CHROM. 4346

Improvement of the chromatographic properties of Sephadex G-15

Tightly cross-linked dextran gels are useful for the separation of substances of low molecular weight. However, the general utility of this chromatographic medium is somewhat limited by the poor resolution which it affords. A substantial improvement in chromatographic resolution may be achieved by boiling the gel in hydrochloric acid.

Experimental

One hundred grams of Sephadex G-15 xerogel (Pharmacia Fine Chemicals, Piscataway, N.J.) were expanded to 300 cm^3 in distilled water by allowing it to stand at room temperature for 24 h. One hundred and fifty cubic centimeters of the gel were packed by sedimentation in a 1.5×85 cm column at room temperature. The eluent, 0.15 *M* saline–0.01 *M* acetic acid, was passed through the column at 6 ml/h for 48 h before use. One hundred and fifty milliliters of 1 *N* HCl were added to an equal volume of the remaining expanded xerogel and placed in a boiling water bath for 2 h with intermittent stirring. After acid boiling, the gel was allowed to settle and the supernatent decanted. Repeated washes with distilled water were performed until the supernatent acidity was reduced to less than 0.01 *M*. This material was packed in an identical column in the same manner as the former procedure and washed with eluent until the refractive index of the effluent returned to that of the eluent. A test sample containing the materials listed in Table I was layered on the gel surface of each column and chromatographed at 6 ml/h.

TABLE I

COMPOSITION OF THE TEST SAMPLE CHROMATOGRAPHED IN FIG. 1

Compound	*Quantity*	M_w	*Source*
Blue Dextran 2000	1 mg	10^6	Pharmacia Fine Chemicals
Stachyose tetrahydrate	50 mg	666	Sigma Chemical Co.
Maltose monohydrate	50 mg	342	Eastman Kodak Co.
Glucose	50 mg	180	Baker Chemical Co.
Ethylene glycol	100 µl	62	Fisher Chemical Co.
Deuterium oxide	900 µl	20	Volk Radiochemical Co.
Sodium chloride	9 mg	—	Baker Chemical Co.

The elution position of the test molecules was indicated by the maximum deflection of a refractive index monitor (Ec211, E-C Apparatus Co., Philadelphia, Pa.) operating at a sensitivity of 0.00068 refractive index units per cm of pen deflection. The void volume (V_0) was measured as the peak elution of blue dextran and the internal volume (V_i) was estimated by the elution of deuterium oxide ($V_i = V_e(D_2O) - V_0$). The diffusion coefficients (K_d) were computed by the relationship[1]:

$$K_d = \frac{V_e - V_0}{V_i} = \frac{V_e - V_e(\text{blue dextran})}{V_e(D_2O) - V_e(\text{blue dextran})} \quad (1)$$

The number of theoretical plates (N) was computed from the standard deviation (σ) of the stachyose elution peak by the relationship[2]:

$$N = \left(\frac{V_e}{\sigma}\right)^2 \qquad (2)$$

The height equivalent to a theoretical plate (HETP) is obtained by dividing N by the column height.

Results and discussion

Tightly cross-linked Sephadex gels exhibit a remarkable resistance to acid hydrolysis. Unlike the more loosely cross-linked gels, Sephadex G-15 and G-10 withstand boiling in 1 N HCl for up to 10 h without noticeable deterioration; complete hydrolysis could be achieved by boiling in 6 N HCl for a comparable length of time. The resistance of these gels to acid hydrolysis suggested that gentle hydrolysis might improve the chromatographic characteristics by the removal of hydrolyzable contaminants. The existence of non-dextran contaminants in Sephadex may be inferred from its anomolous adsorption of aromatic[3] and alphatic substances[4], the presence of fixed negative charges[3] and the release of UV-absorbing material[5].

Acid hydrolysis resulted in three major effects: an increased number of theoretical plates, an increased internal volume and an increased effective pore size. From a practical standpoint, the most important modification is a 127% increase in the total number of theoretical plates (N, Table II). By doubling the number of theoretical plates, the resolution becomes equivalent to that of a column twice its length without the dilution and prolonged elution time which occur as the column length is increased. A factor contributing to improved resolution is a 17% increase in internal volume (V_t, Table II). However, this factor alone does not explain the major improvement seen above. The increase in effective pore size is seen in Fig. 1 as a displacement of the relative elution positions of the test molecules. Fig. 2 indicates that this increase in

TABLE II

THE ELUTION VOLUMES (V_e) AND DIFFUSION COEFFICIENTS (K_d) OF TEST MOLECULES CHROMATOGRAPHED UNDER TWO DIFFERENT CONDITIONS

Columns: Sephadex G-15, 1.5 × 85 cm. (A) V_0 = 49.6 ml; V_t = 61.7 ml; N (stachyose)[a] = 1100 plates; HETP = 0.77 mm/plate. Expanded in distilled water for 24 h. (B) V_0 = 51.8 ml; V_t = 72.2 ml; N (stachyose) = 2500 plates; HETP = 0.34 mm/plate. Expanded as column A and then boiled in hydrochloric acid for 2 h.

	Column A		*Column B*	
	V_e	K_d	V_e	K_d
Blue dextran	49.6	0.0	51.8	0.0
Stachyose	68.5	0.31	81.2	0.41
Maltose	81.5	0.52	95.5	0.60
Glucose	88.0	0.62	102.3	0.70
Ethylene glycol	96.0	0.75	109.8	0.80
Deuterium oxide	101.3	1.0	124.0	1.0

[a] Number of theoretical plates as determined for stachyose.

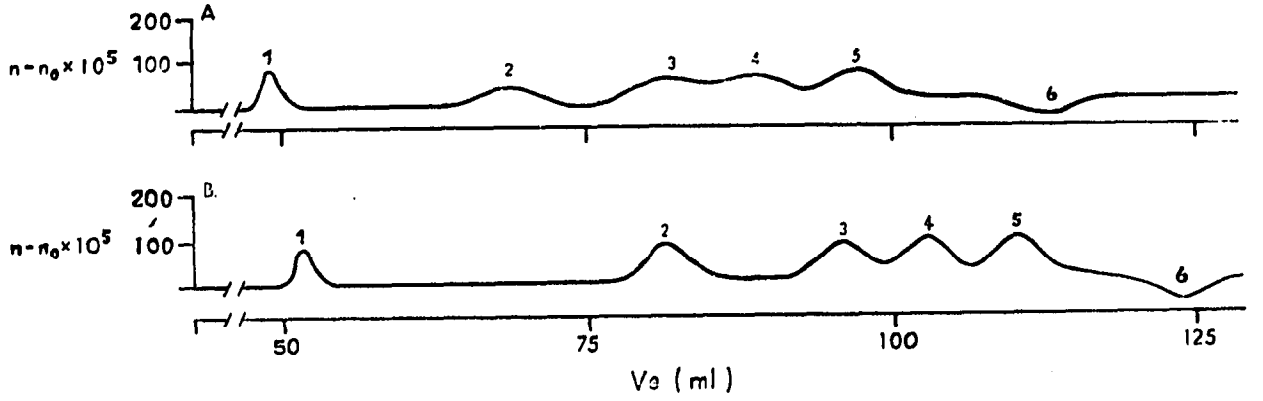

Fig. 1. The elution volume (V_e) of blue dextran (1), stachyose (2), maltose (3), glucose (4), ethylene glycol (5) and deuterium oxide (6) from 1.5 × 85 cm columns of Sephadex G-15. (A) Expanded in distilled water at room temperature for 24 h. The better resolution seen in (B) is obtained by boiling in hydrochloric acid for 2 h before packing. The change in refractive index of the column effluent ($n - n_0$) is used to indicate the elution position of the test molecules.

effective pore size is not associated with a change in the basic geometry but represents an overall enlargement. Thus, the effect of partial acid hydrolysis of Sephadex G-15 appears to be a significant increase in gel swelling as indicated by the larger effective pores and increased internal volume along with an unexplained increase in resolution which may result from decreased gel interaction or improved flow characteristics.

Two methods for gel swelling are suggested by the manufacturer[6]. For tightly cross-linked gels either 3 h at room temperature or 1 h in a boiling water bath is recommended. In our experience, either method produces results comparable to those

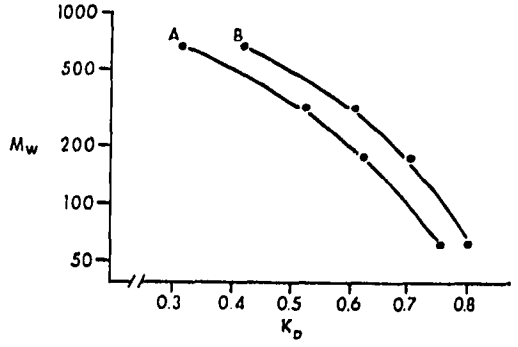

Fig. 2. The change in diffusion coefficients (K_d) for molecules of a given molecular weight (M_w) resulting from partial acid hydrolysis of Sephadex G-15 (B) compared with that obtained by conventional swelling procedures (A) indicating an enlargement of the effective pore size.

seen in Fig. 1A. The increased internal volume seen after boiling in hydrochloric acid suggests that gel swelling is significantly greater by this procedure than that seen with the recommended procedures. It has been noted by several authors[3,4,7,8] that the theoretical relationship $V_i = aW_r$, which is used to estimate the internal volume from the weight of xerogel (a) and the solvent regain (W_r) is in error by approximately 20%.

By this computation, a column containing 50 g G-15 xerogel should have an internal volume of 75 ml. This is very close to the internal volume measured by deu-

terium oxide (72.2 ml) on the acid-treated column and 22% greater than that of the untreated column (61.7 ml) suggesting that the theoretically available diffusion space is not achieved by normal swelling techniques due to internal hindrance which is largely eliminated by acid hydrolysis.

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1 H. DETERMANN, *Angew. Chem.*, 76 (1964) 635.
2 P. FLODIN, *J. Chromatog.*, 5 (1961) 103.
3 B. GELOTTE, *J. Chromatog.*, 3 (1960) 330.
4 N. V. B. MARSDEN, *Ann. N.Y. Acad. Sci.*, 125 (1965) 428.
5 G. K. ACKERS, *Biochemistry*, 3 (1964) 723.
6 *Pharmacia Fine Chemicals Technical Data*, Sheet No. 11.
7 J.-C. JANSON, *J. Chromatog.*, 28 (1967) 12.
8 H. DETERMANN, *Gel Chromatography*, Springer Verlag, New York, 1968.

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Neue Erkenntnisse bei der Verwendung von Ninhydrin–Hydrindantin als Farbreagenz zur Aminosäure-Autoanalyse

Die Nachweisempfindlichkeit einer Aminosäure im Eluat automatisch betriebener Ionenaustauschergeräten (Analyzer) konnte laufend erhöht werden und liegt heute bei 10^{-11} Mol. Derartige Empfindlichkeitssteigerungen waren möglich:

(1) Durch Variation des Säulendurchmessers.

(2) Durch Verwendung besonders einheitlicher Ionenaustauscherharze.

(3) Durch Bereitstellung möglichst einer einzigen, sehr kleinen, kugelförmigen Austauscherfraktion, wobei die Teilchendurchmesser um nicht mehr als 1 μ voneinander abweichen sollten.

(4) Durch Verstärkung der elektrischen Signale, am Colorimeterausgang.

(5) Durch empfindlichere und weniger oxydationsanfällige Anfärbereagentien.

Die Parameter 1–4 sind heute soweit optimiert, dass hier die Grenzen des Möglichen so gut wie erreicht worden sind. Anders beim Nachweisreagenz. Diesem Problem werden wohl in jedem analytischen Laboratorium, das mit Aminosäureautoanalyzern arbeitet, die meisten Untersuchungen gewidmet.

Im allgemeinen werden eluierte Aminosäuren mit einem Ninhydrinreagenz angefärbt. So auch bei allen bekannten, automatischen Aminosäureanalyzern. Wir selbst