

Thin-Layer Chromatography of Malto-Oligosaccharides

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ABSTRACT

Thin-layer chromatographic experiments have been conducted on the homologous series of malto-oligosaccharides with various proportions of two different solvent systems (t-butyl alcohol:water and n-butyl alcohol:pyridine:water) on two support media, Silica Gel G and Kieselguhr G. A plot of $\log_{10} R_f/(1 - R_f)$ against the number of monomer units in the oligosaccharide yields a series of straight lines for the various proportions of each of the solvent systems. The height of the common intercept of these lines is dependent more on the nature of the support medium than on the nature of the solvent systems. Suggestions are made to help govern the selection of the proper support medium and solvent mixture.

The theoretical approach to partition chromatography was originally presented by Martin and co-workers (1,2). French and Wild (3) and Thoma (4,5,6) modified the equations for their studies of the paper chromatography of homologous series of oligosaccharides. Thoma (4) developed a graphical method using the equation:

$$\log_{10} [R_{f_a} / (1 - R_{f_a})] = \log_{10} (A_m/A_s) - (0.434 \Delta\mu_y)/RT [(\Delta\mu_x/\Delta\mu_y) + (n - 1)]$$

R_{f_a} is the R_f of an n-mer, A_m and A_s are the areas of the mobile and stationary phases, $\Delta\mu_x$ and $\Delta\mu_y$ are the free energies necessary to transfer a mole of monomer x and the added monomer y from the stationary to the mobile phase, R is the gas constant, and T is the absolute temperature. The term n refers to the number of monomer units in the oligosaccharide.

A semilogarithmic plot of $R_f/(1 - R_f)$ against one less than the number of monomer units in the polysaccharide yielded a straight line. When the proportions of the components in the irrigant (t-butyl alcohol: water) were altered, Thoma obtained other straight lines. The series of lines had a common intercept. The numerical value of the ordinate of the intercept was $\log_{10} (A_m/A_s)$, the numerical value of the abscissa of this point was $-(\Delta\mu_x/\Delta\mu_y)$, and the slope of the lines was $-(0.434 \Delta\mu_y)/RT$.

While the slope of the lines varied with the proportion of solvent, the vertical height of the intercept depended on the nature of the support medium. Thus the values for several grades of paper were around 3, regardless of the solvent system used (6).

Thoma demonstrated that these graphs could be useful in the selection of irrigation systems for paper chromatography.

The malto-oligosaccharides can also be separated on thin-layer plates (7). The n-butyl alcohol:pyridine:water systems previously used, and the t-butyl alcohol:water system used by Thoma, have been investigated to determine whether the same sort of useful graphs could be obtained.

MATERIALS AND METHODS

Kieselguhr G was obtained from Brinkmann Instrument Co. (Westbury, N.Y.). Silica Gel G was obtained from Warner Chilcott Laboratory Instrument Division

(Morris Plains, N.J.), but some later experiments were conducted with material from Brinkmann Instrument Co.

Plates were prepared to give a thickness of 250 μ .

The plates were irrigated in a standard tank suspended in a constant-temperature bath at $26^\circ \pm 0.2^\circ\text{C}$. The compounds were developed with ethyl alcohol:concentrated sulfuric acid (19:1, v/v). They were heated at 105°C . to develop the colors.

The malto-oligosaccharides were isolated from a hydrolysate of amylose by the charcoal-Celite column technique of Whistler and co-workers (8,9,10).

RESULTS AND DISCUSSION

The results obtained with four different n-butyl alcohol:pyridine:water mixtures on Kieselguhr G are shown in Fig. 1. The A_M/A_S ratio in this case is about 25.

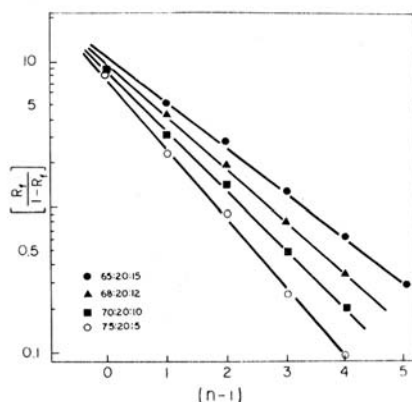


Fig. 1. $\log_{10} R_f/(1 - R_f)$ plotted against $(n-1)$ for malto-oligosaccharides with n-butyl alcohol:pyridine:water solvent mixtures at 26° on Kieselguhr G. n = number of monosaccharide units in oligosaccharide.

The same solvent mixtures were used on Silica Gel G. When these $R_f/(1 - R_f)$ values, shown in Table I, are plotted, the A_M/A_S ratio is around 3. This is lower than the ratio for Kieselguhr G by an order of magnitude.

TABLE I. $R_f/(1 - R_f)$ FOR n-BUTYL ALCOHOL:PYRIDINE:WATER ON SILICA GEL G

	Solvent Proportions			
	65:20:15	68:20:12	70:20:10	75:20:5
Glucose	2.33	2.03	1.86	1.63
Maltose	1.63	1.38	1.22	1.00
Maltotriose	1.13	1.00	0.89	0.64
Maltotetraose	0.85	0.70	0.59	0.39

The two-component system, t-butyl alcohol:water, was used by Thoma (4,5) for many of his paper chromatograms. Several combinations of these two components were used to irrigate the malto-oligosaccharides on both Kieselguhr G and Silica Gel G (Table II). When plotted as in Fig. 1, the A_M/A_S ratio for Silica Gel G is again around 3, whereas the same ratio for Kieselguhr G is around 100, even higher than that previously obtained for Kieselguhr G in Fig. 1.

TABLE II. $R_f/(1 - R_f)$ FOR *t*-BUTYL ALCOHOL:WATER MIXTURES

	Alcohol: Water Proportions				
	98:2	95:5	90:10	85:15	75:25
Kieselguhr G					
Glucose	11.50	19.00	32.30	49.00	
Maltose	02.70	05.25	13.30	24.00	
Malto- triose	00.56	01.95	04.88	13.30	
Malto- tetraose	00.30	00.56	02.23	05.67	
Silica Gel G					
Glucose	01.17	01.44		01.86	1.94
Maltose	00.49	00.85		01.22	1.22
Malto- triose	00.33	00.43		00.79	0.96
Malto- tetraose	00.11	0.234		00.53	0.75
Malto- pentaose		0.124		00.35	0.54

The utility of graphs like Fig. 1 was extended by Thoma (4), who demonstrated that a plot of $\log_{10} R_f/(1 - R_f)$ against the mole fraction of water in a two-component system also yielded a series of straight lines. The same figures can be drawn from the data in Table II and would allow one to choose the solvent proportions best suited for separation of a series of oligosaccharides.

A graph like Fig. 1 can be useful even if the later theoretical treatment is eliminated. The high A_M/A_S ratio and steep slopes of the lines suggest that such a solvent mixture may be valuable. The irrigation mixture which yields the line with the steep slope should give maximum separation of a few components, whereas a solvent mixture which has a lower slope should be useful in scanning a multi-component mixture.

The data presented also demonstrate the utility of Kieselguhr in the scanning of mixtures of these oligosaccharides. It has the advantage of the A_M/A_S ratio as well as the advantage of speed. Solvent development requires about 1 hr. for the *n*-butyl alcohol:pyridine: water mixtures and about 2 hr. for the *t*-butyl alcohol:water. The same solvent systems on Silica Gel G require about twice as long to reach the 10-cm. mark.

The TLC technique is probably not the equal of paper chromatography for many purposes, but it has been found extremely helpful for scanning hydrolysis mixtures, effluents from columns, and those enzymatic transfer reactions which lead to a series of oligosaccharides.

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[Received September 1, 1967]