

Analytical methods for the determination of sugars in marine samples: A historical perspective and future directions

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Abstract

Analytical techniques employed over the past three decades for sugar determination in marine samples are reviewed. This review first summarizes the different hydrolysis protocols used by marine biogeochemists to extract sugars from various marine matrices including sinking particulate organic matter (POM), dissolved organic matter (DOM), ultrafiltered dissolved organic matter (UDOM), and sediments. The most commonly used methods for total sugar estimation are phenol sulfuric acid (PSA), 3-methyl-2-benzothiazoline hydrochloride (MBTH), and 2,4,6-tripyridyl-s-triazine (TPTZ). For individual sugars, gas chromatography-flame ionization detection (GC-FID), liquid chromatography-borate complexes (LC), high performance liquid chromatography-ethylenediamine derivatives (HPLC-EDA), high performance liquid chromatography-*p*-aminobenzoic acid derivatives (HPLC-*p*-AMBA), and high performance anion exchange chromatography-pulsed amperometric detection (HPAEC-PAD) have been employed, the last method being the most widely used. This study demonstrated that mild and strong hydrolysis give comparable results for open ocean samples, including POM, DOM, and UDOM (except for sediments) using chromatographic or colorimetric techniques. A survey of 130 sugar data sets revealed that more than half of the sugar-C escapes analysis using chromatographic or colorimetric techniques compared to ¹H and ¹³C NMR techniques. The reconciliation of the NMR and colorimetric or chromatographic measurements is likely to be the key to the understanding of the composition of DOM. This study also demonstrated that most of the published sugar data obtained by chromatographic techniques are related to suspended or sinking POM and sediments, and that there is a need for data on sugar composition in DOM and UDOM, which involve further analytical difficulties for chromatographic analysis.

Marine biomass constitutes a multifaceted conglomeration of low and high molecular weight products, including carbohydrates, amino acids, lipids, and biopolymers such as cellulose, chitin, chondroitin, laminarin, and proteins. The second most abundant group of these organic compounds are the carbohydrates, which comprise 20 to 40 dry wt.% of plankton and 17 dry wt.% of bacteria (Stouthamer 1977; Parsons et al. 1984). In a similar way to terrestrial organisms and vascular

plants, carbohydrates are common structural (chitin, mureine, and chondroitin; Brock et al. 1994; Benner and Kaiser 2003) and storage (glucans; Painter 1983) compounds in marine planktonic organisms, including bacteria. In contrast to some classes of lipids, the ubiquitous character of carbohydrates does not allow a distinction between potential marine sources such as phytoplankton, zooplankton, and bacteria, which contribute to sinking (>10 μm), suspended (0.7–10 μm), or dissolved (<0.7 μm) organic material (Cowie and Hedges 1984a, 1984b; Saliot et al. 1982; Wakeham et al. 1983; Grimalt et al. 1990). Therefore, very limited information on the source of organic matter can be derived from carbohydrate analysis.

Although particulate sugar determination has been greatly improved in the last decade (Kerhervé et al. 1995; Hernes et al. 1996; Panagiotopoulos et al. 2001), dissolved carbohydrate measurements are analytically challenging. Difficulties begin with quantitatively extracting and concentrating sugars (typically low nanomolar level) from seawater with high salt content. Additionally, carbohydrates exhibit multiple charge states at

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seawater pH including neutral sugars (Mopper et al. 1992; Borch and Kirchman 1997; Skoog and Benner 1997), positively charged amino sugars (Kaiser and Benner 2000), and negatively charged uronic acids (Mopper et al. 1995; Hung et al. 2001), making the isolation of these molecules difficult. Simple and sensitive methods for sugar analysis subsequent to isolation have been lacking. As a result of analytical difficulties and poor biogeochemical information, this class of organic molecules has therefore received little attention so far, despite its importance for biochemical cycling. Within the last two decades, some of these analytical problems have been overcome due to the advent of sophisticated and sensitive chromatographic apparatus such as high-performance anion-exchange chromatography with pulsed amperometric detection (HPAEC-PAD; Mopper et al. 1992). Similarly, the use of anion exchange resins or dialysis techniques to remove salts from seawater samples has improved the detection of dissolved marine sugars (Mopper et al. 1992; Rich et al. 1996; Borch and Kirchman 1997; Skoog and Benner 1997; Amon and Benner 2003). Furthermore, studies based on ^1H and ^{13}C NMR measurements have highlighted the importance of carbohydrates in seawater since these compounds were found to form an important fraction of acyl polysaccharide, a polymer isolated from high molecular weight dissolved organic matter (HMWDOM > 1 kDa; Aluwihare et al. 1997; Aluwihare and Repeta 1999). This biopolymer is mainly composed of neutral and N-acetyl amino sugars, although its full structure is not yet known. Together these two components contribute 60% to 70% of the total carbohydrate, 40% to 50% of the total organic carbon, and an equal amount of the total new carbon in HMWDOM (< 200 years old compared to the old dissolved organic compound [DOC] of the Atlantic or Pacific, which ranges between 4000 and 6000 years; Druffel et al. 1992; Guo et al. 1994). These recent findings suggest that we still have limited information on the structure, distribution, and cycling of carbohydrates in marine environment, and that the demand for the development of new techniques will increase. It is also important to note that comparison of carbohydrate concentrations reported in marine samples is difficult, given the wide range of hydrolysis protocols used by investigators and the lack of intercomparison studies between the various analytical procedures (Mopper 1977; Burney and Sieburth 1977; Borch and Kirchman 1997; Skoog and Benner 1997; Kerhervé et al. 2002).

Although several general reviews on sugar analytical methods have been published, these have mainly focused on carbohydrate determination in foods, fibers, beverages, vegetables, and pharmaceutical products. To our knowledge, only three review studies on carbohydrate analytical techniques in marine samples have been carried out to date (Dawson and Liebezeit 1981; Liebezeit 1985; Moal et al. 1985). However, these reviews did not systematically examine the extraction protocols of sugars from marine samples and mainly focused on particulate organic matter (POM) and/or sediments.

This article presents an overview of all analytical methods (colorimetric and chromatographic techniques) used in sugar determination and includes an assessment of their advantages and limitations to illustrate the existing data set for marine environments. Special emphasis is given to extraction protocols of carbohydrates from marine matrices (POM, seawater, sediments). This article concludes with a discussion of new analytical directions and major problems related to carbohydrate analysis.

Nomenclature of sugars—In chemical oceanography, the term “neutral sugars” (Aluwihare et al. 1997; Borch and Kirchman 1997; Keil et al. 1998; Burdige et al. 2000; Amon and Benner 2003) or neutral carbohydrates (Hernes et al. 1996; Ogier et al. 2001) is synonymous to neutral monosaccharides (Rich et al. 1996; Kerhervé et al. 2002), including aldohexoses (glucose, galactose, and mannose), aldopentoses (arabinose, xylose, and ribose), and deoxysugars (fucose and rhamnose), commonly analyzed using chromatographic techniques (Cowie and Hedges 1984a; McCarthy et al. 1996; Skoog and Benner 1997). Typical examples of some monosaccharides are shown in Fig. 1. These compounds comprise the majority of carbohydrate building blocks, which are constituents of cellulose, hemicelluloses, and storage and intracellular metabolites (Aspinall 1970) and are commonly released after acid hydrolysis.

Properties of sugars—One of the most important chemical properties of monosaccharides is that they can act as mild reducing/oxidizing agents, since the aldehyde group can be either oxidized to form a carboxylic acid group (in the presence of Cu^{2+} , Ag^+ , and Fe^{3+}), or reduced to the corresponding alcohol (in the presence of NaBH_4 , KBH_4). Colorimetric methods used to analyze sugars are based on these properties. For example, in the phenol-sulfuric, *N*-ethyl carbazole, anthrone, *L*-cysteine, *L*-tryptophan, or 2,4,6-tripyridyl-*s*-triazine (TPTZ) methods, monosaccharides act as reducing agents, whereas in the 3-methyl-2-benzothiazoline hydrazone hydrochloride (MBTH) method, they act as oxidizing agents. The latter property is also used in gas chromatography-flame ionization detection (GC-FID), where sugars are reduced to the corresponding alcohols prior to their derivatization. Another important property of sugars is that they act as weak acids in alkaline media, hence permitting their separation on anion exchange columns, despite their close dissociation constants ($\text{pK}_a = 12\text{--}13$).

Experimental protocols: Methods in carbohydrate analysis

Extraction protocols of sugars from different marine matrices—Analysis of carbohydrates is generally initiated with a hydrolysis step that yields a pool of monomers (monosaccharides). Monosaccharides are subsequently detected by either colorimetric or chromatographic methods. Hydrolysis is a chemical decomposition process that uses water to split chemical bonds (α or β glycosidic bonds) of the carbohydrate polymers. There are two types of hydrolysis: enzymatic and acid. Enzymatic hydrolysis of carbohydrate polymers is carried out by extra-

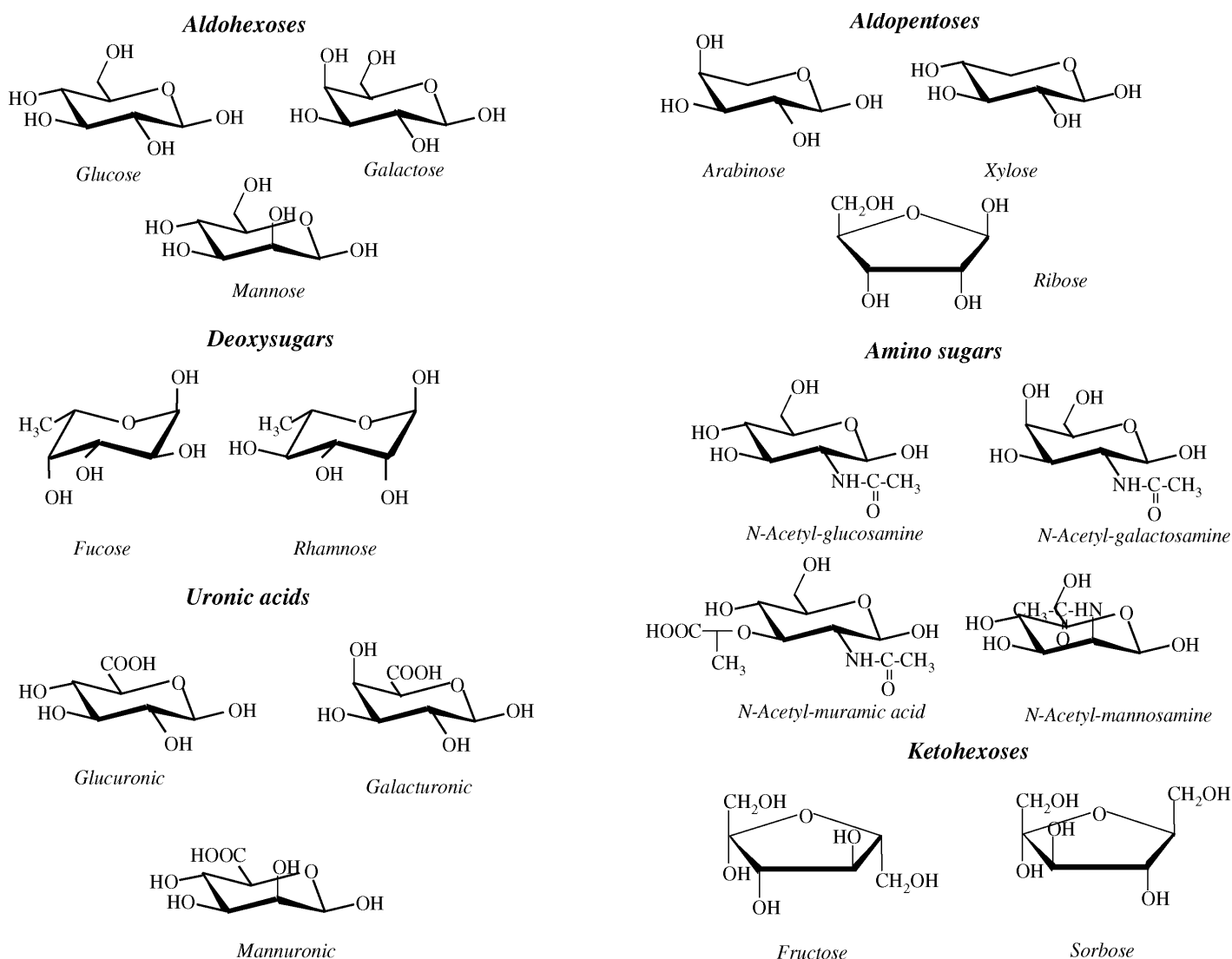


Fig. 1. Some common monosaccharides (aldohexoses, aldopentoses, amino sugars, deoxysugars, and uronic acids) measured in seawater or particulate organic matter after acid hydrolysis.

cellular enzymes of marine bacteria and is beyond the scope of this review (see Arnosti [2000] and references therein). Acid hydrolysis of carbohydrate polymers in marine environmental samples generally employs hydrochloric (HCl) or sulfuric acid (H₂SO₄), however trifluoroacetic (TFA), and *p*-toluenesulfonic acid (ptsa) are used to a lesser extent (Table 1).

Yields of monosaccharides released by acid hydrolysis depend on the type of acid used (HCl, H₂SO₄, etc.), acid strength (pH), duration, and temperature of the hydrolysis. Other parameters that influence hydrolysis yield are associated with the nature of the carbohydrate polymer (homopolysaccharide or heteropolysaccharide), type of glycosidic bond (α or β anomer), the position of the glycosidic bond, cyclic structure of the sugar (pyranose or furanose), degree of cross-linking, associations with other biopolymers

(proteins, lipids, etc.), and number of hydrogen bonds between sugars. Although many exceptions exist, in general, β glycosidic linkages are more stable than α linkages and are consequently more difficult to break down (e.g., maltose is more easily hydrolyzed than cellobiose). The glycosidic bond α (1-6) is more stable than the α (1-4, 1-3, 1-2; e.g., starch is hydrolyzed more easily than glycogen). The pyranoses are more stable than the furanoses (e.g., ribose and fructose in a furanose ring are generally destroyed during acid hydrolysis). Cellulose is more stable than glycogen, not only because of the β glycosidic linkages, but also because of its ability to make fibers stabilized by hydrogen bonds.

Table 1 provides a summary of published carbohydrate measurements from different marine environments in POM, DOM, ultrafiltrated dissolved organic matter (UDOM), sedi-

Table 1. Summary of published carbohydrate measurements in suspended and sinking particulate organic matter (POM), dissolved organic matter, ultrafiltered dissolved organic matter (UDOM), superficial sediment, sediment porewaters, marine colloids, and transparent exopolymer polysaccharides (TEP) taken from different locations*

Sample and location	Method	Hydrolysis conditions	Sugar concentration	Sugar Majors sugars	contribution to the carbon pool	Reference
Suspended POM						
Northern North Sea	LC-vis†	1.8 N HCl 3.5h, 100°C	50-600 nM	Glc > Fru, Gal, Man	Euphotic zone ND (tot. neutral aldo.)	Ittekkot et al. (1982)
Bering Sea	GC-FID	0.5 M H ₂ SO ₄ 4 h, 100°C	154-370 nM	Glc > Man > Gal, Ara	0-3500 m 7% to 14% (tot. neutral aldo.-C/POC)	Tanoue and Handa (1987)
North Pacific	GC-FID	0.5 M H ₂ SO ₄ 4 h, 100°C	20-293 nM	Glc > Man > Gal, Xyl	0-2500 m 7% to 13% (tot. neutral aldo.-C/POC)	Tanoue and Handa (1987)
Bransfield Strait	LC-vis†	4 M HCl 3 h, 110 °C	8-205 nM	Glc > Gal > Rib	0-100 m 0.3% to 4.6% (PCHO-C/POC)	Liebezeit and Bölter (1991)
Amazon River	GC-FID	12 M H ₂ SO ₄ ; 1.2 M H ₂ SO ₄ 3 h, 100°C	~1250 nM	Glc > Man > Gal	9-23 mg tot. neutral aldo./100 mg OC	Hedges et al. (1994)
Western Med. Sea	HPAEC-PAD¶	1 M H ₂ SO ₄ 4 h, 90°C	60 mg g ⁻¹	Xyl > Glc > Ara	30 m ND (tot. neutral aldo. + GlcN)	Kerhervé et al. (1995)
Potomac Estuary	GC-FID/MS	0.5 M HCl 1 h, 100 °C	0.1-5 µM	Glc > Gal > Rha	0-10 m 9-31 mg tot. neutral aldo.-C/100 mg OC	Sigleo (1996)
Equatorial Pacific	HPAEC-PAD	12 M H ₂ SO ₄ ; 1.2 M H ₂ SO ₄ 3 h, 100°C	2-20 nM	Glc > Gal > Man or Xyl	2-4000 m 4% to 14% (tot. neutral aldo.-C/OC)	Skoog and Benner (1997)
Trieste Gulf	MBTH#	2 M HCl 3.5 h, 100°C	195-1555 nM	Glc equivalents	0-20 m 11% to 31% (PCHO-C/POC)	Terzié et al. (1998)
São Francisco River	LC-vis†	2 N HCl 3.5 h 105°C	7-25 mg g ⁻¹	Glc > Gal > Xyl	2 m 6% to 18% (tot. neutral aldo.-C/OC)	Jennerjahn and Ittekkot (1999)
Gironde Estuary	PSA**	Concentrated H ₂ SO ₄	~1.3 mg g ⁻¹	Glc equivalents	1m ND (PCHO)	Burdloff et al. (2001)
Dona Paula Bay	GC-FID	72% H ₂ SO ₄ ; 1.2 M H ₂ SO ₄ 3 h, 100°C	570-4000 nM	Glc > Gal > Man	Surface 2% to 16% (tot. neutral aldo.-C/POC)	D'Souza and Bhosle (2001)
Tech River (Spain)	GC-FID	12 M H ₂ SO ₄ ; 1.2 M H ₂ SO ₄ 3 h, 100°C	0-430 nM	Ara > Glc, Gal or Rha	0.04-94 mg tot. neutral aldo.-C/100 mg OC	Cotrim da Cunha et al. (2002)
Danube River	LC-vis†	2 N HCl 3.5 h, 105°C	750-2300 nM	Glc > Gal > Rib	0-16 m 8% to 14% (tot. neutral aldo.-C/OC)	Reschke et al. (2002)
Pacific Ocean	HPAEC-PAD	3 M HCl 5 h, 100°C	0.2-2 nM	GlcN > GalN > MA	2-4000 m 0.3% to 1.3% (amino sugars-C/POC)	Benner and Kaiser (2003)

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TABLE 1—Continued

Gulf of Mexico	TPTZ††	1.2 M H ₂ SO ₄ 3 h, 100°C	30-187 nM	Glc equivalents	Euphotic zone av. 9% to 22% (PCHO- C/POC)	Hung et al. (2003)
Gulf of Mexico	UA assay‡‡	Concentrated H ₂ SO ₄	1-17 nM	Av. GlcA, GalA, ManA	Euphotic zone av. 0.6-0.7% (PUA-C/ POC)	Hung et al. (2003)
Sink. POM						
Dabob Bay	GC-FID	72% H ₂ SO ₄ ; 1.2 M H ₂ SO ₄ 3 h, 100°C	ND	Glc > Gal or Man	30-90 m 11-31 mg tot. neutral aldo./ 100 mg OC	Cowie and Hedges (1984b)
Sargasso Sea	LC-vis†	2 N HCl 3.5 h, 100°C	4-7 mg g ⁻¹	Glc, Gal or Man	3200 m 3% to 6% (tot. neutral aldo. -C/POC)	Ittekkot et al. (1984a)
Panama Basin	LC-vis†	2 N HCl 3.5 h, 100°C	4-10 mg g ⁻¹	Gal > Glc or Man	890-3560 m 2% to 18% (tot. neutral aldo.-C/POC)	Ittekkot et al. (1984b)
North Pacific	GC-FID	0.5 M H ₂ SO ₄ 4 h, 100°C	7-16 mg g ⁻¹	Glc > Man, Gal, Xyl	100-5250 m 7% to 10% (tot. neutral aldo.-C/POC)	Tanoue and Handa (1987)
Itasca Lake	HPLC- Fluo§§	72% H ₂ SO ₄ ; 1.2 M H ₂ SO ₄ 3 h, 100°C	3-20 mg g ⁻¹	Glc > Man > Gal	4-10 m ND (tot. neutral aldo.)	Hicks et al. (1994)
Laurentian Trough	PSA	Concentrated H ₂ SO ₄	6-24 mg g ⁻¹	Glc equivalents	150 m 8% to 16% (PCHO-C/POC)	Colombo et al. (1996)
Equatorial Pacific	GC-FID	72% H ₂ SO ₄ ; 1.2 M H ₂ SO ₄ 3 h, 100°C	ND	Glc > Gal > Man	105-4000 m 5-18 mg tot. neutral aldo./ 100 mg OC	Hernes et al. (1996)
Dabob Bay	GC-FID	0.5 N TFA 2 h, 135°C	ND	Glc > Gal or Xyl	30-90 m 7-25 mg tot. neutral aldo. + UA + O-Me sug. /100 mg OC	Bergamaschi et al. (1999)
Saanich Inlet	GC-FID	0.5 N TFA 2 h, 135°C	ND	Glc > Gal > Man	50-170 m ~22 mg tot. neutral aldo. + UA + O-Me sug. /100 mg OC	Bergamaschi et al. (1999)
São Francisco river	LC-vis†	2 N HCl 3.5 h, 105°C	4-13 mg g ⁻¹	Glc > Gal > Man	1550 m 4% to 10% (tot. neutral aldo. -C/POC)	Jennerjahn and Ittekkot (1999)
South Aegean Sea	HPAEC-PAD	1 M H ₂ SO ₄ 4 h, 90°C	4-7 mg g ⁻¹	Glc or Gal > Man	965 m 4% to 8% (tot. neutral aldo.-C/OC)	Kerhervé et al. (1999)
South Ionian Sea	HPAEC-PAD	1 M H ₂ SO ₄ 4 h, 90°C	4-8 mg g ⁻¹	Glc or Gal > Man	880-1345 m 3% to 10% (tot. neutral aldo.-C/OC)	Kerhervé et al. (1999)
Aydat Lake	GC-FID	12 M HCl; 1.2 M HCl 3 h, 100°C	4-30 mg g ⁻¹	Rha > Fuc or Glc	5-14 m 3-24 mg neutral aldo. /100 mg OC	Ogier et al. (2001)

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TABLE 1—Continued

Gulf of Mexico	TPTZ	1.2 M H ₂ SO ₄ 3 h, 100°C	ND	Glc equivalents	65-120 m ~ 9% (PCHO-C/POC)	Hung et al. (2003)
Gulf of Mexico	UA assay	Concentrated H ₂ SO ₄	ND	Av. GlcA, GalA, ManA	65-120 m 0.9% to 1.1% (PUA-C/POC)	Hung et al. (2003)
Polar front zone	HPAEC-PAD	0.1 M HCl 20 h, 100°C	5 μM	Glc > Rib > Gal	200 m 8% (tot. neutral aldo.-C + GlcN- C/POC)*	Sempéré and Panagiotopoulos (unpublished results)
Sub-Antarctic region	HPAEC-PAD	0.1 M HCl 20 h, 100°C	1 μM	Glc > Gal or Man	200 m 5% (tot. neutral aldo.-C + GlcN- C/POC) *	Sempéré and Panagiotopoulos (unpublished results)
Polar front zone	HPAEC-PAD	0.1 M HCl 20 h, 100°C	ND	Gal > Rib > Glc	200 m av. 3% (tot. neutral aldo.-C + GlcN-C/POC)	Panagiotopoulos and Sempéré (2005)
Sub-tropical zone	HPAEC-PAD	0.1 M HCl 20 h, 100°C	ND	Rib > Glc > Man	200 m av. 4% (tot. neutral aldo.-C + GlcN-C/POC)	Panagiotopoulos and Sempéré (2005)
Ligurian Sea	HPAEC-PAD	0.1 M HCl 20 h, 100°C	ND	Glc > Gal > Xyl or Rib	100-200 m av. 12% to 13% (neutral aldo. -C + GlcN-C/POC)	Panagiotopoulos and Sempéré (2005)
South Ionian Sea	HPAEC-PAD	0.1 M HCl 20 h, 100°C	ND	Glc > Gal > Fru	250 m av. 14% (neutral aldo.-C + GlcN- C/POC)	Panagiotopoulos and Sempéré (2005)
Atlantic upwelling	HPAEC-PAD	0.1 M HCl 20 h, 100°C	ND	Glc > Fru > Gal	50 m av. 4% (neutral aldo.-C + GlcN- C/POC)	Panagiotopoulos and Sempéré (2005)
DOM						
North Sea	LC-vis‡	0.75 M pta 4 h, 100°C (+ glycerin)	4 μM (filt.)	Glc > Man > Xyl	Surface 2% (tot. neutral aldo.-C/DOC)	Mopper (1977)
North Sea	LC-vis‡	No hydrolysis	0.3 μM (filt.)	Glc > Fru > Rib	Surface 0.15% (tot. neutral aldo. -C/DOC)	Mopper (1977)
Black Sea	LC-vis‡	0.75 M pta 4 h, 100°C (+ glycerin)	2 μM (filt.)	Glc > Xyl > Fru	Surface 2% (tot. neutral aldo.-C/DOC)	Mopper (1977)
Black Sea	LC-vis‡	No hydrolysis	52-590 nM (filt.)	Glc > Fru > Gal	Surface-500 m 0.08% to 0.62% (tot. neutral aldo.-C/DOC)	Mopper (1977)
North Sea	LC-vis‡	0.75 M pta 4h, 100°C (+ glycerin)	260 nM (filt.)	GlcA > 4-O Me GlcA	Surface 0.12% (UA-C/DOC)	Mopper (1977)
Black Sea	LC-vis§	0.75 M pta 4h, 100°C (+ glycerin)	75 nM (filt.)	GulA > GalA	Surface 0.08% (UA-C/DOC)	Mopper (1977)
Narraganset Bay	MBTH	0.1 M HCl 20 h, 100°C	2-8 μM (filt.)	Glc equivalents	Surface 6% to 18% (TDCHO-C/DOC)	Burney and Sieburth (1977)

Continued

TABLE 1—Continued

Narraganset Bay	MBTH	No hydrolysis	0.8-4 μM (filt.)	Glc equivalents	Surface 2% to 5% (DFCHO-C/DOC)	Burney and Sieburth (1977)
Northern North Sea	LC-vis†	1.8 N HCl 3.5 h, 100°C	0.3-2 μM (filt.)	Glc > Fru > Man or Gal	0-150 m 1% to 5% (total neutral aldo.-C/DOC)	Ittekkot (1982)
Williamson River	GC-FID	3 M HCl 1h, 100°C	0.1-8 μM (filt.)	Xyl, Glc, Gal, Ara	Av. 2% (total neutral aldo.-C/DOC)	Sweet and Perdue (1982)
<i>J. effuses beds</i> (Lake)	HPAEC-PAD	No hydrolysis	~ 620 nM (filt.)	Glc > Ara > Fru	< 5% (tot. free neutral aldo.-C + GlcN-C + Suc-C + UA-C/DOC)	Wicks et al. (1991)
Fresh water swamp	HPAEC-PAD	No hydrolysis	~ 890 nM (filt.)	Glc > Fru > GalA	< 5% (tot. free neutral aldo.-C + GlcN-C + Suc-C + UA-C/DOC)	Wicks et al. (1991)
Salt marsh	HPAEC-PAD	No hydrolysis	2300 nM (filt.)	Glc > Fru > Rib	< 5% (tot. free neutral aldo.-C + GlcN-C + Suc-C /DOC)	Wicks et al. (1991)
Elorn estuary	HPAEC-PAD	HCl (30%) 3.5 h, 100°C	0.3-8 μM (filt.)	Glc equivalents	2% to 23% (TDCHO-C/DOC)	Senior and Chevelot (1991)
Elorn estuary	MBTH	No hydrolysis	0-2.5 μM (filt.)	Glc equivalents	0% to 5% (DFCHO-C/DOC)	Senior and Chevelot (1991)
Furesø Lake	MBTH	1.5 M HCl 4h, 100°C	1-2 μM (filt.)	Glc > Gal > Cel	ND (total neutral aldo. + Cel. + Mel.)	Jørgensen and Jensen (1994)
Furesø Lake	HPAEC-PAD	No hydrolysis	0.1-0.2 μM (filt.)	Glc > Fru > Cel	ND (total free neutral aldo. + Mel.)	Jørgensen and Jensen (1994)
Equatorial Pacific	MBTH	12 M H ₂ SO ₄ ; 1.2 M H ₂ SO ₄ 3 h, 100°C	0.9-5 μM (unfilt.)	Glc equivalents	3-4000 m 10% to 46% (TDCHO-C/DOC)	Pakulski and Benner (1994)
North Pacific	MBTH	12 M H ₂ SO ₄ ; 1.2 M H ₂ SO ₄ 3 h, 100°C	1-4 μM (filt.)	Glc equivalents	10-4000 m 16% to 32% (TDCHO-C/DOC)	Pakulski and Benner (1994)
Gulf of Mexico	MBTH	12 M H ₂ SO ₄ ; 1.2 M H ₂ SO ₄ 3 h, 100°C	2-5 μM (filt.)	Glc equivalents	10-500 m 14% to 34% (TDCHO-C/DOC)	Pakulski and Benner (1994)
North Atlantic	MBTH	12 M H ₂ SO ₄ ; 1.2 M H ₂ SO ₄ 3 h, 100°C	1-2 μM (filt.)	Glc equivalents	4-4000 m 13% to 18% (TDCHO-C/DOC)	Pakulski and Benner (1994)
Gerlache Strait	MBTH	12 M H ₂ SO ₄ ; 1.2 M H ₂ SO ₄ 3 h, 100°C	2-4 μM (filt.)	Glc equivalents	2-200 m 13% to 19% (TDCHO-C/DOC)	Pakulski and Benner (1994)
Constance Lake	MBTH	0.85 M H ₂ SO ₄ , 24 h, 100°C	2-6 μM (filt.)	Glc equivalents	ND (TDCHO)	Hanisch et al. (1996)
Equatorial Pacific	HPAEC-PAD	No hydrolysis	19-81 nM (filt.)	Glc > Fru > Ara	0-80 m ND (total free neutral aldo.)	Rich et al. (1996)
Equatorial Pacific	HPAEC-PAD	0.85 M H ₂ SO ₄ 24 h, 100°C	18-280 nM (unfilt.)	Glc > Gal > Fuc	10-1000 m ND (total neutral aldo.)	Borch and Kirchman (1997)

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TABLE 1—Continued

Oregon in/offshore	HPAEC-PAD	0.85 M H ₂ SO ₄ 24 h, 100°C	0.12-2.2 μM (filt.)	Glc > Gal > Man + Xyl	10-250 m ND (total neutral aldo.)	Borch and Kirchman (1997)
Sargasso Sea	HPAEC-PAD	0.85 M H ₂ SO ₄ 24 h, 100°C	180 nM (unfilt.)	Glc > Gal > Fuc	Surface ND (total neutral aldo.)	Borch and Kirchman (1997)
Stream water	HPAEC-PAD	0.24 M HCl 12 h, 100°C	1-13 μM (filt.)	Glc > Gal or Rha	3% to 12% (tot. neutral aldo.-C + GlcN-C + GalN-C + Ino- C/DOC)	Gremm and Kaplan (1997)
Stream water	HPAEC-PAD	No hydrolysis	50-380 nM (filt.)	Glc > Fru > Rib	0.06-0.33% (total free neutral aldo. -C/DOC)	Gremm and Kaplan (1997)
Central Arctic	HPAEC-PAD	0.85 M H ₂ SO ₄ 24 h, 100°C	35-2860 nM (filt.)	Glc > Man > Gal	0-40 m 2% to 20% (total aldo.-C/DOC)	Rich et al. (1997)
Central Arctic	HPAEC-PAD	No hydrolysis	42-90 nM (filt.)	Glc > Fru > Ara	ND (total free neutral aldo.)	Rich et al. (1997)
Equatorial Pacific	HPAEC-PAD	12 M H ₂ SO ₄ ; 1.2 M H ₂ SO ₄ 3 h, 100°C	128-804 nM (unfilt.)	Glc > Gal or Man	2-4000 m 2% to 4% (total free neutral aldo.-C/DOC)	Skoog and Benner (1997)
Arabian Sea	MBTH	12 M H ₂ SO ₄ ; 1.2 M H ₂ SO ₄ 3 h, 100°C	7-13 μM (filt.)	Glc equivalents	0-1000 m ND (TDCHO)	Bhosle et al. (1998)
Arabian Sea	MBTH	No hydrolysis	2-4 μM (filt.)	Glc equivalents	0-1000 m ND (DFCHO)	Bhosle et al. (1998)
Bengal Bay	MBTH	12 M H ₂ SO ₄ ; 1.2 M H ₂ SO ₄ 3 h, 100°C	5-7 μM (filt.)	Glc equivalents	0-1500 m ND (TDCHO)	Bhosle et al. (1998)
Bengal Bay	MBTH	No hydrolysis	0.4-3 μM (filt.)	Glc equivalents	0-1500 m ND (DFCHO)	Bhosle et al. (1998)
Trieste Gulf	MBTH	2 M HCl 3.5 h, 100°C	2-21 μM (filt.)	Glc equivalents	0-20 m 10% to 63% (TDCHO-C/DOC)	Terzić et al. (1998)
Trieste Gulf	MBTH	No hydrolysis	0.3-2 μM (filt.)	Glc equivalents	0-20 m 1% to 11% (DFCHO-C/DOC)	Terzić et al. (1998)
Trondheimsfjord	TPTZ	0.1 M HCl 20 h, 100°C	2-6 μM (filt.)	Glc equivalents	0-400 m 15% to 21% (TDCHO-C/DOC)	Børshheim et al. (1999)
Northern Adriatic Sea	MBTH	0.1 M HCl 20 h, 100°C	1-12 μM (filt.)	Glc equivalents	0-63 m 5% to 50% (TDCHO-C/DOC)	Pettine et al. (1999)
San Francisco Bay	MBTH	1 M HCl 20 h, 100°C	1-4 μM (filt.)	Glc equivalents	Surface 9% to 24% (TDCHO-C/DOC)	Murrell and Hollibaugh (2000)
San Francisco Bay	MBTH	No hydrolysis	0.2-0.3 μM (filt.)	Glc equivalents	Surface 3% to 6% (DFCHO-C/DOC)	Murrell and Hollibaugh (2000)
Gulf of Mexico	TPTZ	0.1 M HCl 1 h, 150°C	~ 5 μM (filt.)	Glc equivalents	Surface ~ 14% (TDCHO-C/DOC)	Hung and Santschi (2001)
Gulf of Mexico	UA assay	Concentrated H ₂ SO ₄	0.5 μM (filt.)	Av. GlcA, GalA, ManA	Surface ~ 2% (TDUA-C /DOC)	Hung and Santschi (2001)
Galveston Bay	TPTZ	0.1 M HCl 1 h, 150°C	4-16 μM (filt.)	Glc equivalents	Surface 13% to 24% (TDCHO-C/DOC)	Hung et al. (2001)

Continued

TABLE 1—Continued

Galveston Bay	TPTZ	No hydrolysis	2-10 μM (filt.)	Glc equivalents	Surface 8% to 15% (DFCHO-C/DOC)	Hung et al. (2001)
Galveston Bay	UA assay	Concentrated H_2SO_4	0.17-1.4 μM (filt.)	Av. GlcA, GalA, ManA	Surface 1% to 3% (TDUA-C/DOC)	Hung et al. (2001)
Ross Sea	HPAEC-PAD	0.85 M H_2SO_4 24 h, 100°C	50-500 nM (unfilt.)	Glc > Man + Xyl > Gal	550-750 m 1% to 11% (total neutral aldo.-C/DOC)	Kirchman et al. (2001)
Polar Front Zone	HPAEC-PAD	0.85 M H_2SO_4 24 h, 100°C	0.22-0.28 μM (unfilt.)	Glc > Gal > Man + Xyl	1000-3400 m ND (total neutral aldo.)	Kirchman et al. (2001)
Black Sea	TPTZ	0.1 M HCl 20 h, 100°C	2.5-6 μM (filt.)	Glc equivalents	0-20 m 10% to 24% (TDCHO-C/DOC)	Cauwet et al. (2002)
Middle Atlantic Bight	TPTZ	0.1 M HCl 1 h, 150°C	0.7-2.8 μM (unfilt.)	Glc equivalents	10-1000 m 7% to 14% (TDCHO-C/TOC)	Witter and Luther III (2002)
Middle Atlantic Bight	TPTZ	No hydrolysis	0.35-0.93 μM (unfilt.)	Glc equivalents	10-1000 m 2% to 5% (DFCHO-C/TOC)	Witter and Luther III (2002)
Delaware Estuary	TPTZ	0.1 M HCl 1 h, 150°C	0.72-2.7 μM (unfilt.)	Glc equivalents	10-1000 m 6% to 14% (TDCHO-C/TOC)	Witter and Luther III (2002)
Delaware Estuary	TPTZ	No hydrolysis	0.48-1.2 μM (unfilt.)	Glc equivalents	10-1000 m 2% to 5% (DFCHO-C/TOC)	Witter and Luther III (2002)
UDOM						
North Pacific	MBTH	12 M H_2SO_4 ; 1.2 M H_2SO_4 3 h, 100°C	333-2000 nM	Glc equivalents	10-4000 m 18% to 49% (tot. neutral aldo.-C/OC) (> 1kDa)	Benner et al. (1992)
Amazon River	GC-FID	12 M H_2SO_4 ; 1.2 M H_2SO_4 3 h, 100°C	~ 970 nM	Glc > Rha > Gal	3-5 mg tot. neutral aldo./100 mg OC (> 1 kDa)	Hedges et al. (1994)
North Pacific	GC-FID	72% H_2SO_4 ; 1.2 M H_2SO_4 3 h, 100°C	ca. 100-650 nM	Glc, Gal, Fuc, Rha	10-4000 m 5-31 mg tot. neutral aldo./100 mg UDOM OC (> 1 kDa)¶¶	McCarthy et al. (1996)
Sargasso Sea	GC-FID	72% H_2SO_4 ; 1.2 M H_2SO_4 3 h, 100°C	ca. 80-300 nM	Glc, Gal, Fuc, Rha	2-2400 m 9-25 mg tot. neutral aldo./100 mg UDOM OC (> 1 kDa)¶¶	McCarthy et al. (1996)
Gulf of Mexico	GC-FID	72% H_2SO_4 ; 1.2 M H_2SO_4 3 h, 100°C	ca. 0-550 nM	Glc, Gal, Fuc, Rha	10-750 m 3-25 mg tot. neutral aldo./100 mg UDOM OC (> 1 kDa)¶¶	McCarthy et al. (1996)
Equatorial Pacific	HPAEC-PAD	12 M H_2SO_4 ; 1.2 M H_2SO_4 3 h, 100°C	20-360 nM	Glc, Gal, Fuc, Rha	2-4000 m 2% to 10% (HMW tot. neutral aldo.-C/DOC) (> 1 kDa)¶¶	Skoog and Benner (1997)
Equatorial Pacific	HPAEC-PAD	12 M H_2SO_4 ; 1.2 M H_2SO_4 3 h, 100°C	89-661 nM	Glc > Man, Gal, Ara	2-4000 m 1% to 2% (LMW tot. neutral aldo.-C/DOC) (< 1 kDa)¶¶	Skoog and Benner (1997)

Continued

TABLE 1—Continued

UDOM						
Delaware Estuary	MBTH	12 M H ₂ SO ₄ ; 1.2 M H ₂ SO ₄ 3 h, 100°C	1-2 µM	Glc equivalents	1m 7.5% to 19% (TDCHO-C/UDOM) (1-30 kDa)	Mannino and Harvey (2000)
Delaware Estuary	MBTH	12 M H ₂ SO ₄ ; 1.2 M H ₂ SO ₄ 3 h, 100°C	58-300 nM	Glc equivalents	1m 30% to 56% (TDCHO-C/UDOM) (30 kDa-0.2 µm)	Mannino and Harvey (2000)
Mississippi River	GC-FID	12 M H ₂ SO ₄ ; 1.2 M H ₂ SO ₄ 3 h, 100°C	0.2-1 µM	Glc > Rha > Xyl	0-5 m 1% to 24% (tot. neutral aldo.-C/OC) (> 1 kDa)	Benner and Opsahl (2001)
Missis. adjac. waters	GC-FID	12 M H ₂ SO ₄ ; 1.2 M H ₂ SO ₄ 3 h, 100°C	180-270 nM	Gal > Fuc > Glc	0-5 m 6% to 11% (tot. neutral aldo.-C/OC) (> 1 kDa)	Benner and Opsahl (2001)
Arctic Ocean	HPAEC-PAD	12 M H ₂ SO ₄ ; 1.2 M H ₂ SO ₄ 3 h, 100°C	31-409 nM	Glc, Gal, Fuc	12-4000 m 1% to 10% (HMW tot. neutral aldo.-C/DOC) (> 1 kDa)¶¶	Amon and Benner (2003)
Arctic Rivers	HPAEC-PAD	12 M H ₂ SO ₄ ; 1.2 M H ₂ SO ₄ 3 h, 100°C	912-1823 nM	Glc > Rha > Gal	0-2 m 2% to 4% (HMW tot. neutral aldo.-C/ DOC) (> 1 kDa)¶¶	Amon and Benner (2003)
Pacific Ocean	HPAEC-PAD	3 M HCl 5 h, 100°C	6-93 nM	GlcN > GalN > MA	2-4000 m 0.5% to 2.5% (tot. amino sugars- C/UDOM) (1-100 nm)	Benner and Kaiser (2003)
Atlantic Ocean	HPAEC-PAD	3 M HCl 5 h, 100°C	10-49 nM	GlcN > GalN > MA	1-2400 m 0.6% to 1.8% (tot. amino sugars- C/UDOM) (1-200 nm)	Benner and Kaiser (2003)
Arctic Ocean	HPAEC-PAD	3 M HCl 5 h, 100°C	10-34 nM	GlcN > GalN > MA	10-1600 m 0.5% to 0.8% (tot. amino sugars- C/UDOM) (1-200 nm)	Benner and Kaiser (2003)
Superficial sediment						
Black Sea	LC-vis‡	72% H ₂ SO ₄ ; 1.86 M H ₂ SO ₄ 4 h, 100°C	36 mg g ⁻¹	Gal > Glc > Rha	19% (tot. neutral aldo. -C/TOC)	Mopper (1977)
Black Sea	LC-vis§	72% H ₂ SO ₄ ; 1.86 M H ₂ SO ₄ 4 h, 100°C	2 mg g ⁻¹	GalA > GlcA	1% (UA-C/TOC)	Mopper (1977)
Dabob Bay	GC-FID	72% H ₂ SO ₄ ; 1.2 M H ₂ SO ₄ 3 h, 100°C	ND	Glc > Man > Gal	13-15 mg tot. neutral aldo./100 mg OC	Cowie and Hedges (1984b)
Sabkha Gavich	GC-FID/MS	0.25 M H ₂ SO ₄ 18 h, 100°C	16 mg g ⁻¹	Glc > Gal > Xyl	10% (tot. neutral aldo. -C/POC)	Klok et al. (1984)
Solar Lake	GC-FID/MS	0.25 M H ₂ SO ₄ 18 h, 100°C	104 mg g ⁻¹	Glc > Xyl > Gal	21% (tot. neutral aldo. -C/POC)	Klok et al. (1984)

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TABLE 1—Continued

Namibian shelf	GC-FID/MS	0.25 M H ₂ SO ₄ 18 h, 100°C	7 mg g ⁻¹	Gal > Xyl or Glc	2% (tot. neutral aldo. -C/POC)	Klok et al. (1984)
Black Sea	GC-FID/MS	0.25 M H ₂ SO ₄ 18 h, 100°C	5-20 mg g ⁻¹	Glc > Gal > Man	3% to 5% (tot. neutral aldo. -C/POC)	Klok et al. (1984)
Bering Sea	GC-FID	0.5 M H ₂ SO ₄ 4 h, 100°C	2 mg g ⁻¹	Glc > Gal > Man	5% (tot. neutral aldo. -C/POC)	Tanoue and Handa
Itasca Lake	HPLC-Fluo	72% H ₂ SO ₄ ; 1.2 M H ₂ SO ₄ 3 h, 100°C	4-7 mg g ⁻¹	Glc, Man, Fuc	ND (tot. neutral aldo.)	Hicks et al. (1994)
Ligurian Sea	PSA	Concentrated H ₂ SO ₄	0.1-0.7 mg g ⁻¹	Glc equivalents	7% to 31% (PCHO-C /POC)	Fabiano et al. (1995)
Gulf of Lions	PSA	Concentrated H ₂ SO ₄	0.3-1.6 mg g ⁻¹	Glc equivalents	5% to 25% (PCHO-C /POC)	Buscail et al. (1995)
Gulf of Lions	HPAEC-PAD	72% H ₂ SO ₄ ; 1.2 M H ₂ SO ₄ 4 h, 100°C	0.2-0.4 mg g ⁻¹	Ara > Gal > Glc	3% to 8% (tot. neutral aldo.-C/POC)	Buscail et al. (1995)
Equatorial Pacific	GC-FID	72% H ₂ SO ₄ ; 1.2 M H ₂ SO ₄ 3 h, 100°C	ND	Glc > Gal > Man	5-26 mg neutral aldo. /100 mg OC	Hernes et al. (1996)
Washington coast	GC-FID	72% H ₂ SO ₄ ; 1.2 M H ₂ SO ₄ 3 h, 100°C	0.17-0.32 mg g ⁻¹	Glc, Gal, Man	~1.5 mg tot. neutral aldo./100 mg OC (bulk sediment)##	Keil et al. (1998)
Dabob Bay	GC-FID	0.5 N TFA 2 h, 135°C	ND	Glc > Man > Gal	18 mg tot. neutral aldo. + UA + O-Me sugars /100 mg OC	Bergamaschi et al. (1999)
Saanich Inlet	GC-FID	0.5 N TFA 2 h, 135°C	ND	Glc > Man > Gal	16 mg tot. neutral aldo. + UA + O-Me sugars /100 mg OC	Bergamaschi et al. (1999)
São Francisco River	HPLC-vis†	2 N HCl 3.5 hour 105°C	0.9-1.4 mg g ⁻¹	Rib > Glc or Gal	3% to 6% (tot. neutral aldo. -C/POC)	Jennerjahn and Ittekkot (1999)
Chesapeake Bay	MBTH	1.2 M H ₂ SO ₄ 3 h, 100°C	1-46 mg g ⁻¹	Glc equivalents	5% to 24% (PCHO-C /POC)	Burdige et al. (2000)
Mid-Atlantic shelf	MBTH	1.2 M H ₂ SO ₄ 3 h, 100°C	~ 3 mg g ⁻¹	Glc equivalents	~ 6% (PCHO-C/POC)	Burdige et al. (2000)
Arcachon lagoon	PSA	Concentrated H ₂ SO ₄	~ 15 mg g ⁻¹	Glc equivalents	ND	Burdloff et al. (2001)
Japanese bays/slopes	PSA	Concentrated H ₂ SO ₄	ND	Glc equivalents	9% to 10% (PCHO- C/POC) (< 3 kDa) extracted fraction	Miyajima et al. (2001)
Japanese bays/slopes	PSA	Concentrated H ₂ SO ₄	ND	Glc equivalents	11% to 14% (PCHO- C/POC) (3-10 kDa) extracted fraction	Miyajima et al. (2001)
Japanese bays/slopes	PSA	Concentrated H ₂ SO ₄	ND	Glc equivalents	31% to 37% (PCHO-C/ POC) (10-100 kDa) extracted fraction	Miyajima et al. (2001)

Continued

TABLE 1—Continued

Japanese bays/slopes	PSA	Concentrated H ₂ SO ₄	ND	Glc equivalents	39% to 42% (PCHO-C/ POC) (> 100 kDa) extracted fraction	Miyajima et al. (2001)
Japanese bays/slopes	PSA	Concentrated H ₂ SO ₄	ND	Gal > Glc or Xyl	12% to 13% (PCHO-C/ POC) residue ***	Miyajima et al. (2001)
Aydat Lake	GC-FID	12 M HCl ; 1.2 M HCl 3 h, 100°C	5 mg g ⁻¹	Rha > Glc > Xyl	~ 4 mg tot. neutral aldo./100 mg OC	Ogier et al. (2001)
Northwestern Med.Sea	HPAEC-PAD	1 M H ₂ SO ₄ 4 h, 90°C	0.5-1.0 mg g ⁻¹	Gal > Glc or Ara	3% to 5% (tot. neutral aldo.-C/OC)	Kerhervé et al. (2002)
Northwestern Med.Sea	HPAEC-PAD	1 M H ₂ SO ₄ 4 h, 90°C	0.5-1.1 mg g ⁻¹	Gal > Glc or Ara	3% to 6% (tot. neutral aldo.-C + GlcN-C/ OC) †††	Kerhervé et al. (2002)
Sediment porewaters						
Chesapeake Bay	MBTH	1.2 M H ₂ SO ₄ 3 h, 100°C	ca. 8-25 µM	Glc equivalents	~5% to 15% (TDCHO- C/DOC)	Burdige et al. (2000)
Mid-Atlantic shelf	MBTH	1.2 M H ₂ SO ₄ 3 h, 100°C	ca. 17-67 µM	Glc equivalents	~20% to 30% (TDCHO- C/DOC)	Burdige et al. (2000)
Colloids						
Potomac Estuary	GC-FID/MS	0.5 M HCl 1 h, 100°C	1-3 µM	Gal > Fuc or Xyl	0-10 m 17-40 mg total neutral aldo.-C/ 100 mg OC	Sigleo (1996)
Galveston Bay	TPTZ	0.1 M HCl 1 h, 150°C	1-9 µM	Glc equivalents	Surface 11% to 24% (CCHO-C/COC)	Hung et al. (2001)
Galveston Bay	UA assay	Concentrated H ₂ SO ₄	0.1-1 µM	Av. GlcA, GalA, ManA	Surface 1% to 4% (CUA-C/COC)	Hung et al. (2001)
TEP						
Mesocosm experiment	MBTH	72% H ₂ SO ₄ ; 1.2 M H ₂ SO ₄ 4 h, 100°C	ND	Glc equivalents	17% to 46% (TDCHO-C/TOC)	Mopper et al. (1995)
Shannon point	MBTH	72% H ₂ SO ₄ ; 1.2 M H ₂ SO ₄ 4 h, 100°C	45 nM (< 1 µm)	Glc equivalents	ND	Zhou et al. (1998)
Shannon point	MBTH	72% H ₂ SO ₄ ; 1.2 M H ₂ SO ₄	13 nM (< 0.1 µm)	Glc equivalents	ND	Zhou et al. (1998)
Shannon point	MBTH	72% H ₂ SO ₄ ; 1.2 M H ₂ SO ₄ 4 h, 100°C	< 19 nM (< 100 kDa)	Glc equivalents	ND	Zhou et al. (1998)
Shannon point	MBTH	72% H ₂ SO ₄ ; 1.2 M H ₂ SO ₄ 4 h, 100°C	< 21 nM (< 10 kDa)	Glc equivalents	ND	Zhou et al. (1998)
East sound	MBTH	72% H ₂ SO ₄ ; 1.2 M H ₂ SO ₄ 4 h, 100°C	91 nM (< 1 µm)	Glc equivalents	ND	Zhou et al. (1998)

Continued

TABLE 1—Continued

TEP						
East sound	MBTH	72% H ₂ SO ₄ ; 1.2 M H ₂ SO ₄ 4 h, 100°C	42 nM ($< 0.1 \mu\text{m}$)	Glc equivalents	ND	Zhou et al. (1998)
East sound	MBTH	72% H ₂ SO ₄ ; 1.2 M H ₂ SO ₄ 4 h, 100°C	36 nM ($< 100 \text{ kDa}$)	Glc equivalents	ND	Zhou et al. (1998)
East sound	MBTH	72% H ₂ SO ₄ ; 1.2 M H ₂ SO ₄ 4 h, 100°C	57 nM ($< 10 \text{ kDa}$)	Glc equivalents	ND	Zhou et al. (1998)

*Data were taken from tables, estimated from graphs, or obtained from the authors.

†The LC-vis technique (partition chromatography) in borate medium on anion exchange resins detects the following sugars: 2-deoxy ribose, cellobiose, maltose, lactose, fucose, rhamnose, arabinose, galactose, glucose, mannose, xylose, fructose, ribose, gentiobiose, and melibiose (Mopper 1978c). Analyses of sinking or suspended POM using this technique indicated that the major sugars were fucose, rhamnose, arabinose, galactose, glucose, mannose, xylose, and ribose (Ittekkot et al. 1982, 1984a, 1984b) whereas studies of seawater DOM also indicated the presence of fructose (Ittekkot 1982). However all of these monosaccharides (fucose, rhamnose, arabinose, galactose, glucose, mannose, xylose, fructose, and ribose) are considered neutral aldoses (see text) and are presented as such in this table.

‡The LC-vis technique (partition chromatography) using ethanol as eluant on anion exchange resins detects the following sugars: digitoxose, 2-deoxy ribose, 2-deoxy ribose galactose, 3-O-methylglucose, 6-deoxy glucose, fucose, rhamnose, arabinose, galactose, glucose, mannose, xylose, fructose, ribose, lyxose, tagatose, sorbose, and gulose (Mopper 1978a). Analyses of sediments or seawater (DOM) using this technique indicated that the major sugars were fucose, rhamnose, arabinose, galactose, glucose, mannose, xylose, fructose, and ribose. Trace amounts of gulose and sorbose were also reported (Mopper 1977). Again the previous sugars (fucose, rhamnose, arabinose, galactose, glucose, mannose, xylose, fructose and ribose) are listed in this table as neutral aldoses.

§The LC-vis technique (partition chromatography) in acetate medium on anion exchange resins detects the following sugars: mannuronic, glucuronic, guluronic, galacturonic, 4-O-methyl glucuronic, and cellobiuronic acids (Mopper 1977, 1978b). Additional information is presented in Mopper and Larsson (1978).

||The GC-FID technique detects the following sugars: fucose, rhamnose, arabinose, galactose, glucose, mannose, xylose, lyxose and ribose (Cowie and Hedges 1984a). Ribose is generally destroyed during acid hydrolysis and is not always reported in GC-FID measurements. Lyxose does have a record of natural occurrence and is considered as an epimerization product of xylose formed during hydrolysis (Cowie and Hedges 1984a). As above, these sugars are described as neutral aldoses.

¶A variety of monosaccharides including neutral aldoses (fucose, rhamnose, arabinose, galactose, glucose, mannose, xylose, fructose, and ribose), amino sugars (glucosamine, galactosamine, mannosamine, and muramic acid), uronic acids (glucuronic, mannuronic, and galacturonic acids), and some disaccharides such as sucrose, cellobiose, and melibiose have been detected by HPAEC-PAD (Wicks et al. 1991; Mopper et al. 1992; Jørgensen and Jensen 1994; Kerhervé et al. 1995; Mopper et al. 1995; Gremm and Kaplan 1997; Kaiser and Benner 2000; Panagiotopoulos et al. 2001). With the exception of Wicks et al. (1991) (these authors analyzed simultaneously, neutral aldoses, amino sugars, and uronic acids), most investigators only analyzed neutral aldoses by HPAEC-PAD (Rich et al. 1996; Skoog and Benner 1997; Borch and Kirchman 1997; Kirchman et al. 2001). Ribose and fructose were generally found to be destroyed when strong hydrolysis (use of H₂SO₄) was used (Borch and Kirchman 1997; Skoog and Benner 1997; Amon and Benner 2003). They are usually detected when milder conditions (use of HCl or TFA) are employed (Wicks et al. 1991; Jørgensen and Jensen 1994; Gremm and Kaplan 1997; Panagiotopoulos and Sempéré 2005). Kerhervé et al. (1995) and Panagiotopoulos and Sempéré (2005) indicated that glucosamine (GlcN) could also be detected in the pool of the neutral aldoses. Recent investigations suggested that amino sugars may be detected separately from the pool of neutral aldoses (Kaiser and Benner 2000; Benner and Kaiser 2003).

#The MBTH method detects the following categories of sugars: sugar alcohols (mannitol, dulcitol, sorbitol, xylitol, and arabitol), hexoses (fructose, sorbose, galactose, mannose, and glucose), pentoses (arabinose, xylose, and ribose), deoxysugars (2-deoxy galactose, 2-deoxy glucose, fucose, and rhamnose), uronic acids (glucuronic, galacturonic, and gluconic), amino sugars (glucosamine and N-acetyl glucosamine), and disaccharides (maltose) (Johnson and Sieburth 1977; Pakulski and Benner 1992; Table 2). Although few studies have addressed the reactivity of amino sugars with the MBTH reagent (Pakulski and Benner 1992), we believe that other monosaccharides in the amino sugar pool such as galactosamine, mannosamine, and muramic acid also react positively because they contain an aldehyde functional group. The sum of the above categories (sugars alcohols, hexoses, pentoses, etc.), if present in natural samples, is indicated as total dissolved monosaccharides (TDCHO). When samples are analyzed without acid hydrolysis, sugars are listed as dissolved free monosaccharides (DFCHO).

**The PSA method detects the following categories of sugars: hexoses (fructose, galactose, glucose, and mannose), pentoses (arabinose and xylose), deoxy-sugars (fucose and rhamnose), disaccharides (sucrose, maltose, and lactose), trisaccharides (raffinose), uronic acids (galacturonic acid), furaldehydes (5 hydroxy methyl-2-furaldehyde), and some di, tri, or tetra O-Me monosaccharides (2-O-Me xylose, 2,3 Di-O-Me xylose, 2,3,6 Tri-O-Me glucose, etc.) (Dubois et al. 1956; Table 2). It is important to note that this method does not distinguish between free and combined monosaccharides because strong acid (H₂SO₄) is employed as part of the protocol. Consequently results are listed as total dissolved monosaccharides (TDCHO) (see text).

††The TPTZ method detects the following categories of sugars: hexoses (fructose, galactose, glucose, and mannose), pentoses (ribose, arabinose, and xylose), deoxy-sugars (fucose and rhamnose), and uronic acids (glucuronic, galacturonic, and mannuronic acids) (Myklestad et al. 1997; Table 2). Although we have no evidence to suggest that the TPTZ method also detects amino sugars (glucosamine, galactosamine, mannosamine, muramic

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TABLE 1—Continued

acid, and their N-acetyl derivatives), we believe that these compounds are also react positively with the TPTZ reagent because they contain an aldehyde functional group. However, further investigations should estimate their relative absorbance compared to the glucose standard. The sum of the above categories are presented in this table as total dissolved monosaccharides (TDCHO). When samples were measured without acid hydrolysis, sugars are listed as dissolved free monosaccharides (DFCHO).

‡‡The UA assay detects as carbohydrates the following uronic acids: glucuronic, galacturonic, and mannuronic acid. This spectrophotometric method (absorbance 525 nm) is based on the reaction of UA with the sulfamate/m-hydroxydiphenyl reagents and has a high selectivity (> 50-fold) over neutral sugars. The result is given by using an average slope of the three main uronic acids (glucuronic, galacturonic, and mannuronic acids).

Analytical conditions are described in detail elsewhere (Filisetti-Cozzi and Carpita 1991; Hung and Santschi 2001).

§§The HPLC-fluo technique using Dns-hydrazine as fluorimetric labeling reagents detects the following categories of sugars: disaccharides (gentiobiose, lactose, maltose, and cellobiose), hexoses (fructose, galactose, glucose, and mannose), pentoses (arabinose, xylose, and ribose) and deoxy-sugars (fucose, rhamnose, 2- deoxy glucose, and 2- deoxy ribose) (Mopper and Johnson 1983). Analysis of sinking POM or sediment using this technique indicated that the major sugars were fucose, rhamnose, arabinose + xylose (coeluted), galactose, glucose, mannose, and ribose (Hicks et al. 1994). As indicated above, these monosaccharides (fucose, rhamnose, arabinose, galactose, glucose, mannose, xylose, fructose, and ribose) are listed in this table as neutral aldoses.

||Sinking particles were collected using in situ pumps (ISP) equipped with 60- μ m pore diameter filters; in all the other studies sediment traps were used. Additional information provided in Panagiotopoulos et al. (2002).

¶¶The order of relative abundance of aldoses changes with depth. Thus only the major aldoses are presented in this table. McCarthy et al. (1996) indicated that galactose was the most abundant sugar in surface water followed by glucose and fucose or rhamnose, whereas in deep waters, glucose was the major aldose followed by galactose, fucose, rhamnose, and mannose. Similar results for HMWDOM were also reported by Skoog and Benner (1997), and Amon and Benner (2003).

##The order of the relative abundance of aldoses changes with sampling site. Thus, in the sedimentary plume, mid-shelf silt deposit, and Columbia river estuary, glucose was the major aldose followed by galactose and mannose. On the continental slope and in the oxygen minimum, mannose or galactose were the major aldoses, followed by glucose (Keil et al. 1998).

***Miyajima et al. (2001) analyzed the molecular composition of aldoses in the total extracted fraction and the residue by gas chromatography using the method of Walter and Hedges (1988). In the extracted fraction mannose, glucuronic acid and deoxy sugars were the major aldoses while galactose or arabinose were the major aldoses in the residue.

†††Results estimated by authors' data.

ments, sediment porewaters, marine colloids, and transparent exopolymer polysaccharides (TEP), using different extraction protocols and analytical techniques. This data highlights the fact that several protocols of hydrolysis have been proposed, which make direct comparison of results very difficult. Also, it appears that optimal hydrolysis conditions for one type of sample may not be suitable for another (i.e., different hydrolysis protocols between seawater and sinking POM or sediments samples). Additionally, hydrolysis efficiencies varied widely even between samples of the same nature (i.e., particles, sediments, or seawater). For example, Mopper (1977) indicated that 2 N HCl (3 h, 105°C; rotary evaporation or freeze-dry) hydrolysis gave higher yields than 2 N H₂SO₄ (4 h, 100°C) in ancient sediments, whereas use of the same 1.8 or 2 N HCl had a more destructive effect than 2 N H₂SO₄ on organic rich anoxic sediment. Mopper (1977) also indicated that the use of H₂SO₄ or HCl in oxic sediments produced similar carbohydrate yields.

Both strong and mild hydrolysis has been used by marine biogeochemists. Strong hydrolysis is usually performed in two steps (Mopper 1977; Cowie and Hedges 1984a; Pakulski and Benner 1992): samples are treated with concentrated H₂SO₄ (72 wt % ~ 12 M H₂SO₄) for 2 h at ambient temperature (pretreatment). The solution is then diluted to 1.86-1.2 M H₂SO₄ and heated at 100°C for 3 to 4 h (Mopper 1977; Cowie and Hedges 1984a). Although the two-step hydrolysis may induce losses of some monosaccharides (notably of rhamnose, fucose, and xylose; Mopper 1977), several investigators reported that concentrated H₂SO₄ gave higher total aldose yields than clas-

sical 1.86-1.2 M H₂SO₄ hydrolysis (Cowie and Hedges 1984a; Pakulski and Benner 1992). According to Mopper (1977), this is due to the swelling of polysaccharides and partial cleavage of glycosidic bonds, which greatly enhances the effectiveness of subsequent hydrolysis steps. Cowie and Hedges (1984a) analyzed carbohydrates in wood, plankton, and sediment trap samples by GC-FID and suggested that these higher total yields were mainly due to the higher yield of glucose. Glucose appeared to be more efficiently released from cellulose (the primary form of refractory glucose in terrestrial plants) since no significant changes in the concentration of the other monosaccharides were observed (with and without pretreatment). The same observation was also made by Skoog and Benner (1997) in HMWDOM samples analyzed by HPAEC-PAD. Strong hydrolysis is generally recommended for terrestrial samples rich in α -cellulose (the portion of cellulosic material that does not dissolve in a 17.5% solution of NaOH; the portion that dissolves in alkaline solution and precipitates upon acidification is β -cellulose). This two-step hydrolysis may provide important information (by difference in glucose yield) concerning the presence or absence of α -cellulose in samples (Cowie and Hedges 1984b). Strong hydrolysis may also be used for marine samples containing "refractory" structural polysaccharides such as alginic acid, chitin, or cellulose (Pakulski and Benner 1992).

By contrast, mild hydrolysis (0.09-2 M) is usually performed without pretreatment using various acids (H₂SO₄, HCl, TFA, pta, etc.). Mopper (1977) reported for sediments, the hydrolysis efficiency of several acids in the following order: H₂SO₄

(2 N) > HCl (2 N) > CHCl₂COOH (1 N) > H₃PO₄ (15 N) > HOOC-COOH (2 N). Other investigators suggested that H₂SO₄ (0.85 M, 100°C, 24 h, or 0.85 M, 100°C, 4 h) gave the same or higher concentrations for dissolved carbohydrates in natural samples than hydrolysis in dilute HCl (0.09 M, 100°C 20 h or 0.25 M, 24 h, 100°C; Mopper 1977; Hanisch et al. 1996; Borch and Kirchman 1997). Borch and Kirchman (1997) also suggested that 0.85 M HCl hydrolysis (100°C, 24 h) resulted in similar yields to the pretreatment method (Pakulski and Benner 1992). Burdige et al. (2000) decided to eliminate the pretreatment procedure in sediments and sediment porewaters because sugar yields were similar with and without pretreatment (12 M H₂SO₄, 2 h and 1.2 M H₂SO₄ at 100°C for 3 h versus 1.2 M H₂SO₄ at 100°C for 3 h). However, it should be noted that mild hydrolysis clearly does not attack cellulose and thus is not recommended for land-derived material when analyzing carbohydrates in rivers, lakes, or estuaries. As mentioned previously, pentoses are more fragile than hexoses and are partially or completely destroyed after acid hydrolysis. Thus, in particular, fructose and ribose concentrations in environmental samples are only occasionally reported, being generally close to the detection limits of the method employed. However, when mild hydrolysis conditions are used, these sugars are generally detected (Mopper 1977; Walters and Hedges 1988; Sigleo 1996) and, in some cases, may represent the dominant monosaccharides (Panagiotopoulos and Sempéré 2005).

Trifluoroacetic acid (TFA) has also been used by marine biogeochemists for mild hydrolysis of environmental samples (Aluwihare et al. 1997; Repeta et al. 2002). Walters and Hedges (1988) indicated that 0.5 M TFA at 135°C for 2 h hydrolyzed neutral aldoses and uronic acid polymers such as alginic acid (co-polymer of glucuronic and mannuronic acid), and polygalacturonic acid in plankton, sediment, and wood (*see also* Bergamaschi et al. 1999; Table 1). Wicks et al. (1991) reported that use of 2 M TFA at 121°C for 1 h may sufficiently hydrolyze neutral sugars, amino sugars, and uronic acids. However, no direct comparison of TFA and H₂SO₄ or HCl hydrolysis efficiencies have been made so far.

Recoveries of monosaccharide standards using mild hydrolysis conditions (2 M TFA at 121°C for 1 h; 0.1 M HCl at 100°C for 20 h; 0.85 M H₂SO₄ at 100°C for 24 h) fall into the range of 70% to 95% (Wicks et al. 1991; Borch and Kirchman 1997). In agreement with this observation, Cowie and Hedges (1984b) reported that strong hydrolysis, including desalting steps, resulted in losses (on average 15%) for each neutral sugar spiked into a variety of matrices (woods, plankton, and bacteria) as reflected by the adonitol internal standard. However, one should take into consideration that the accuracy of carbohydrate hydrolysis is best tested with an authentic reference carbohydrate polymer (starch, xylan, mannan, cellulose, etc.). Only a few studies have investigated hydrolysis efficiencies on standard polysaccharides with different glycosidic bonds (α or β ; Walters and Hedges 1988; Borch and Kirchman 1997). Borch and Kirchman (1997) indicated that strong hydrolysis was

more efficient for xylan and microcrystalline cellulose (structural polysaccharides) than mild hydrolysis (75% to 80% versus 10% to 40%, respectively), but that the two methods gave comparable yields for laminarin and mannan. It is interesting to note that whether mild or strong hydrolysis is used, sugar loss will occur. Additionally, losses occur during neutralization and deionization procedures. The most common sugars used as spiked internal standards for correction of these losses are adonitol and 2-deoxyribose (Cowie and Hedges 1984a; Borch and Kirchman 1997). With a few exceptions, biogeochemical data are generally not corrected for such losses.

Each acid (H₂SO₄, HCl, or TFA) has advantages and disadvantages that should be considered independently from the effects on hydrolysis yields. For example, H₂SO₄ is not volatile compared to HCl or TFA acids. In this context, hydrolysis employing sulfuric acid requires additional neutralization steps (use of Ba(OH)₂ or precombusted CaCO₃ powder). These steps involve precipitate formation of BaSO₄ or CaSO₄ salts that may provide a substrate for absorption and possible sugar loss. In general, marine biogeochemists prefer CaCO₃ over Ba(OH)₂ because the pH remains lower with CaCO₃ (pH ~ 6), and hydrolyzed sugars do not undergo rearrangements occurring at pH > 7 that would lower the recovery (Borch and Kirchman 1997; Skoog and Benner 1997). Some investigators have reported sugar losses as high as 60% during HCl evaporation (Mopper 1977) whereas others have not (Sweet and Perdue 1982; Jørgensen and Jensen 1994; Sigleo 1996; Cheng and Kaplan 2001). In our laboratory, we have performed HCl hydrolysis followed by evaporation using a rotary evaporator at ambient temperatures (25–30°C), reducing the volume of sample to a few microliters (50–200 μ L). Repetition of the evaporation procedure 2–3 times by adding 200 μ L of Milli-Q H₂O until pH ~7 did not produce detectable sugar losses (Panagiotopoulos and Sempéré 2005). On the other hand, the advantage of using of H₂SO₄ acid rather than HCl acid is that the precipitation of CaSO₄ reduces the ionic strength of the solution, thereby facilitating subsequent desalting steps in the analysis of dissolved samples.

Detection of sugars—Sugars, like lipids and some amino acids, do not have chromogenic or fluorogenic group(s) and consequently do not absorb visible or ultraviolet radiation (sugars generally absorb in < 200 nm; Rocklin and Pohl 1983). Additionally, they are insufficiently volatile for direct analysis by gas chromatography. Thus, most assays rely on derivatization by a chromogen or other molecules such as trimethylsilyl ethers (Me₃Si) or trifluoroacetate (TFA) esters that render the sugars volatile. The name of the chromogen used is generally attributed to the method name. In contrast to other methods, detection by pulsed amperometry (PAD) is unique in that it is performed without prior derivatization (Johnson and LaCourse 1990).

Colorimetric methods—Historically, the first colorimetric methods for environmental carbohydrate determination used chromogens such as anthrone (Antia and Lee 1963; Walsh and Dou-

glass 1966), *N*-ethyl carbazole (Zein-Eldin and May 1958), phenol (Dubois et al. 1956; Handa 1966a; Liu et al. 1973), orcinol (Larsson and Samuelson 1967), and *L*-tryptophan (Josefsson et al. 1972), producing molecules that strongly absorb in the visible spectra. Burney and Sieburth (1977) introduced another colorimetric method based on derivatization with MBTH, which became very popular for its high specificity and low detection limit but involves several procedural steps (see later discussion). Recently, Mykkestad et al. (1997) introduced an "indirect" method (the sugar is not directly analyzed; the product that reacts with sugar is measured) using TPTZ as a chromogen. Total sugar content in environmental samples is generally expressed in glucose equivalents. The major advantages and drawbacks of these methods will be examined in the following sections and comparisons will be made in the assessment section.

Phenol-sulfuric acid (PSA) or Dubois method—In this method, sugars are dehydrated in the presence of concentrated H_2SO_4 at high temperature, forming furfurals (from pentoses) or hydroxymethylfurfurals (from hexoses). Condensation of the latter compounds with a phenol group produces orange-yellow substances that absorb at 480–490 nm (Fig. 2). The color produced at a constant phenol concentration is proportional to the amount of sugar originally present. This method has good precision (< 20% at the 50 μM level) and a detection limit of 25–50 μM (Table 2; Dubois et al. 1956; Gerchakov and Hatcher 1972). The PSA method has mostly been used for quantitative determination of total sugars (TCHO; free + combined) in POM and sediments (Artem'yev 1969, 1970, 1974; Handa 1966b, 1967; Liu et al. 1973).

Dubois et al. (1956) suggested that this method was suitable not only for monosaccharides but also for their methyl derivatives including uronic acids, some oligosaccharides (raffinose and sucrose), and polysaccharides (dextran and starch). Additionally, this method is simple and rapid. However, when used for environmental samples, reproducibility may be low. Color development depends on the sample matrix, as well as the order of addition of the reagents (Artem'yev 1970; Gerchakov and Hatcher 1972). Josefsson et al. (1972) reported that this method shows different molar responses (variable absorbances for equal concentrations of different sugars) for four different sugars tested.

Other investigators indicated that this method cannot distinguish between mono- and polysaccharides (due to the strong acid used) and reported poor detection limits regarding the sugar concentration in seawater (Burney and Sieburth 1977; Dawson and Liebezeit 1981). Additionally, the use of a strong acid may also produce other degradation products (noncarbohydrate compounds) that strongly absorb in the same spectral band with sugars, leading to overestimation of the carbohydrate signal (Josefsson et al. 1972; Dawson and Liebezeit 1981). For instance, when Gelbstoff material (humic material) is treated with H_2SO_4 , it also absorbs in the range of 350–500 nm and may interfere with the determination of total carbohydrates (Sieburth and Jensen 1969; Meadows and Campbell 1978). Similar interference was reported for certain types of flavonoids such as catechin (Rahman and Richards 1987).

Because the PSA method is affected by the presence of salts and has low sensitivity, it has mostly been applied to particulate or sedimentary organic materials and has only rarely been used on seawater samples (Handa 1966b; Janse et al. 1996). Despite these drawbacks, the method is still in use for studies on sediment trap contents (Danovaro et al. 2000), suspended organic matter (Burdloff et al. 2001), algae cultures (Volkman et al. 1993; Janse et al. 1996), and sediments (Fabiano et al. 1995; Underwood et al. 1995; Blanchard et al. 2000; Miyajima et al. 2001).

MBTH method—The lack of specificity and the poor detection limit of the PSA method may be overcome by the MBTH method that combines three well-known reactions of monosaccharides to analyze only one final compound (formaldehyde). The assay relies on reduction of monosaccharides to the corresponding alditols with potassium borohydride (KBH_4) (Abdel-Akher et al. 1951). The produced alditols are treated with periodic acid (HIO_4) and their terminal alditol glycol groups ($-\text{CH}_2\text{OH}$) produce two moles of formaldehyde (Sawicki et al. 1961). The latter compound further reacts with the chromogen 3-methyl-2-benzothiazoline hydrazone hydrochloride (MBTH), producing a blue complex that absorbs at 635 nm (Fig. 2).

In contrast to the PSA method, this method does not present any problems associated with salts or differential responses between sugars. Specificity is enhanced because only one compound is measured, and this compound is formed specifically from sugars via a series of coupled reactions. Furthermore, as there is no requirement for strong acid conditions, a hydrolysis step can be added before analysis to permit determination of both mono- and polysaccharides (free and combined monosaccharides). Additionally, its low detection limits (420–500 nM) and precision (<10% at the μM level) allow its application not only to particulate (Kovac et al. 1998; Murrell and Hollibaugh 2000; Harvey and Mannino 2001) and sediment organic material (Burdige et al. 2000), but also to dissolved samples (free/combined carbohydrates) (Dawson and Liebezeit 1981; Johnson et al. 1981; Harvey 1983; Henrichs and Williams 1985; Burney 1986; Senior and Chevelot 1991; Pakulski and Benner 1994; Harvey et al. 1995; Hanisch et al. 1996; Biddanda and Benner 1997; Bhosle et al. 1998; Terzić and Ahel 1998; Terzić et al. 1998; Pettine et al. 1999; Murrell and Hollibaugh 2000) and ultrafiltered organic matter (Biersmith and Benner 1998; Liu et al. 1998; Mannino and Harvey 2000; Harvey and Mannino 2001). The major classes of sugars analyzed by this method are neutral sugars (pentoses, hexoses, and deoxysugars), amino sugars, uronic acids, and some disaccharides (maltose) (Johnson and Sieburth 1977; Pakulski and Benner 1992).

Despite its potential, the MBTH method is quite laborious since it involves three chemical reactions (Fig. 2; Table 2). The efficiency of each reaction step is fundamental, and it is crucial that any excess reagent (KBH_4 , HIO_4) is eliminated at the end of each reaction before proceeding onto the next step.

Other techniques such as ^{13}C nuclear magnetic resonance (NMR) provide comparable or higher carbohydrate

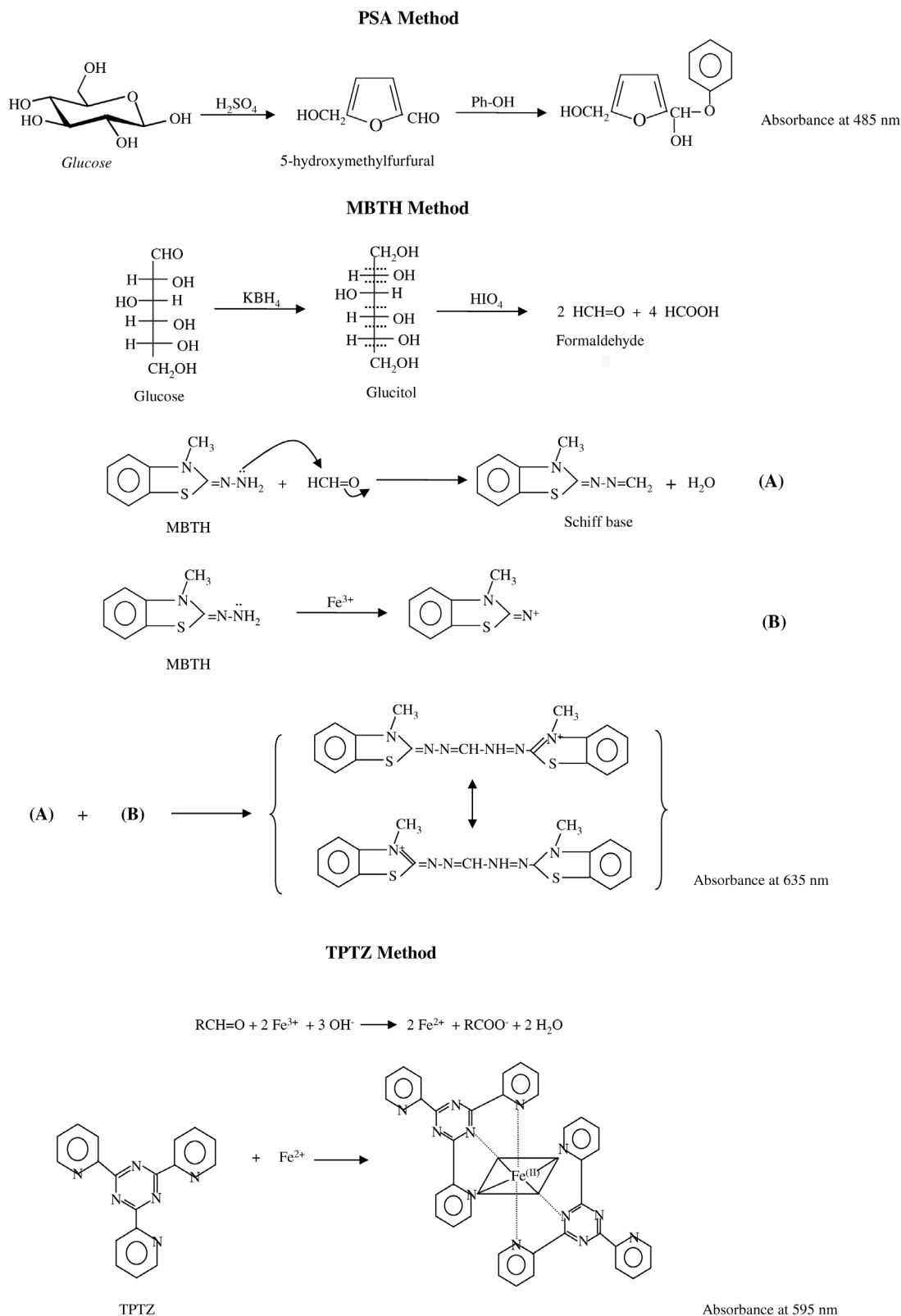


Fig. 2. Schematic reactions for the PSA, MBTH, and TPTZ methods. Note that the unreacted reagents KBH_4 and HIO_4 for the MBTH are quenched using HCl and NaAsO_2 , respectively.

Table 2. Comparison and major characteristics of the colorimetric methods used for environmental carbohydrate determination

	Colorimetric methods		
	PSA	MBTH	TPTZ
Analysis characteristics			
Detection limit	~ μM	~ 0.5 μM	~ 0.4 μM
Precision	<20% (50 μM)	<12% (1 μM)	10% (580 nM)
Derivatization	Visible derivatives (485 nm)	Visible derivatives (635 nm)	Visible derivatives (595 nm)
Pretreatment of samples	Hydrolysis/desalting	Hydrolysis	Hydrolysis
Number of reactions prior to the analysis of final product	2	3	2
Stability of final product and reagents	Medium	Good	Medium
Time for blank preparation	Rapid	Quite long	Rapid
Interferences			
Salts	High	None	None
Humic Substances	High	No sufficient data	No data
Compounds with RCH=O functions	No data	Glyceraldehyde	Glyceraldehyde
Amino acids	No data	Yes*/No†	Only tryptophan and tyrosine
Other	Dissimilar responses between sugars. Substances that absorb in the same spectral band with sugars.	Low signal by tannins and lignins	The final product is very sensitive in light
Applicability			
Neutral aldoses	+	+	+
Uronic acids	+	+	+
Amino sugars	+	+	No data
Methylated sugars	+	No data	No data
Sugar alcohols	No data	+	No data
Oligosaccharides	+	Only reducing sugars	Only reducing sugars
Sum of the above	+	+	+
Type of environmental sample			
Particulate monosaccharides (PCHO)	+	+	+
Sediment	+	+	No study until present
Dissolved free monosaccharides (DFCHO)	–	+	+
Total dissolved monosaccharides (TDCHO)	+	+	+
Characteristics of method			
Simplicity	Easy	Medium	Easy
Rapidity	60 samples/d	15 samples/2 d	60 samples/d
Optimization	Easy	Medium	Easy
Implementation	Simple	Simple	Simple
Cost	Low	Low	Low

*According to Johnson and Sieburth (1977), serine gives a positive reaction.

†According to Opsahl and Benner (1999), bovine serum albumin and lysozyme do not give a positive reaction after acid hydrolysis.

concentrations than MBTH determination (Benner et al. 1992; Pakulski and Benner 1992; McCarthy et al. 1993; Repeta et al. 2002). Such comparisons highlight the suitability of the MBTH method for carbohydrate determination. Opsahl and Benner (1999) tested the cross reactions of the MBTH method in a number of natural compounds including lignins, tannins, proteins, and purified cuticles, and their results indicated that these substances do not interfere with the MBTH method. The method is therefore

likely to be suitable for application on a broad range of environmental samples.

TPTZ method—The TPTZ method was recently introduced (Myklestad et al. 1997) to analyze marine carbohydrates. It combines the low detection limits and precision of the MBTH method with the rapidity and simplicity of the PSA method. This method uses the reducing properties of sugars (*see above*) and is based on the oxidation of sugars by Fe^{3+} ($\text{K}_3[\text{Fe}(\text{CN})_6]$) in alkaline media (Avigad 1968). The resultant Fe^{2+} reacts with

the chromogen TPTZ, producing a violet complex that absorbs at 595 nm (Fig. 2). The TPTZ method is inherently less selective than the MBTH method, however it also has no salt dependence and permits the determination of both mono- and polysaccharides (free and combined monosaccharides).

Its low detection limits ~ 400 nM and precision (< 10%) allow its application to many types of environmental samples including dissolved (free/combined carbohydrates) (Myklestad et al. 1997; Børsheim et al. 1999; Hung and Santschi 2001; Witter and Luther III 2002; Van Oijen et al. 2005) and particulate organic material (Witter and Luther III 2002; Panagiotopoulos and Sempéré unpubl. data unref.). The major classes of sugars analyzed by this method are neutral sugars (pentoses, hexoses, and deoxysugars) and uronic acids (Table 2). Sugar alcohols, some amino acids (glutamic acid, serine, and arginine) and nonreducing oligosaccharides, such as trehalose and sucrose, do not yield significant absorbance (Myklestad et al. 1997). Although no data are currently available on amino sugars measurements by this method, we believe that amino sugars will also react with TPTZ because they are considered reducing sugars.

It is important to note that most of the reagents, as well as the final complex ($\text{Fe}(\text{TPTZ})_2^{2+}$), are very light sensitive. In our laboratory, we tried to minimize light exposure by covering reagent tubes with aluminum foil and by conducting the entire analytical procedure in the dark (Panagiotopoulos et al. 2002). Nevertheless, we found that each sample should be analyzed at least 2 to 3 times because of slight variations in the absorbance of the final complex due to erratic light penetration.

Determination of individual monosaccharides—The methods discussed above provide an estimate of the total carbohydrates present in marine samples. The result is generally given as glucose equivalents, due to the different responses of the various sugar classes (aldohexoses, aldopentoses, etc.), and no information on the individual sugars (mannose, glucose, xylose etc.) is provided. Compositional analyses or determination of individual sugars in marine samples requires additional techniques. Chief among the nonchromatographic techniques are enzymatic assays, while most common chromatographic methods rely upon GC and HPLC.

Enzymatic techniques—Enzymatic techniques for the analysis of sugars are based on a specific reaction between an enzyme and a particular monosaccharide (i.e., glucose, fructose) or a specific glycosidic bond (α or β) of an oligosaccharide (i.e., sucrose) and are therefore potentially very selective (Olechno et al. 1987, and references therein). However, when applied to marine samples, they suffer from interference since metals, salts, or other contaminants may deactivate the enzymes (Dawson and Liebezeit 1981). To our knowledge, these techniques have only been used for glucose determination and not for the estimation of other sugars present (Hicks and Carey 1968; Hanson and Snyder 1979). The choice of enzyme is critical. For example, the use of a nonspecific hexokinase by Hicks and Carey (1968) may result in phosphorylated hexoses

other than glucose, leading to an overestimation of glucose concentration (Hanson and Snyder 1979). The detection limit of the enzymatic assays ranges from 10 to 30 nM, which falls within the concentration range of free dissolved sugars as it is reported by chromatographic techniques. However, blank values can be as high as 80% of the experimental value, which render this method unreliable (Hicks and Carey 1968).

Chromatographic techniques—Paper chromatography was the first method to be routinely employed for the separation of sugars in sediment hydrolysates. It was eventually replaced by thin-layer chromatography (TLC) in silica-gel or cellulose plates (Plunket 1957). This method allows the separation of most monosaccharides as well as their derivatives using different solvent systems (Ghebregzabher et al. 1976). The separated sugars form spots on the TLC and their position is compared with pure sugar standards, thereby permitting their identification. The surface of the spot, after removal, provides a quantitative estimation of the individual sugar. These techniques were mainly applied in freshwater, seawater, POM, and sediments (Degens et al. 1964; Handa and Tominaga 1969; Artem'yev 1974). However, both approaches require hours to days to achieve good separation of the monosaccharides, whereas separation of oligosaccharides is often impossible (Olechno et al. 1987, and references therein). Sugar concentrations on the spots may be estimated by colorimetric methods after reaction with tetrazolium blue (Mopper and Degens 1972). Nevertheless, this involves additional steps, which may include removing sugar spots from the TLC plates and may lead to contamination (Ghebregzabher et al. 1976 and references therein). Furthermore, estimation of sugar concentrations is unreliable due to uncertainties associated with quantifying sugar spots. Thus, these techniques are now only of historic interest (Dawson and Liebezeit 1981).

The first chromatographic methods were based on low pressure partition chromatography (Larsson and Samuelson 1967) using anion exchange resins and ethanol-water as eluants. Detection was carried out using chromogens such as orcinol-sulphuric acid (Josefsson 1970), tetrazolium blue (Mopper and Degens 1972), or Cu-bicinchoninate (Mopper and Gindler 1973; Mopper 1978a). These approaches were successfully applied for the determination of dissolved free or combined sugars in seawater and marine sediments (Mopper 1977). However, these procedures require purification steps (i.e., neutralization, desalting) and the subsequent formation of derivatives may induce losses or contamination of the samples (Mopper 1977, 1978a).

The introduction of borate complexes on strong anion exchange resins in conjunction with the Cu-bicinchoninate (Mopper 1978c) or ethylenediamine detection (Mopper et al. 1980) was the first liquid chromatography method for the analysis of environmental samples, including seawater (Mopper 1978c; Meyer-Reil et al. 1979; Liebezeit et al. 1980; Mopper et al. 1980; Ittekkot et al. 1982, 1984a, 1984b; Jennerjahn and Ittekkot 1999). Despite its potential (minimal sample

pretreatment), this method did not become popular because of the long run times required (2.5 h/sample). Purification procedures of samples may be simplified using reverse-phase chromatography. However, the high polarity and chemical similarity of simple sugar isomers do not allow their separation on reversed-phase columns unless their polarity is drastically decreased by derivatization. Derivatization will also permit their fluorimetric/UV detection (Mopper and Johnson 1983). Although several reagents for derivatization may be found in the literature (Honda 1996; Meyer et al. 2001 and references therein), 5-dimethylamino-naphthalene-sulfonyldiazine (DNS, Mopper and Johnson 1983), and *p*-amino benzoic acid (*p*-AMBA, Meyer et al. 2001) are those most commonly employed for environmental samples (see later discussion).

The advent of high-performance anion-exchange chromatography (HPAEC) in the early 1980s revolutionized sugar analysis, mainly due to two developments in sugar separation and detection. First, the synthesis of anion-exchange resins in pellicular form (Rocklin and Pohl 1983) enormously facilitated sugar separation in alkaline media compared to classical separation techniques using borate complexes (Mopper 1978c; Mopper et al. 1980). Second, the introduction of pulsed amperometric detection (PAD), permitting carbohydrate detection (even those without reducing groups) at high sensitivity (down to 10 pmol) without pre- or post-column derivatization (Rocklin and Pohl 1983; Olechno et al. 1987; Johnson and LaCourse 1990; Lee 1990 and references therein). The first environmental applications using HPAEC-PAD were carried out in the 1990s (Mopper et al. 1992; Jørgensen and Jensen 1994; Rich et al. 1996; Borch and Kirchman 1997; Skoog and Benner 1997); prior to this considerable effort put into sample cleanup (i.e., removal of salts and metals by resins; Wicks et al. 1991; Mopper et al. 1992).

The first gas chromatographic (GC) assays for environmental sugar determination began in the early 1970s. Various compounds were employed to form volatile derivatives. Detection was most frequently performed using flame-ionization (FID). In contrast to liquid chromatographic techniques, GC can be easily coupled to mass spectrometry (MS) providing important structural information (Klok et al. 1984; Sigleo 1996). However, the multiplicity of sugar peaks in the chromatograms and the potentially complex chemical manipulations required (derivatization, desalting) demand careful laboratory techniques and interpretation. In the sections that follow, each of the major chromatographic techniques (GC, borate complexes chromatography, DNS and *p*-AMBA derivatives, and HPAEC-PAD) will be compared and contrasted.

Gas chromatography-flame ionization detection (GC-FID)—As mentioned previously, sugars cannot be directly analyzed by GC because they are not volatile. A variety of compounds such as trimethylsilyl ethers (Modzeleski et al. 1971; Cowie and Hedges 1984a, 1984b; Li and Andrews 1986; Hernes et al. 1996), trifluoacetate esters (Eklund et al. 1977), and acetyl derivatives (Klok et al. 1984; Sakugawa and Handa 1985;

Bhosle et al. 1992; Tanoue and Handa 1987) have been used to form volatile sugar derivatives. The precision of the GC-FID method ranges from 10% to 20% depending on the sugar concentration (Cowie and Hedges 1984a; McCarthy et al. 1996; Sigleo 1996; D'Souza and Bhosle 2001; Ogier et al. 2001). The detection limit ranges from 100 to 150 nM (Table 3; Sweet and Perdue 1982; Cowie and Hedges 1984a; Cheng and Kaplan 2001 and references therein).

The most common monosaccharides analyzed by GC-FID are fucose, rhamnose, arabinose, galactose, glucose, mannose, and ribose (Cowie and Hedges 1984a), but this method is also suitable for the determination of uronic acids (glucuronic, manuronic, guluronic, and galacturonic acids; Walters and Hedges 1988). However, few studies have used this technique to simultaneously measure neutral and acidic sugars (Bergamaschi et al. 1999). Despite its widespread use, GC-FID has two main drawbacks. First, free sugars in solution may exist in five different forms: one acyclic form and two anomers for each of the five- and six-membered ring forms. This multiplicity of forms leads to a complex of peaks for each sugar that has proven difficult to resolve. Second, it is reported that some volatile derivatives (notably TFA derivatives) have low stability and can be lost on the chromatographic column (Eklund et al. 1977).

Removing the carbonyl group involved in ring formation eliminates the problem of multiple peaks for each sugar. Carbonyl group removal can be accomplished by reducing the sugars to their corresponding alditols and is generally followed by formation of Me₃Si ethers or acetate derivatives. However, this approach requires substantial chemical manipulation. Some compositional information may also be lost because certain sugar pairs—lyxose-arabinose and gulose-glucose—yield the same alcohol. Additionally, ketoses present in a sample may also yield the same alcohol (i.e., sorbitol for fructose or glucose). These problems may be partly resolved using a catalyst (e.g., LiClO₄) to bring sugars into mutarotation equilibrium in an organic solvent (e.g., pyridine) prior to derivatization (Cowie and Hedges 1984a; Fig. 3). As an example, Cowie and Hedges (1984a) identified twenty-four peaks from a mixture of 10 monosaccharides standards. This high number of peaks indicates that internal standards (adonitol and sorbitol are commonly employed) are critical in obtaining accurate results. GC-FID has been widely employed for analysis of marine samples, including suspended (Hedges et al. 1994; Cotrim da Cunha et al. 2001; D'Souza and Bhosle 2001) and sinking POM (Cowie and Hedges 1984b; Tanoue and Handa 1987; Hernes et al. 1996; Bergamaschi et al. 1999; Ogier et al. 2001), UDOM (Hedges et al. 1994; McCarthy et al. 1996; Aluwihare et al. 1997; Benner and Opsahl 2001; Repeta et al. 2002), and sediments (Modzeleski et al. 1971; Klok et al. 1984; Bergamaschi et al. 1999; Ogier et al. 2001). Because of its poor detection limit (100-150 nM) compared to modern chromatographic techniques (HPAEC-PAD), data on concentrations of dissolved free monosaccharides are not reported in literature.

Liquid chromatography (LC)—Low-pressure and high-pressure chromatography has been used to measure individual monosac-

Table 3. Comparison and major characteristics of the chromatographic methods used for environmental carbohydrate determination

	Chromatographic methods					
	LC borate complexes		Reversed phase HPLC			
	Cu ²⁺ bicinchoninate	EDA	DNS	p-AMBA	GC-FID	HPAEC-PAD
Analysis characteristics						
Detection limit	4-20 µM	5-20 µM	200-500 nM	100-150 nM	100-150 nM	2-10 nM
Precision	<10% (50 µM)	<10% (50 µM)	<10% (1 µM)	<10% (280 nM)	< 20% (120 nM)	5% to 10% (50 nM)
Derivatization	Visible derivat. (562 nm)	Fluo. derivat. (Ex. 320-365; Em. 410-460 nm)	Fluo. derivat. (Ex. 360-380; Em. 540 nm)	Fluo. derivat. (Ex. 313 ; Em. 358 nm)	Volatile deriv.	No derivatization
Pretreatment of samples	Hydrolysis, desalting*†	Hydrolysis	Hydrolysis	Hydrolysis	Hydrolysis, desalting	Hydrolysis, desalting
Number of reactions prior to the analysis of final product	1	1	1	1	1	0
Stability of final product and reagents	1 week	No data	No data	No data	High	High
Interferences						
Salts	Yes*/No†	None	None	None	High	High
Compounds with RCH = O functions	No data	No data	High	No data	None	None
Amino acids	No data	No data	No data	None	None	No data
Other	Ara. and Fuc. coeluted	Ions: Ag, Hg, Cu, Pd, Ca, I ⁻	Ara and Xyl. coeluted	No data	Multiplicity of peaks	Stability of base line
Applicability						
Neutral aldoses	+	+	+	+	+	+
Uronic acids	No data	+	-	+	+	-
Amino sugars	No data	No data	-	Only glucosamine	No data	+
Methylated sugars	No data	+	-	-	+	-
Sugar Alcohols	No data	-	-	-	+	-
Oligosaccharides	Only reducing oligosac.	+	Only reducing oligosac.	Only lactose	-	+
Sum of the above	-	-	-	-	-	-
Type of environmental sample						
Particulate monosaccharides (PCHO)	+	+	+	No data	+	+
Sediment	+	+	+	No data	+	+
Dissolved free monosaccharides (DFCHO)	No data	No data	-	No data	No data	+
Total dissolved monosaccharides (TDCHO)	+	No data	+	No data	No data	+
Characteristics of method						
Simplicity	Quite difficult	Quite difficult	Medium	Simple	Medium	Difficult
Rapidity (HPLC run)	150 min/sample	100 min/sample	30 min/sample	30 min/sample	30-40 min/ sample	40 min/sample
Optimization	Medium	Medium	Medium	Medium	Medium	Difficult
Implementation	Medium	Medium	Simple	Simple	Medium	Medium
Cost	Medium	Medium	Low	Low	Low	High

*According to Mopper (1978c) and Mopper et al. (1980), purification and desalting steps are not required.

†According to Ittekkot (1984a, 1984b) and Jennerkahn and Ittekkot (1999), POM samples must be desalted by electro dialysis prior to injection.

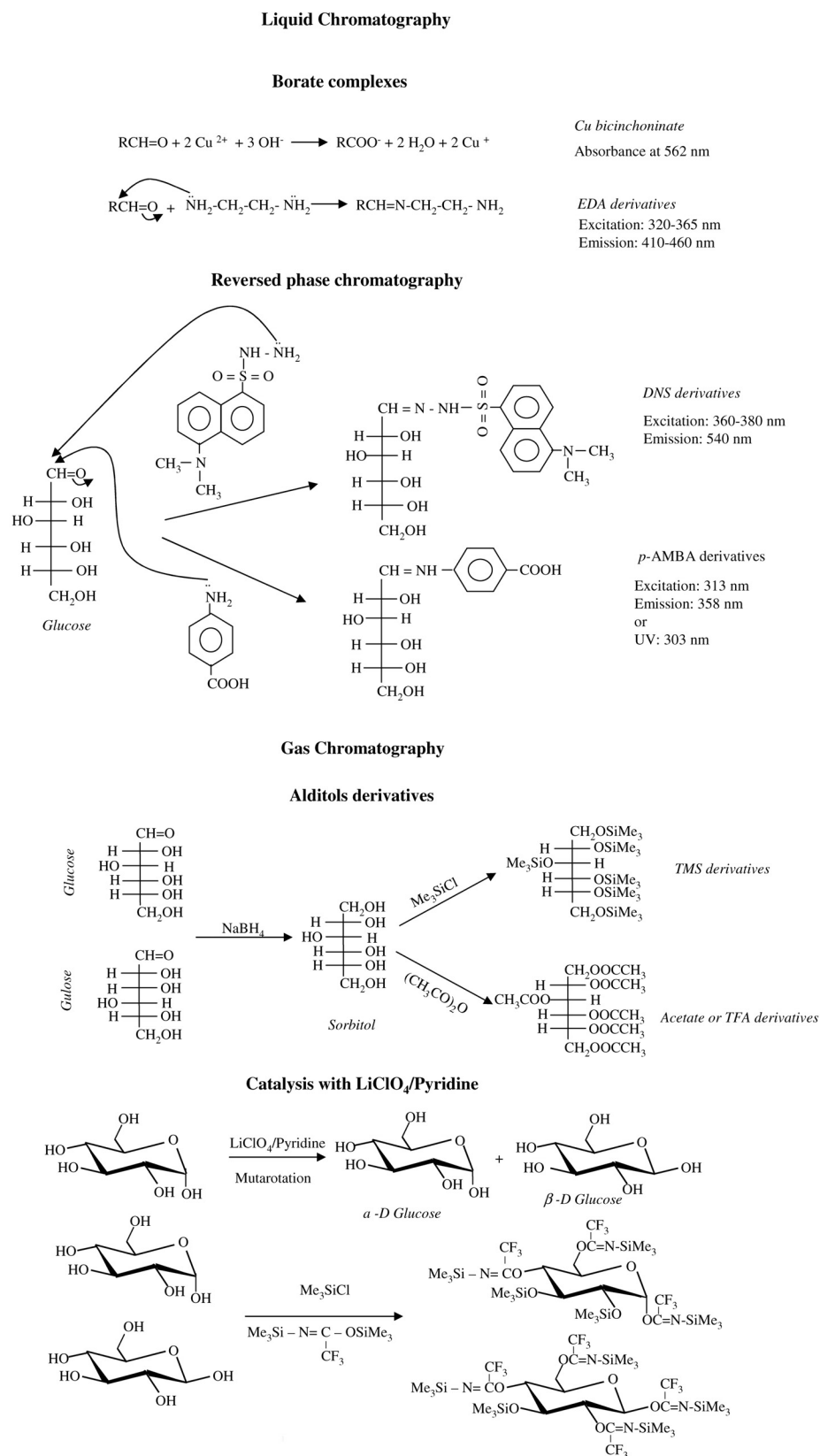


Fig. 3. Schematic reactions of the detection mode of the major chromatographic techniques used for environmental sugar determination: LC-borate complexes, reversed phase chromatography, GC-FID, and HPAEC-PAD. (Continued)

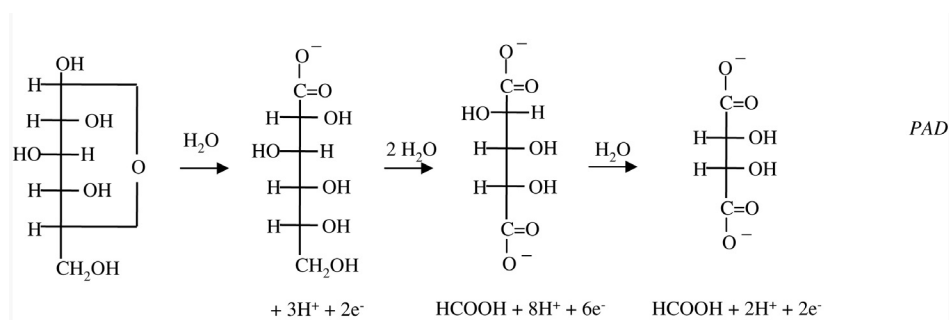


Fig. 3. Schematic reactions of the detection mode of the major chromatographic techniques used for environmental sugar determination: LC-borate complexes, reversed phase chromatography, GC-FID, and HPAEC-PAD.

charides in environmental samples. Low-pressure chromatography with anion exchange resins has been used to separate borate-sugar complexes. High-pressure chromatography includes high-performance liquid chromatography with separation performed on reversed phase columns (use of DNS or *p*-amino benzoic acid derivatives) or anion exchange columns (HPAEC).

Borate complexes—In this method, sugars are separated on strong anion exchange resins by forming complexes with H_3BO_3 , which is added to the eluant. Detection is carried out by spectrophotometry after reaction with Cu^{2+} bichinchoninate (absorbance of Cu^+ at 562 nm), or by fluorescence after reaction with ethylenediamine (EDA; excitation 320-365 nm; emission 410-460 nm; Fig. 3). The precision of the method is $< 10\%$ at the $50\ \mu\text{M}$ level with a detection limit of 4-20 μM (Table 3; Mopper 1978c; Mopper et al. 1980).

Chromatography of sugar-borate complexes has been applied to analysis of sinking or suspended POM (Ittekkot et al. 1982, 1984a, 1984b; Liebezeit 1987; Liebezeit and Bölter 1991; Jennerjahn and Ittekkot 1999; Reschke et al. 2002), sediments (Mopper 1978c; Jennerjahn and Ittekkot 1999), and seawater samples, though to a lesser extent than other methods (Liebezeit et al. 1980; Ittekkot et al. 1982). The most common sugar categories detected by this technique are neutral sugars, methylated sugars, uronic acids, and some reducing oligosaccharides (cellobiose, lactose, maltose, gentiobiose, and melibiose). Mopper et al. (1980) indicated that the EDA-derivatization method was also suitable for nonreducing disaccharides such as raffinose, sucrose, trehalose, and stachyose, although the response factors for nonreducing disaccharides were very low compared to the other sugars. However, nonreducing disaccharides have not been reported in environmental samples by this method.

The major advantage of the borate complex approach is that samples require little or no pretreatment, which makes for easier application in seawater samples, or other aqueous matrices (Mopper et al. 1980; Mopper and Johnson 1983). However, time gained in reduced pretreatment is offset by longer analysis time of 100-150 min/sample, which compares unfavorably with modern HPLC techniques (Table 3). Several parameters such as the flow rate and molarity of the buffer, the pH of the

eluants, the particle size, and the degree of cross linkage of the resins, as well as column temperature, should be taken into account to produce reliable results (Mopper 1978c, 1980). Few recent studies have used this method due to the popularity of modern HPLC for analysis of environmental samples (Jennerjahn and Ittekkot 1999; Reschke et al. 2002).

Reversed phase HPLC, DNS, and *p*-AMBA derivatization—In reversed phase chromatography, the polarity of sugars is decreased during precolumn derivatization, allowing analysis on octadecylsilyl or C_{18} columns. Sugar elution is improved and the time of analysis is shorter, whereas interference from salts or other contaminants is minimized. Several reagents have been used for precolumn derivatization (see review by Honda [1996]); however, environmental applications have used 5-dimethylaminonaphthalene-sulphonylhydrazine (DNS) and *p*-AMBA.

Several investigators improved (Avigad 1977; Alpenfels et al. 1982; Mopper and Johnson 1983) and applied the DNS-derivatization method to seawater (Senior et al. 1985), sinking or suspended POM (Compiano et al. 1993; Hicks et al. 1994), and sediment samples (Hicks et al. 1994). The derivatization reaction is performed by adding trichloroacetic acid and DNS to the sugar sample at 65°C for 20 min (Fig. 3). These pre-analytical steps are simpler and chemically less aggressive than those used in GC analysis, which generally requires higher temperatures. The elution has been performed either by gradient mode and subsequent fluorimetric detection (excitation 360-380 nm; emission 540 nm; Mopper and Johnson 1983) or by isocratic mode and UV detection (230 nm; Senior et al. 1985).

Precision of the method is less than 10% at the μM level with a 200-500 nM detection limit (Mopper and Johnson 1983; Compiano et al. 1993; Table 3). This method detects neutral sugars and also some reducing oligosaccharides such as cellobiose, lactose, maltose, and gentiobiose. Although no description of measurements of amino sugars and uronic acids by this method is currently available, these sugars should also give a positive reaction with DNS reagent because they also contain the aldehyde group. However, environmental applications report only neutral sugars. Mopper and Johnson (1983) indicated that carbonyl compounds such as simple aldehydes

and ketones or ketosteroids react positively with the DNS reagent. Because of their hydrophobicity, the elution of these derivatives does not overlap with those of sugars; however, the presence of these compounds may interfere with sugar derivatization in that they consume reagent (DNS). Consequently, such interfering compounds should either be eliminated prior to derivatization or the unreacted DNS should be quenched by additional purification steps. The latter option is possible using an off-line cleanup procedure as proposed by Mopper and Johnson (1983) that provides excellent sugar recovery (>90%). Finally, some monosaccharides (xylose and arabinose) are poorly resolved while others (e.g., fructose) elute as two chromatographic peaks (Mopper and Johnson 1983; Hicks et al. 1994). Despite these drawbacks, this method is suitable for the trace analysis of reducing sugars in any kind of environmental samples with minimum pretreatment.

The *p*-AMBA method (Meyer et al. 2001) is a promising alternative for the determination of sugars in environmental samples although its use to date has been limited. Like the DNS method, the derivatization reaction with *p*-AMBA reagent forms Schiff bases (Fig. 3). Detection is either photometric (303 nm) or fluorimetric (excitation 313 nm; emission 358 nm). Precision of the method is less than 10% at the 280 nM level with a 100 to 150 nM detection limit (Meyer et al. 2001; Table 3). As for the DNS method, parameters such as the pH of the eluent, temperature of derivatization, and eluant composition should be carefully chosen and optimized to achieve optimal sugar separation. This method detects neutral sugars (xylose, arabinose, galactose, glucose, and mannose), uronic acids (galacturonic and glucuronic acid) and some amino sugars (*N*-acetyl glucosamine).

HPAEC-PAD—In this method, sugar separation is based on anion exchange mechanisms, and the order of elution depends mainly on their dissociation constants ($pK_a = 12-13$). Thus, using a strong base such as NaOH (range 12-25 mM) as an eluant, sugars are either partially or completely ionized, permitting their separation (Rocklin and Pohl 1983). Detection is performed without derivatization by a pulsed amperometric detector (PAD) that applies a triple sequence of potential to a gold electrode and allows the determination of sugars at nM levels (Johnson and LaCourse 1990; Mopper et al. 1992). Before detection (i.e., between the column and the detection cell), a strong base (1M-NaOH) is added to the eluent stream to increase PAD sensitivity and minimize baseline drifting.

Precision of the method falls in the 5% to 10% range (50 nM level) with a detection limit of 2 to 10 nM (Mopper et al. 1992; Table 3). This method generally detects neutral sugars (Mopper et al. 1992) and some disaccharides (Jørgensen and Jensen 1994; Gremm and Kaplan 1997). A recent study indicated that detection of amino sugars was also feasible using different elution conditions (Kaiser and Benner 2000). Although acceptance of the HPAEC-PAD method is widespread, this should not be taken to imply that all major problems associated with sugars analysis in seawater have been resolved. The remaining dif-

iculties fall into two broad categories; problems and inadequacies with the HPAEC-PAD method itself, and problems related to sample pretreatment.

Theoretically, all monosaccharides should be separated on an anion exchange column since they have different pK_a values; nevertheless, this appears not to be the case. Several investigators have reported coelution of sugars, notably for closely eluting pairs of sugars, such as rhamnose/arabinose and xylose/mannose (Jørgensen and Jensen 1994; Kerhervé et al. 1995; Borch and Kirchman 1997). Panagiotopoulos et al. (2001) suggested that small changes in temperature ($\pm 5^\circ\text{C}$) can be found in uncontrolled laboratory conditions and may significantly modify the retention times of sugars, including coelution and poor reproducibility. Coelution of sugars was avoided by fixing the temperature of the column at 17°C and using a flow rate of 0.7 mL min^{-1} (Panagiotopoulos et al. 2001). These findings clearly suggest that temperature control is fundamental to anion exchange separation in order to obtain consistent results.

An additional problem is the presence of carbonate in the eluants or the chromatographic column. Carbonate decreases column performance by lowering the retention times of sugars. Although eluents in all modern chromatographic HPAEC-PAD systems are continuously sparged with inert gases (He or N_2), this can be insufficient and columns should be flushed with a strong base (i.e. 200 mM NaOH; Dionex, Technical note 20). The carbonate problem is most pronounced with eluants < 20 mM NaOH, the range of NaOH concentrations used by marine biogeochemists for neutral sugar determination by HPAEC-PAD. Carbonates can be trapped either online, using carbonate removal traps, or by adding $\text{Ba}(\text{OH})_2$ into the eluants (Cataldi et al. 1998). In our laboratory, we found that the use of $\text{Ba}(\text{OH})_2$ decreased the resolution factor of closely eluted sugars (mannose/xylose). Thus, we preferred not to add $\text{Ba}(\text{OH})_2$ into the eluants, but instead applied a more rigorous cleanup procedure (i.e., flushing the column at the end of each analysis by 1 M NaOH for 30-40 min; Panagiotopoulos and Sempéré 2005). This treatment not only regenerated the column by eliminating carbonate traces, but also re-established its performance by removing other organic or inorganic contaminants associated with sugar samples, hence greatly facilitating the comparison of samples with standards.

In our laboratory, we used a Pd reference electrode (Antec) for more than 3 y with results comparable (detection limit 10 nM; $S/n = 3$, loop = 200 μL) to those obtained with other PAD detectors (E.G.&G. or Dionex). This type of electrode requires no maintenance, unlike the Ag/AgCl reference electrode, which showed progressive deterioration within the mobile phase concentration range (< 20 mM NaOH) used.

Desalination of samples before HPAEC-PAD analysis is of major importance to obtain valid results. Particulate samples (after hydrolysis) do not exhibit serious problems because of the low amount of salts and the high quantity of material. However, the salts present when dissolved sugar are analyzed can decrease sugar retention times because of the high affinity

of Cl^- with the anion exchange column. Two procedures exist for desalting seawater samples (Mopper et al. 1992). The first desalination procedure is based on anion exchange (mixture of AG2-X8 resin in the carbonate form and AG50-X8 resin in the hydrogen form) and is generally preferred by marine biogeochemists because it is milder and less expensive. Nevertheless, this procedure has two main drawbacks. First, the recovery of individual monosaccharides from seawater is variable. For example, Borch and Kirchman (1997) reported sugar losses of 40% to 50% during deionization; other investigators reported comparable sugar losses (Rich et al. 1996). The second problem is the loss of charged sugars (i.e., amino sugars and uronic acids). Analysis of some charged sugars (amino sugars) is feasible using other types of resins (AG11 A8-Biorad for neutralization and AG50-X8 in the Na^+ form for purification of the sample) but no study has simultaneously analyzed both neutral and amino sugars (Kaiser and Benner 2000). Furthermore, so far no attempt has been made to analyze uronic acids by HPAEC-PAD in seawater samples. The second desalination procedure uses silver cartridges to remove Cl^- from samples in the form of AgCl . Although this is faster and easier than using anion exchange resins (commercially available cartridges are easily found), limited data concerning the use of silver cartridges exist in literature.

In marine studies, HPAEC-PAD has been mainly used to analyze sugars in DOM or UDOM (Jørgensen and Jensen 1994; Rich et al. 1996; Borch and Kirchman 1997; Gremm and Kaplan 1997; Rich et al. 1997; Skoog and Benner 1997; Amon et al. 2001; Kirchman et al. 2001; Amon and Benner 2003; Benner and Kaiser 2003) and to a lesser extent in POM or sediment samples (Buscail et al. 1995; Kerhervé et al. 1995; Skoog and Benner 1997; Kerhervé et al. 1999; Kerhervé et al. 2002; Panagiotopoulos and Sempéré 2005).

Nuclear magnetic resonance spectroscopy (NMR)—NMR spectroscopy is a very good complement to the colorimetric or chromatographic techniques since it is capable of defining the major structural characteristics (functional groups) of natural organic mixtures. The technique is nondestructive and provides information on the chemical environment surrounding individual hydrogen, carbon, and nitrogen atoms, from which molecular structures are inferred. As an example, the major functional groups that have been identified in HMWDOM are COOH (resonance at 180 ppm), $\text{C}=\text{C}$ (resonance at 140 ppm), OCO (resonance at 110 ppm), HCOH (resonance at 70 ppm), and CH_x (resonance at 20-30 ppm) (Benner et al. 1992; Repeta et al. 2002). OCO and HCOH functional groups are typical of carbohydrates (Fig. 1). The information given by NMR is also quantitative (integration of peaks), and the results may be compared to those obtained by colorimetric or chromatographic techniques.

The major advantage of this technique is that it can be expanded to two-dimensional resolution to reveal the linkages and proximities of atoms (C, H, and N) in their natural sample matrices. Examples of the NMR potential are correlation

spectroscopy (COSY), total correlation spectroscopy (TOSCY), and heteronuclear correlation spectroscopy (HETCOR). However, NMR spectroscopy has much poorer detection limits than HPLC or MS techniques (samples quantities in the order of several mg are required), thus limiting its application to marine samples. Therefore, it is not surprising that most of the carbohydrate data obtained by NMR do not concern the bulk of DOM, but only the HMWDOM samples which are obtained by ultrafiltration of hundreds to thousands liters of seawater, and generally represent 20% to 35% of DOM (Benner et al. 1992; Aluwihare et al. 1997; Repeta et al. 2002).

Assessment

Comparison of the colorimetric methods—The major characteristics of the colorimetric methods are shown in Table 2. Included in the table are factors that can interfere with analysis, the classes of sugars analyzed, and the different types of environmental samples that can be analyzed by each method. Colorimetric methods are simple and rapid (except the MBTH method) with low analytical cost, but all require derivatization prior to detection. The most important advantage of the colorimetric methods is that several sugar classes can be analyzed simultaneously, including neutral sugars, amino sugars, uronic acids, and sugar alcohols. Modern chromatographic methods do not always allow simultaneous analysis of different sugar classes (see later discussion). Furthermore, most colorimetric methods are not sensitive to salts, facilitating their use for dissolved marine samples.

A major drawback of colorimetric methods is their poor detection limit, ranging from 0.4 to 1 μM (Table 2), which does not allow analysis of dissolved free carbohydrates. Thus, it is not surprising that most studies of dissolved free carbohydrates reported concentrations near the detection limit of the method despite the use of different hydrolysis conditions (H_2SO_4 or HCl ; Pakulski and Benner 1994; Witter and Luther III 2002). From the perspective of specificity and sensitivity, the most reliable methods are MBTH and TPTZ, although the PSA method is still in use. Witter and Luther III (2002) reported that the TPTZ and MBTH methods give similar results for dissolved carbohydrates. However, the same authors have also reported discrepancies between the two methods when particulate carbohydrates are analyzed. It is possible that monosaccharides react differently with the TPTZ reagent than with the MBTH reagent when associated with colloidal or POM (Witter and Luther III 2002). However, the simplicity and rapidity of the TPTZ method allows shipboard use, which can facilitate analysis (Luther and Witter III 2002).

Comparison of chromatographic methods—The major characteristics of the various chromatographic methods described in this review, including possible chemical interferences of each method, the class of sugars analyzed, and the types of environmental samples each method has been applied to are shown in Table 3. Chromatographic methods have a medium

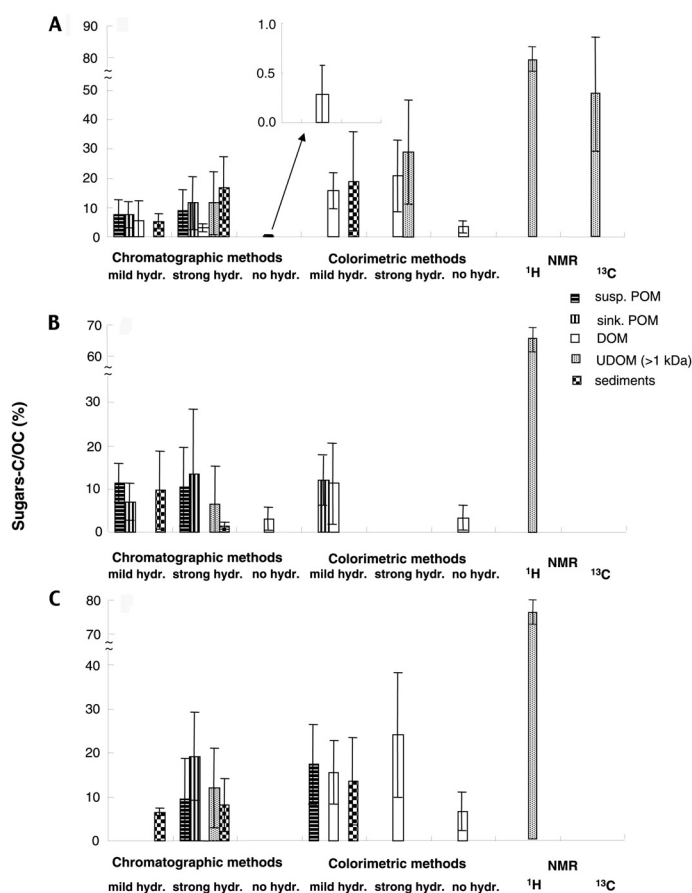


Fig. 4. Sugar contribution to the total organic carbon pool using different extraction protocols (strong or mild hydrolysis) in (A) open ocean/seas, (B) lakes/rivers/estuaries/, and (C) gulfs/bays analyzed by chromatographic (HPLC-vis, HPLC-fluo, GC-FID, and HPAEC-PAD) or colorimetric techniques (PSA, MBTH, and TPTZ). Figure data are obtained from Table 1. ¹³C NMR data are obtained from Benner et al. (1992) and Repeta et al. (2002). ¹H NMR data are from Aluwihare et al. (1997) and Repeta et al. (2002).

implementation with low cost of analysis (except for the HPAEC-PAD technique) but optimization of these techniques is not easily achieved. Most of the chromatographic techniques are similar to colorimetric techniques in that they require derivatization steps prior to detection. In contrast, HPAEC-PAD does not require prior derivatization. However, the detector (PAD) is expensive and delicate to operate, with lengthy system equilibration times (35–50 min). Also, the working electrode requires regular clean up.

The most important advantage of chromatographic methods over colorimetric methods is their low detection limit as well as their potential to analyze individual sugars. The low detection limits permit analysis of dissolved free or combined sugars in marine environmental samples. However, the presence of salts in marine samples requires additional pretreatment (desalination) steps, unless reversed phase HPLC is employed. All of these techniques allow the analysis of all

seven neutral sugars (fucose, rhamnose, arabinose, galactose, glucose, mannose, and xylose) commonly found in marine samples after acid hydrolysis (fructose and ribose are generally destroyed), although concentrations of some other monosaccharides (i.e., glucosamine, muramic acid, sugar alcohols, methylated monosaccharides) or disaccharides (lactose, sucrose) are sometimes reported. Among these techniques, HPAEC-PAD would seem to be best for environmental applications as it combines high sensitivity (nM level) with minimal pretreatment.

Sugar content in suspended and sinking POM—Literature data indicate that sugar concentrations in suspended POM are generally higher in estuaries/lakes/rivers (100–5000 nM) or in gulfs/bays (30–4000 nM) relative to open ocean/sea samples (2–600 nM; Table 1). However, it is interesting to note that whatever the extraction protocol employed, the relative contribution of sugars to the POC pool (PCHO-C/POC) estimated by chromatographic methods is approximately 8% to 11% for samples from all aquatic environments (Table 1; Fig. 4). These results suggest that there is little difference between strong and mild hydrolysis conditions when analyzing suspended POM by chromatographic techniques. Additionally, glucose is the major aldose in most suspended POM samples, whereas xylose or arabinose are occasionally found as predominant monosaccharides (Table 1).

Sugar concentrations in sinking POM range from 4–24 mg g⁻¹ and from 3–30 mg g⁻¹ for samples from open ocean/seas and estuaries/lakes/rivers, respectively. Similar to observations on suspended POM, sugar yields in open ocean/sea samples analyzed chromatographically showed a close agreement between mild (range 3% to 18%) and strong hydrolysis (range 5% to 18%) for all environmental samples; Fig. 4). However, strong hydrolysis resulted in higher yields for samples from estuaries/lakes/rivers or gulfs/bays (range 3% to 31%). The higher yields following strong hydrolysis of nearshore samples of sinking POM may be due to the presence of cellulose from terrestrial sources. With few exceptions, glucose was the dominant sugar in sinking POM samples.

Sugar content in DOM and UDOM—Concentrations of dissolved combined sugars in DOM for gulf/bay samples range from 2–21 μM and are higher than those reported in the open ocean/sea (range 0.035–13 μM) or in estuaries/lakes/rivers (range 0.1–8 μM). Similar patterns were also observed for dissolved free sugars, with higher concentrations in gulf/bay samples (range 0.3–10 μM) than in open ocean/sea (range 0.019–4 μM) or estuary/lakes/river samples (range 0.1–3 μM). Regardless of the hydrolysis protocol used, dissolved combined sugar yields estimated by colorimetric techniques were higher (range 11% to 24%) than those estimated by chromatographic analyses (3% to 6%) for samples in all environments (ocean/seas, estuaries/lakes/rivers, and gulfs/bays). The same has been observed for dissolved free sugars (0.3% to 3% for chromatographic techniques versus 4% to 7% for colorimetric techniques). These results are consistent with observations of sus-

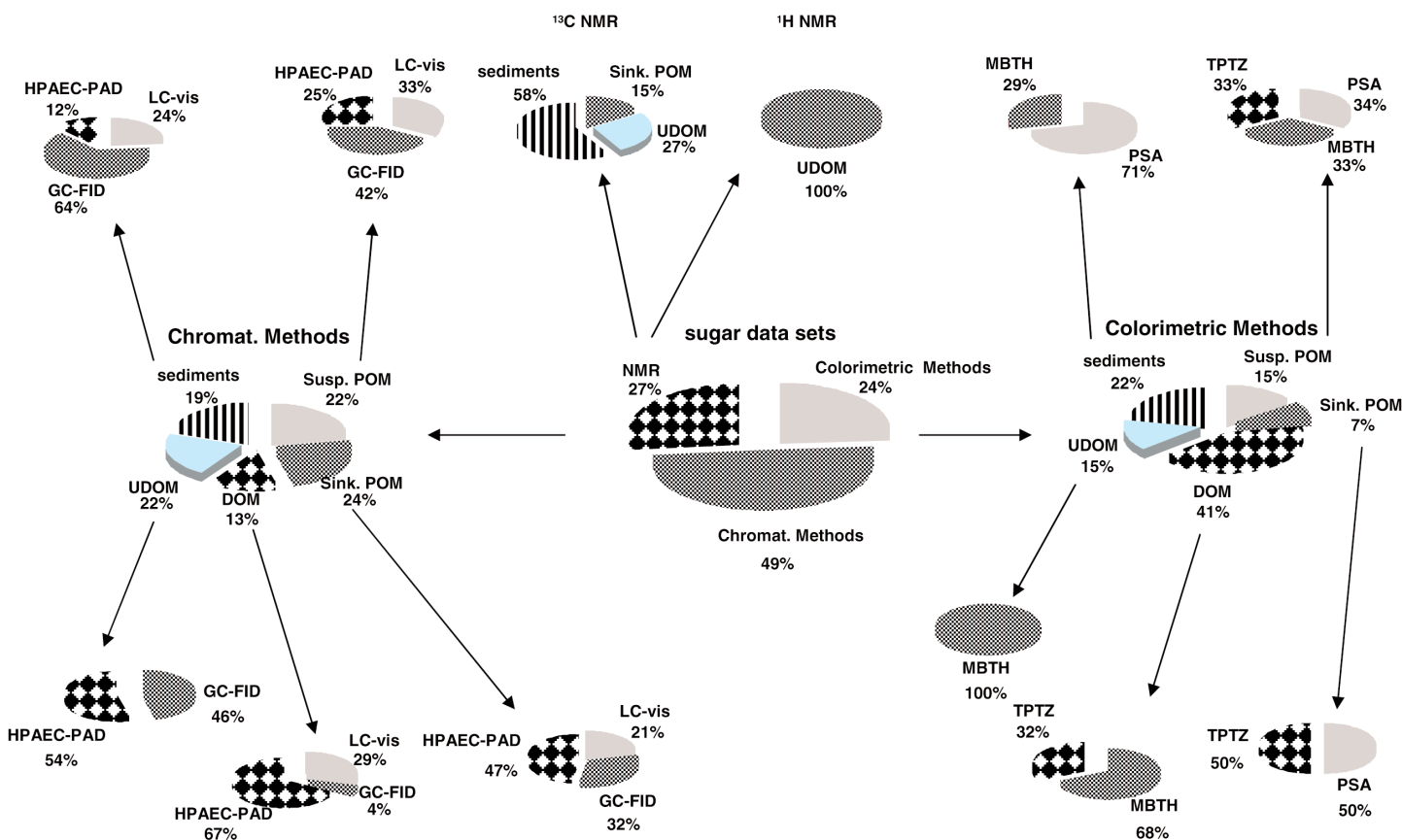


Fig. 5. Sugar data sets using NMR, spectrophotometric, and chromatographic techniques in suspended and sinking POM, DOM, UDOM, and superficial sediments. Spectrophotometric and chromatographic data are those presented in Table 2. NMR data are from Benner et al. (1992), Aluwihare et al. (1997), Gélinas et al. (2001), Hedges et al. (2001), and Repeta et al. (2002).

pendent POM samples and suggest that either a significant fraction of the carbohydrate pool is underestimated using chromatographic techniques, or that molecules with similar functional groups ($-CH=O$) as sugars may give positive reactions in the colorimetric techniques (Fig. 4). Thus, it is not surprising that several authors employing the MBTH method have used the term “carbohydrate-like compounds” when describing their results (Senior and Chevelot 1991; Harvey et al. 1995).

Figure 4 indicates that strong hydrolysis yields more monosaccharides (yields: 21% to 24%) than mild hydrolysis (yields: 11% to 16%) when samples are analyzed by colorimetric techniques, but not when analyzed by chromatographic techniques (yields 3% to 5.5%). Glucose and fructose are the major aldoses in unhydrolyzed samples (dissolved free aldoses), while glucose, galactose, mannose, and xylose are the predominant aldoses in hydrolyzed samples (dissolved combined aldoses).

The concentrations of dissolved combined sugars in UDOM for gulf/bay samples range from 0.18 to 0.55 μM , lower than those reported in open ocean/sea (range 0.03–2 μM) or estuary/lake/river samples (range 0.058–1.8 μM). Sugar yields from colorimetric techniques after strong acid hydrolysis (~ 29%) were

higher than those obtained by chromatographic techniques (range 7% to 12%). Glucose, galactose, fucose, rhamnose, xylose, and mannose were the dominant aldoses in the UDOM pool (Aluwihare et al. 1997). However, their relative abundance changes as a function of depth (McCarthy et al. 1996; Skoog and Benner 1997).

Sugar content in sediments—The concentration of combined sugars in sediments for gulf/bay samples (range: 0.2–46 mg g^{-1}) were similar to those in the open ocean/sea (range 0.1–36 mg g^{-1}). Lower values were reported in estuaries/lakes/river samples (range 0.9–7 mg g^{-1}) although a value as high as 104 mg g^{-1} has also been recorded. Sugar yields estimated by colorimetric techniques after acid hydrolysis were higher (range 14% to 19%) than those obtained by chromatographic techniques (range: 5% to 17%; Fig. 4). Fig. 4 indicates that strong hydrolysis conditions lead to higher yields for sediment samples (5% to 10% mild hydrolysis versus 2% to 17% for strong hydrolysis). A low yield (~4%) from strong hydrolysis in estuary/lake/river samples (Ogier et al. 2001) may be due to pretreatment and hydrolysis by HCl. Glucose, galactose, mannose, and xylose were the dominant aldoses in sediments.

Discussion

The major points of this review are illustrated in Fig. 5. The fact that nearly half of the data in Fig. 5 were obtained by chromatographic techniques further highlights the level of interest within the marine biogeochemical community in the molecular composition of sugars. The results also show that most of the chromatographic data (65%) deal with suspended or sinking POM and sediments, which exhibit fewer analytical difficulties than seawater samples (DOM or UDOM). This result suggests that there is a lack of data on sugars in DOM and UDOM and additional measurements on concentrations of these compounds will be well-accepted in the future. Among available techniques, the ones most often used for environmental samples were GC-FID and HPAEC-PAD. However, it is important to note that molecular-level analyses are highly selective for individual classes or subclasses of sugars (e.g., the HPAEC-PAD method only provides information on neutral sugars or amino sugars), which may comprise a small, and not necessarily representative, portion of the bulk carbohydrate pool.

On the other hand, colorimetric and NMR techniques contribute similar amounts of data to the marine sugar literature (24% and 27%, respectively) although the use of NMR for sugar determination only started a decade ago. In contrast to chromatographic techniques, colorimetric techniques have produced more data (56%) on sugar concentration in DOM or UDOM. The more frequent use of colorimetric techniques for DOM and UDOM is due to the lack of any interference by salts in the analysis (except for the PSA method). The most commonly used techniques for environmental samples are the MBTH and TPTZ methods. Sugar analysis by colorimetric or NMR techniques has the major advantage of yielding characteristics that are representative of the entire carbohydrate pool, but provides no information at the molecular level.

Carbohydrate measurements of UDOM using chromatographic methods indicate that more than half of the sugar-C escapes analysis, compared to ^1H and ^{13}C NMR techniques (Fig. 4). Similar inconsistencies have also been reported for plankton and sinking POM (Wakeham et al. 1997; Hedges et al. 2001, 2002). There is no clear explanation for these inconsistencies, however, two suggestions are made: First, other carbohydrate compounds, such as sugar alcohols, methylated sugars, uronic acids sulfonated/phosphorylated sugars, furfurals, glyoxal, etc, may contribute considerably to the carbohydrate pool, but are outside the analytical window of the HPLC or GC. These compounds may either be present in the samples or be formed through acid hydrolysis. For example, when sugars are treated with concentrated acid, it is well known that they produce furfurals (see PSA method). Amino acids may also give side reactions with sugars during hydrolysis (e.g., formation of Schiff bases). To our knowledge, no study has tested for the presence of Schiff bases after hydrolysis in marine samples.

A second possible explanation is that the rigid structure of the carbohydrate polymers in seawater is not broken down

after acid hydrolysis. Recent ^1H and ^{13}C NMR studies indicated that 60% to 80% of the HMWDOM carbon is composed of acyl polysaccharides, which are highly branched (Aluwihare et al. 1997). However, previous authors have only succeeded in characterizing only 15% to 20% of the HMWDOM carbon as individual monosaccharides after acid hydrolysis using chromatographic techniques. Assuming that NMR measurements do not overestimate the sugar content in marine samples, as suggested Repeta et al. (2002) and Eglinton and Repeta (2004), it seems likely that the reconciliation of NMR, colorimetric, and chromatographic measurements is a key to the understanding of the composition of a major DOM fraction.

Comments, recommendations, and future directions

This study demonstrated that mild and strong hydrolysis give comparable results for open ocean samples, including POM, DOM, and UDOM (except for sediments) using chromatographic or colorimetric techniques (Fig. 4). Fig. 4 also indicates that colorimetric techniques show much higher concentrations of reducing sugars than are observed using molecular separation methods such as GC-FID or HPAEC-PAD. As mentioned above, chromatographic techniques cannot provide information about the entire carbohydrate pool. To our knowledge, no studies have analyzed sugar alcohols, uronic acids, or methylated sugars by HPAEC-PAD in seawater, although this study should be feasible, since these compounds have different pKa values from neutral sugars. In the three studies that analyzed such compounds by GC-FID, these classes of sugars constituted less than 12% of the carbohydrate pool in sinking POM or sediments (Bergamaschi et al. 1999). It is interesting to note that more than 40 varieties of *O*-Me sugars were identified in Black Sea sediments after acid hydrolysis and subsequent GC-FID analysis (Klok et al. 1984).

Analysis of uronic acids by HPAEC-PAD will be an important analytical challenge for marine biogeochemists, not only because of the high amount of salts in samples and the negative charge of these compounds, but also because acid hydrolysis produces lactones (internal condensation of the carboxylic group and the hydroxyl group of sugars). Although lactones are readily split in an aqueous NaOH solution (pH = 8-9 at 35°C), the Lobry de Bryan-Alberda van Ekenstein transformation (isomerization reaction) may occur, altering the uronic acid content of the sample. This transformation is well known for sugars, and uronic acids are even more labile than sugars (Mopper 1977; Angyal 2001).

Furthermore, there is a lack of data comparing different hydrolysis protocols using the same material in literature, while no intercalibration exercise has been conducted between HPLC and GC. Other classes of anionic carbohydrates for which little up-to-date information exists are phosphorylated and sulfated sugars. Phosphorylated sugars, which are intermediate metabolites, are key compounds in a variety of biological processes, such as synthesis of oligosaccharides and nucleotides (Marquardt and Freeze 2001). These compounds

are also allosteric regulators for a whole range of enzymes and are involved in signal transduction and catabolite regulation (Saier et al. 1996; Helenius and Aebi 2001). Sulfated sugars are important components of surface-active polysaccharides and mucopolysaccharides, which are binding agents in marine aggregates and are the major exudates of algae and bacteria (Mopper et al. 1995 and references therein; Zhou et al. 1998). Sulfated sugars and 5-thio-sugars have also been reported in marine sponges (Capon and McLeod 1987; Zierer and Mourão 2000). The biochemical and the bioanalytical literature contains a plethora of methods related to the analysis of sulfonated (Karlsson et al. 1996; Siegel et al. 1997; Descroix et al. 2003; Nika et al. 2003) or phosphorylated sugars (Swezey 1995; Feurle et al. 1998; de Bruijn et al. 1999; Leavell and Leary 2003), however, these techniques have never been applied to marine samples. For example, phosphorylated sugars can be separated on anion exchange columns and detected by pulsed amperometry or on β -cyclodextrin bonded phases and detected by electron ionization tandem mass spectroscopy (de Bruijn et al. 1999; Feurle et al. 1998). Although amperometry enables detection of very small amounts of carbohydrates (nM level) without extensive purification or derivatization, online mass spectroscopy (MS) allows reliable identification of compounds. Thus, phosphorylated sugars, such as 1-phosphate glucose and 6-phosphate that are difficult to differentiate merely by chromatographic means could be identified by characteristic product ions (Feurle et al. 1998).

Another interesting field for future research is the development of techniques that allow the simultaneous measurement of D/L enantiomeric sugars in marine samples. While most naturally occurring sugars are assumed to have the D-configuration, the less common L-forms have also been found in the leaves of jute and *Grindelia* species (L-glucose), algal mucilages (L-galactose), and bacterial polysaccharides (L-mannose, galactose, fucose, and galactose; Kennedy and White 1983). Similarly, although most amino acids occur in the L-form, D amino acids are found in the bacterial cell wall polymer (peptidoglycan) and have been reported as important components of UDOM (McCarthy et al. 1998). This finding has recently been challenged (Aluwihare et al. 2005), nevertheless other studies indicated that amino acids found in environments of low biological activity exhibit high D/L ratios (Dittmar et al. 2001). Is the D/L ratio of enantiomeric sugars similar to or different from that of amino acids? Do L-sugars constitute a fraction of the low molecular weight DOM? Do bacteria also uptake L-sugars? These questions cannot be answered without the development of chromatographic techniques applicable to marine samples. Earlier investigations indicated that enantiomeric sugars can be analyzed by GC-FID on permethylated α -, β -, or γ -cyclodextrin capillary columns as trifluoroacetylated alditols or as aldose diethyl dithioacetals derivatives (Lindqvist and Jansson 1997). However, this technique does not allow the separation of D/L forms of galactose, xylose, and ribose, while the FID is not as sensitive as the PAD

method (see previous discussion and Table 1). Alternatively, enantiomeric sugars can be separated by capillary electrophoresis either as borate complexes or as complexes with linear or cyclic dextrans (Stefansson and Novothy 1993). The detection is carried out after derivatization with fluorescent reagents. Another promising technique is based on the reaction of permethylated monosaccharides with a chiral fluorescent reagent (2-methyl-2 β -2naphthyl-1,3-benzodioxole-4-carboxylic acid) and HPLC fluorimetric detection of the derivatives (Bai et al. 1997). It is worth mentioning that this method is able to determine D, L enantiomeric sugars at the picomolar level.

Acid hydrolysis provides little information on the secondary and tertiary structures of marine carbohydrates. For example, although glucose is abundant in POM and seawater samples, we do not have information on the structure of the original glucose-containing polysaccharides. Is it a β -1,3 glucan (storage polysaccharide), cellulose (structural polysaccharide), glycoprotein, glycolipid, or another sugar polymer? These are fundamental questions, since these polymers, depending on their structure, can be more or less amenable to breakdown by heterotrophic bacteria involving sugars in the global carbon cycling. There appears to be a paucity of data on oligosaccharides and polysaccharides present in seawater (Sakugawa and Handa 1985) and as the full structure of these polysaccharides is not known, they cannot be compared with pure reference sugars polymers. Additionally, there are only limited numbers of reference sugar polymers commercially available, and their cost is often prohibitive for standard research budgets. Furthermore, there is a need to establish methods for predicting chromatographic parameters (i.e., elution time) for known oligo-polysaccharide structures and, conversely, for deducing the structure of unknown oligo-polysaccharides from their chromatographic parameters. To our knowledge very few attempts have been made regarding this issue using HPAEC (Sasagawa et al. 1989).

In a recent study, Penna et al. (2003) characterized oligosaccharides in the water-soluble fraction of mucilage, using HPLC (C18 column) and refractive index (RI) detection. Their results suggested that the mucilage material mainly consisted of maltose and maltopentaose. This technique appears to be promising, although the detected sugars were not further characterized by MS. Alternatively, polysaccharides can be separated on cation exchange HPLC on a sulphonated styrene-divinylbenzene gel in the silver or lead form and detected with RI (Angyal 1989; Angyal 2002; Aluwihare et al. 2002). Although the detection limit of RI is high (0.2 mg L⁻¹), these methods can be applied in concentrated POM, HMWDOM, or mucus material. The use of mild eluants (Milli-Q, H₃PO₄) is an advantage, as they can be removed easily from the sample. Separation and purification of polysaccharides by HPAEC using NaOH and/or CH₃COONa as eluants requires additional steps to eliminate the NaOH/CH₃COONa (Lee 1996). Furthermore, these techniques do not destroy the sample (RI detection vs PAD), which can be recovered at the output of the detector.

The biochemical literature, therefore, appears to contain a large number of publications on the structural determination of the secondary and tertiary structure of polysaccharides, some of which could be adapted to marine samples. In most of these studies polysaccharide characterization is carried out using a combination of chemical and physical techniques including methylation analysis, reductive cleavage, partial hydrolysis, low-pressure gel filtration, anion exchange chromatography, gas chromatography, capillary electrophoresis, mass spectroscopy, and 1- and 2D NMR spectroscopy. For example, in the reductive cleavage method, sugars are first permethylated and then the glucosidic bonds are cleaved with Et_3SiH using $\text{Me}_3\text{SiOSO}_2\text{CF}_3$ as catalyst producing anhydroalditols. The latter compounds are further analyzed by GC-FID or GC-MS (Rolf and Gray 1982; Ahrazem et al. 2001; Colquhoun et al. 2001; Hashii et al. 2003). NMR spectroscopy is a powerful tool in providing information on the linkages (α or β), geometry, and functional groups ($-\text{NH}_2$, $-\text{OME}$, $-\text{CH}_3$, etc.) of the monosaccharides constituting polysaccharides, lipopolysaccharides, or glycoproteins (Bailey et al. 1997; Deepak et al. 1999; Bystrova et al. 2003; Mao et al. 2003). In addition, NMR spectroscopy can be used to check the purity of the isolated compound(s). However, most NMR studies are carried out on HMWDOM and hence very little data on POM or sediments are available. The combination of HPAEC with analytical tools such as MS or NMR may be useful in the future for resolving the structures of sugar polymers in seawater. HPLC-MS and HPLC-NMR analyses have become commonplace in many laboratories for routine analysis of vitamins, metabolites, alkaloids, flavonoids, sesquiterpenes, and saponins. Application of these tools to the analysis of sugar mixtures should become a priority for marine biogeochemists.

Finally, very little up-to-date information exists on the isotope geochemistry of carbohydrates. Despite the fact ^{14}C -depleted DOC dominates the average bulk pool, the few compound-class and compound-specific $\Delta^{14}\text{C}$ measurements that have been made to date in the HMWDOC, appear to indicate that the sugars fraction has a modern radiocarbon age (Aluwihare et al. 2002; Loh et al. 2004). This result suggests rapid cycling of sugars, highlighting their importance as a substrate for marine bacteria. However, more information on carbohydrate isotopes is required to make any generalizations about their cycling in seawater. Nevertheless, techniques for isolating, purifying, and measuring individual monosaccharides from HMWDOM, POM, and sediments for subsequent radiocarbon analyses have been lacking or are in progress of development (the same holds for amino acids). Therefore, the development of such techniques should be one of the main future concerns of biogeochemists.

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