
Communications

Influence of humic substances on results of the spectrophotometric (TPTZ) analysis of monosaccharides

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Abstract

A spectrophotometric method of monosaccharide analysis was assessed with regard to its non-selectivity towards humic substances. Analysis of model solutions showed that it responds positively to both marine and terrestrial humics. The systematic error in monosaccharide analysis (in glucose equivalents) was 0.18–0.20 mg per 1 mg of Aldrich humic acid and 0.11–0.12 mg per 1 mg of humic substances isolated from Gulf of Gdańsk water.

1. Introduction

Methods of monosaccharide analysis in natural waters can be divided into chromatography-based techniques permitting the molecular identification of monosaccharides and those quantifying the whole class of monomers via color-producing reactions characteristic of monosaccharides. The former include capillary gas chromatography (Cowie & Hedges 1984) and liquid chromatography with pulsed-amperometric detection (Mopper et al. 1992). The colorimetric techniques suffer from potential non-selectivity but have the advantage of measuring the whole class of monosaccharides in a single

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measurement. A commonly used representative of that group is the MBTH method (3-methyl-2-benzothiazolinone hydrochloride) (Johnson & Sieburth 1977) based on a color-producing reaction of formaldehyde with MBTH. In addition, there are methods using the reducing properties of monosaccharides towards metal ions (e.g. Dawson & Liebzeit 1980). These methods have recently been supplemented by the so-called TPTZ method (2,4,6-tripirydył-s-triazine) (Myklestad et al. 1997) based on ferricyanide reduction (Avigad 1968). The TPTZ method has been used in several studies, mostly in seawater analysis (Myklestad et al. 1997, Bhosle et al. 1998, Børshheim et al. 1999, Grzybowski & Maksymiuk 2001, Hung & Santschi 2001, Cauwet et al. 2002, Guo et al. 2002, Quigley et al. 2002, Hung et al. 2003, Penna et al. 2003, Santschi et al. 2003, van Oijen et al. 2003). Its advantage over the MBTH method lies in the fact that no pre-concentration or desalting is necessary, and measurements are unaffected by salinity. Moreover, the results are comparable with those obtained using the MBTH method (Penna et al. 2003).

Myklestad et al. (1997) demonstrated that in typical seawater, interference from components other than carbohydrates is negligible. In the present study, we have verified the selectivity of the TPTZ method in solutions with high concentrations of humic substances (encountered, for example, in areas like the Gulf of Gdańsk and the Vistula river mouth). The idea was that a complex structure of humic substances contains functionalities able to reduce iron under the reaction conditions and, at sufficiently high concentrations, to interfere with the TPTZ monosaccharide analysis. Some of these reducing groups may originate from the 'true' carbohydrate, the existence of which in the humic substances has been reported in the literature (see McKnight & Aiken (1998), Benner (2002) and references therein).

In our experiment we tested model solutions made up from commercially available Aldrich humic acid (as a proxy for terrigenous substances) and marine humic substances isolated from Gulf of Gdańsk water.

2. Experimental

Humic substances were isolated according to the operational definition of aquatic substances (Thurman & Malcolm 1981, Aiken 1985). Briefly, acidified Gulf of Gdańsk water (pH~2–3) was passed through pre-cleaned XAD-8 resin and subsequently eluted with 0.1 M NH₄OH. The commercially available Aldrich humic acids were used as delivered.

Absorbance spectra were measured with a Perkin-Elmer Lambda 3B dual-beam spectrophotometer in 100-mm long quartz cuvettes with Milli-Q water as a reference. Monosaccharide analysis was as given in Myklestad

et al. (1997) with modifications by Hung & Santschi (2001). The reagents and standard solutions were made up in Milli-Q water. The ferric chloride solution was prepared directly before analysis. The addition of reagents and the subsequent manipulations of samples and colorimetric measurements were performed in subdued red light. The analyses of one set of samples included a Milli-Q water blank and at least one standard solution. Colorimetric measurements were made in 10 mm cuvettes with Milli-Q water as a blank sample.

The precision of the analysis, expressed as a relative standard error, was below 4% for 1 mg dm^{-3} glucose standards and ranged from 2% to 6% for humus solutions. The possibility of contamination from iron bound to humic Aldrich acids and Gulf of Gdańsk humic substances was ruled out by repeating the analysis at the room temperature.

The starch solution was prepared by adding starch powder to cold water and stirring to create a fluid paste. This was then poured into warm water and the solution heated to near boiling-point.

The total carbohydrate (monosaccharides + polysaccharides) concentration was determined in hydrolyzed samples: 4 ml of solution and 0.4 ml of 1 M HCL were added to an 8 ml glass ampoule, and the sealed ampoules heated in an oven at 150 C for 1 h. Next, the solution was neutralized with 0.4 ml of 1 M NaOH and then subjected to the TPTZ monosaccharide analytical procedure.

3. Results and discussion

Fig. 1 presents UV-VIS spectra of the analyzed solutions of marine and terrestrial humus in comparison with their natural water 'counterparts': water from the Gulf of Gdańsk and the Vistula river.

The shapes of the spectra of the Aldrich humic acids and the marine humic substances differ, which suggests qualitative differences. In addition, the former are significantly 'darker' as seen from a comparison of the mass-normalized absorbances: 0.13 units of absorbance at 350 nm per 1 mg of Aldrich humic acids versus 0.04 for marine humic substances (absorbance measured in 100 mm long optical cuvettes).

Fig. 2 shows the results of putting solutions of Aldrich humic acids, marine humic substances (isolated from Gulf of Gdańsk water) and, for comparative purposes, a glucose standard, through the TPTZ analytical procedure.

As can be seen in Fig. 2, humic substances respond positively to the TPTZ analysis. Concentrations of TPTZ-detectable moieties increased linearly with concentrations of humus, the increase being faster for the

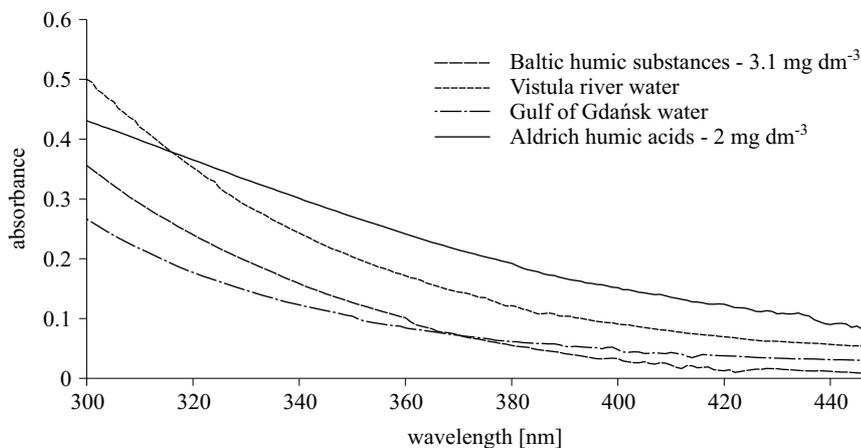


Fig. 1. Comparison of the optical characteristics of Gulf of Gdańsk water, Vistula river water, and solutions of Aldrich humic acids and humic substances isolated from the Gulf of Gdańsk (spectra recorded using 100 mm long optical cuvettes)

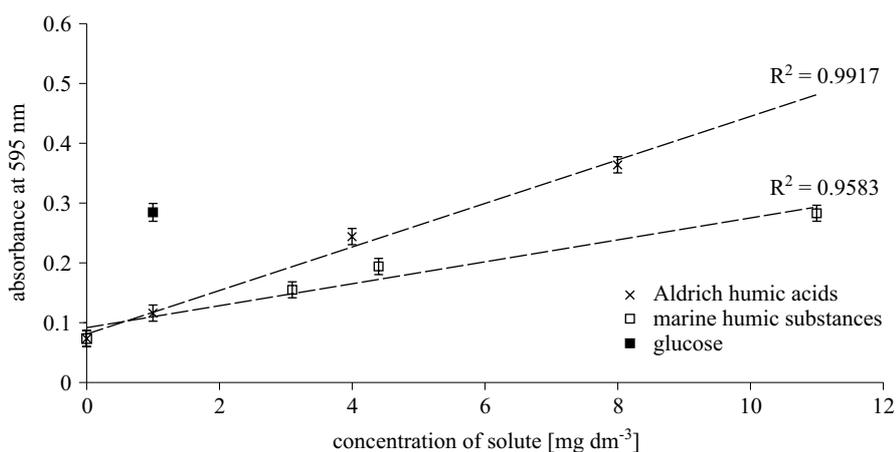


Fig. 2. Response of the TPTZ analysis to the presence of humic substances (absorbance measured in a 10 mm long optical cuvette, error bars represent ± 1 s.d.)

Aldrich humic substances. The measured absorbance values corresponded to 0.18–0.20 mg of glucose per 1 mg of Aldrich humic acid and 0.11–0.12 mg per 1 mg of marine humic substances.

Because of the well-known low bioreactivity of humic substances it seems unlikely that the detected moieties were ‘true’ monosaccharides. There is a possibility, however, that the method detected residual carbohydrates

built-in and/or bound to humic substances (the presence of carbohydrates in humic structures has been demonstrated using NMR techniques, e.g. by Hedges et al. 1992). To check for this, analyses were carried out for solutions subjected to acid hydrolysis. The results are presented in Fig. 3.

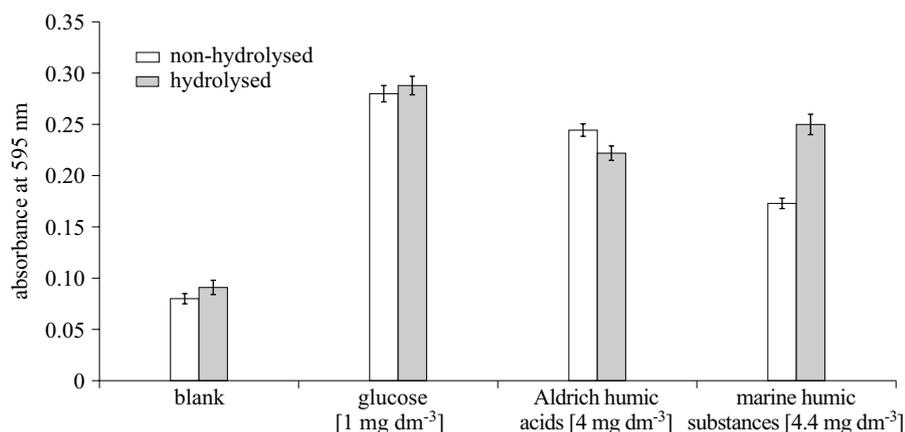


Fig. 3. Comparison of the response of the TPTZ analysis to hydrolyzed and non-hydrolyzed solutions (absorbance measured in a 10 mm long optical cuvette, error bars represent ± 1 s.d.)

Interestingly, hydrolysis measurably decreased the reducing potential of the Aldrich humic acids. In contrast, marine humic substances showed an increase in concentration of the TPTZ-measurable moieties after hydrolysis, which suggests the presence of polysaccharides (it should be noted, however, that polysaccharides are here defined on the basis of hydrolysis, after Myklestad et al. (1997)).

A test of the sensitivity of the TPTZ method to polysaccharides, carried out in a batch of polysaccharide solutions, showed that the TPTZ method detects 0.11–0.13 mg dm⁻³ glucose in an 8 mg dm⁻³ starch solution. It is possible, then, that some of the TPTZ-measurable moieties recorded in non-hydrolyzed solutions of marine humic substances are due to polysaccharides. However, the nature of the link between polysaccharides and marine humic substances remains an open question. While polysaccharides have been postulated as precursors in aquatic humus formation (see McKnight & Aiken (1998) and the references therein), there is a possibility that our results were, to some extent, artifacts resulting from the method of isolating humic substances from seawater. It has recently been demonstrated that solid

phase extraction of humic substances (an operational definition of aquatic humic substances) is non-selective and that polysaccharides are co-extracted with humic substances (Boult et al. 2001).

In contrast to marine humic substances, the solution of Aldrich humic acids did not contain hydrolyzable polysaccharides.

The reasons notwithstanding, the results obtained here show clearly that the TPTZ method responds positively in samples apparently devoid of monosaccharides. The humus-induced systematic error is higher for terrigenous substances. It should be stressed that these results do not permit a quantitative assessment of the non-selectivity of the TPTZ method in natural water samples owing to the problems involved in determining the concentration of humic substances. It is extremely difficult to assess the quantity of humic substances by means of solid-phase extraction because of non-quantitative sorption and desorption (Fu & Pocklington 1983, Amador et al. 1990).

A commonly used approach to the semi-quantitative determination of humic substances in natural water is to use absorbance values. Taking into account the 350 nm absorbance values from Fig. 1, humic substances would be responsible for the TPTZ-measured glucose concentration of $\sim 0.2 \text{ mg dm}^{-3}$ and $\sim 0.1 \text{ mg dm}^{-3}$ in Vistula river and Gulf of Gdańsk water respectively. This very rough assessment is based on the following assumptions: (a) river water humus is dominated by substances resembling Aldrich humic acids (terrigenous), (b) marine humic substances retain their properties after the isolation procedure, and (c) seawater is not affected by terrigenous input (and vice versa). Comparison of absorbance spectra in Fig. 1, however, suggests that qualitative differences exist between the humus substances in solutions and those contained in natural water. Moreover, the optical properties of humic substances in the aquatic environment are indeed affected by terrigenous input and photobleaching (Vodacek et al. 1997).

4. Conclusions

The TPTZ spectrophotometric method of monosaccharide analysis is liable to interference from humic substances present in natural water samples. The impact of humic substances varies for substances of different origin and its magnitude cannot be easily assessed owing to the problems involved in the determination of their concentration in natural waters. It would be advisable to take such interference into account when analyzing waters where a high absorbance suggests a high concentration of humic substances.

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