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Note

Determination of dissolved carbohydrates in natural water by gas–liquid chromatography

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Dissolved carbohydrates (DCHO) are *ca.* 10–40% of the dissolved organic matter in natural water and play an important role in the aquatic ecosystem. DCHO in natural water have been measured by the anthrone–sulphuric acid, phenol–sulphuric acid and orcinol–sulphuric acid methods[1–5]. However, it was not possible to obtain information concerning the monosaccharide composition from the results of these colorimetries, and compounds other than DCHO may interfere with the colour development.

Monosaccharides can be determined by gas–liquid chromatography (GLC) after the formation of their acetyl, trimethylsilyl or trifluoroacetyl derivatives[6–8]. Josefsson[9] and Stabel[10] estimated the monosaccharides of DCHO in natural water by the use of trimethylsilylation. Eklund *et al.*[8] developed a method for sensitive GLC analysis of monosaccharides in sea-water using trifluoroacetyl derivatization and electron capture detection. It was difficult to determine accurately the monosaccharide concentrations using this method because a number of chromatographic peaks result from the anomers of each monosaccharide. Alditol-acetylation is suitable for natural water samples containing a complex mixture of organic matter, because only one peak appears for each monosaccharide. Good results were obtained with GLC using alditol-acetylation after hydrolysis of the DCHO in lake-water.

EXPERIMENTAL

The analysis of monosaccharides by GLC followed the method of Crowell and Burnett[7]. To determine its usefulness for the analysis of DCHO in natural water, the time of hydrolysis was studied.

The water samples were filtered through Whatman GF/C glass fibre filter, previously baked in a furnace at 450°C for 2 h. A 100- or 200-ml volume of filtered water sample was dried in a freeze dryer. Inositol was added to the dried sample as internal standard and dissolved in 1 *N* HCl. This material was transferred to a 10-ml glass ampoule and sealed under nitrogen. The sample was hydrolysed under nitrogen in 1 *N* HCl at 100°C. The hydrolysate was again dried and reduced with sodium borohydride for 1 h at 60°C. The reduced sample was applied to the top of a column (150 × 8 mm) of Dowex 50W-X8 cation exchange resin, which had previously been

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cleaned with 1 *N* sodium chloride solution, regenerated (to H^+) with 1 *N* HCl and rinsed to neutrality with distilled water. The monosaccharides were eluted with 25 ml of distilled water. The solution was evaporated to dryness, 20 ml of methanol added and re-evaporated to dryness. The dehydrated residue was transferred to a 1-ml glass ampoule with methanol and evaporated to dryness under vacuum. The residue was acetylated with 100 μl of acetic anhydride–pyridine (1:1) for 2 h at 100°C. The acetylation mixture was evaporated and the residue dissolved in 30 μl of chloroform. An aliquot of this solution was injected into the gas chromatograph for analysis.

The gas chromatograph used was a Shimadzu Model GC-6AM instrument equipped with a flame ionization detector. Chromatographic peak area measurements were made with a Shimadzu Model C-R1A chromatopac integrator. A glass column (2 m × 3 mm I.D.) packed with 5% OV-275 on Chromosorb W was employed at a nitrogen flow-rate of 40 ml/min. A temperature-programmed analysis from 160°C to 240°C at 2°C/min required 40 min to eluate the acetyl derivatives of eight monosaccharides and the internal standard inositol.

RESULTS

The alditol acetates of monosaccharides have been determined generally by GLC using Gas-Chrom Q with ECNSS-M as column packing[7,11]. Chromosorb W with silicone OV-275 was used in this experiment. The maximum temperature of use was 220°C in the case of ECNSS-M and 250°C for OV-275. Since the gas chromatography could be performed at higher temperature, the determination was completed in a shorter time.

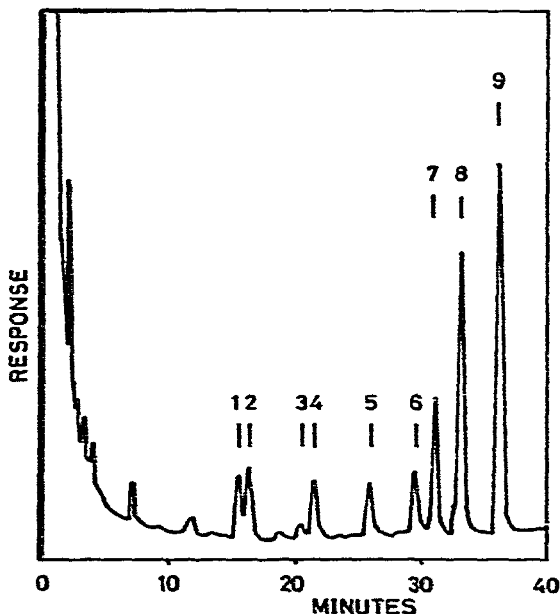

Fig. 1. Gas–liquid chromatography of alditol acetates of monosaccharides, formed by hydrolysis of DCHO in lake-water (taken from the surface of Lake Nakanuma, Japan, on May 26th, 1978). Peaks: 1 = rhamnose; 2 = fucose; 3 = ribose; 4 = arabinose; 5 = xylose; 6 = mannose; 7 = galactose; 8 = glucose; 9 = inositol internal standard.

Although there are lipids, amino acids, humic substances, etc., in addition to carbohydrates in natural water, the monosaccharides of the DCHO could be measured without clean-up. A typical chromatogram of monosaccharides of the DCHO in natural water is shown in Fig. 1.

The hydrolysis times of the DCHO in lake-water with 1 *N* HCl are shown in Table I. Stabel[10] analysed monosaccharide trimethylsilyl derivatives after hydrolysis of the DCHO in distrophic lake-water for 12 h with 1 *N* HCl. Handa[12] measured monosaccharide acetyl derivatives after hydrolysing the carbohydrates in lake sediment for 15 h with 1 *N* HCl. Hecky *et al.*[13] analysed the sugar composition of diatoms after hydrolysis for 4 h with 1.8 *N* HCl. After 7 h the hydrolysate of the DCHO with 1 *N* HCl had little effect on the colour developing activity of anthrone–sulphuric acid, but after 12 h it slightly decreased the activity. Three hydrolysis times, 7, 12 and 24 h, were studied. The results indicated that hydrolysis times of 12 and 24 h were too long for the DCHO in the lake-water. Thus, the concentrations of total monosaccharides after 12 and 24 h of hydrolysis were only 76% and 33% of those after 7 h.

TABLE I

LIBERATION OF MONOSACCHARIDES FROM DCHO IN LAKE-WATER AFTER DIFFERENT TIMES OF HYDROLYSIS WITH 1 *N* HCl

R = Rhamnose; F = fucose; Rb = ribose; A = arabinose; X = xylose; M = mannose; Ga = galactose; G = glucose.

Time (h)	*% liberated*								
	Total	*R*	*F*	*Rb*	*A*	*X*	*M*	*Ga*	*G*
7	100	100	100	100	100	100	100	100	100
12	76	91	93	11	70	70	89	98	83
24	33	43	39	10	66	15	36	40	30

The reproducibility of the concentrations of monosaccharides in the DCHO of Lake Suwako surface water by this method is shown in Table II. The average of the total monosaccharide concentrations was 4350 μg/l, the standard deviation 80 μg/l and the coefficient of variation 1.8%. The reproducibility for each mono-

TABLE II

REPRODUCIBILITY OF DETERMINATION OF MONOSACCHARIDES OF DCHO IN LAKE-WATER

$\bar{x}$ = Average; S.D. = standard deviation; C.V. = Coefficient of variation.

Sample No.	*Concentration (µg/l)*								
	Total	*R*	*F*	*Rb*	*A*	*X*	*M*	*Ga*	*G*
1	4270	523	287	140	64	238	259	1059	1698
2	4340	518	289	156	62	212	261	1085	1757
3	4430	546	296	167	69	264	287	1034	1766
$\bar{x}$	4350	529	291	154	65	238	269	1059	1740
S.D.	80	14.9	4.7	13.6	3.6	26.0	15.6	25.5	36.9
C.V. (%)	1.8	2.8	1.6	8.8	5.5	10.9	5.8	2.4	2.1

saccharide, fucose, glucose, galactose and rhamnose, was good; that of xylose was poorer but satisfactory. The results indicated the potential of this analytical method for the determination of dissolved carbohydrates in natural water.

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