Journal of Chromatography, 268 (1983) 187–195
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROM. 15,965

PREPARATION OF WHOLE-FIBER COTTON GEL-FILTRATION CHROMATOGRAPHY COLUMNS

CLINTON P. WADE
Southern Regional Research Laboratory, Agricultural Research Service, U.S. Department of Agriculture, New Orleans, LA 70179 (U.S.A.)
(Received May 5th, 1983)

SUMMARY

Methods of preparing whole-fiber gel-filtration columns are described. Reproducible results were obtained with columns prepared by packing with randomized fiber mat type discs (such as cut from absorbent cotton mat rolls). Results from these columns compared favorably with Sephadex columns. The columns had a useful life exceeding one year. Techniques for reducing void volume and increasing the accessible pore volume are described.

INTRODUCTION

In gel-filtration chromatography, the pores of the gel act as a molecular sieve separating molecules of different molecular weight and size. Conversely, solutes of different molecular weight and size can be used to characterize the pores of the substrate.

An understanding of the penetration of the components used to modify chemically cotton fibers and fabrics for desired textile properties such as durable-press and flame-retardancy is needed. Understanding of interactions between solutes in aqueous media and cellulose can improve the chemical modification, reduce energy requirements and enhance efficiency to achieve improved performance qualities.

Fabrics are usually made of whole fibers which are spun into yarns and woven into the fabric form. Durable-press and flame retardant fabrics are created by reacting the fabrics with mixtures containing reagents, catalysts and other modifying chemicals. The reaction occurs within or on the fibers of the fabric. Knowledge of the pore characteristics and pore water are needed to understand reactions.

Since no one has devised a method of making whole-fiber or fabric gel-filtration columns, Sephadex, chopped fiber and decrystallized cotton fiber columns have been used to estimate the interactions taking place. The interaction of solutes and pores, as well as internal pore water accessible to the solutes in Sephadex and fibrous cotton have been characterized[1–3]. The cotton and modified cotton used in the columns were in the form of chopped (Wiley-milled) fibers and also in the decrystallized (vibratory ball-milled) form[4–8]. Slurry packing was used for preparation of the

columns which facilitated uniform packing of the columns. Decrystallization by ball-milling decreases the degree of crystallinity and reduces the degree of polymerization of cotton to approximately 500. Thus the pore structure of ball-milled cotton results in greater accessibility and larger differences in relative elution volumes of solutes. Changes produced by cross-linking this material are not representative of the corresponding properties of fibrous, crystalline cotton[7]. Satisfactory columns were not obtained consistently by Wiley-milling through 60-mesh screen, as judged by uniformity of packing, constancy of flow-rate and reproducibility of measurements of the relative elution volumes of the solute. Consistently suitable columns could be prepared only by Wiley-milling through the 80-mesh screen, resulting in very slight loss of crystallinity[9]. Elution rates of 13–15 ml/h were used. Columns of chopped cotton had relatively short operating life, often permitting only a few measurements before plugging occurred[3]. Because of these limitations, columns containing whole fibers would be advantageous.

Uniform packing is a prerequisite for good column performance. This could only be accomplished by a slurry packing procedure using Sephadex beads or finely chopped or ball-milled cotton fibers. Glass fiber columns have been prepared[10] by suspending fibers in a large quantity of water with appropriate dispersing agents to keep the individual fibers separated, and allowing the stirred glass fibers to slowly settle while withdrawing the water. Whole cotton fibers are not only non-uniform in length, but are very convoluted and exhibit crimp. Such entanglement precludes separating them into isolated single fibers capable of being settled into a column.

Until now, ways to prepare such columns had not been devised. The technique for doing so is the subject of this report. When the columns proved satisfactory, selected solutes such as sugars and polyglycols were used to determine the pore characteristics of the column. Estimates of the lifetimes of the columns were obtained.

MATERIALS AND METHODS

Materials

Absorbent cotton was U.S.P. grade roll from American White Cross Laboratories*. Print cloth was 3.2 oz. per yard, 80 ends by 80 picks per inch, desized, scoured and bleached cotton. Sliver used was raw cotton fibers Soxhlet extracted three times with 95% ethanol (8 h each time). The die used for cutting cotton discs was precision 1 in. (2.54 cm) diameter for NAEF Model B Hand Punch Press. Dextran T40 was obtained from Pharmacia, ^2H_2O from Stohler Isotope Chemical Co. and glucose from Mallinckrodt Chemical Works. Tap water from the Mississippi River at New Orleans, LA, U.S.A., was filtered and deionized through a Continental Model 300 deionizer unit, and routinely filtered through a 0.45-μm Millipore filter to cold sterilize and remove particulate matter which would clog up the column pores, followed by deaeration by boiling under house vacuum.

Methods

Columns were Chromtronix precision bore, 2.54 cm (1 in.) diameter by *ca.*

* Use of a company and/or product named by the Department does not imply approval or recommendation of the product to the exclusion of others which may also be suitable.

43.0–46.0 cm length of packing between the top and bottom bed supports. Solutes were introduced through a 0.50-ml sample loop as separate 1 % solutions in water or 0.02 % sodium azide. Injection of individual samples was spaced to prevent overlapping of elution curves. Elution was carried out with deaerated deionized water alone or containing 0.02 % sodium azide at a flow-rate of *ca.* 1.75 ml/min, pumping with a Milton Roy Mini-pump. The temperature was 20 ± 0.5°C. The eluate concentrations were recorded continuously by a differential refractometer, Model 300L, Pharmacia refractive index (RI) monitor collecting with a LKB 7000 Ultrarac Fraction Collector into tared 10-ml test-tubes. Volumes were determined gravimetrically by collecting fractions in tared test-tubes, then summing the weights and proportional parts of fractions between injection and the peak of the elution curve for each solute. The peak was determined by tangent lines drawn on the slopes of the peak.

The simplified procedure of Haglund[11] was used to determine the fraction of internal water, A_w, that is accessible to a given solute. A_w for a solute can be determined from V_e, V_0, $V^2_{H_2O}$ (peak elution volumes of the test solute, void volume indicator and ^2H_2O, respectively) and the equation:

$$A_w = 1.075\,(V_e - V_0)/(V^2_{H_2O} - V_0)$$

A_w can be calculated from the equation

$$A_w = (V_e - V_0)/V_w$$

where V_w is obtained from the dimensions of the column, weight of the dry substrate in the column and the specific volume of the substrate.

The void volume, V_0, was determined by use of Dextran T40 (mol.wt. ≈ 40,000) or Carbowax 6000. The internal water volume, V_w, was determined with ^2H_2O. Standard test solute for elution volume (V_e) was glucose for the initial tests on a column, and to serve as a running reference for column performance in determining fraction of internal water accessible to solute (A_w).

After each column was packed, it was flushed with the deaerated water to eliminate adsorbed air, swell the fibers (in the case of dry pack), fill void spaces and wash fine particles out. This usually took several days or more to reach equilibrium. Then evaluation proceeded with dye injection and/or the use of the Dextran T40, glucose and ^2H_2O.

EXPERIMENTAL METHODS OF PACKING AND RESULTS

Whole fiber columns could be prepared with the fibers perpendicular to, parallel to or random to the direction of flow. Experimental columns were made using different techniques to orient fibers in these configurations.

Exploratory columns packed with cotton fiber tufts

An absorbent cotton roll or mat contains fibers in a random distribution, but primarily in the horizontal mode. Three columns were prepared wherein small, medium and large tufts, columns T1, T2 and T3 respectively, were pulled from the roll, immersed in boiling water to wet out, deaerate and swell the fibers. The tufts

assumed a ball shape in the water upon agitation. After being allowed to cool, the wet cotton tufts were inserted into the Chromatronix glass column and packed with a small wooden dowel as tight as possible until the column was filled to approximately 1 in. from top. Excess water was allowed to overflow from the top of the tube or drained from the bottom. The other end plug was then placed in column and pressed down as far as possible. This method while intended to give random fiber orientation, resulted in a (visually) random fiber orientation in the vertical direction, and somewhat stratified across the direction of flow. The dye test showed a somewhat irregular band which diffused as it proceeded down the column. However tests with Dextran T40, glucose and ^2H_2O showed peaks which could be determined. Column T1 was evaluated by the dye test only. Column T3 (Fig. 1) gave double humped peaks, consisting of a small skewed peak with a larger, broad, relatively normal peak. Column T2 (Fig. 1) gave a skewed curve wherein the peak position did not coincide with the peak determined by the tangents to the peak slope. Although the total volume accessible to water, $V_c = V_0 + V_w$, remained constant for the column, the void volume and internal pore volume accessible to water changed with time (Table I). The broad peaks (covering *ca.* 40–45 min intervals) would allow few runs per day.

Column packed with fibers oriented in direction of flow of solute

Dewaxed sliver. A column was prepared by taking many strands of dewaxed sliver and combining them in a tight roll until sufficient to fill the column, and packing by twisting, pulling and pushing into the column. After conditioning the column, test runs with 2% Dextran T40 and 4% glucose disclosed large double humped peaks,

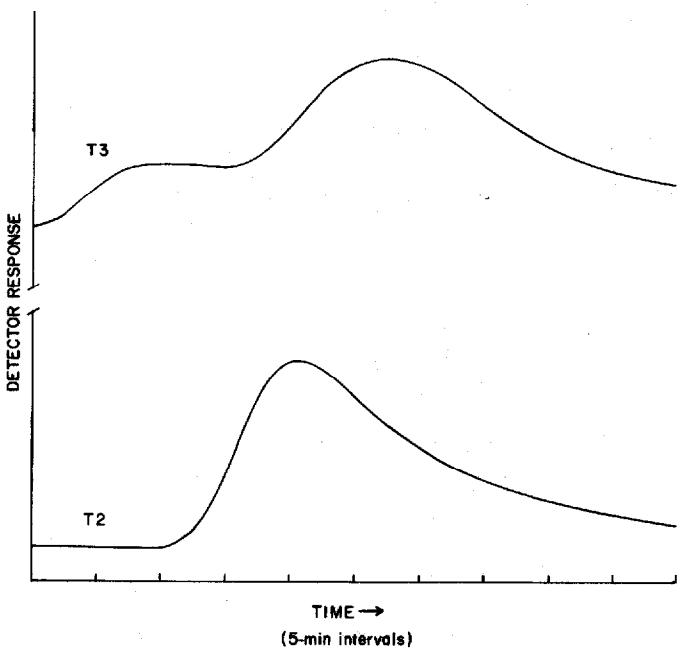

Fig. 1. Elution of glucose from exploratory cotton tuft columns —fibers random oriented to direction of solute flow. T2 = Medium tufts; T3 = large tufts; both columns packed and compressed wet.

TABLE I

PERFORMANCE OF WHOLE FIBER ABSORBENT COTTON TUFTS COLUMN

Column T2: medium size tufts wet out in boiling water, packed wet and compressed wet. Peaks determined by tangent slope method.

Date	V_0 *(ml)*	V_w *(ml)*	$V_c = V_0 + V_w$ *(ml)*	A_w *(glucose)*
May 20	111.43	21.45	132.88	0.688
June 3	117.76	19.30	137.06	0.527
June 4	116.48	20.76	137.24	0.672
June 5	106.83	30.88	137.71	0.765
June 9	117.01	20.32	137.32	0.665

which were not skewed and required long elution times (70–80 min). V_0 was estimated at 136.11 ml, V_w as 25.76 ml and A_w (glucose) as 0.68. The column had been packed dry. It was discovered that the column could easily be compressed further while wet and although the elution curve shape did not change, V_0 was reduced to 112.16 ml, V_w changed to 23.01 ml and A_w (glucose) was 0.61.

Roll of cotton printcloth. Two columns were prepared by hand rolling a length of printcloth (47.5 cm wide) around a 6-mm glass or steel rod cut *ca.* 0.32 cm shorter than the fabric. The fabric was rolled as tightly and evenly as possible to a diameter that would just fit into the column with great difficulty. The end column plugs were inserted tightly and the column was conditioned. It was anticipated that upon wetting, the fibers would expand considerably. When the test solutes were injected, no peaks were detected, but the RI value rose after 5 min and remained up. Upon disassembly, the roll of fabric slipped out readily and was extremely hard to the squeeze touch, indicating that although it had swollen, contraction from the walls had occurred rather than the anticipated expansion.

Column packed with fibers oriented perpendicular to the direction of solute flow

Experience from the prior packings indicated that a more uniform packing might be achieved by cutting and packing with discs which would be stratified across the direction of flow but thoroughly randomized in the disc. This would give a uniform random solute flow and minimum channeling.

Cotton printcloth discs. A column was packed dry to a depth of 47.0 cm with 2.54-cm discs cut by precision die from the cotton printcloth. After conditioning of the packing, test solutes were injected. After about 15 min, there was a gradual rise and fall of the eluate curve which extended for 80 min and then another gradual rise, etc., which indicated that the column was not functioning properly. Upon disassembly, the discs readily dropped out because they also had shrunk away from the cylinder walls upon wetting out.

Cotton mat discs. Fibers in an absorbent cotton roll mat are randomly oriented, but upon packing in a column, the discs are stratified perpendicular to the direction of the solute flow. Duplicate columns (MD 1 and MD 2) were prepared using the 2.54-cm absorbent cotton discs, and compressing them in the dry state as much as possible. One end plug of the column was fixed in place and after several cotton mat disc plugs were inserted into the column, a large wooden dowel was used

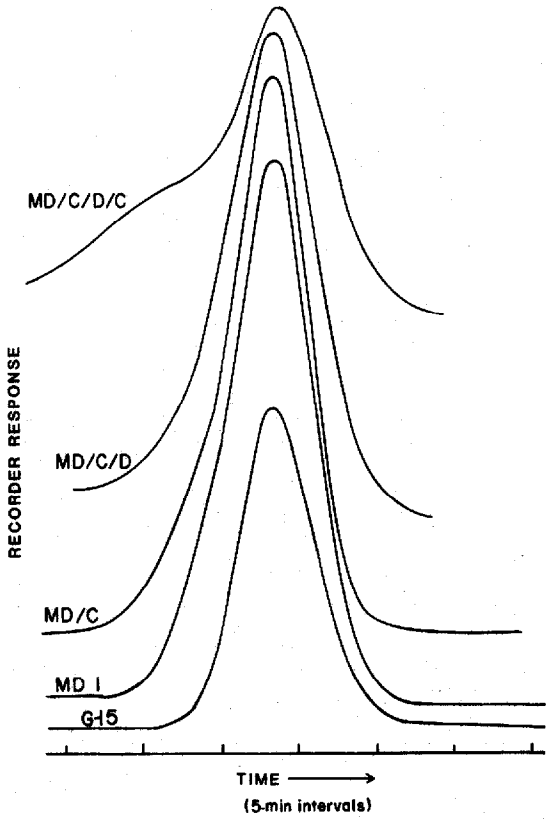

Fig. 2. Elution of glucose from cotton mat disc columns —fibers stratified across direction of flow. G-15 = desired performance elution curve; MD 1 = packed and compressed dry, 43.8 cm depth; MD/C = MD 1 compressed wet to 33.3 cm depth; MD/C/D = 7.6-cm compressed dry discs added to each end of MD/C, 48.6 cm depth; MD/C/D/C = MD/C/D column further compressed wet to 42.9 cm depth.

to push and compress the plugs as much as possible. Care was used in selecting, inserting and keeping the plugs even. The other column end plug was then inserted and pressed down to compress the column as much as possible while dry. Deaerated deionized water was pumped through for sufficient time to remove entrapped air and to allow swelling to reach equilibrium. Test results of Dextran T40 and glucose showed sharp clean peaks similar to those from a Sephadex G-15 column (Fig. 2). This results in a large void volume so one column (MD 1) was compressed in the wet state from 43.8 to 33.3 cm (column MD/C) which reduced V_0 from 172.9 to 120.4 ml or a 30.3% reduction in void volume. V_w and A_w remained the same (Table II). An additional 7.6 cm of compressed dry discs were added to each end (to 48.6 cm depth, column MD/C/D) of this column to increase the capacity of the column's internal pores. The column was wet out and evaluated. V_0 increased to 182 ml and V_w increased to 25.4 ml. It was compressed again wet (42.9 cm, column MD/C/D/C) lowering V_0 to 154.8 ml and V_w remained around 25.9 ml. Some detriment to the overall elution curve occurred upon this compression (Fig. 2). A small broad shoulder occurred on the forefront of the larger elution peak indicating a shorter pathway or

TABLE II

PERFORMANCE OF WHOLE FIBER COTTON MAT DISC COLUMNS

Column	*Date*	V_0		V_w *(ml)*	$V_e = V_0 + V_w$ *(ml)*	A_w	
		ml	*S.D.*			*Glucose*	*C.V.*
MD 2*	June 22	172.96	0.51	19.37	192.33	0.750	3.93
	June 24	172.75	0.20	19.14	191.89	0.750	1.43
	June 26	172.54	0.27	18.93	191.47	0.733	3.71
MD 1**	June 10	151.50	–	15.64	167.14	0.678	–
	June 11	165.81	0.26	19.76	185.57	0.718	2.74
	June 12	172.99	0.33	17.93	190.92	0.733	2.42
	June 15	172.89	0.10	17.89	190.78	0.764	0.61
MD/C***	June 17	120.45	0.07	18.26	138.71	0.746	1.36
	June 18	121.63	–	17.34	138.97	0.716	–
MD/C/D§	July 7	181.70	0.05	25.42	207.42	0.750	–
	July 8	180.86	0.74	25.29	206.15	0.745	1.00
MD/C/D/C§§	July 10	154.53	0.09	25.89	180.42	0.743	0.80
	July 14	154.73	0.33	25.97	179.70	0.754	2.16
	July 22	154.80	0.01	25.53	180.33	0.738	0.06
	July 27	154.89	0.02	26.07	180.90	0.752	0.19
	August 5	155.42	0.11	26.17	181.59	0.740	0.21
	August 10	155.58	0.07	26.17	181.75	0.732	0.56
	August 13	155.68	0.05	26.02	181.69	0.740	0.20
	(27 days)	155.22	0.35	25.99 ± 0.42	181.21		

* Absorbent cotton discs compressed dry, 44.0 × 2.54 cm, total column volume, V_t = 222.95 ml.
** Same as *, but 43.8 cm height, V_t = 221.94 ml.
*** Column MD 1 compressed wet to 33.3 cm, V_t = 168.73 ml.
§ 7.6-cm compressed dry discs added to each end of MD/C 48.6 cm height, V_t = 246.26 ml.
§§ MD/C/D column compressed wet to 42.9 cm height, V_t = 217.38 ml.

non-uniform spreading of sample onto column had occurred. Resolution, however, was still acceptable for determining elution volume. After several days use, the column became very stable and permitted good reproducibility. Some of the improvements in the data occurred as a result of improvements in the operation of the equipment. The other column (MD 2) was packed to 44.1 cm height and was not compressed wet. Although the V_0 and V_w for this column are not the same as for the other column, the values of A_w (glucose) are not statistically different. Thus two columns packed from the same parent stock give the same fraction of internal water available to the same solute.

Accessible internal pore water (V_w) for comparable height Sephadex G-15 columns were about 90 ml, whereas in the whole-fiber cotton mat columns, V_w was 18–20 ml for MD 1, 18–19 ml for MD 2 and 25–26 ml for the compressed MD/C/D/C or about 20% of the capacity of the Sephadex. An increased accessible internal pore water elution volume would help improve determination of retention values.

These columns had low back pressures, ranging from 2–3 p.s.i. for columns MD 1 and MD 2 up to 16 p.s.i. for the compressed MD/C/D/C column.

DISCUSSION

The goal of preparing whole-fiber cotton gel-filtration columns has been achieved. Effective gel-filtration columns made from plugs of whole-fiber mats represents a significant advance in research technology and considerable simplification in column preparation. The characterization of whole cotton and other fibers as they may behave in yarn and fabric form should now be possible. Since columns are very stable and easily prepared, they could be used for other conventional and preparative work.

The most satisfactory procedure consists of precision cutting plugs from a mat made up of highly random, uniform, loose fibers such as that generated from a garnet machine, opening and picking line or carding machine. These provide the uniformity of solute flow through the substate which is necessary for acceptable elution peak formation. The plugs are fragile and have no mechanical strength to hold them together, but with reasonable care can be easily packed dry and compressed dry. If there is need to reduce the magnitude of their void-volume value, they can be further compressed after wet conditioning. The internal pore volume can be increased by adding additional dry plugs to a column after it is wet conditioned and compressed. Compression of the added plugs in our case gave a slight degeneration of the elution curve peak, but since this was probably due to uneven sample distribution by the grid upon wet compression, the insertion of several additional dry plugs could have eliminated this problem. In subsequent preparation of whole-fiber columns it was noted that the use of a thicker mat results in fewer plugs, faster packing and sharper, cleaner peaks with visibly fewer void spaces in the column.

Column life was over 2 months duration and tended to improve with age. The life of these columns would probably be limited only by deterioration caused by microbial contamination. The useful life is definitely much longer than that of the chopped or ball-milled cotton columns. Once made, they do not suffer fractures such as the Sephadex columns do if they should go dry. It is reported that column MD/C/D/C and one made from ammonia mercerized absorbent cotton are not only functional after 1.5 years but give the same solute elution volumes for a sugar series consisting of glucose, raffinose, maltose and stachyose[12].

Columns prepared of whole cloth fabric would also be desirable from the experimental viewpoint. Those prepared here were from a roll of cloth, which was not tension relieved. Wetting or washing the cloth allows tension release (commonly used by the seamstress before cutting out the pattern). It is suggested that discs cut from stress relieved fabrics would likely work. Alternatively, some method of radial compression could be used.

ACKNOWLEDGEMENTS

I thank S. Malanez, P. Howley and N. Bertoniere for their technical assistance, N. Meadows for illustrations and S. Rowland for suggestions.

REFERENCES

1 L. F. Martin, F. A. Blouin, N. R. Bertoniere and S. P. Rowland, *Tappi*, 52 (1969) 708.
2 L. F. Martin, N. R. Bertoniere and S. P. Rowland, *J. Chromatogr.*, 64 (1972) 263.

3 S. P. Rowland and N. R. Bertoniere, *Text. Res. J.*, 46 (1976) 770.
4 L. F. Martin and S. P. Rowland, *J. Chromatogr.*, 28 (1967) 139.
5 L. F. Martin and S. P. Rowland, *J. Polym. Sci.*, 5 (1967) 2563.
6 L. F. Martin, F. A. Blouin and S. P. Rowland, *Separ. Sci.*, 6 (1971) 287.
7 L. F. Martin, N. R. Bertoniere, F. A. Blouin, M. A. Brannon and S. P. Rowland, *Text. Res. J.*, 40 (1970) 8.
8 F. A. Blouin, L. F. Martin and S. P. Rowland, *Text. Res. J.*, 40 (1970) 959.
9 F. A. Blouin, L. F. Martin and S. P. Rowland, *Text. Res. J.*, 40 (1970) 809.
10 J. Zurcher, personal communication.
11 Å. C. Haglund, *J. Chromatogr.*, 156 (1978) 317.
12 N. R. Bertoniere, personal communication.