose residue and the 6-OH group in some particular states have been estimated. A possible use of such thermodynamic functions in medicinal chemistry is suggested.

Keywords——HPLC; high performance liquid chromatography; liquid chromatography; thermodynamic functions; saccharides; hexoses; deoxyhexoses; monosaccharides; oligosaccharides; malto-oligosaccharides

In this paper, we briefly show that, from a chromatographic experiment, some parameters can be obtained which may characterize the molecule in question in a unique manner. One such parameter, we propose, is the difference ($\Delta G°$) in the standard free energy of the molecule in the stationary phase ($G_s°$) from that in the mobile phase ($G_m°$).

Free Energy of Retention

The isocratic elution mode, the adjusted retention time t'_R is given as

$$t'_R = t_R - t_0 = t_0 \frac{C_s}{C_m} \frac{V_s}{V_m}, \tag{1}$$

where t_R is the retention time, t_0 the hold-up time, C_s/C_m the equilibrium concentration ratio of the sample in the stationary phase *versus* that in the mobile phase, and V_s/V_m the effective volume ratio of the stationary phase *versus* the mobile phase. The equilibrium constant $C_s/C_m=K$ is related to the difference $\Delta G°=G_s°-G_m°$ of the standard free energy $G_s°$ in the stationary phase from that in the mobile phase $G_m°$ by $\Delta G°=-\boldsymbol{R}T\ 1nK$, where $\boldsymbol{R}$ is the gas constant and T the absolute temperature. Therefore,

$$\log_{10} t'_R = \log_{10} t_0 \frac{V_s}{V_m} - \frac{0.4343}{\boldsymbol{RT}} \Delta G°. \tag{2}$$

Thus the difference in ΔG° of two molecules 1 and 2 is readily given by

$$(\log_{10} t'_R)_2 - (\log_{10} t'_R)_1 = \frac{0.4343}{RT}(\Delta G^\circ_1 - \Delta G^\circ_2). \quad (3)$$

Retention Free Energy of Monosaccharides

From the chromatogram shown in Fig. 1, the values of t'_R were obtained for four aldohexoses and the corresponding 6-deoxy derivatives, and the logarithms of these values are given in Fig. 2. It is interesting to find that the H→OH change at position 6 always causes an increase of the free energy of staying in the particular stationary phase now in question (Hitachi #3013-N) by 2.7 kJ mol^{-1}, and that this value does not depend upon the structure of the other part of the hexose molecule. It is also noteworthy that a definite value of free energy is found assignable to a particular structure (geometrical isomer) of the six-membered ring and that the value does not depend upon whether the position 6 carbon has the OH group or not. We would like to point out here that such ΔG° values, in general, may be useful in mapping molecules for establishing quantitative structure–activity relationship in medicinal chemistry. It is suggested that such an approach would be effective if the mapping is done in combination with various types of affinity chromatography.

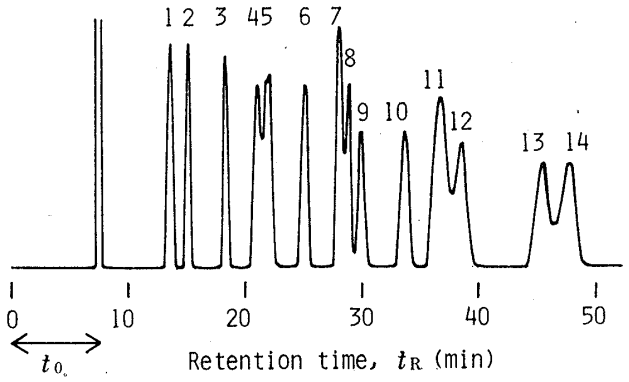

Fig. 1. A Chromatogram of a Mixture of Monosaccharides

Stationary phase: Hitachi #3013-N. Mobile phase: 70% CH_3CN.[a] Temperature: 50°C.
Peak No.: 1, 2-deoxyribose; 2, 6-deoxytalose; 3, rhamnose; 4, fucose; 5, 6-deoxyglucose, ribose; 6, lyxose; 7, talose, arabinose; 8, xylose; 9, idose; 10, altrose; 11, allose, mannose; 12, gulose; 13, galactose; 14, glucose.
a) In this paper, 70% CH_3CN means the mixture of CH_3CN, H_2O, and 1 M phosphate buffer (pH 7) in the ratio 70: 29: 1 (v/v), and 65%, 60%, 50%, 40%, 30%, and 0% CH_3CN have similar meanings.

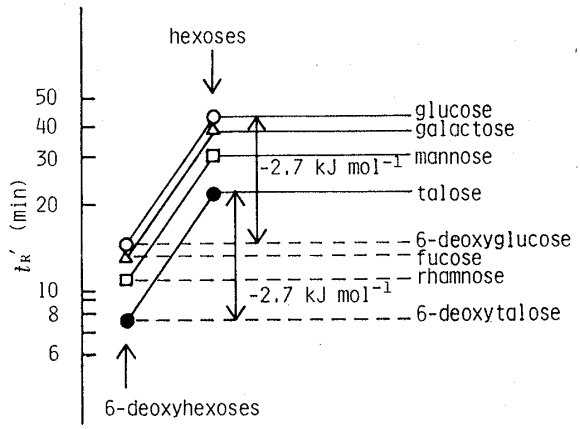

Fig. 2. The Values of Adjusted Retention Time, t'_R, of Monosaccharides in the Chromatogram shown in Fig. 1 are given on a Logarithmic Scale

Retention Free Energy and Enthalpy of Oligosaccharides

Another example of molecular characterization was carried out with malto-oligosaccharides on the basis of chromatograms, one of which is illustrated in Fig. 3. This time, capacity ratio,

$$k' = \frac{C_s}{C_m}\frac{V_s}{V_m} = t'_R/t_0 \quad (4)$$

was used instead of t'_R. This is also related to ΔG° as follows

$$\ln k' = \ln \frac{V_s}{V_m}\frac{1}{RT}\Delta G^\circ. \quad (5)$$

The values of k' were obtained from chromatograms (such as Fig. 3) for several malto-oligosaccharides and they are given in Fig. 4. As may be seen here, a constant free energy value (−0.55

kJ mol^{-1} with 50% CH_3CN eluent at 50°C, for example) can be assigned to one glucose residue. The value does not depend upon the position of the residue in the chain. It depends, however, upon the temperature; at 30°C $\Delta G^\circ = -0.48$ kJ mol^{-1}. This means that the enthalpy of retention $H_s^\circ - H_m^\circ = 0.41$ kJ mol^{-1} per glucose residue. It is rather surprising that a greater amount of free energy is assigned to the glucose residue at a higher temperature (50°C) than at a lower temperature (30°C), so that a positive value of enthalpy $H_s^\circ - H_m^\circ$, rather than a negative value, must be given to every glucose residue. In other words, the retention time of glucose residues on Hitachi #3013-N must be caused by a certain entropy factor, not by any attractive force.

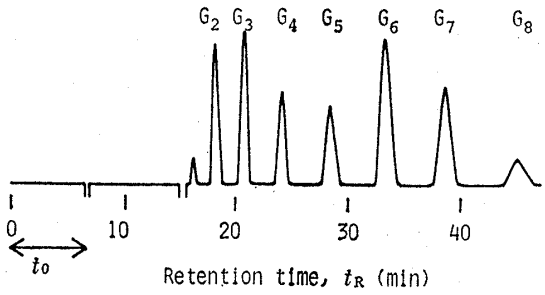

Fig. 3. A Chromatogram of a Mixture of Malto-oligosaccharides, G_n

Stationary phase: Hitachi #3013-N. Mobile phase: 50% CH_3CN. Temperature: 50°C.

Effect of Eluent

In the oligosaccharide chromatography just mentioned, the free energy value $\Delta G^\circ = G_s^\circ - G_m^\circ$ assignable to one glucose residue depends upon the CH_3CN content in the eluent. It is -1.28 kJ mol^{-1} with 65% CH_3CN, -0.93 kJ mol^{-1} with 60% CH_3CN, -0.27 kJ mol^{-1} with 40% CH_3CN, and nearly zero with 30% CH_3CN. With higher CH_3CN content in the eluent, the glucose residue would become less stable, so that the relative stability in the stationary phase becomes greater. The stability is considered to be caused by an entropy factor here again; for example, a smaller number of conformations may be possible in the solvent with a greater CH_3CN content.

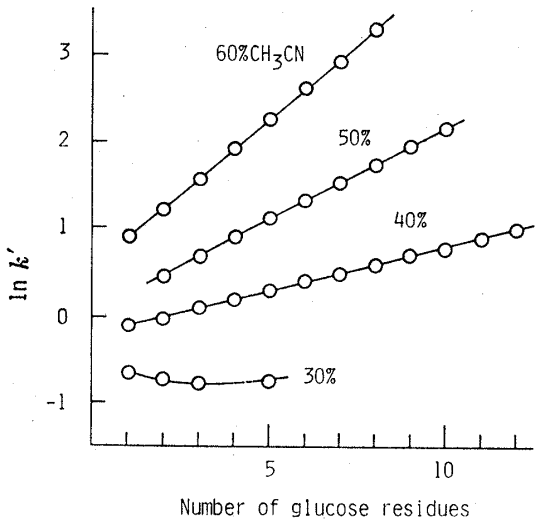

Fig. 4. The Values of Capacity Ratio, $k' = \frac{C_s}{C_m}\frac{V_s}{V_m}$, of Malto-oligosaccharides in Chromatography (the Chromatogram illustrated in Fig. 3) are plotted on a Logarithmic Scale against the Number of Glucose Residues involved in Each Saccharide

Stationary phase: Hitachi #3013-N. Mobile phase: CH_3CN (60%, 50%, 40%, or 30%). Temperature: 50°C.

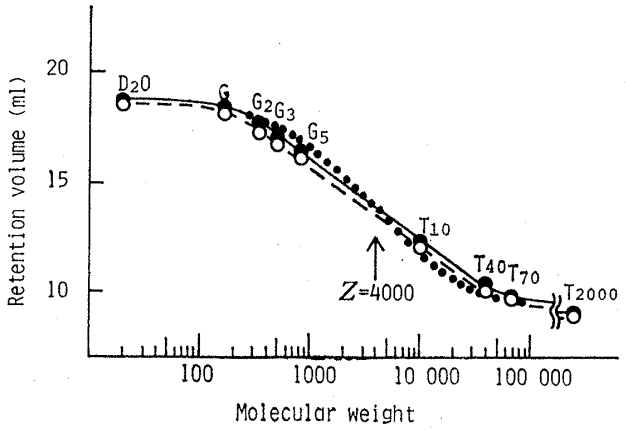

Fig. 5. A Plot of the Retention Volume in Chromatography of Polysaccharides against the Molecular Weight

Stationary phase: Hitachi #3013-N. Mobile phase: 0.01 M phosphate buffer, pH 7.0. Temperature: 23.5°C (—●—) and 50°C (—○—). G: Glucose. G_n: Malto-oligosaccharides with n glucose residues. T_{10}—T_{2000}: Dextran. (····): theoretical curve based on Eq. 8.

Chromatography of oligosaccharides has been carefully investigated by Samuelson.[1] For example, malto-oligosaccharides were separated on Dowex 1×8 (sulfate form) in aqueous ethanol at 75°C.[2] We have estimated the free energy values ΔG° assignable to one glucose residue at various ethanol contents in the eluent on the basis of their data shown in Fig. 3 of ref. 2; they are -0.40 kJ mol^{-1} in 65% ethanol, 0 kJ mol^{-1} in 60% ethanol, 0.74 kJ mol^{-1} in 50% ethanol, and 1.41 kJ mol^{-1} in 40% ethanol. These ΔG° values are different from the corresponding values estimated from our data; the difference can be attributed to the difference in the solvent, and especially to the difference in the salt concentration.

Gel Filtration

With eluent containing no CH_3CN, the chromatographic system works as a gel filtration; the longer saccharides emerge earlier than the shorter ones. In Fig. 5, the retention volume, which is proportional to the retention time t_R, is plotted against the molecular weight of the sample.

It would be worthwhile to propose here a very simple formulation for the case of gel-filtration. Let us assume that the probability of each molecule in the mobile phase (volume $V_m = V_{D_2O} - V_0$) being pushed into the stationary phase (its effective volume is assumed to be V_s) is given by

$$P = \frac{Z/M}{1+(Z/M)}, \tag{6}$$

where M is the molecular weight of the molecule in question and Z is a constant which gives the effective size (in the molecular weight unit) of the holes in the gel. Thus, the concentration C_s in the stationary phase depends upon the concentration C_m in the mobile phase, so that

$$C_s = PC_m. \tag{7}$$

Therefore, retention volume V_R is given as

$$V_R = V_0\left[1+\frac{V_s}{V_m}\frac{C_s}{C_m}\right] = V_0\left[1+\frac{V_s}{V_m}\frac{Z/M}{1+(Z/M)}\right], \tag{8}$$

where V_0 is the hold-up volume. In other words, the free energy ΔG° in this case is mostly due to $-T\Delta S^\circ$ where ΔS° is the change in the standard entropy on going from the mobile phase to the stationary phase. Thus,

$$\frac{C_s}{C_m} = e^{-\Delta G^\circ/R} = e^{\Delta S^\circ/RT} \tag{9}$$

where

$$\Delta S^\circ = R \ln P. \tag{10}$$

In Fig. 5, the theoretical curve given by the simple formula (8) (with the assumption that $z=4000$) is compared with what was actually observed.

Experimental

Chromatographic Conditions——Apparatus; Hitachi 635 high-speed liquid chromatograph equipped with a Showa Denko SE-11 refractive index monitor. Column; 8ϕ×500 mm containing Hitachi Gel #3013-N. Eluents; aqueous acetonitrile containing 0.01 M phosphate buffer (pH 7.0). Flow rate; 2 ml/min.

Determination of t_0 Value——The retention time (t_R) of the solvent front peak after injection of a $CH_3CN + H_2O$ mixture with a different composition from that of the eluent was observed, and this retention time was used as the t_0 value. Such a "t_0 value" has been found to be independent of the composition of the mixture, as long as the eluent composition is kept constant.

Samples——β-D-Allose, D-altrose, D-arabinose, 6-deoxy-D-glucose, D-gulose, D-idose, and D-ribose were purchased from Sigma Chem. Co., 2-deoxy-D-ribose, D-fucose, D-lyxose, D-mannose, D-talose, L-rhamnose, and D-xylose from Tokyo Kasei Kogyo Co., D-galactose, D-maltose, and maltopentaose from Wako Pure

Chem. Industries Co., Dextran T-10, T-40, T-70, and T-2000 from Pharmacia Co., D-glucose from Koso Chem. Co., and maltotriose from Hayashibara Biochem. Lab. Malto-oligosaccharide mixture was kindly supplied by Showa Denko Co. 6-Deoxy-L-talose was synthesized according to Collins *et al.*[3] and Brimacombe *et al.*[4]

References

1) O. Samuelson, *Adv. Chromatog.*, **16**, 113 (1978).
2) J. Havlicek and O. Samuelson, *Anal. Chem.*, **47**, 1854 (1975).
3) P.M. Collins and W.G. Overend, *J. Chem. Soc.*, **1965**, 1912.
4) J.S. Brimacombe, F. Hunedy, and A.K. Al-Radhi, *Carbohydrate Res.*, **11**, 331 (1969).