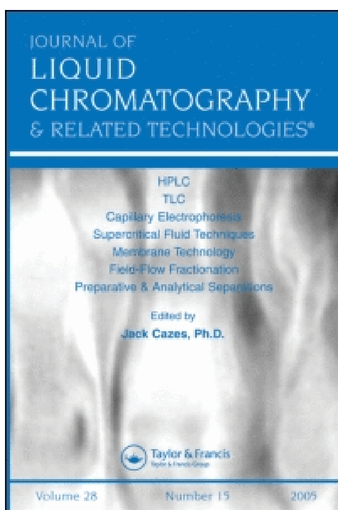
This article was downloaded by:
On: *24 January 2011*
Access details: *Access Details: Free Access*
Publisher *Taylor & Francis*
Informa Ltd Registered in England and Wales Registered Number: 1072954 Registered office: Mortimer House, 37-41 Mortimer Street, London W1T 3JH, UK



Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

Differential Binding of Sugars and Polyhydric Alcohols to Ion Exchange Resins: Inappropriateness for Quantitative HPLC

John G. Baust[a]; Richard. E. Lee Jr.[a]; Howie James[b]
[a] Department of Biology, University of Houston, Houston, TX [b] Waters Associates Milford, MA

To cite this Article Baust, John G. , Lee Jr., Richard. E. and James, Howie(1982) 'Differential Binding of Sugars and Polyhydric Alcohols to Ion Exchange Resins: Inappropriateness for Quantitative HPLC', Journal of Liquid Chromatography & Related Technologies, 5: 4, 767 — 779

To link to this Article: DOI: 10.1080/01483918208060585

URL: http://dx.doi.org/10.1080/01483918208060585

PLEASE SCROLL DOWN FOR ARTICLE

Full terms and conditions of use: http://www.informaworld.com/terms-and-conditions-of-access.pdf

This article may be used for research, teaching and private study purposes. Any substantial or systematic reproduction, re-distribution, re-selling, loan or sub-licensing, systematic supply or distribution in any form to anyone is expressly forbidden.

The publisher does not give any warranty express or implied or make any representation that the contents will be complete or accurate or up to date. The accuracy of any instructions, formulae and drug doses should be independently verified with primary sources. The publisher shall not be liable for any loss, actions, claims, proceedings, demand or costs or damages whatsoever or howsoever caused arising directly or indirectly in connection with or arising out of the use of this material.

JOURNAL OF LIQUID CHROMATOGRAPHY, 5(4), 767-779 (1982)

DIFFERENTIAL BINDING OF SUGARS AND POLYHYDRIC ALCOHOLS TO ION EXCHANGE RESINS: INAPPROPRIATENESS FOR QUANTITATIVE HPLC

John G. Baust and Richard E. Lee, Jr.
Department of Biology
University of Houston
Houston, TX 77004

and

Howie James
Waters Associates
Milford, MA 01757

Downloaded At: 18:09 24 January 2011

ABSTRACT

The use of hydroxyl exchange resins (Dowex AG 1-X8 and AG 501-X8) as a component in the preparative clean up of biological samples for HPLC sugar and polyhydric alcohol analysis is inappropriate. On a weight specific basis, these resins bind 95-100% of monosaccharides (fructose and glucose), 45-85% of disaccharides (sucrose and trehalose) and 15-50% of polyhydric alcohols (glycerol and adonitol) present in sample solutions.

INTRODUCTION

Strongly basic ion exchange resins such as Dowex-1X8(OH^-) have been utilized for the selective binding of monosaccharides and presumed separation of alditols (1,2,3). Recently, it was demonstrated (4) that aldi-

Copyright © 1982 by Marcel Dekker, Inc.

tols are also selectively bound by this and other cationic exchange resins. Attempts to completely elute glucose and alditols by effecting the partitioning characteristics of the column with distilled water (up to 20 column volumes) were only partially successful.

Both the qualitative and quantitative analysis of these saccharides are facilitated by HPLC (5). However, the use of HPLC for carbohydrate analysis of biological extracts (plasma and animal/plant tissue extracts) is severely compromised by competitive binding of proteinaceous, lipid and divalent inorganic ion contaminants. Even trace levels of these contaminants may result in appreciable loss in either reverse phase (carbohydrate analysis column-Waters) or ion exchange columns (Bio Rad HPX-87) (column life= 15-50 injections). Sample preparation of biologics must therefore ensure the removal of each category of contaminant. Lipid extraction and partial deproteinization can be routinely accomplished with both solvent (i.e., chloroform:methanol) and heat (60-100°C) treatment. Complete deproteinization however requires the addition of Zn^{++} and Ba^{++} salts. Therefore, the levels of divalent cations are increased over already high endogenous levels (i.e., plasma: Ca^{++}= 9-11 mg/dl and Mg^{++}= 1-3 mg/dl). Removal of disruptively high levels of cations can be simply and efficiently accomplished by sample pretreatment with an anionic exchanger [Dowex -1X8 (H^{+})]. To avoid complexing pH changes, mixed bed resins [Dowex AG -501-X8 (50% OH^{-}:50% H^{+})] are routinely used in the purification of biological samples (6).

This paper describes the degree of differential binding by a strongly basic cation exchanger (Dowex

Downloaded At: 18:09 24 January 2011

AG-1X8 (OH^-) of polyhydric alcohols, mono- and disaccharides as effected by time and resin volume.

MATERIALS & METHODS

A Waters Model 6000A pump, Model 710B WISP automated injection system and Model R401 differential refractometer were used in conjunction with a Waters Model 730 Date Module. A Radial pak silica cartridge (10cm x 8mm ID) (10u particle size) was employed in a RCM-100 radial compression module to effect carbohydrate separation. The cartridge was initially conditioned by pumping 50 ml acetonitrile: water (70:30) containing 0.1% (v/v) tetraethylenepentamine (TEPA) (pH 9.2) at 2.0 ml/min. Following conditioning, a recirculating eluent of acetonitrile: water (81:19) containing 0.02% TEPA (pH 8.9) was introduced to the cartridge and stabilization allowed to proceed overnight. Carbohydrate and polyhydric alcohol standards were dissolved in distilled water (5mg/ml) and injected (50ul) at 26°C. A flow rate of 2.0 ml/min generated a back pressure of 300-400 psi. Acetonitrile was Fisher HPLC grade (Pittsburg, PA). Technical grade TEPA was obtained from Eastman Chemicals (Rochester, N.Y.), and carbohydrates and polyhydric alcohols from Sigma Chemical (St. Louis, Mo.). The water phase of the eluent was prepared by deionization and glass distillation. Eluents were degassed and filtered through Millipore 0.22um filter (GSWP) (Bedford, Ma.). Ion exchange resins are listed in Table I.

A 0.65 ml sample of the seven component standard (Table 2) was exposed to each resin (0-300 mg) for

Downloaded At: 18:09 24 January 2011

TABLE I

Ion Exchange Resins

Nomenclature	Type	Source
AG 1-X8 (OH^-)	anion exchanger (20-50 mesh)	Bio Rad
AG 50-X8 (H^+, OH^-)	mixed bed exchanger (20-50 mesh)	Bio Rad
Dowex 1-X8-400 (Cl^-)	anion exchanger (200-400 mesh)	Sigma
Dowex 50-X8-400 (Cl^-)	cation exchanger (200-400 mesh)	Sigma
CX/Corasil Bondapak	cation exchanger	Waters
AX/Corasil Bondapak	anion exchanger	Waters

Downloaded At: 18:09 24 January 2011

TABLE II

Retention Time of Standards

Order	Component	Retention Time (min)
1	Water	1.95
2	Ethylene Glycol	2.83
3	Glycerol	3.75
4	Adonitol (Ribitol)	6.33
5	Fructose	7.29
6	Glucose	9.29
7	Sucrose	14.70
8	Trehalose	20.37

periods of 3-120 min, with or without vortexing (15 sec). Samples were then filtered (0.45um) (HAWP), degassed in a vacuum for 5 min and injected. All samples were run in duplicate with mean values plotted. Reproducibility of duplicate samples was less than $\pm 2\%$.

RESULTS & DISCUSSIONS

Separation of polyhydric alcohol-carbohydrate mixtures at pH 8.9 is illustrated in Figure 1. Elution times ranged between 2.8 and 20.4 min and separation appeared related to carbon number (Table 2). Addition of 75 mg AG 1-X8 (OH^-) for 10 min resulted in the illustrated decreased amplitude (concentration) of each component. Attempts to elute the components by successive distilled water washes did not result in uniform recovery (4).

The time dependent binding of simple carbohydrates and polyhydric alcohols to the hydroxyl resin AG 1-X8 is illustrated in Figure 2. Polyols were not as effectively bound (∿15-25%) as monosaccharides (75-85%). Disaccharides demonstrated a wide range of binding. Trehalose, a 1-a-D-glucopyranosyl-a-D-glucopyranoside, and sucrose, a 1-a-D-glucopyranosyl-B-D-fructofuranoside, are both non-reducing sugars and lack the properties characteristic of the free sugar group. The similarities in overall reactiveness between these two sugars would suggest similar binding capacities. This however was not the case. After 2 min exposure to the resin, nearly 70% of the sucrose was bound but only 25% of the trehalose. The binding

Downloaded At: 18:09 24 January 2011

Downloaded At: 18:09 24 January 2011

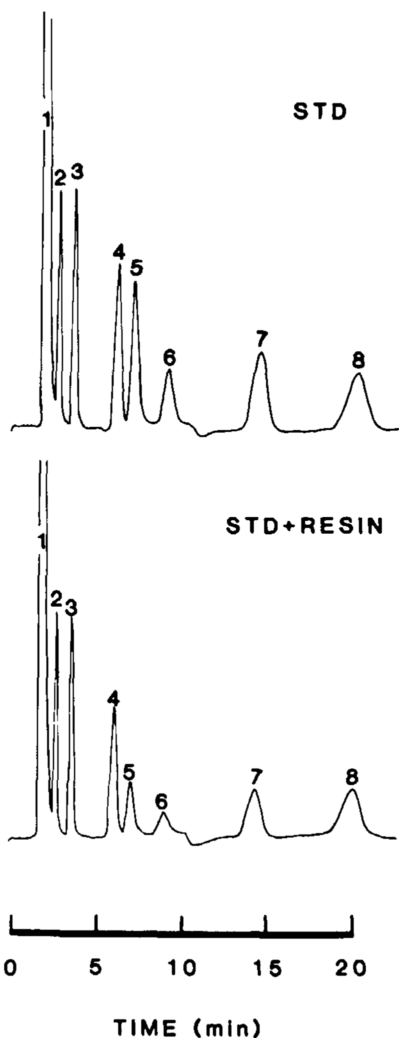


FIGURE 1. Effect of treatment with AG1-X8 Anion Exchange Resin (hydroxyl form) on polyol/saccharide recovery. Chromatogram represent polyol/saccharide separation on a hydrostatically compressed 10μ Radial-pak silica cartridge (10cm X 8mm I.D.). Elution solvent: Acetonitrile-water (81:19), pH 9.2, modified with 0.02% TEPA, flow rate = 2.0m/min., 300-450 psi. Top: Untreated standard solution. Bottom: 0.65ml of standard solution treated with 75mg of resin for 10 min. 1 = water; 2 = ethylene glycol; 3 = glycerol; 4 = adonitol; 5 = fructose; 6 = glucose; 7 = sucrose; 8 = trehalose.

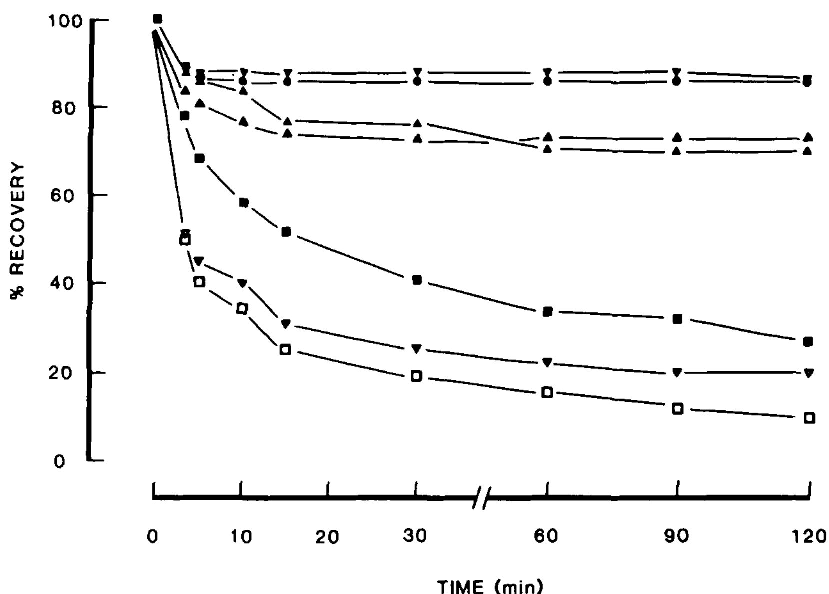


FIGURE 2. Effect of exposure time in the presence of *AG1-X8 Anion Exchange Resin (hydroxyl form) on the* recovery of polyol/saccharide standard mixture. ▼ = ethylene glycol; ● = glycerol; ▲ = adonitol; □ = fructose; ▼ glucose; ■ = sucrose; ▲ = trehalose.

Downloaded At: 18:09 24 January 2011

characteristics of trehalose were similar to those of the polyols and sucrose to those of the monosaccharides.

Vortexing enhanced sample removal from the test solution. Rates of binding of saccharides and polyols were maximized within 1-5 minutes following brief vortexing (0.25 or 1.0 min.) (Figure 3). Equilibrium binding occurred more rapidly than would be predicted by simple probabilistic considerations.

Per cent recoveries were also lower for all components. The integrity of the binding differential observed between the non-reducing disaccharides (Δ= 42-52%) and trehalose:ethylene glycol (Δ=18-20%) suggests that

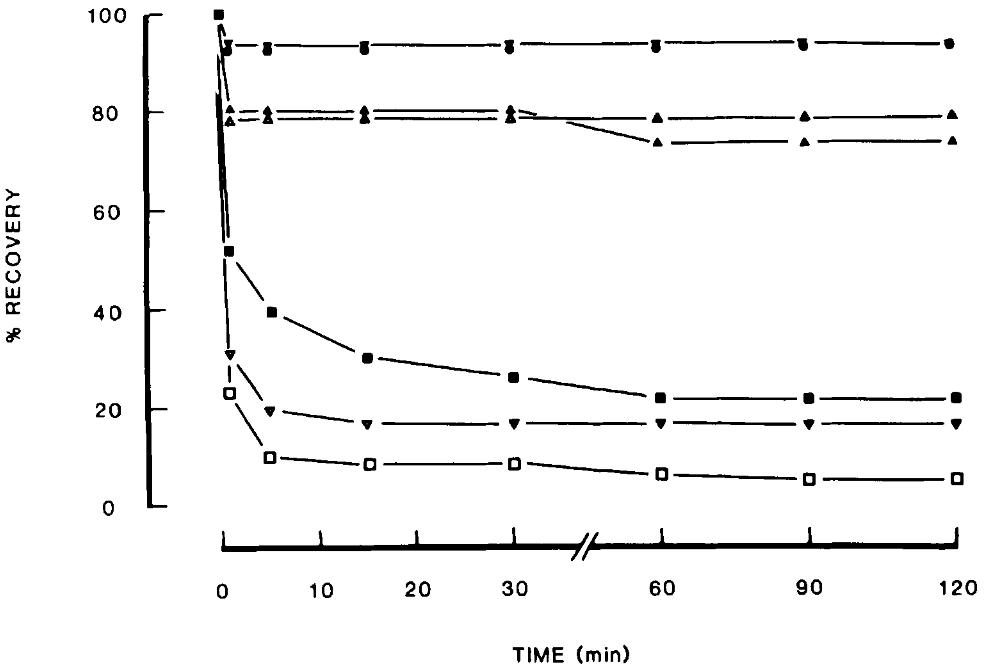


FIGURE 3. Effect of variable shaking time (240 cycles/min.) on recovery of polyols and saccharides treated with 75 mg of AGI-X8 Anion Exchange Resin (hydroxyl form). ▼= ethylene glycol; ●= glycerol; △ = adonitol; □= fructose; ▽= glucose; ■= sucrose; ▲= trehalose.

Downloaded At: 18:09 24 January 2011

molecular density, solubility or exchange site spacing are not critical determinants of binding.

Incorporation of a cationic exchanger (H^+) with an anionic exchanger (OH^-) (Dowex AG 501-X8) (150mg) did not result in a modification of sample recovery as compared to the use of the anion exchanger only (Figure 2). Also, the use of a cationic exchanger only (Dowex 50W) (H^+) resulted in 100% sample recovery. Accordingly, only the anionic species participated in binding.

In addition to time, sample binding is dependent on resin weight (Figure 4). Interestingly, however, 100% binding (0% recovery) was encountered only for glucose in the shaken samples. Shaking for 10 minutes augmented the separation on a weight specific basis (Figure 5). However, complete binding was not observed except for glucose, fructose and sucrose. Since only 75% of trehalose was bound as compared to sucrose, binding site saturation does not appear to be the explanation for this observation. Differential binding appears due to affinity relationships

Downloaded At: 18:09 24 January 2011

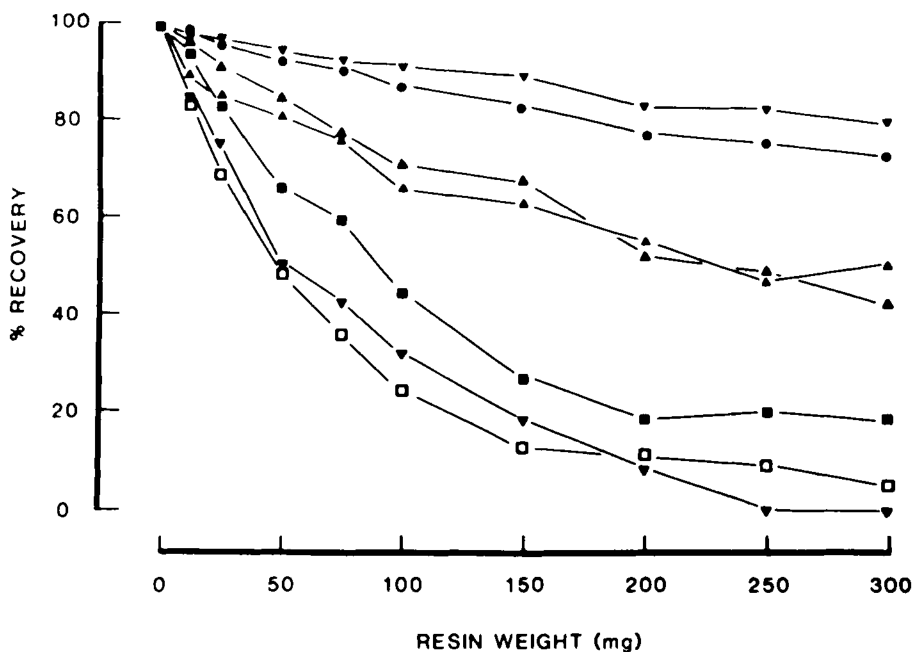


FIGURE 4. Effect of treatment with varying weights of AG1-X8 Anion Exchange Resin (hydroxyl form) on the recovery of polyol/saccharide standard mixture. ▼ = ethylene glycol; ● = glycerol; △ = adonitol; □ = fructose; ▽ = glucose; ■ = sucrose; ▲ = trehalose.

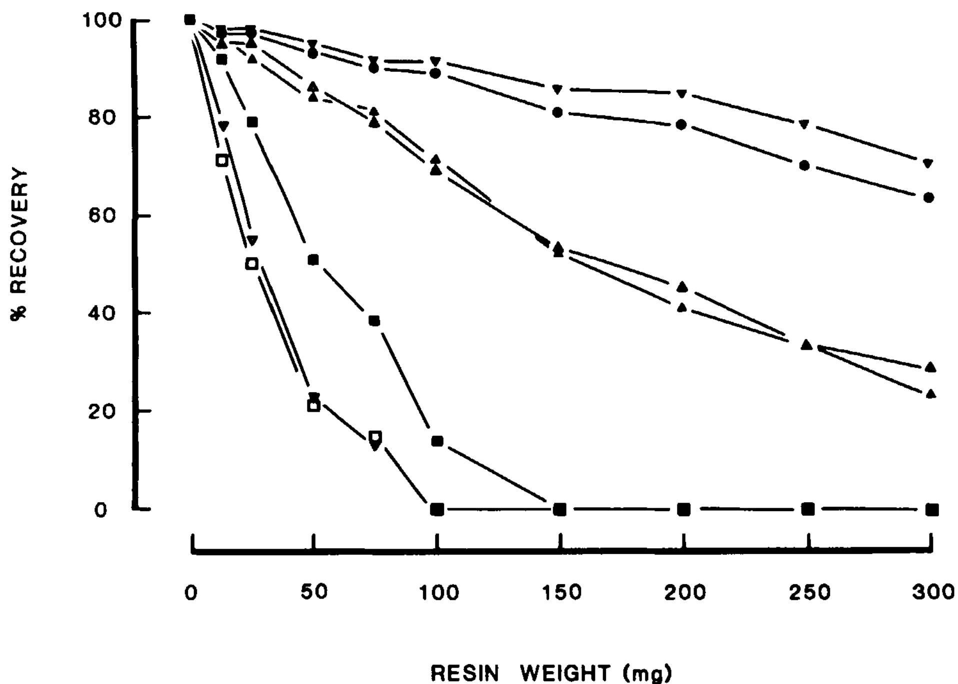


FIGURE 5. Effect of treatment with varying weights of AG1-X8 Anion Exchange Resin (hydroxyl form) on the recovery of polyol/saccharide standard mixture after shaking (240 cycles/min.) for 10 min. ▼= ethylene glycol; ●= glycerol; △= adonitol; □= fructose; ▽= glucose; ■= sucrose; ▲= trehalose.

Downloaded At: 18:09 24 January 2011

between resin and carbohydrate. The comparatively high recovery of glycerol ("1/2 glucose") when considered with respect to the overall binding profile further supports this statement. Simple saturation kinetics would suggest that at resin concentrations that completely bind glucose (150mg), 50% of the glycerol ought to be bound and at a resin concentration of 300mg, 100% of the glycerol would be bound. At

these concentrations, only 19% and 37% of the glycerol was bound.

The possibility of differential binding being matrix dependent was considered. Figure 6 illustrates that recovery from a seven component test standard was solute specific and not dependent on matrix effects.

The use of hydroxyl ion exchangers is not recommended during the preparative phase of sample clean up for HPLC analysis of polyhydroxy compounds due to the pronounced differential binding. In addition, sample handling is made more complex by the

Downloaded At: 18:09 24 January 2011

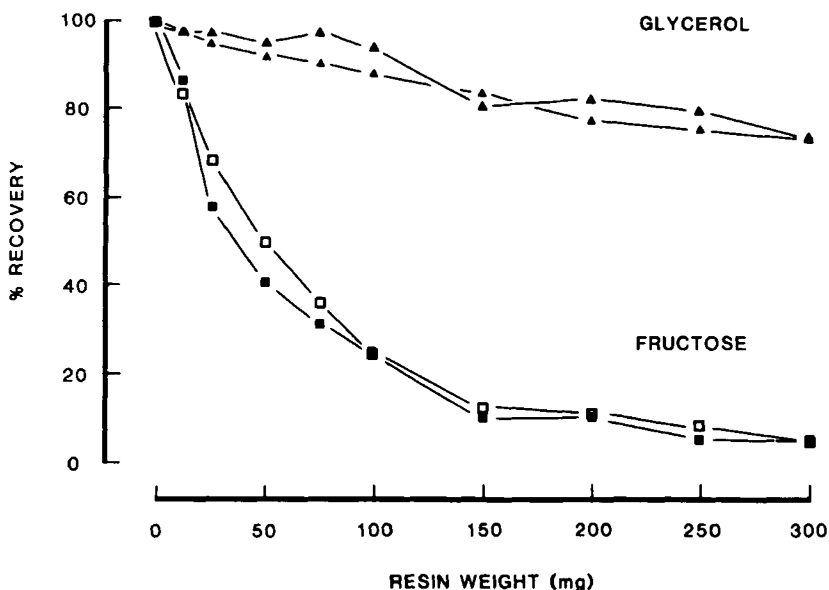


FIGURE 6. Matrix effects: Recovery of glycerol and fructose in single versus multi-solute solutions after treatment with AG1-X8 Anion Exchange Resin (hydroxyl form). Open symbol for single solute solutions; solid symbol for multi-solute solutions.

specific time/weight dependencies (exposures). The use of cationic exchangers or chloride ion anion exchangers (Table) did not effect the recovery of carbohydrates or polyhydric alcohols.

ACKNOWLEDGMENTS

This work was supported in part by NSF grants PMC 78-09589 and DPP 78-21116 and by Waters Associates, Milford, Mass.

Downloaded At: 18:09 24 January 2011

REFERENCES

1. Yamaguchi, H., Namura, S.I., and Makino, K., Studies on a microchemical method for the determination of the degree of polymerization of neutral oligo- and polysaccharides. J. Biochem. (Tokyo), 79, 299 (1976).

2. Yamaguchi, H. and Makino, K., Studies on a microchemical method for determination of the degree of polymerization of neutral oligo- and polysaccharides. J. Biochem. (Tokyo), 81, 563 (1977).

3. Yamaguchi, H. and Okamoto, K., Structural studies on the reducing -end sugar residues of polysaccharides by GLC and mass spectrometry. J. Biochem. (Tokyo), 82, 511 (1977).

4. Tanaka, M., Binding of alditols to the hydroxyl form of Dowex-1X8, a strongly basic ion exchange resin: an improved method for estimation of the degree of polymerization of neutral oligo- saccharides and polysaccharides and polysaccharides. Carboh. Res., 88, 1 (1981).

5. Hendrix, D.L., Lee, Jr., R.E., Baust, J.G. and James, H., Separation of carbohydrates and polyols by radially compressed high-performance liquid chromatographic silica column modified with tetraethylene pentamine. J. Chromat., 210, 45 (1981).

6. Baust, J.G., and Edwards, J.S., Mechanisms of freezing tolerance in an antarctic midge, *Belgica antarctica*. Physiol. Entomol., 4, 1 (1979).

Downloaded At: 18:09 24 January 2011