

CHROM. 4944

Increased resolution of polymers through longitudinal compression of agarose gel columns

EDWARDS AND HELFT¹ recently reported to have improved resolution through compressed beds of cross-linked dextran. Concurrently, we were measuring the effects of compression on a much more easily deformable gel, agarose, as a prelude to other investigations involving the molecular weight distributions of water soluble polymers. Since our results differed somewhat from those of EDWARDS AND HELFT, it was decided to make our findings the subject of this note; and, in addition, to introduce an alternate concept to resolution, namely, unit resolution.

Materials and methods

The components of the chromatographic system consisted of a 5-gallon polyethylene carboy (Nalge)*, a sample-injection valve (Chromatronix Model R60SV), a column fitted with one flow adapter (Sephadex type K25/45, I.D. 25 mm), a peristaltic pump (Buchler Instruments, type polystatic), and a differential refractive index monitor (Waters Model R-4 equipped with microcell). These components were connected in series with small bore (teflon or tygon tubing), approximately 0.031 in. I.D. between injection valve and detector. Chromatronix fittings joined separate pieces of tubing where necessary. The output of the refractive index monitor was fed into a potentiometric recorder (Leeds and Northrup Model W Speedomax).

Polyethylene glycol (PEG) 20,000 and 6,000 (Union Carbide) were mixed. The numbers following the name give the number average molecular weight specified by the manufacturer. A certain fraction of the PEG 20,000 (approximately 14% of the area under the refractive index against time curve) eluted at the void volume (V_0), while the remainder together with the PEG 6,000 polymer eluted at the total volume (V_T).

The column packing material was agarose gel (Sephacrose 6B, lot No. 1929, purchased from Pharmacia Fine Chemicals, Inc.) which, according to specifications, has a molecular weight exclusion limit of 1×10^6 based on the elution of polysaccharides.

According to a procedure suggested by the manufacturer², the column was packed using a final head pressure of 30 cm of water. The column packing was permitted to stabilize by passing two column volumes of water through it and then lowering the flow adapter into place. The freely-settled height of the gel was 42.3 cm. The pump was set to deliver eluent in the range of 21.0–23.1 ml/h.

In each experiment, a 0.5 ml sample containing 2.5 mg of each polymer was dissolved in and eluted with singly-distilled water.

The gel remained stable for 166 h, during which time several samples were percolated through the gel. From this time until the end of the experiment, the gel appeared to compress in a stepwise manner after the sample had completely eluted through the column. Beyond 26% compression of the gel bed, the flow rate at the same pump setting began to decrease rapidly, so the experiments were discontinued.

* Mention of commercial items is for your convenience and does not imply endorsement by the U.S. Department of Agriculture over others of a similar nature.

Results

Chromatographers commonly use the parameters resolution (R_S) and height equivalent to a theoretical plate (H) to describe separating power and peak broadening in chromatographic columns.

Eqns. 1 and 2 define these parameters in terms of measurable quantities³.

$$R_S = \Delta Z / 4\sigma_{AV} \quad (1)$$

$$H = \sigma^2 / L \quad (2)$$

where

σ is the quarter width of an elution peak

σ_{AV} is the arithmetic mean for σ 's of two peaks

ΔZ is the distance between the means of two elution peaks

L is the column length.

The results of Table I indicate that a decrease in column length by compression is not equivalent to making a series of shorter columns by adding smaller amounts of the same gel under a constant packing procedure. These experiments altered the ratio of accessible internal volume to void volume for the gel. Hence, H is no longer a proportionality constant between σ^2 and L . Nevertheless, R_S as defined by eqn. 1 remains virtually constant, despite compression of the gel. Since a constant flow rate was maintained, even while compressing the gel, an appreciable saving of time was

TABLE I

PARAMETERS OF AN AGAROSE COLUMN AS A FUNCTION OF GEL COMPRESSION

L (cm)	V_0 (ml)	V_T (ml)	H_{AV} (mm)	$4\sigma_{AV}$ (ml)	t (h) ^a	ΔZ (ml)	R_S	L_R (cm)
38.8	51.3	149.6	0.718	17.7	7.82	98.3	5.55	6.99
38.8	51.3	152.0	0.870	20.0	7.95	100.7	5.04	7.70
37.0	42.9	139.2	0.803	17.5	7.55	96.3	5.50	6.78
34.9	34.1	128.4	0.949	16.5	6.88	94.3	5.72	6.10
31.2	23.3	112.2	1.37	16.6	6.71	88.9	5.36	5.82

^a Measured from time of sample injection until trailing edge of last peak reaches baseline.

obtained as indicated in Table I. The values reported in the table are based on the commonly-accepted definition of R_S (ref. 4), whereas those in ref. 1 are based on the quarter width. In the course of these experiments, the column decreased in length from 38.8 to 31.2 cm. It appears we have effected this economy in time without causing significant diminution of the molecular weight range over which the gel separates. This is inferred from the relatively small change in σ_{AV} which we have obtained, but confirmation is needed by a separation of compounds within the complete operating range of the gel.

As an aid in comparing the gel compression experiments, the parameter unit resolution (L_R) may be used.

$$L_R = L/R_S \quad (3)$$

Table I shows that L_R decreases with gel compression, which indicates a more efficient separation. In addition, one can use L_R to calculate the minimum length of column required to separate two peaks, using data from an experiment in which incomplete or too great a separation occurs.

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Thin-layer chromatography of adenine and its metabolites

When large quantities of adenine are administered to various mammalian species, a portion of it is oxidized by xanthine oxidase to 2,8-dihydroxyadenine (DHA) which is extensively deposited in the kidney^{1,2}. 2-Hydroxyadenine (2HA) and 8-hydroxyadenine (8HA) have been shown to be the minor and major intermediate, respectively, in this metabolic pathway³. In this communication, a thin-layer chromatographic (TLC) procedure for their separation and identification is described.

Materials and methods

Adenine (Kay Fries) and DHA (Aldrich) were commercial products. Adenine was further purified by recrystallization from water. 2HA (isoguanine) was prepared from isoguanine sulfate (Sigma). 8HA was synthesized by the reaction of phosgene on 4,5,6-triamino pyrimidine⁴. Solutions of approximately 1 mg/ml were prepared. DHA and 2HA were dissolved in boiling mixture of morpholine-water (50:50) while adenine and 8HA were dissolved in dimethylsulfoxide. Solutions were prepared fresh prior to use. Samples were applied with 3 μ l capillaries* (Microcaps, Drummond Scientific Co., Broomall, Pa.) and solvent evaporated by using a hot air gun.

TLC was carried out on precoated Avicel, microcrystalline Cellulose powder F glass plates with fluorescent indicator (Merck, Darmstadt, G.F.R.) using the following solvent systems: (1) *n*-butanol-methanol-water-conc. ammonia (60:20:20:1); (2) isopropanol-water-conc. ammonia (70:30:1); (3) *n*-butanol-morpholine-diethylene glycol (MCB)-water (45:15:10:30). All solvents used were reagent grade. Spots were located using a short wave UV lamp.

* Use of microsyringes with metallic parts should be avoided as additional spots are observed due to degradation.

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