Journal of Chromatography, 190 (1980) 448–451

CHROM. 12,557

Note

Distribution ratios and elution behavior of carbamates and polyhydroxy compounds from Sephadex G-15

DAVID J. STANONIS and STANLEY P. ROWLAND

Southern Regional Research Center, AR, SEA, U.S. Department of Agriculture, New Orleans, La. 70179 (U.S.A.)

(Received November 22nd, 1979)

We have shown that during gel filtration from chopped cotton or Sephadex G-15 columns there is an increase in elution volume as the molecular weight (size) of an *n*-alkyl carbamate is increased by the addition of methylene groups[1]. We concluded that the reason for the increase was hydrophobic sorption. Eaker and Porath[2] earlier suggested that hydrophobic sorption accounted for this increase in elution volume as the size of *n*-alkanols was increased by the addition of methylene groups. In 1976 Di Gregorio and Sinibaldi[3] suggested that the sorption of thiocyanate ion on Sephadex gel can be attributed to a "hydrophobic interaction".

In 1977 Kura *et al.*[4] correlated the elution behavior of thiocyanate ion from Sephadex G-15 at 20° with the adsorption isotherm of thiocyanate ion on Sephadex G-15 at 20°. For this paper we have determined the distribution ratios of several *n*-alkyl carbamates, sugars and vicinal polyols on Sephadex G-15 by static batch experiments. We have correlated these ratios with the elution behavior of these same compounds in dynamic gel filtration using a Sephadex G-15 column.

EXPERIMENTAL*

Materials

Sephadex G-15 was obtained from Pharmacia (Uppsala, Sweden); samples were dried at 105° for 30 min. The *n*-alkyl carbamates were furnished by Proctor (Salisbury, N.C., U.S.A.). All of the carbamates were recrystallized from deionized water. The polyhydroxy compounds were obtained commercially and were used as received.

Sorption of water by Sephadex G-15

The volume of water sorbed per gram of dry Sephadex G-15 was obtained by adding 40 ml of a solution containing 3 mg/ml of Carbowax 6000, a solute that is totally excluded from the Sephadex G-15–water gel, to a 10-g sample of dry Sephadex in a glass-stoppered erlenmeyer flask. The flask was placed in a boiling water bath

* Names of companies or commercial products are given solely for the purpose of providing specific information; their mention does not imply recommendation or endorsement by the U.S. Department of Agriculture over others not mentioned.

and was shaken from time to time. After 1 h, the flask containing the water-swollen Sephadex was placed in a constant-temperature room at 20°. After equilibration, a portion of the supernatant liquid was drawn through a 5-μm filter into a hypodermic syringe. The concentration of solute in the supernatant liquid was determined by differential refractometry as described below. The excluded Carbowax 6000 (120 mg) was divided by the concentration of Carbowax in the supernatant to obtain the volume of excluded solution. The volume of sorbed water was found by subtracting the volume of supernatant liquid from the original volume of liquid added to the Sephadex.

Analysis by differential refractometry

The column in a liquid chromatograph equipped with a Pharmacia differential refractive index monitor was replaced by a length of small-bore polyethylene tubing. The sample loop in the injector was filled successively with aqueous solutions containing known concentrations of a given solute. For each injected sample, the output from the detector was recorded as a sharp peak on a strip chart. Peak heights were related in a linear fashion to the concentrations of a given solute.

Penetration and sorption of solutes by Sephadex G-15 (static method)

Exactly 40 ml of solution containing a known concentration of solute was added to a weighed sample of dry Sephadex G-15. Before being dried, the Sephadex weighed approximately 10 g. After heating and cooling as described above, the equilibrium concentration of solute in the supernatant liquid was determined by differential refractometry. We assumed that the volume of solution entering per gram of dry Sephadex was the same as the volume of water that entered or was sorbed in the Carbowax 6000 experiment. By substracting the volume of solution that entered the sample of Sephadex from the original volume of 40 ml, the volume of supernatant solution was determined. From this volume and the known original concentration, the weight of solute in the supernatant was calculated. When this weight was subtracted from the total weight of solute in the original 40 ml, the weight of solute sorbed by the Sephadex was obtained. The weight of solute sorbed divided by the weight of the dry Sephadex used gave the weight of solute sorbed per gram of dry Sephadex. By using several concentrations of each solute, we obtained the milligrams of solute in the gel phase per gram of dry Sephadex as a function of the final equilibrium concentration of the solute in the supernatant; D is the slope of the line representing this relationship.

Gel filtration measurement of A_w (dynamic method)

Methods of operation of the Sephadex columns and determination of A_w (the fraction of internal water available to the solute as solvent) were those of Rowland and Bertoniere[5].

RESULTS AND DISCUSSION

Each value of D considered in subsequent paragraphs was obtained by measuring several times the distribution of each solute between the Sephadex gel phase and the supernatant solution phase. The milligrams of solute in the gel phase was plotted against that in the supernatant solution phase. The slope of the regression

line running through the origin obtained by the method of Strong[6] is the distribution ratio, D, which provides a measure of the amount of solute that is in the gel phase. Solute in the gel phase consists of that which finds large enough pores for penetration in the dissolved state plus that which is sorbed on pore surfaces.

Values of D (from static experiments) and A_w (from dynamic gel filtration) for three classes of solutes under study are summarized in Table I. Both D and A_w

TABLE I

DISTRIBUTION AND ELUTION DATA FOR WATER-SOLUBLE SOLUTES ON SEPHADEX G-15

Solute	*Mol. wt.*	D*	A_w**
n-Amyl carbamate	131	3.19	2.00
n-Butyl carbamate	117	2.58	1.69
Ethyl carbamate	89	1.73	1.25
Methyl carbamate	75	1.58	1.17
Stachyose	666	0.60	0.33
Raffinose	504	0.79	0.42
Maltose	342	0.88	0.56
Sorbitol	182	0.93	0.61
Inositol	180	0.85	0.59
Glucose	180	1.02	0.66
Xylitol	152	0.97	0.66
Erythritol	122	1.00	0.70
Glycerol	92	1.04	0.74
Ethylene glycol	62	1.13	0.80

* Distribution ratio: (mg of solute per g of dry Sephadex G-15) (mg of solute per ml of equilibrium solution) from static equilibrium measurements.

** Fraction of internal water accessible to the solute during dynamic gel filtration with water as eluent. An A_w value greater than 1.0 is an indication of sorption.

decrease with increasing molecular weight of solute in the sugar series and the polyol series. This situation is normal, indicative of penetration into pores that is limited by size of the solute. There is no evidence in our study or preceding studies that these two types of solutes are sorbed on polysaccharidic surfaces. Nevertheless, there is a significant difference in the relationship of D to A_w for sugars and for polyols (Fig. 1). The difference is intriguing to us (a) because we are concerned with identifying differences in the way in which various types of solutes penetrate and interact with cellulosic surfaces, for which Sephadex (in this case) is a model, and (b) because these two types of solutes differ in only small degree in chemical structure. The regression equations for the individual lines through the data for sugars and those for polyols and corresponding correlation coefficients (r) are:

Sugars: $D = 1.174\,A_w + 0.244$; $\quad r = 0.977$

Polyols: $D = 1.174\,A_w + 0.184$; $\quad r = 0.978$

The third type of solute differs substantially from the sugars and polyols (Fig. 1). The values of D and A_w increase with increasing molecular weight of the solute, and the slope of the relationship between D and A_w is substantially different. The regression equation for this line and the correlation coefficient are:

Carbamates: $D = 1.943\,A_w - 0.702$; $\quad r = 0.999$

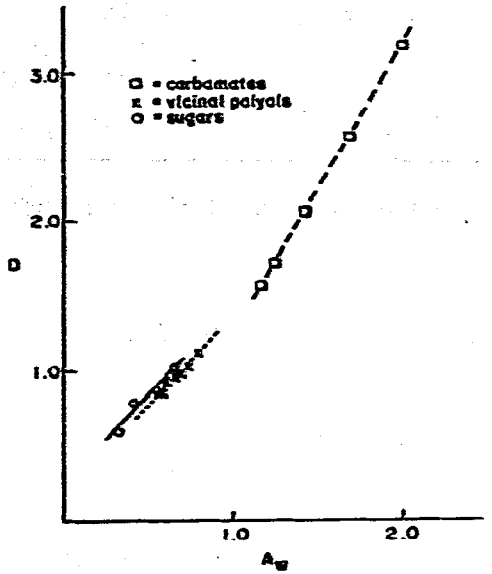

Fig. 1. Equilibrium distribution ratio (D) in relation to fraction of accessible water (A_w) for three series of solutes identified in Table I.

We conclude from these measurements and comparisons of D and A_w that the equilibrium batch method for assessing penetration–sorption of solutes into the Sephadex gel provides slightly to substantially different degrees of information than the dynamic gel filtration operation with Sephadex gel. The former method appears to provide a more sensitive means for assessing sorption; the two methods taken together substantiate differences in penetration of sugars and polyols into the Sephadex gel.

ACKNOWLEDGEMENT

The authors acknowledge the helpful assistance of Mildred Black during a part of this research.

REFERENCES

1 D. J. Stanonis and S. P. Rowland, *Text. Res. J.*, 49 (1979) 72.
2 D. Eaker and J. Porath, *Sep. Sci.*, 2 (1967) 507.
3 V. Di Gregorio and M. Sinibaldi, *J. Chromatogr.*, 129 (1976) 407–411.
4 G. Kura, A. Koyama and T. Tarutani, *J. Chromatogr.*, 144 (1977) 245.
5 S. P. Rowland and N. R. Bertoniere, *Text. Res. J.*, 46 (1976) 770.
6 F. C. Strong, *Anal. Chem.*, 51 (1979) 298.