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The use of solid-phase extraction with graphitised carbon for the fractionation and purification of sugars

John W. Redmond, Nicolle H. Packer *

Macquarie University Centre for Analytical Biotechnology, Macquarie University, North Ryde, NSW 2109, Australia

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Abstract

The unique selectivity, high preparative capacity and chemical inertness of graphitised carbon make it an ideal medium for the solid-phase extraction (SPE) of sugars from dilute solution, and for their analytical and preparative separation from salt, alkali or mineral acid. Graded elution with water containing an organic modifier (such as an alcohol or acetonitrile) permits the separation of groups of oligosaccharides. Acidic sugars are retained by graphitised carbon, while comparable neutral and amino sugars are eluted by water containing an organic modifier; the acidic components are then eluted by the same eluant containing trifluoroacetic acid. As such, graphitised carbon presents a much-needed solid-phase packing for the general clean-up and separation of sugars. © 1999 Elsevier Science Ltd. All rights reserved.

Keywords: Chromatography; Graphitised carbon; Solid-phase extraction

1. Introduction

Activated carbon (charcoal) has long been used for the preparative chromatographic fractionation of mixtures of oligosaccharides obtained by partial acid hydrolysis of polysaccharides [1–4]. In this procedure, an acid hydrolysate of a polysaccharide is neutralized and applied to a column of activated carbon, typically dispersed in diatomaceous earth to improve its flow characteristics, and the oligosaccharides are recovered, in order of increasing size, by batch elution with water containing increasing proportions of ethanol as organic modifier [1]. With the availability of a graphitised carbon adsorbent, this mode of separation has now been adapted to analytical high-performance liquid chromatography (HPLC) of sugars [5–8], typically using acetonitrile as organic modifier. The oligosaccharides are eluted in order of increasing size [6], as with the older charcoal adsorbent, and acidic sugars are retained more than neutral compounds [5,9]. We describe here the use of graphitised carbon for preparative procedures, such as the solid-phase extraction (SPE) of oligosaccharides from dilute solutions, the removal of salt and the fractionation of mixtures of sugars on the basis of size or ionic character. The technique provides a solution to many traditional difficulties in the isolation and purification of sugars and can be used in a wide range of applications in the field of carbohydrate chemistry. This paper describes some of these applications using standard sugars to demonstrate the potential of this simple procedure.

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* Corresponding author. Present address: Proteome Systems Ltd, Locked Bag 2073, North Ryde, 1670, Sydney, NSW, Australia. Fax: + 61-2-9889-1830.

E-mail address: nicolle.packer@proteomesystems.com (N.H. Packer)

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2. Results and discussion

The graphitised carbon SPE of oligosaccharides from aqueous solutions was illustrated by applying an aqueous solution of glucose, glucosamine HCl, maltose and raffinose to a graphitised carbon cartridge, followed by rinsing with water. Elution with acetonitrile–water gave recoveries of 0, 0, 97 and 99% glucose, glucosamine HCl, maltose and raffinose, respectively, indicating that maltose and raffinose are retained by the cartridge, while glucose is not. This outcome is consistent with previous observations [1–4] that glucose is eluted from activated carbon with water, whereas glucose oligosaccharides require the addition of an organic modifier. The retention of maltose by graphitised carbon is marginal: when applied to a 0.5 mL cartridge in a volume of up to 5 mL, it was effectively retained, while the application in larger volumes of water led to reduced recoveries. Larger oligosaccharides were retained, even on extensive washing with water. As an example, when 100 mL of water containing 1 ppm (1 μg mL^{-1}) of raffinose was percolated through a 0.5 mL graphitised carbon cartridge, 98% of the sugar was retained and recovered by elution with water containing acetonitrile. This demonstrated the effectiveness of graphitised carbon SPE for the concentration and isolation of small amounts of sugars of DP of 3 or more from mixed solutions.

The potential for graphitised carbon SPE for purification on a preparative scale was demonstrated by application of a swamping amount of raffinose (200 mg) to a 0.5 mL graphitised carbon cartridge, which contained 150 mg of packing. The recovery of 41 mg of raffinose on elution with acetonitrile–water was consistent with the high loading reported for activated carbon [1–4] and demonstrated the feasibility of preparative separations of sugars with limited amounts of the carbon adsorbent. This was illustrated by the preparative fractionation of mixtures of oligosaccharides in a partial hydrolysate of dextran [10]. Such oligosaccharides are typically used to calibrate size-exclusion separations of glycans from glycoproteins [10]. Fractions containing specified higher oligosaccharides are presently very expensive but, using SPE, they are now very simple to prepare. The dextran hydrolysate was diluted with water and applied to an SPE cartridge without neutralization. The oligosaccharides were then separated into groups by batch elution with water containing graded amounts of 1-butanol as an organic modifier (Fig. 1). All the oligosaccharides in the unfractionated hydrolysate, up to a DP of at least 24, were present in the eluted fractions

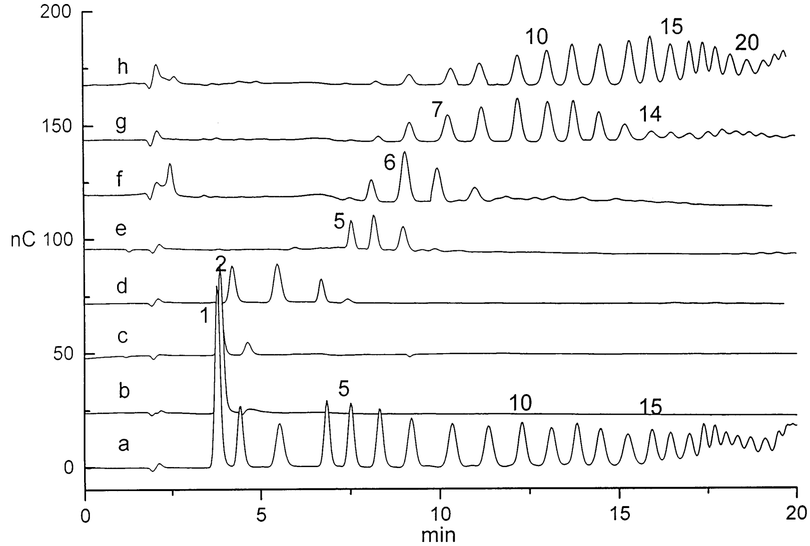

Fig. 1. Preparative fractionation of a partial hydrolysate of dextran using batch elution from a Carbograph cartridge with dilutions of BSW. (a) Unfractionated hydrolysate; (b) unretained sugars; (c–h) fractions obtained by elution with 5 mL of BSW/32, BSW/16, BSW/8, BSW/4, BSW/2 and BSW, respectively. The numbers refer to DP of selected oligosaccharides. Analysis was by HPAEC using a gradient of 0–400 mM NaOAc in 100 mM NaOH over 25 min and a flow rate of 1 mL min^{-1}.

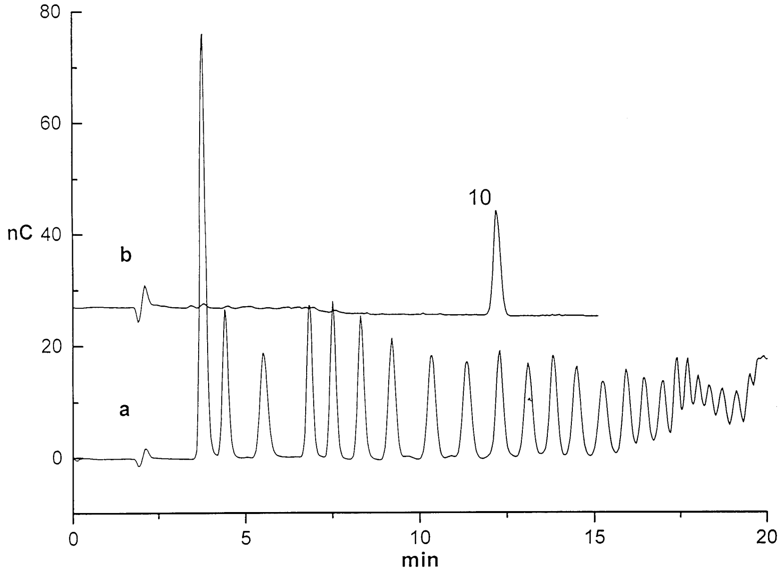

Fig. 2. Preparative isolation of a pure oligosaccharide from a partial hydrolysate of dextran using HPAEC and SPE on a Carbograph cartridge. (a) Unfractionated hydrolysate; (b) oligosaccharide (DP 10) obtained from a collected fraction. Conditions as in Fig. 1.

as judged by HPAEC of the eluent. Butanol was chosen for this application as it is an effective organic modifier at low concentrations, and the eluants therefore do not have a depressing effect on the solubility of the larger oligosaccharides. In general, other modifiers, such as methanol, acetonitrile, propanol or acetone, can be used for such procedures.

The use of graphitised carbon SPE for the fractionation of the dextran oligosaccharides in the presence of acid also demonstrates a more general use of graphitised carbon for the removal of a non-volatile acid (such as hydrochloric or sulfuric acid) from oligosaccharides, without the need for tedious neutralization and desalting. In view of its high chemical stability, graphitised carbon is also particularly useful for the removal of alkali and acetate from fractions obtained from preparative high-performance anion-exchange chromatography (HPAEC), which remains the method of choice for high-resolution analytical and semi-preparative separations of sugars. An alkaline fraction collected from this procedure can now be neutralized with acid and submitted to desalting on graphitised carbon. More conveniently, the alkaline fraction can be applied directly to the adsorbent and the alkali removed by elution with water, followed by a trace of dilute volatile acid such as trifluoroacetic acid, to suppress any remaining charge on the adsorbed sugar. The salt-free sugar can then be eluted as before. The success of this approach is illustrated by the preparative purification of a homogeneous dextran decasaccharide present in a hydrolysate (Fig. 2).

It was possible to carry out graphitised carbon SPE separation and desalting in a single step. For example, graphitised carbon SPE of maltose and raffinose dissolved in 0.1 M sodium chloride, acetate or phosphate gave the same recoveries as in water. We report elsewhere [11] the successful exploitation of graphitised carbon for both batch and in-line removal of salt and other contaminants, such as protein and detergents, in the chromatographic and mass spectrometric analysis of glycans released from glycoproteins and proteoglycans.

The mode of interaction of solutes with graphitised carbon is complex, and is sensitive to both molecular shape and charge [5]. The stronger adsorption of acidic sugars [5,7], relative to comparable neutral and basic sugars, provides an alternative to anion-exchange chromatography for the preparative fractionation of neutral and acidic oligosaccharides; the neutral species are first eluted with water containing an organic modifier, while the acidic species, including phosphorylated and sulfated monosaccharides, are eluted with a similar eluant containing a volatile acid, such as trifluoroacetic acid. This was illustrated by the separation of lactose and neuraminyllactose (Fig. 3) in which the neutral form is easily separated from the sialylated form of lactose by a single-step elution from the graphitised carbon, as monitored by HPAEC. An important practical advantage of this procedure over anion-exchange chromatography [7] is that the sugars are isolated without contaminating salt. Alternatively, if it is necessary to use ion-exchange chromatography, desalting of the fractions collected from the ion-exchange column can be effected with graphitised carbon.

These are only a few examples demonstrating the scope of the application of graphitised carbon SPE to the separation and purification of sugars from solution using a simple, quick and cheap adsorbent and volatile eluants. Individual applications will require optimization of the conditions (e.g., the clean-up of glycans released from glycoproteins [11] and derivatised [12]), but will involve the same basic principles as described in this paper.

3. Experimental

Materials.—Carbograph (non-porous graphitised carbon) cartridges (150 mg, 0.5 mL) were obtained from Alltech Associates, and conditioned before use by washing with acetonitrile or MeOH (1.5 mL), followed by water (3 × 1.5 mL). HPLC-grade solvents were from Ajax Chemicals (Auburn, Australia), Dextran 500 from Pharmacia (Uppsala, Sweden) and all other chemicals from Sigma Chemical Co (St Louis, MO, USA). Butanol-saturated water (BSW), which contains approx 8% butanol, was the lower layer obtained after shaking 1-butanol (15 mL) with MilliQ water (100 mL). Serial dilutions, such as BSW/2 and BSW/4, were prepared by dilution of BSW with MilliQ water.

High-performance anion-exchange chromatography.—HPAEC was carried out on a DX500 system (Dionex, Sunnyvale, CA, USA), fitted with a Dionex PA1 guard column and analytical column (250 × 4 mm) and eluted at 1 mL min^{-1}, either isocratically with 100 mM NaOH (procedure A) or with a gradient of 0–400 mM NaOAc in 250 mM NaOH over 25 min (procedure B).

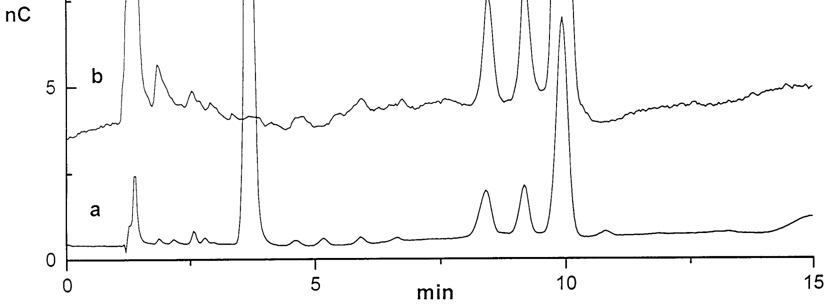

Fig. 3. Separation of lactose and neuraminyllactose by batch elution from a Carbograph cartridge. (a) Fraction eluted with 1:3 acetonitrile–water (2.5 mL); (b) fraction eluted with 1:3 acetonitrile–water (2.5 mL) containing 0.05% trifluoroacetic acid. Analysis was by HPAEC using isocratic elution with 100 mM NaOH at 1 mL min^{-1}. Peak 1: lactose, Peaks 2a, 2b and 2c: the different isomeric forms of neuraminyllactose.

Applications

Solid-phase extraction and desalting of sugars. A solution of glucose, glucosamine hydrochloride, maltose and raffinose (each 100 μg) in 5 mL of water, or in 0.1 M NaCl, acetate buffer (pH 5.0) or phosphate (pH 6.8) was applied to a conditioned graphitised carbon cartridge, and the eluate collected. The cartridge was washed with water (5 × 1 mL) to remove traces of salt, and the adsorbed sugars were eluted from the cartridge with 1:1 acetonitrile–water (5 mL). The eluate was adjusted to 10 mL with water, and an aliquot of this (200 μL) was evaporated in a stream of nitrogen, reconstituted in water (100 μL) and analysed by HPAEC using procedure A. In each case, the recoveries of adsorbed glucose, glucosamine hydrochloride, maltose and raffinose from a mixture were 0, 0, 98 ± 1 and 99 ± 2%, respectively.

Solid-phase extraction of raffinose from dilute solution. A dilute solution of raffinose (1 μg mL^{-1}, 100 mL) was allowed to percolate through a conditioned graphitised carbon cartridge at approx 30 mL h^{-1}, and the cartridge then washed with water (5 × 1 mL). The retained raffinose was eluted in 1:1 acetonitrile–water (5 mL). After diluting the eluate to 10 mL with water, an aliquot (200 μL) was analysed by HPAEC using procedure A. The recovery of raffinose was 98 ± 3%.

Preparative loading for raffinose. A solution of raffinose (200 mg) in water (5 mL) was applied to a conditioned graphitised carbon cartridge, the cartridge rinsed with water (5 × 1 mL) and the retained raffinose eluted with 1:1 acetonitrile–water (5 mL). After diluting the eluate to 10 mL with water, an aliquot (20 μL) was analysed by HPAEC using procedure A. The total raffinose recovered in the eluate was 41 ± 2 mg.

Preparation and fractionation of dextran oligosaccharides. A partial hydrolysate of dextran was prepared by a modification of a published method [10]. Briefly, dextran 500 (25 mg) in 0.1 M hydrochloric acid (0.25 mL) was heated in a sealed tube at 100 °C for 2 h, then cooled and diluted with water (5 mL). An aliquot (100 μL) was freeze-dried directly, and the remainder of the hydrolysate was applied to a conditioned graphitised carbon cartridge. The cartridge was rinsed with water (5 × 1 mL) to remove acid, and groups of oligosaccharides were obtained by sequential batch elution with 5 mL each of BSW/32, BSW/16, BSW/8, BSW/4, BSW/2 and BSW. Aliquots of the eluates were analysed by HPAEC (Fig. 1) using procedure B.

Desalting of fractions from HPAEC. A sample of hydrolyzed dextran 500 (1 mg) was separated by HPAEC using procedure B (Fig. 2). The fraction (approx 0.5 mL) corresponding to the oligosaccharide of degree of polymerization (DP) 10 was collected, diluted with water (5 mL) and applied to a conditioned graphitised carbon cartridge. The cartridge was washed with water (5 × 1 mL), 0.05% trifluoroacetic acid (2 mL) and water (3 mL), and the oligosaccharide was recovered by elution with BSW (5 mL). An aliquot (100 μL) of the eluate was evaporated, reconstituted in water (50 μL) and analysed by HPAEC (Fig. 2) using procedure B.

Fractionation of neutral and anionic sugars. A solution of lactose and *N*-acetylneuraminyllactose (each 100 μg) in water (5 mL) was applied to a conditioned graphitised carbon cartridge, and the cartridge rinsed with water (5 × 1 mL). The lactose was eluted with 1:3 acetonitrile–water (1.5 mL) and the *N*-acetylneuraminyllactose was then eluted with 1:3 acetonitrile–water (1.5 mL) containing 0.05% trifluoroacetic acid. The starting solution and eluates were analysed by HPAEC using procedure A (Fig. 3), showing complete separation of the neutral and acidic oligosaccharides. Glucuronic acid, glucose-6-phosphate, *N*-acetylglucosamine 6-phosphate, glucosamine 2-sulfate and glucosamine 3-sulfate were all retained by graphitised carbon on elution with 1:3 acetonitrile–water, but eluted quantitatively when trifluoroacetic acid (0.05%) is added to the eluant.

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