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Note

Measurements of the variation of distribution coefficients (K_d) of glucose and fructose with on-column sugar concentration in chromatography columns

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Distribution coefficients may be determined[1] from the basic chromatographic equation as shown below:

$$V_R = V_m + K_d V_s \quad (1)$$

where V_R = elution volume of the component, V_m = total volume of the mobile phase in the column and V_s = volume of the stationary phase.

With an analytical chromatographic column, the feed sample is so small, approximately 20 μl compared to the column volume of about 10 ml that the elution can be considered to take place at infinite dilution. In preparative and production chromatography a feed volume as high as 25% of the total column volume is used[2]. This produces a considerably higher on-column sugar concentration and has an effect on the distribution coefficient of the components.

When simulating a semicontinuous chromatographic process previous workers[3,4] have found it necessary to estimate the values of the distribution coefficients of the components to fit the simulated results with the experimental results. The object of this experimental work was to investigate the effect of on-column sugar concentrations on the distribution coefficients in order to improve the mathematical modelling of the system and hence obtain a better fit to the experimental data.

EXPERIMENTAL

Equipment

A relatively simple chromatographic system was used for measuring the distribution coefficients, consisting of a pump sample introduction device, a column, detector and recorder. All results were obtained by manual measurement of the chromatograms.

The eluent was pumped into a stainless steel column of 500 × 5 mm I.D., packed with zerolit SRC14 (150–300 μm) with 4% DVB cross linking, charged in the calcium form, and pressure sealed at both ends. The filtered sample was injected into the column via a six port sample injection valve fitted with a constant volume sample loop.

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The eluate from the column passed into a differential refractometer. From the resulting chromatogram the K_d values could be calculated.

Determination of distribution coefficient values

From eqn. 1 the K_d can be defined as a function of the retention volume of a solute.

$$K_d = \frac{V_R - V_m}{V_s} \tag{2}$$

The void volume was taken to be the elution volume of dextran. Since dextran (polyglucose) molecules were too large to enter the pore volume they travelled only through the interstitial or void volume. Consequently the distribution coefficient of a component can be defined as

$$K_d = \frac{V_R - V_d}{V_T - V_d}$$

where V_T = total column volume and V_d = retention volume of dextran = void volume. Hence for glucose

$$K_{dg} = \frac{V_g - V_d}{V_T - V_d} \text{ and } K_{df} = \frac{V_f - V_d}{V_T - V_d}$$

Materials

The measurement of the distribution coefficient was performed with three different types of eluent namely: (i) Various glucose solutions within the concentration range of 0 to 50% w/v; (ii) Various fructose solutions within the concentration range of 0 to 50% w/v; (iii) Various dextran solutions within the concentration 0–20% w/v.

The sample was prepared by dissolving a known amount of sugar in the eluent sugar solution. Three sample solutions were prepared for each experiment: (i) 10 g of dextran dissolved in eluent to make 100 ml of sample; (ii) 10 g of glucose dissolved in eluent to make 100 ml of sample and (iii) 10 g of fructose dissolved in eluent to make 100 ml of sample.

Experimental procedure

Three chromatograms per eluent were run: dextran, glucose and fructose.

The reference and the sample cells in the refractometer were balanced while filled with the eluent to be used, and the pen position marked on the chart recorder. The sample cell was then connected to the outlet of the column. The eluent was pumped through the column until the cell was balanced again, *i.e.* pen reached the marked position.

On establishing a steady base line on the chart recorder, the sample was injected through the sample valve and the time was marked on the chart recorder. A peak appeared on the chart recorder as the sample was eluted. All the chromatograms produced were analysed to obtain the distribution coefficient data for glucose and fructose.

TABLE I

STATISTICAL ANALYSIS OF EXPERIMENTAL DATA

C_g, C_d, C_f are concentration in w/v fraction, *i.e.*, $C_g = \frac{\text{weight of glucose}}{\text{volume of solution}}$.

Eluent medium	*Component*	*Correlation equation for the component*	*Correlation coefficient*	*Confidence level (%)*	*Significance of correlation*
Glucose	Glucose	$K_{dg} = 0.77\ C_g + 0.1237$	0.992	>99.5	Significant correlation
	Fructose	$K_{df} = 0.65\ C_g + 0.3648$	0.991	>99.5	Significant correlation
Fructose	Glucose	$K_{dg} = 0.24\ C_f + 0.129$	0.987	>99.5	Significant correlation
	Fructose	$K_{df} = 0.15\ C_f + 0.386$	0.923	>99.5	Significant correlation
Dextran	Glucose	$K_{dg} = 0.7\ C_d + 0.143$	0.95	>97.5	Significant correlation
	Fructose	$K_{df} = 1.1\ C_d + 0.426$	0.91	>97.5	Significant correlation

RESULTS AND CONCLUSIONS

The statistically analysed results from the experimental work is shown in Table I and Figs. 1–6. From the experimental results the following observations can be made: Δk_d in dextran medium > ΔK_d in glucose medium > ΔK_d in fructose medium.

The mechanism by which a component is retained in the stationary phase contributes significantly to the variation in the distribution coefficient.

The dextran molecules cannot be retained in the stationary phase because the molecules are too large to enter the pores, the glucose molecules however are small

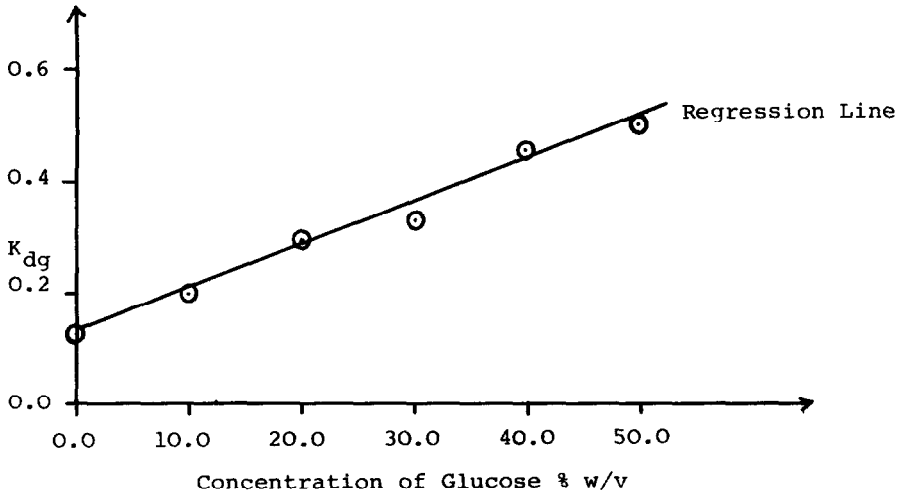

Fig. 1. Variation of distribution coefficient of glucose with glucose concentration at ambient temperature.

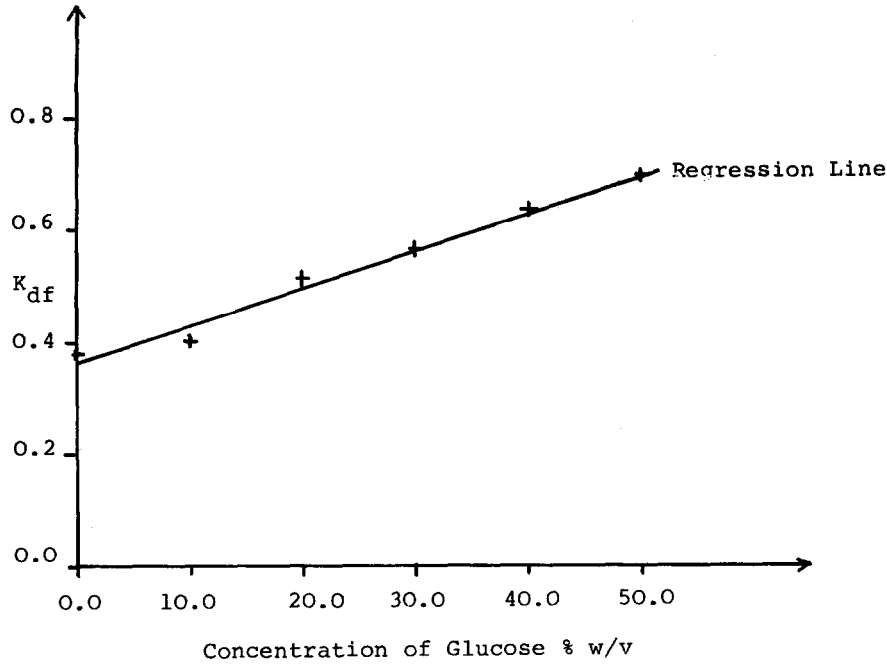

Fig. 2. Variation of distribution coefficient of fructose with glucose concentration at ambient temperature.

enough to enter the pores and can be retained by the stationary phase. For fructose molecules, although the same size as glucose, further retardation is achieved by formation of a chemical complex between the fructose molecules and the calcium ions present within the resin particles.

The results can be explained in terms of three factors:

(i) Viscosity effect: increasing the concentration of sugar increases the viscosity

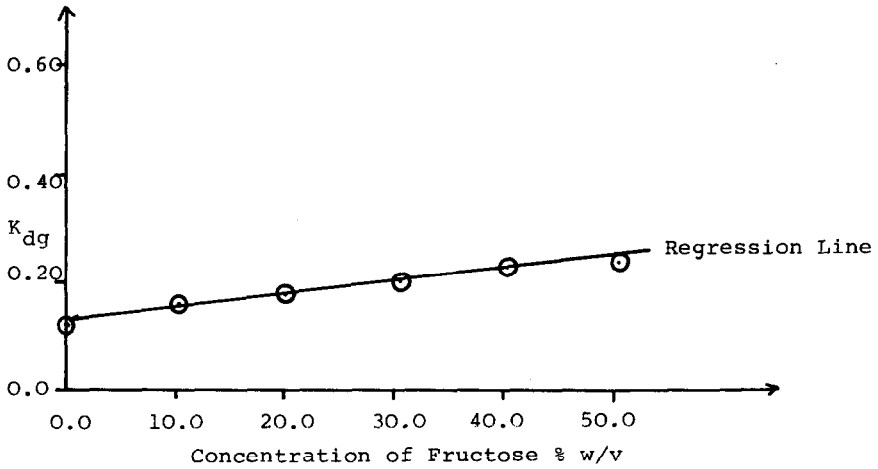

Fig. 3. Variation of distribution of glucose with fructose concentration at ambient temperature.

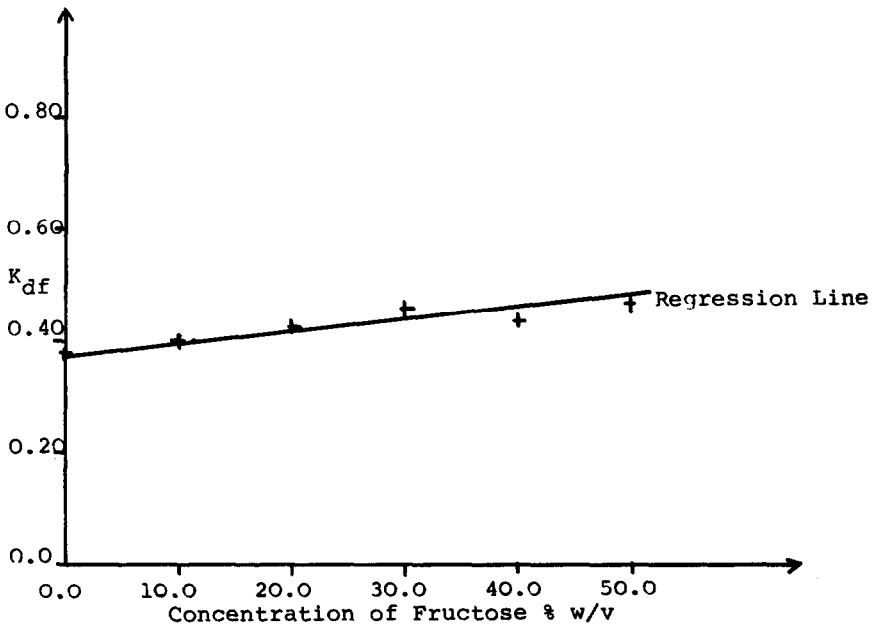

Fig. 4. Variation of distribution coefficient of fructose with fructose concentration at ambient temperature.

of solution and an increase in viscosity increases the elution volume, hence the distribution coefficients of both glucose and fructose increase. There is a marked increase in viscosity of dextran solutions with concentration causing a rapid increase in the distribution coefficient of the components.

(ii) Concentration effect: a concentration gradient between the stationary and the mobile phase is created. The gradient is strong enough to force glucose to stay

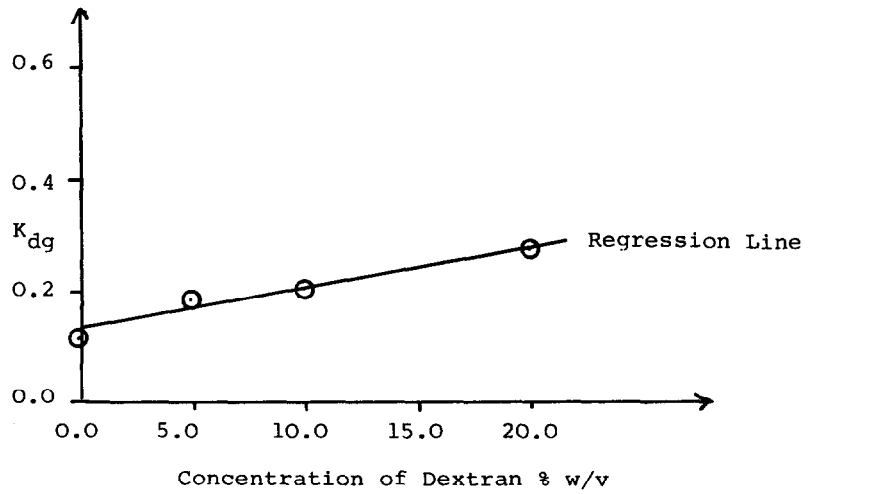

Fig. 5. Variation of distribution coefficient with dextran concentration at ambient temperature.

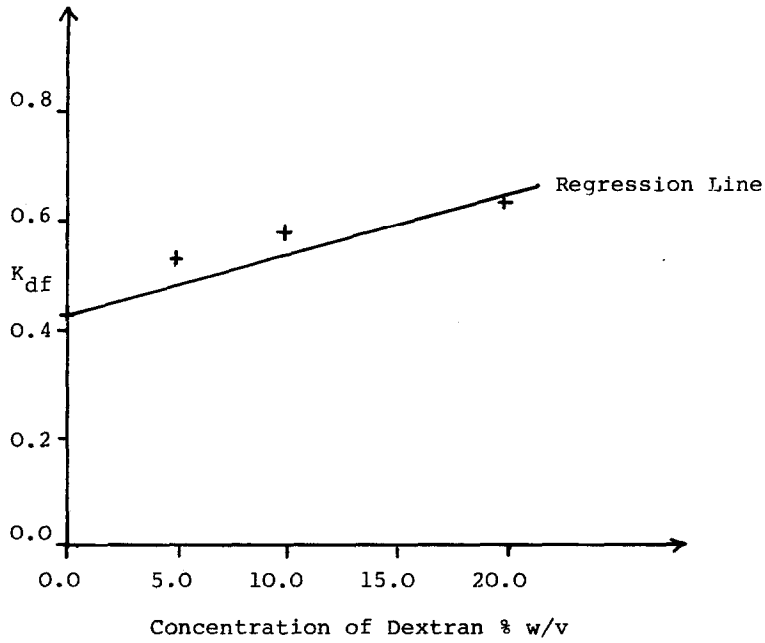

Fig. 6. Variation of distribution coefficient with dextran concentration at ambient temperature.

with the stationary phase either by diffusion or by osmosis. For fructose this effect is reduced since it is already chemisorbed by the resin to its limiting capacity.

(iii) The effect of chemical structure: this applies to fructose only. β-D-fructofuranose, a six-membered ring structure is the only form of fructose that complexes with the calcium ions. The equilibrium concentration of this form of fructose decreases with increasing concentration of fructose, *i.e.* less fructose is likely to be chemisorbed at high concentration, by calcium ions in the resin, causing a decrease in the value of the distribution coefficient.

In dextran solution the viscosity effect is prominent together with the concentration effect causing an overall rapid increase in the distribution coefficient.

The viscosity effect is less marked in the glucose solution, so the increase in the distribution coefficient is mainly because of the concentration effect.

In a fructose medium, the increase in the distribution coefficient is caused by viscosity to some extent and the concentration effect. However the effect of change in chemical structure reduces the K_d value. The overall result is a less rapid increase in K_d value compared with glucose.

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