

USE OF PREPARATIVE HPLC FOR THE ISOLATION OF
OLIGOSACCHARIDES WITH BIOLOGICAL ACTIVITY OR UNIQUE
FOOD APPLICATIONS

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ABSTRACT

High performance liquid chromatography (HPLC) is becoming an indispensable tool for carbohydrate researchers. Many stationary phases and techniques are now readily available for the analytical-scale separation of simple sugars, small oligosaccharides, and their derivatives. There has been a need, however, for the development of improved analytical- and preparative-scale HPLC techniques for medium to large oligosaccharides, which are of intense interest in many areas of current research. In our group, we are interested in identifying oligosaccharides that may be useful in the food and pharmaceutical industry. Additionally, we are interested in determining the roles that oligosaccharides play during ripening, senescence, and defense-responses in fruits and vegetables. In the course of these studies, we have recently developed a number of useful preparative and analytical HPLC techniques. Preparative HPLC was accomplished on stainless steel columns (30 cm X 2.2 or 4.4 cm) filled with either polystyrene- or silica-based stationary phases. Polystyrene-type cation exchange resins, cross-linked with 2, 4, or 8% divinylbenzene, were converted into either H^+ , Ca^{++} , Ag^+ , or Pb^{++} forms prior to packing into columns at 1000 - 3000 psi. These columns were conveniently used on conventional analytical HPLC systems since they required simple mobile phases of H_2O , or 0.01N H_2SO_4 (H^+ -form only) at flow rates of only 1 - 3 ml/min and they ran at nominal back pressures (lower than 200 psi) when they were externally heated at 35 to 85°. Commercial silica-based columns (C_{18} reversed phase and aminopropyl normal phase) required acetonitrile/water or pure water (C_{18} only) mobile phases at flow rates of 2 to 50 ml/min, depending on column size. These various stationary and mobile phases, used in conjunction with a microprocessor-controlled pump, a preparative refractive index detector, an automated sample injector, and a programmable fraction collector, enabled the automated isolation of multi-gram quantities of pure oligosaccharides derived from amylose, chitin, cellulose, pectin, inulin, and cyclodextrins. Several new analytical HPLC techniques for oligosaccharides have also been developed in our laboratory. Analytical scale columns packed with high performance cation or anion exchange resins were used to separate oligosaccharides derived from cellulose, inulin, and polygalacturonic acid that had DP values as large as 10, 35, and 50, respectively. Extremely sensitive (ng) detection was achieved by way of ultra sensitive RI or pulsed amperometric detection.

PRESENTATION

It is a great honor to be in Tsukuba City to attend this Food Forum, held in conjunction with the U.S. Japan Joint Committee on Development and Utilization of Natural Resources. Whereas many of the interesting talks here are about utilization of proteins, I hope that my talk on carbohydrates may also be of some interest to all of you in the audience.

The work I will discuss today, Slide 1, is the result of several years of work by my associates and me at the Eastern Regional Research Center, Agricultural Research Service, in Philadelphia and is entitled, the Use of Preparative HPLC for the Isolation of Oligosaccharides with Biological Activity or Unique Food Applications.

My research projects over the last 5 years have been initiated to solve long-standing problems in American agriculture. Because my background and interests are predominantly in the area of carbohydrate chemistry and biochemistry, my research approaches have generally involved carbohydrate research. Specifically, our recent interest has been in the study of oligosaccharides for food- and biological applications. Whereas much effort has been devoted over the years to studying small monosaccharides and large polysaccharides, the use and properties of the intermediate oligosaccharides have received relatively little attention until

recently. This is unfortunate, since as is shown in Slide 2, oligosaccharides are of great importance in food and in plant systems. In foods they function as sweeteners, caloric and non-caloric ingredients, as flavor encapsulators, and in our lab, we are interested in using them to prevent quality loss in minimally processed fruit and vegetables. This last area was the subject of my earlier presentation to the joint panel. In plant systems, researchers have found that oligosaccharides may play important roles in elicitation of plant defense responses, in inducing ethylene biosynthesis (for ripening, etc.), and for modulating plant gene expression. Certainly, this class of compounds are worthy of study from many of these points of view.

In our studies, as in those of most others, the ability to examine the effects of oligosaccharides in food or biological systems relies on 1) their availability and 2) the ability to separate, detect and analyze them. In order to meet these two needs, we have found hplc to be uniquely useful. Analytical hplc has many advantages over other analytical methods for carbohydrate analysis, Slide 3. The method is generally quantitative, rapid, does not require formation of derivatives prior to analysis, provides relatively high resolution and utilizes non-destructive detection methods, the latter of which are particularly useful for preparative applications. And, since many of the most interesting oligosaccharides are not commercially available, preparative hplc has been very useful for their isolation from biological systems.

Although hplc is an extremely useful technique, early hplc systems had the disadvantages outlined in Slide 4. I would now like to describe some of the recent progress in solving some of these shortcomings of analytical hplc methods and later discuss our results in the area of preparative methods.

First I would like to quickly review the present state of the art in analytical hplc methods for carbohydrates. As seen in Slide 5, there are basically four major systems for carbohydrate hplc analysis. The development of each system has been made possible by hundreds of contributions from many laboratories around the world. We have used the first system, involving separations on cation exchange resins to accomplish several difficult sugar analyses. Separations on these resins occur by a variety of mechanisms, the first of which is ligand exchange. As shown in Slide 6, due to pioneering studies by Angyal and others, the theoretical basis for these interactions are now well known. In our laboratory, we needed a method to separate the cyclitols, Slide 7. The cyclitols are important precursors to plant cell wall polysaccharides and also are components of critically important biological signal molecules. We were trying to synthesize Carbon 13 labelled inositols and needed methods to analyze and isolate the product. This was a very difficult separation since these 8 compounds are all isomers with very similar structures. Although this separation had never been reported, we reasoned that the arrangements of hydroxyl groups were such that separations could be possible by the ligand exchange techniques. As shown in Slide 8, complete

separation of these important compounds was readily carried out. As far as we know, no other method can successfully achieve this separation.

The second mechanism by which separations of carbohydrates occur on cation exchange resins is by gel permeation. The cation exchangers normally used in these separations are made of polystyrene that is cross-linked with divinylbenzene. The percentage of cross-linking has a great effect on the type of carbohydrate separations that can be achieved, as shown in Slides 9 and 10. We were interested in developing a simple hplc system with smaller amounts of cross-linking, that could separate larger oligosaccharides than were currently possible with commercial systems. Slide 11 gives the details for this stationary phase that was developed in conjunction with BioRad laboratories. Slide 12 gives chromatograms that were achieved with this system. As it can be seen, excellent separation of oligosaccharides from amylose, cellulose, and pectin were possible with this simple system. Although the column requires more care than conventional columns, it can be used for many months with little change in efficiency.

The second stationary phase I would like to discuss for analytical hplc is the new type of pellicular anion exchangers, Slide 13. With this system, laboratories around the world are achieving exciting results. For instance, one of the most remarkable chromatograms I have ever seen is shown in Slide 14. This work was

done by Dr. Kyoko Koizumi, of Mukogawa Women's University in Nishinomiya, Japan, who is one of the true leaders in developing hplc methods for carbohydrates. By her method it is now possible to analyze oligosaccharides from starch that have DP values up to 60 or more. This has had a tremendous impact on the study of the structure and function of starch. As I mentioned earlier, we are very interested in analysis of oligosaccharides generated from pectin in plants, due to their proposed roles in regulation of biochemical events in plants. By using the conditions shown in Slide 15, we were able to obtain the first separation, Slide 16 of these important oligogalacturonic acids up to a DP level of 40-50. This method is now being used routinely by several researchers in plant and food science areas.

With these new methods, we feel that some of the earlier mentioned problem areas of carbohydrate hplc have now been eliminated, namely: the inability to analyze higher oligosaccharides and the inability to separate closely related isomers. Slide 17 shows that the earlier discussed problem of detector sensitivity has now been partially solved. Development of new, ultra sensitive refractive index detectors by companies such as ERMA Optical Works and Showa Denko, now provide excellent sensitivity. In addition, the pulsed amperometric detectors now available also allow quite sensitive detection of saccharides.

Now, I would like to spend the rest of the time on preparative hplc methodology.

Although the potential for hplc on the preparative scale is great, as shown in Slide 18 preparative hplc did not become a practical laboratory technique as readily as did the analytical scale method. This was because of the many problems associated with first and second generation preparative hplc systems. For preparative hplc to be a truly useful and practical method for the carbohydrate research laboratory, it must have the characteristics shown on Slide 19. We have worked in this area intensively in the last several years to allow the isolation of a number of carbohydrates of interest. We have had very good success by adopting the type of hardware seen in Slide 20. Our studies, verified by many others now, showed that small, spherical or irregular hplc stationary phases used in analytical scale columns could be packed into preparative columns, resulting in moderate pressure, rapid, high resolution separations, as long as one increased the *diameter* of the column, rather than the *length*.

Preparative columns with dimensions of 2.2 cm X 30 cm, were readily packed with polystyrene-based cation exchangers shown in Slide 21 at a nominal cost. These columns were used on an automated preparative hplc system equipped with two solvent pumps, an automatic injector pump, and an "intelligent" fraction collector which automatically collected peaks detected on the refractive index detector.

Slide 22 shows a 125 mg maltodextrin sample separated on two different

laboratory packed preparative hplc columns, each held at 85° C and eluted at 1.1 ml/min with distilled water. Both columns were packed at 1000 psi with 4% cross-linked Ag⁺-form cation exchange resins. The only difference in the stationary phases is that the average diameter of the resin size is much smaller and more monodisperse in chromatogram "b" than in "a". Although the 20-30 micron-sized resin is somewhat more expensive, it provides significantly greater resolution. The fact that these resins are extremely durable and can be used for several years, also makes the initial cost become a less important factor.

In recent years, more economical and durable preparative hplc columns have become available from commercial vendors. Slide 23 shows examples of some of the commercial phases and approximate costs, for your comparison to the laboratory packed columns. One vendor, Rainin Instruments, produces preparative columns in a cartridge format as shown in Slide 24. This unique system allows re-use of the end fittings and hence saves on hardware costs. Even more important, however, are the threaded end fittings, which allow one to compress the column bed whenever voids occur. This capability can easily extend the lifetime of a column by 300-400%.

Slide 25 shows the preparative injections of maltodextrins on a reversed-phase (C₁₈) and on a normal-phase (aminopropyl silica gel) column. While the reversed-phase column required a very practical mobile phase (water) at a low flow rate,

3 ml/min, its capacity was small (less than 20 mg/injection). The normal-phase column required a more complex mobile phase and faster flow rate (acetonitrile/water (55:45) at 12 ml/min) but allowed rapid separation of large samples. Many factors must be considered when choosing a system for preparative separations. Toxicity or expense of mobile phase, initial expense and durability of stationary phase, and stationary phase capacity and efficiency all must be factored into the decision. A simple comparison of the cation exchange and normal phase separations is given in slide 26.

Another class of oligosaccharides which are of great interest in the area of food bulking agents and in the conversion of cellulosic biomass into fuel alcohol, are the cellodextrins. Although these oligosaccharides are well known, it is difficult to obtain samples of them for research and development activities. We used a published procedure for hydrolysis of cellulose by trifluoroacetic acid, cleaned up the reaction mixture, and injected the mixture onto the Ag^+ form cation exchange resin described earlier. Slide 27 shows the high resolution preparation of these oligosaccharides. Slide 28 shows the approximate amounts of pure oligosaccharides that can be isolated in a given period of time.

The aminopropyl silica gel column was found to be ideal for the isolation of oligosaccharides from chitin, Slide 29. These oligosaccharides and their derivatives are undergoing intense study as wound healing agents and for the

protection of plants and plant products from fungal infection.

The same aminopropyl silica gel column was very useful for isolating samples of pure oligosaccharides from the hydrolysis of polygalacturonic acid, Slide 30. These oligogalacturonides are needed for studies on the mechanism of elicitation of defense responses in plants. By using a gradient approach, Slide 31, we have been able to isolate even larger oligosaccharides in this series. By use of a preparative version of the high performance anion exchange columns discussed earlier, we are now in the process of isolating oligosaccharides with DP values between 15 and 50. We hope to be able to report this information in the near future.

Recently we examined our system for the isolation of fructo-oligosaccharides from Jerusalem artichoke. As many of you probably know, it is being proposed that these compounds provide a number of health promoting effects and I believe they are being aggressively marketed in some parts of the world including Japan. Coors-Biotech Products Company, is now introducing this product to the American market. Very few methods are available for isolating individual oligofructosides of defined molecular weight. We, therefore, examined three different stationary phases as shown in Slide 32. Pure oligofructosides from DP 3 to 13 can now be readily prepared by preparative hplc on one or another of the phases.

Recently a larger commercially available preparative column, shown in Slide 33, has become available. Slide 34 demonstrates the rapid separation of gram quantities of malto-oligosaccharides on this column. The purity of the fraction is shown in Slide 35 and an analysis of the sample throughput is shown in slide 36.

For those who are interested, I include a list of references to our studies in Slide 37.

At this time I would like to acknowledge the following people in Slide 38 and leave you with a picture, Slide 39, of my research institution as it looks at this time of year.

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IN FOOD SYSTEMS:

- * AS SWEETENERS
- * AS CALORIC/NONCALORIC SPECIALTY FOOD INGREDIENTS
- * IN FLAVOR/VOLATILE ENCAPSULATION
- * FOR PREVENTING QUALITY LOSS IN FRESH FRUIT AND VEGETABLE PRODUCTS

IN PLANT SYSTEMS:

- * ELICITORS OF PLANT DEFENSE RESPONSES
- * INDUCERS OF ETHYLENE PRODUCTION IN FRUITS
- * AS MODULATORS OF PLANT GENE EXPRESSION

TRADITIONAL ADVANTAGES OF HPLC

- * Quantitative
- * Rapid
- * Derivatives not required
- * High selectivity/resolution
- * Non-destructive detection

TRADITIONAL DISADVANTAGES OF HPLC

- * Short column lifetimes
- * Expensive analytical columns
- * Expensive preparative columns
- * Poor separations of large oligosaccharides
- * Poor detector sensitivity

PRESENT HPLC SYSTEMS FOR CARBOHYDRATE SEPARATIONS

Stationary Phase

I. PS-DVB Cation Exchangers

II. Amine-bonded Silica Gel

III. Alkylated Silica Gel

IV. Pellicular Anion Exchangers

Mobile Phase

H₂O

ACN/H₂O

H₂O (CH₃OH)

NaOH, NaOAc

THEORETICAL BASIS FOR SEPARATION OF SUGARS ON M⁺-FORM CATION EXCHANGERS

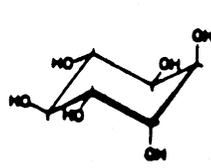
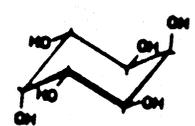
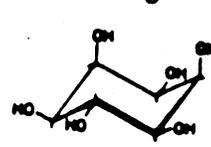
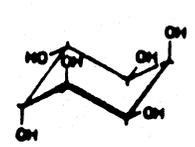
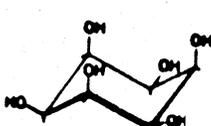
1. Sugars form coordination complexes with certain metal ions.
2. The stability of the coordination complex is governed by the stereochemistry of OH groups.
3. Sugars with more favorable complexation sites for column-bound metal ions will be retained longer on the column than sugars with less favorable sites.
4. For pyranose ring forms, the following arrangements of OH groups form complexes with Ca²⁺ ions:

ax, eq, ax
(strong complex)

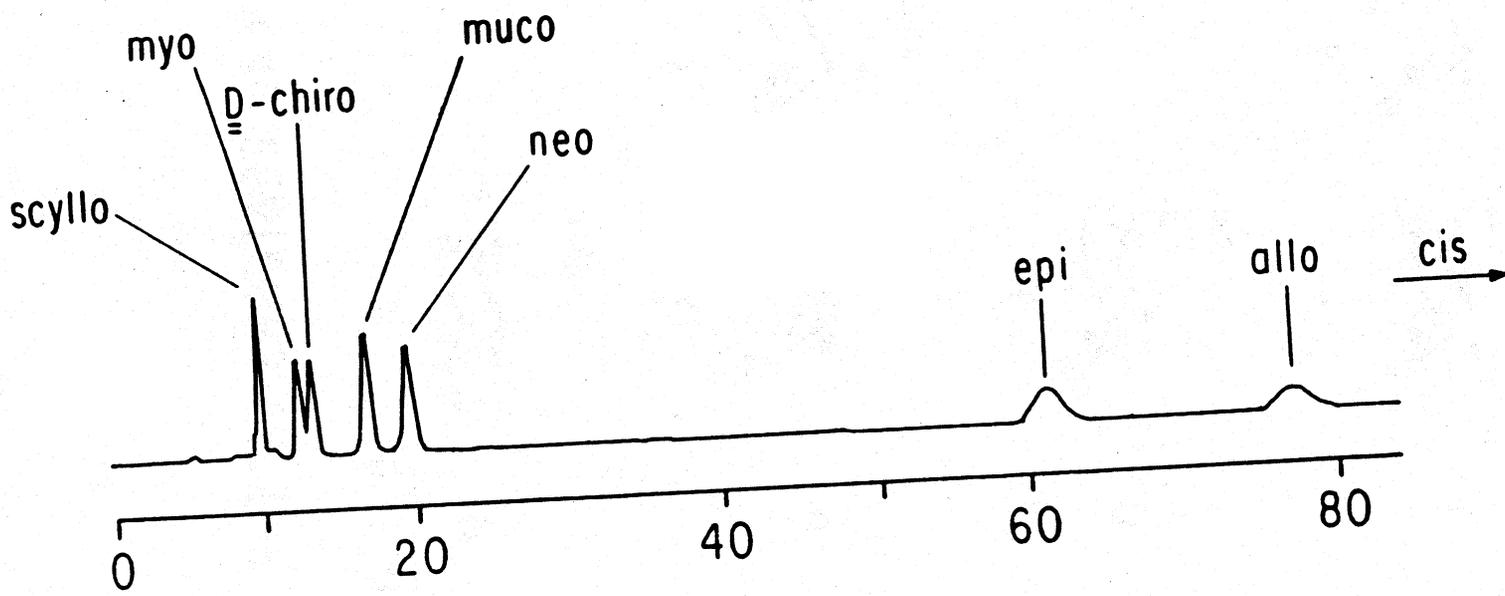
as, eq
(weak)

eq, eq
(weaker)

STEREOCHEMICAL FEATURES OF THE INOSITOLS

		<u>a, e, a</u>	<u>a, e</u>
1		0	0
	scyllo		
2			
	myo		
3			
	D-chiro		
4		0	2
	muco		
5		0	4
	neo		
6			
	epi		
7		1	4
	allo		
8		3	6
	cis		

SEPARATION OF INOSITOLS BY BY LIGAND EXCHANGE HPLC



SASAKI, et al

TIME (min)

ADVANCES IN CATION EXCHANGE RESIN STATIONARY PHASES

Variation in Cross Linking

% X-link

PROPERTIES / APPLICATIONS

35%

Rigid gel, Normal Phase Chromatography

8%

Semi-rigid gel, small pores, allows separation of small carbohydrates such as mono- and disaccharides by size and ion exclusion, and ligand exchange chromatography

ADVANCES IN CATION EXCHANGE RESIN STATIONARY PHASES

Variation in Cross Linking (continued)

<u>% X-link</u>	<u>PROPERTIES / APPLICATIONS</u>
6%	Semi-soft gel, larger pores, allows separation of larger oligosaccharides (< dp 5)
4%	Soft gel, even larger pores, allows separation of oligosaccharides up to dp 8-10

**HPX-22H: AN EXPERIMENTAL
CATION EXCHANGE RESIN FOR HPLC**

POLYSTYRENE BASE

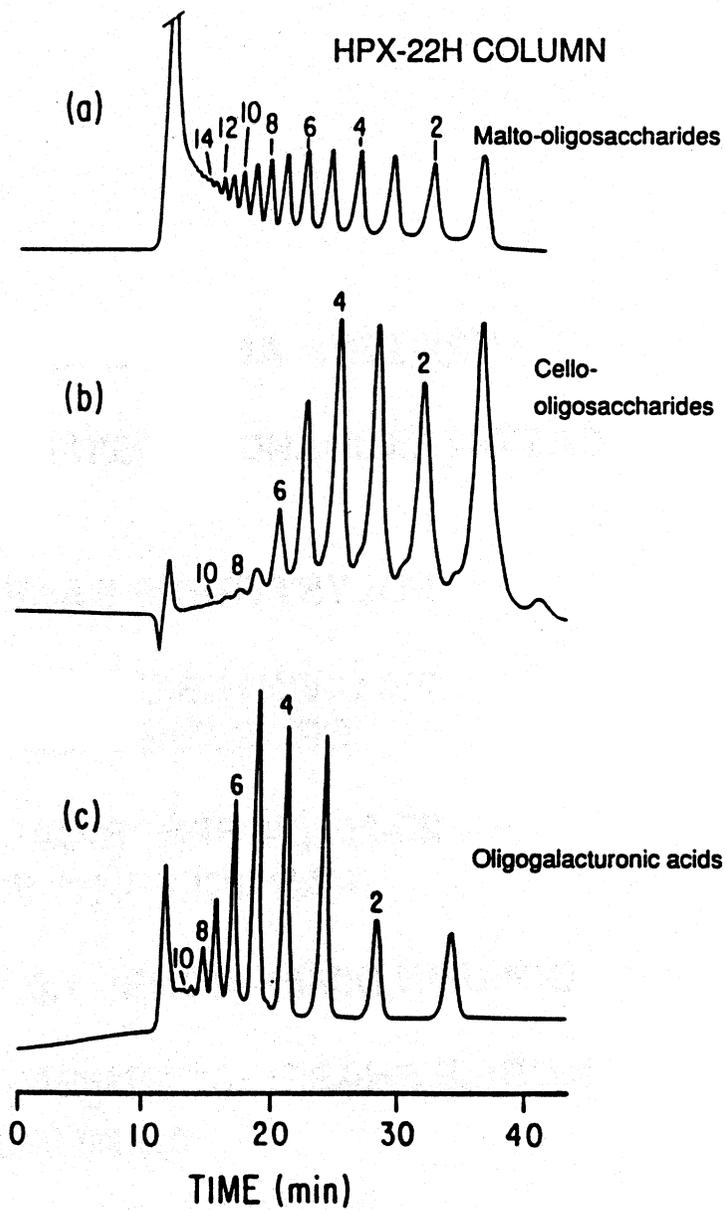
**2% DIVINYLBENZENE
CROSSLINKAGE**

**20-25 μ M SPHERICAL
PARTICLES, H⁺ FORM**

COLUMN DIMENSIONS: 1.0 X 30 CM

**MOBILE PHASE: .01 N H₂SO₄ AT 0.1 -
0.5 ML/MIN**

TEMPERATURE: 35 - 85 ° C



ADVANCES IN ANION EXCHANGE HPLC OF CARBOHYDRATES (HPAE-PAD)

Stationary Phase: Pellicular, strong anion exchangers

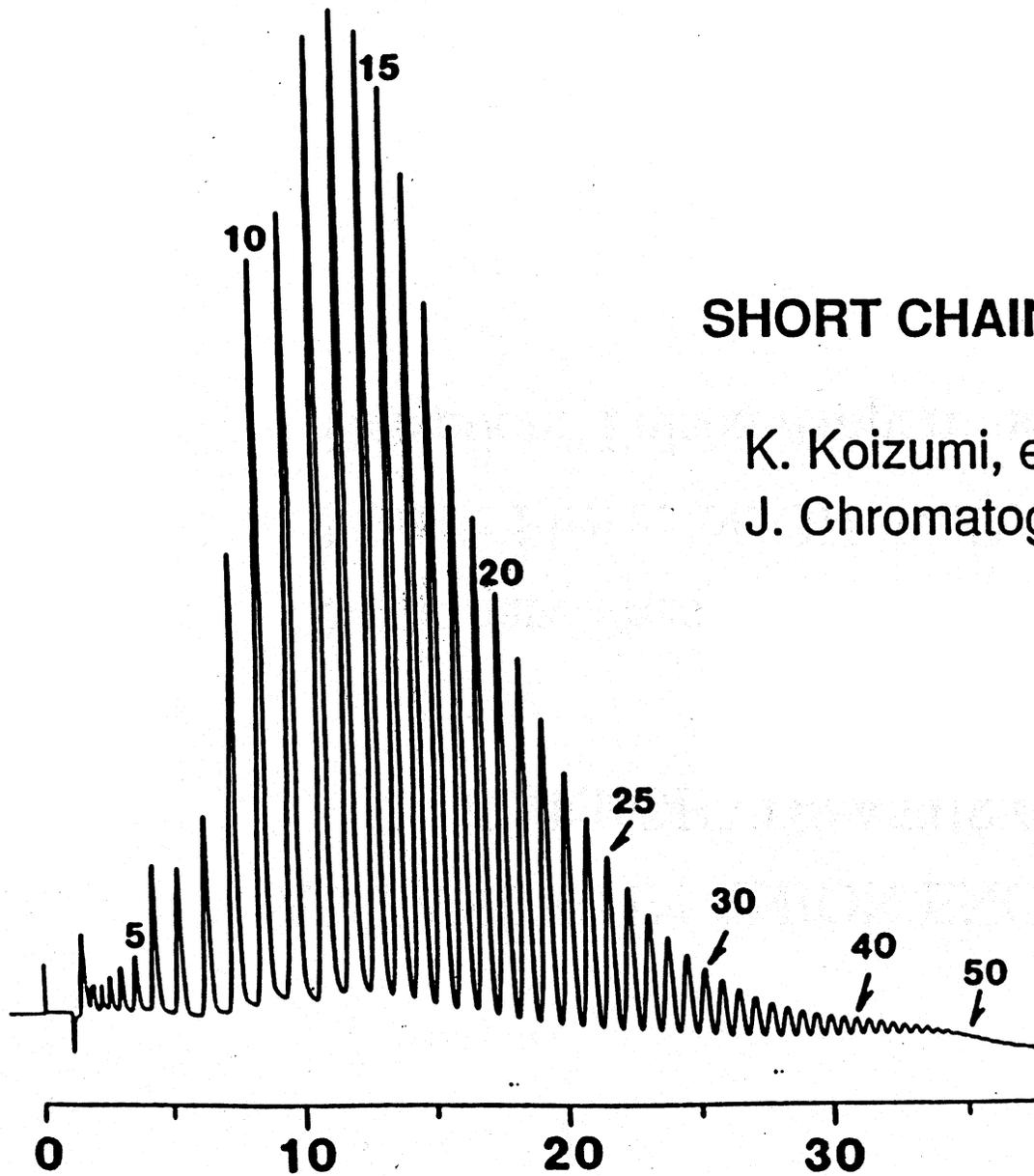
Mobile Phase: NaOH, NaOAc

Detection: Pulsed Amperometry

SHORT CHAIN AMYLOSE

K. Koizumi, et al.

J. Chromatogr., 464 (1989) 365.



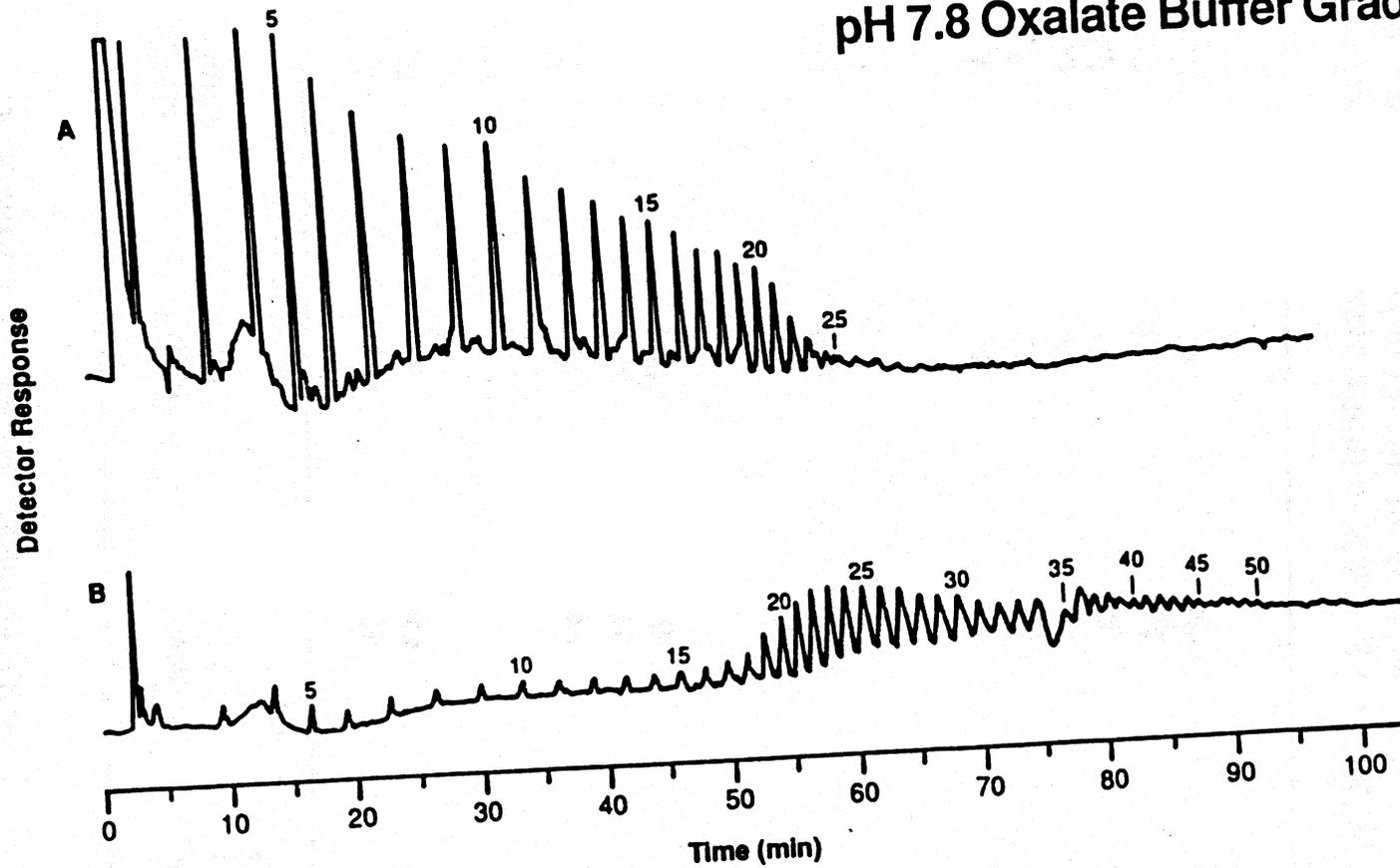
CHROMATOGRAPHIC CONDITIONS

Gradient	Time (min)	Eluant A (%)	Eluant B H ₂ O (%)	Acetate or Oxalate Conc. (M)
Acetate 1 (A = 1.0 M acetate, pH 5)	0	10	90	0.10
	30	60	40	0.60
Acetate 2 (A = 1.0 M acetate, pH 5)	0	27	73	0.27
	30	82	18	0.82
	60	100	0	1.00
Acetate 3 (A = 1.0 M acetate, pH 5)	0	45	55	0.45
	60	100	0	1.00
Oxalate (A = 0.5 M oxalate, pH 6)	0	5	95	0.025
	9	20	80	0.100
	40	40	60	0.200
	65	50	50	0.250
	95	56	44	0.280
	110	70	30	0.350

Flow rate = 0.8 ml/min, post-column addition of 0.5 M OH⁻ (K⁺ or Na⁺ salt) such that final flow rate = 1.6 ml/min from the detector.

H.P.A.E. Separation of Oligogalacturonic Acids

pH 7.8 Oxalate Buffer Gradient



SENSITIVITY OF HPLC DETECTORS FOR CARBOHYDRATE ANALYSIS

DETECTOR	LIMIT OF DETECTION (APPROXIMATE)
Conventional Refractive Index	5- 20 ug
Ultra Sensitive Refractive Index	15-50 ng
Pulsed Amperometric	.1-10 ng

**PREPARATIVE HPLC:
CHARACTERISTICS OF TRADITIONAL
SYSTEMS**

- * Expensive, "dedicated" equipment**
- * Expensive columns with short life-times**
- * Low resolution separations**
- * Labor intensive**

CHARACTERISTICS OF A PRACTICAL PREPARATIVE HPLC SYSTEM

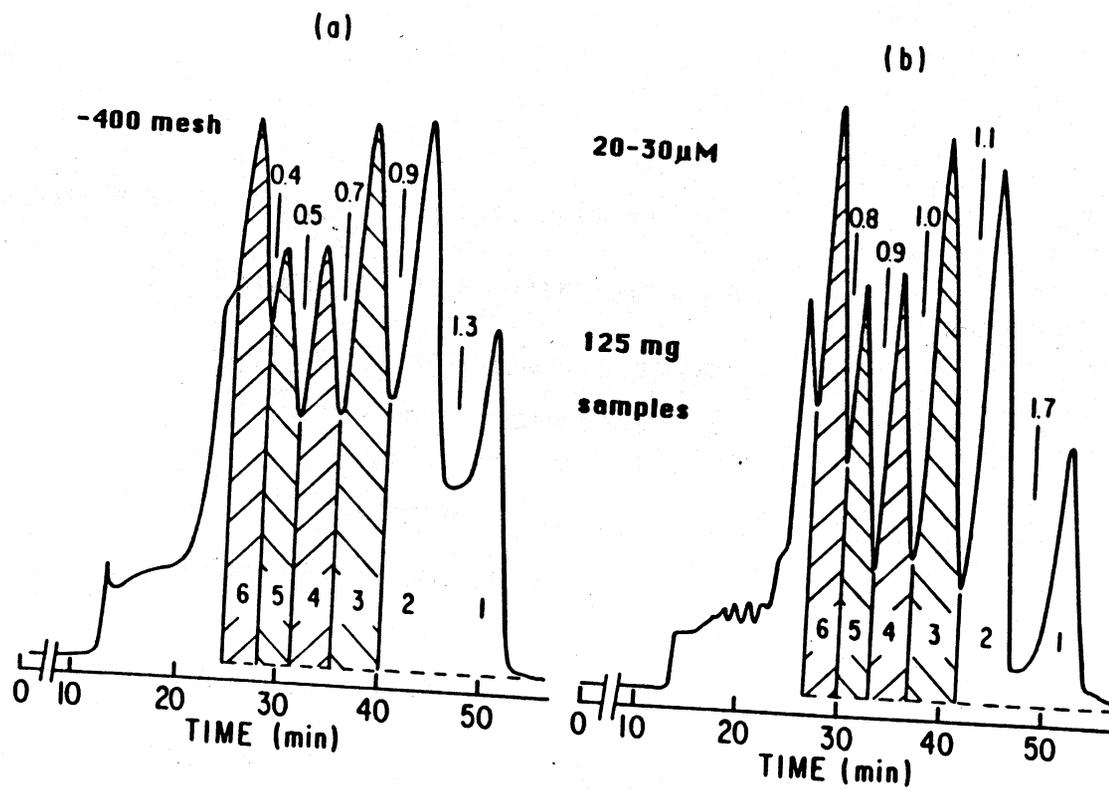
- * RAPIDITY**
- * HIGH RESOLVING CAPABILITY**
- * ECONOMY / PRACTICALITY**
- * HIGH SAMPLE CAPACITY**

LABORATORY-PACKED PREPARATIVE HPLC COLUMNS

(all 2.0 x 30 cm)

<u>Stationary Phase</u>	<u>Cost/column</u>	<u>Mobile Phase</u>
AG-50W-H8, H ⁺ , -400 mesh	Minimal (<\$50)	.01N H ₂ SO ₄
AG-50W-H4, H ⁺ , -400 mesh	Minimal (<\$50)	.01N H ₂ SO ₄
AG-50W-H4, Ag ⁺ , -400 mesh	Minimal (<\$50)	H ₂ O
AG-50W-H4, Ag ⁺ , 20-30μM	<\$1000	H ₂ O
Aminex Q-15S Ca ²⁺ , 20-30μM	<\$1000	H ₂ O

PREPARATIVE HPLC OF MALTO-OLIGOSACCHARIDES: AG-50W-H4-Ag⁺



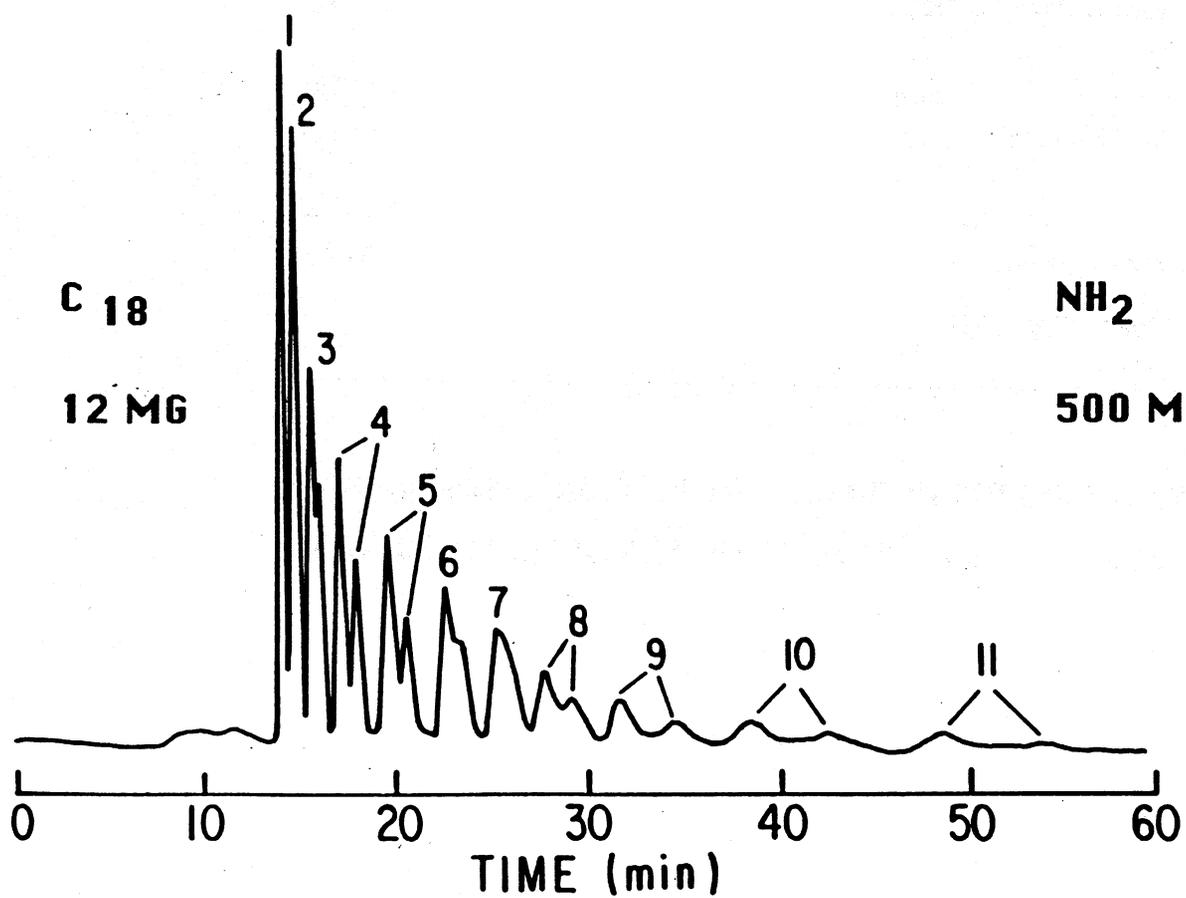
PREPARATIVE HPLC COLUMNS USED FOR CARBOHYDRATE ISOLATIONS

COMMERCIAL COLUMNS

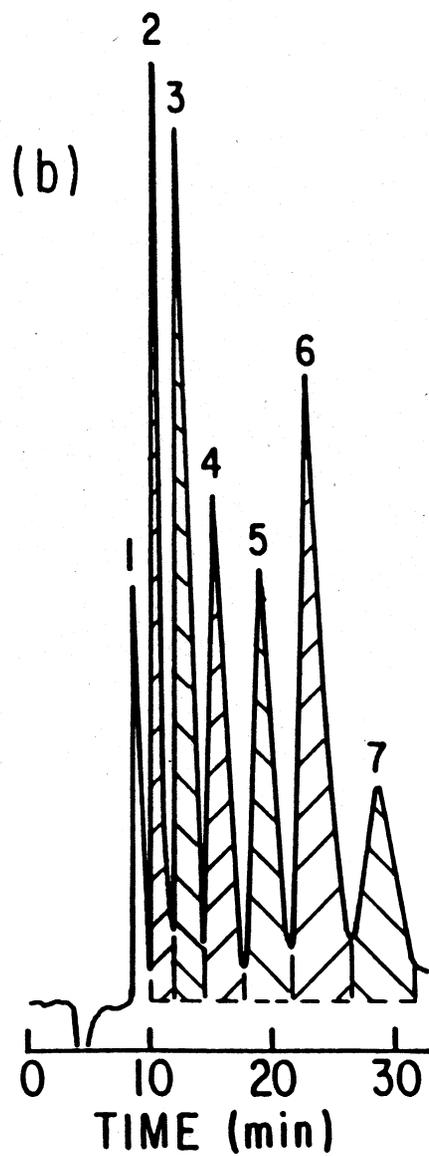
(all = 2 x 30 cm)

<u>Stationary phase</u>	<u>Cost</u>	<u>Mobile phase</u>
Zorbax NH ₂ (7μm)	≈\$2000.00	ACN / H ₂ O at 10-20 ml/min.
Dynamax NH ₂ (7μm)	≈\$450.00*	ACN / H ₂ O at 10-15 ml/min.
Dynamax C-18 (7μm)	≈\$450.00*	H ₂ O at 2 - 4 ml/min.

(a)



(b)



**COMPARISON OF NH₂ AND CER (Ag⁺) PHASES FOR
PREPARATIVE HPLC OF MALTO-OLIGOSACCHARIDES**

PHASE	SAMPLE SIZE (mg)	RUN TIME (min)	<u>ISOLATED OLIGOSACCHARIDES - M6 (% PURITY)</u>				
			DP 3	DP 4	DP 5	DP 6	DP 7
CER (Ag ⁺)	125/ 250 μL	55 (30)	23(88)	13(88)	11(88)	23(92)	-----
			(70 mg Total)				
NH ₂	500/ 2.0 mL	32 (50)	80(98)	48(95)	52(94)	100(96)	46(81)
			(326 mg Total)				

CELLODEXTRINS ISOLATED BY PREPARATIVE HPLC

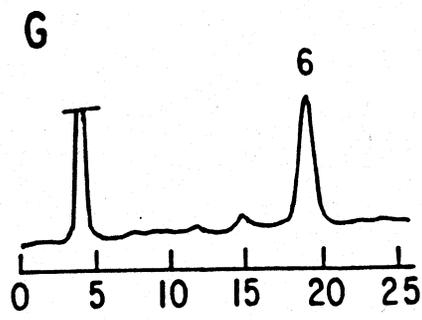
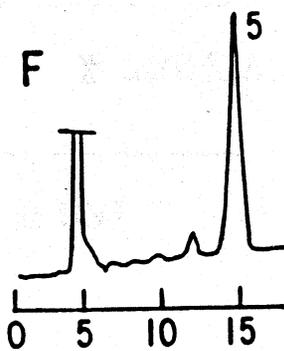
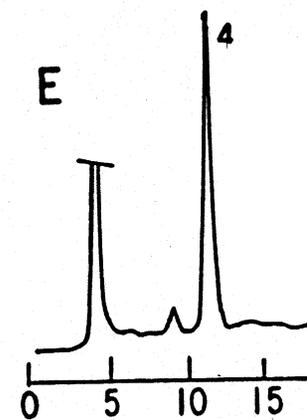
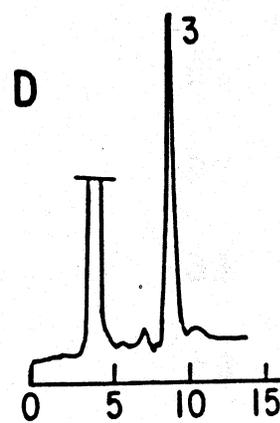
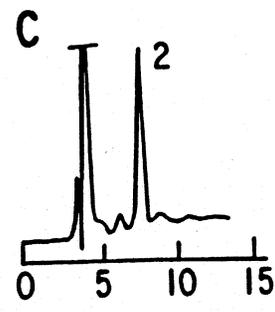
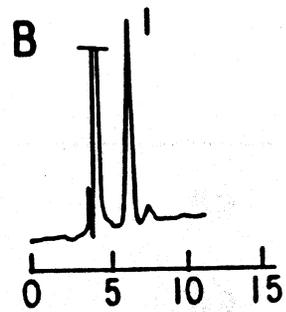
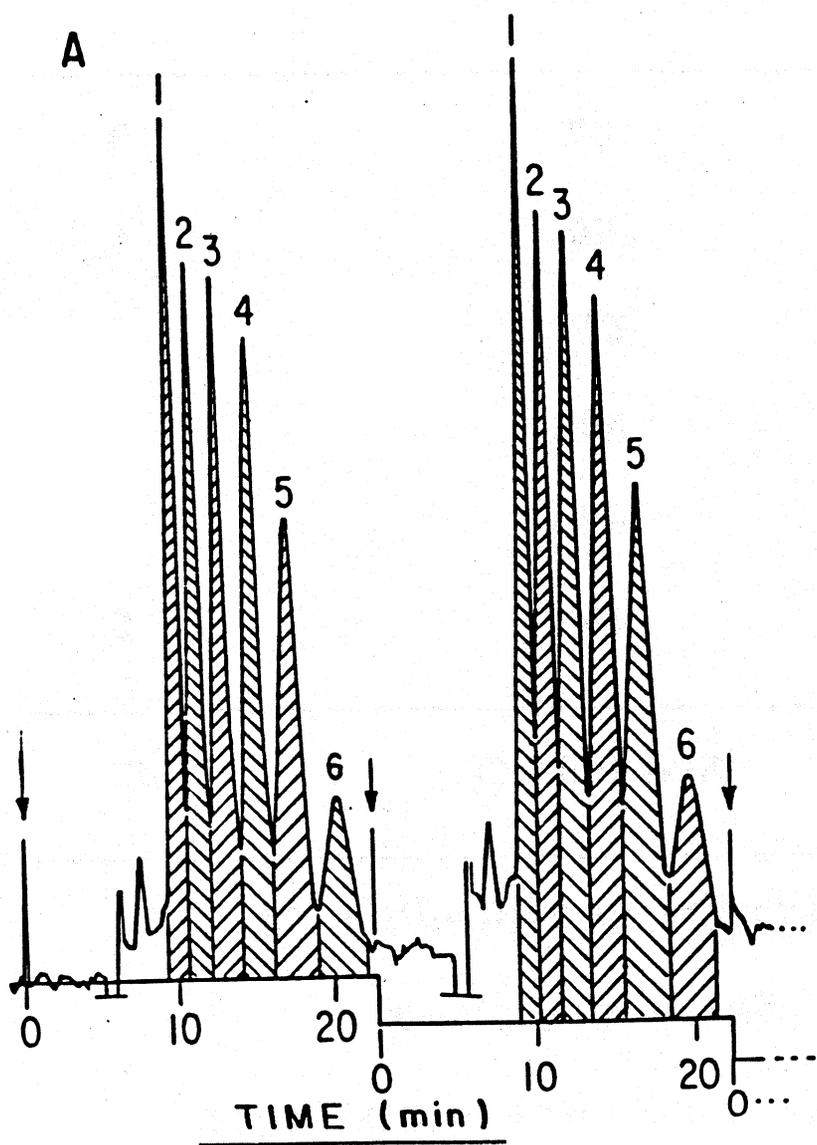
ON AG-50W-X4-Ag⁺ FORM

(29 AUTOMATED INJECTIONS IN 13.5 HR)

DP	WEIGHT (MG)	% PURITY
3	662	99
4	809	97
5	504	96
6	372	81
7	93	93
8	68	85

2,498 MG

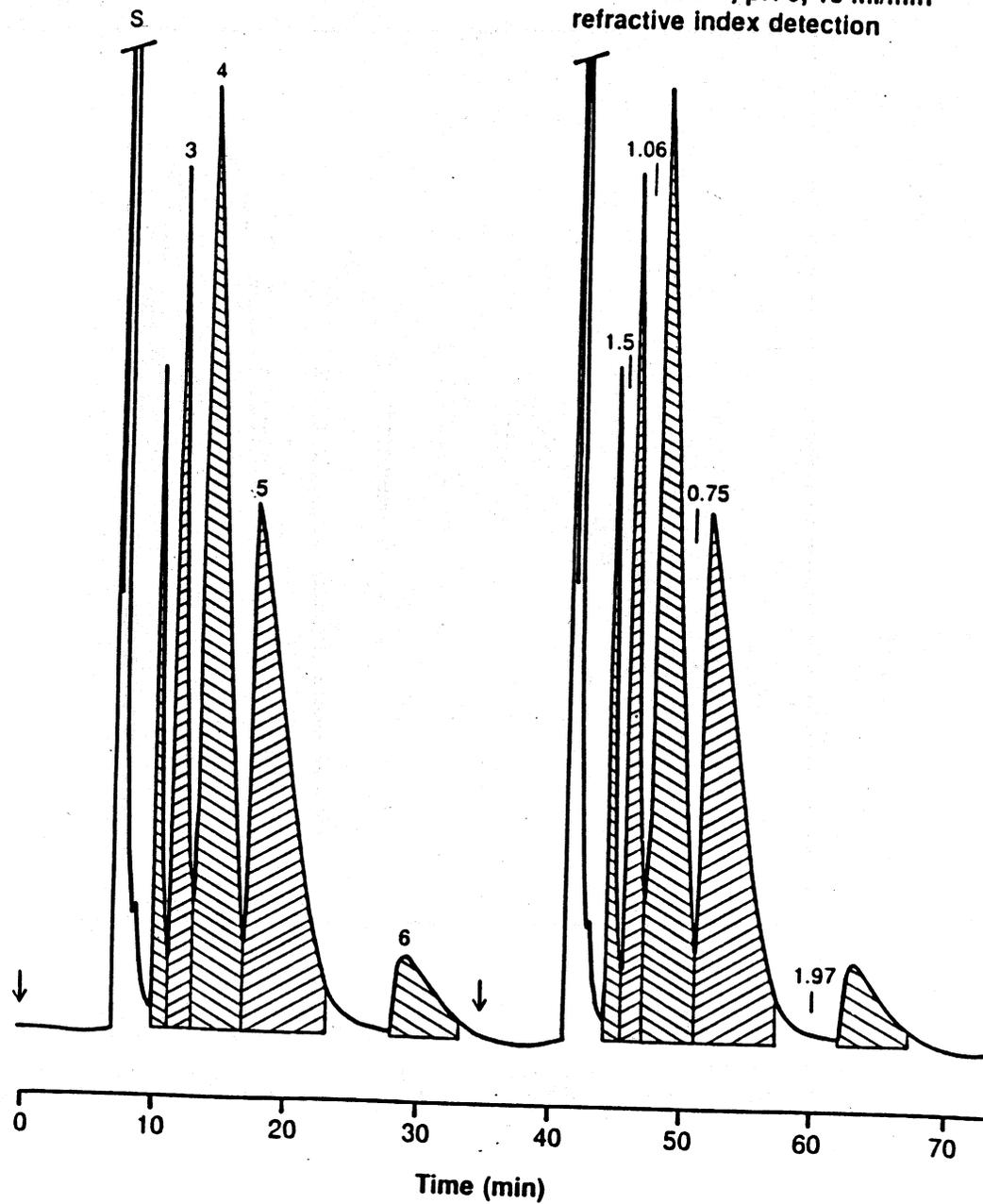
CHITIN OLIGOSACCHARIDE ISOLATION ON DYNAMAX-NH₂



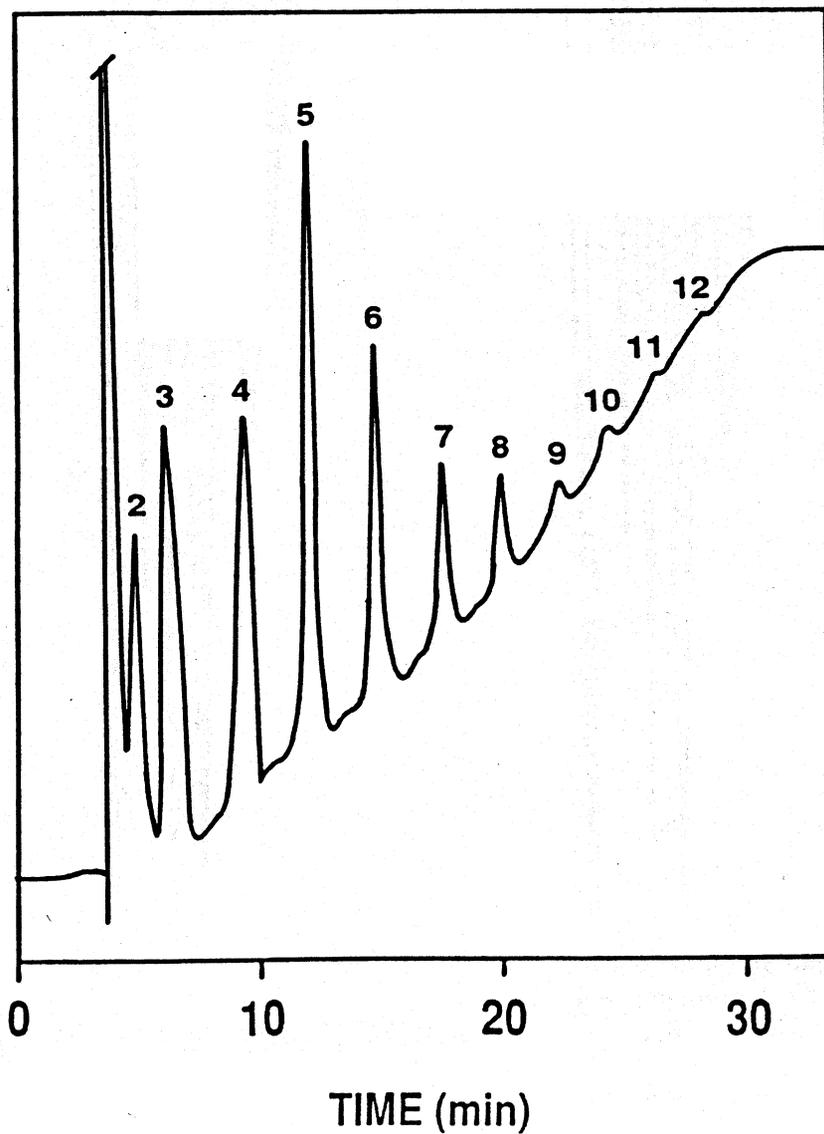
OLIGOGALACTURONIC ACIDS

Automated injections (375 mg)

Dynamax 60A, NH₂ (21.4 mm x 30 cm)
0.9 M acetate, pH 5, 10 ml/min
refractive index detection



PHOSPHATE BUFFER GRADIENT SEPARATION OF OLIGOGALACTURONIC ACIDS



Dynamax-60A, NH₂
analytical column
2 mg injected
0.1 - 0.4 M
pH 5.9, 25 min
gradient, 1 ml/min
mobile phase
UV absorption
(220 nm) detection

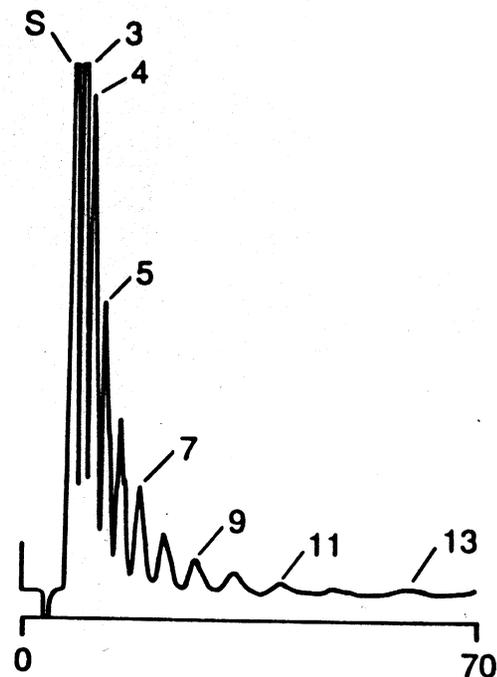
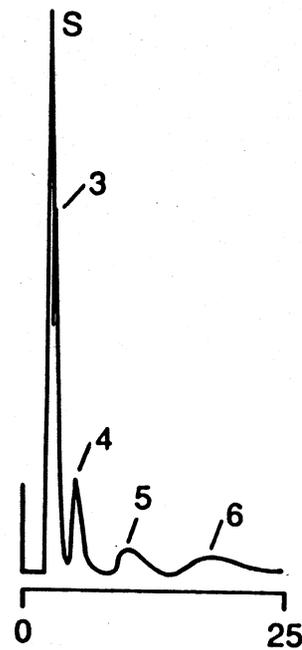
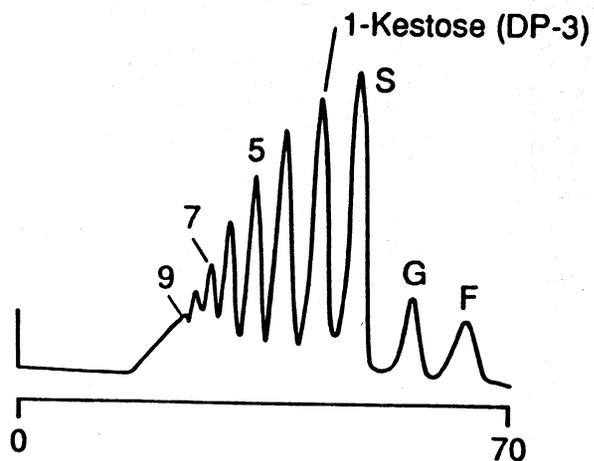
PREPARATIVE HPLC OF INULIN OLIGOSACCHARIDES (FROM JERUSALEM ARTICHOKE)

(ALL COLUMNS 2.0 X 25 OR 30CM)

Phase: AG-50W-X4-Ag⁺ 25uM
Sample: 1 ml (100mg)
Pressure: 5 Bar
Flow: 1ml/min; H₂O
Fractions: DP 3-8; 20-75mg
Purity:

Dynamax C₁₈
2 ml (200mg)
100 Bar
20 ml/min H₂O
DP 4-6; 250-900mg
>95%

Dynamax NH₂
2 ml (500mg)
50 Bar
15 ml/min ACN/H₂O
DP 3-12; 3-250mg
>90%

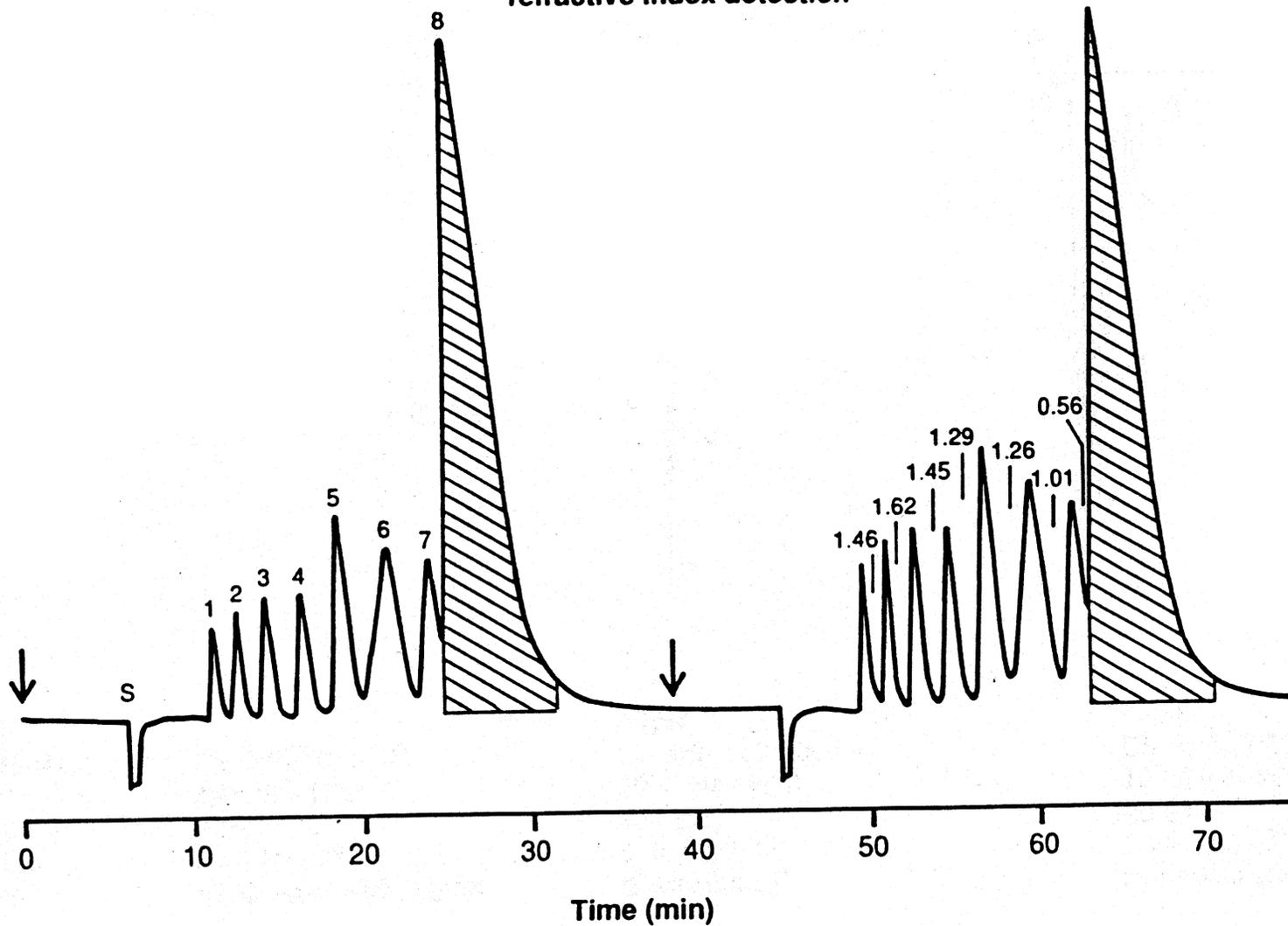


Time (min)

MALTODEXTRINS

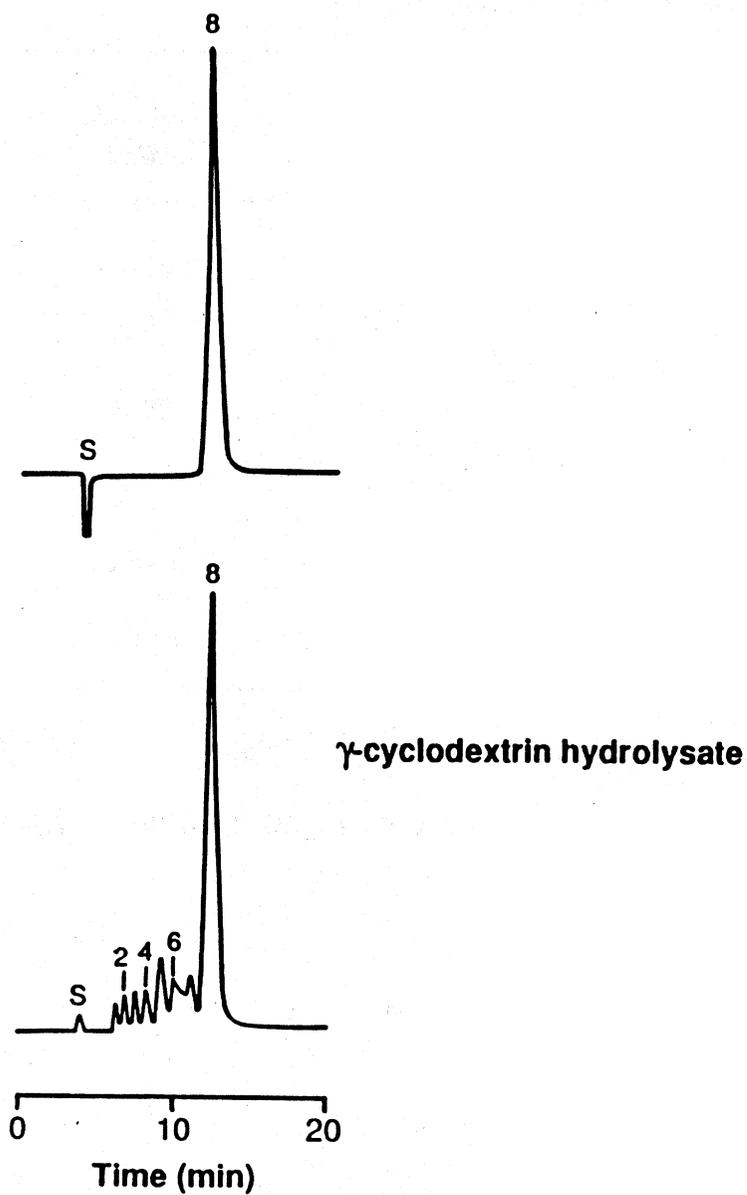
Manual injections (1.6 g, 1.75 g)

Dynamax 60A, NH2 (41.4 mm x 30cm)
50:50 Acetonitrile: H₂O, 40 ml/min
refractive index detection



**Purity of DP 8 gluco-oligosaccharide
isolated by preparative HPLC**

**Dynamax 60A, NH2 (4.6 mm x 30 cm)
50:50 Acetonitrile:H₂O, 0.75 ml/min
refractive index detection**



Malto-oligosaccharide isolation efficiency

<u>d.p.</u>	<u>prep. h.p.l.c. peak resolution</u>	<u>isol. rate (mg/h)</u>	<u>% purity (peak area)</u>
3 ^a	1.4	55.5	91.9
4 ^a	1.0	79.6	91.4
5 ^a	1.2	79.8	91.8
6 ^a	0.9	75.8	92.6
7 ^a	0.6	255.1	98.0
8 ^b		996.3	99.0

^aDynamax 60A, NH2 (21.4 mm x 30cm), 55:45 Acetonitrile:H₂O, 13 mL/min

^bDynamax 60A, NH2 (41.4 mm x 30cm), 50:50 Acetonitrile:H₂O, 40 mL/min

ANALYTICAL HPLC OF:

CYCLITOLS: *Carbohydr. Res.* 183 (1988) 1-9

OLIGOSACCHARIDES: (HPX-22H column) *J. Chromatogr.* 441 (1988) 382-386

OLIGOGALACTURONIC ACIDS: *Anal. Biochem.* 184 (1990) 200-206

PREPARATIVE HPLC OF:

MALTO-OLIGOSACCHARIDES: *J. Chromatogr.*, 389 (1987) 183-194,
for submission to Carbohydr. Res.

MONO- AND DISACCHARIDES: *Carbohydr. Res.*, 168 (1987) 33-45

CHITIN OLIGOSACCHARIDES: *Methods in Enzymology*, 161 (1988) 410-416

OLIGOGALACTURONIC ACIDS: *Carbohydr. Res.*, 215 (1991) 81-90

INULIN OLIGOSACCHARIDES: *for submission to Carbohydr. Res.*

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