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Investigation of photochemical reactions of saccharides during direct ultraviolet absorbance detection in capillary electrophoresis

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ABSTRACT

Direct UV detection of saccharides in capillary electrophoresis is possible due to a base-catalyzed conversion into UV absorbing substances initiated by the light of the UV detector lamp. In the present study the compounds formed during this reaction were investigated with capillary electrophoresis using an additional UV lamp for online irradiation at a certain distance before the detector resulting in a separation of the conversion products. It was found that for all investigated saccharides (glucose, ribose and sucrose) the major portion of the UV absorption in direct UV detection resulted from one and the same substance. By CE–UV, CE–MS as well as HPLC–MS measurements this substance was demonstrated to be malondialdehyde, present as its conjugate base malonenolate under alkaline conditions. Additional experiments revealed that the conversion reaction is highly sensitive to the residence time under the UV light as malondialdehyde can further degrade into non-UV absorbing products. NMR measurements showed that under alkaline conditions this degradation proceeds slowly even without UV irradiation.

1. Introduction

Analysis of saccharides can be demanding due to the great variety of this class of substances. In addition, structural similarity of representatives such as stereoisomers of monosaccharides further complicates analysis. Analytical approaches include gas chromatography after derivatizing the analytes to increase their volatility [1,2], as well as a range of high-performance liquid chromatographic (HPLC) techniques working without derivatization. In the latter field, ligand-exchange chromatography based on cation-exchangers loaded with metal ions has played a major role for many years [3]. In addition, hydrophilic interaction liquid chromatography (HILIC) is widely used with amino-modified silica as the originally preferred stationary phase complemented by a variety of other HILIC stationary phases in recent years [4]. As an alternative, anion-exchange chromatography under strongly alkaline conditions has become a routine tool for separations of saccharides [5–7]. Detectors commonly used range from refractive index detection to pulsed amperometric detection, evaporative light scattering detection and mass spectrometric (MS) detection [8]. Sensitive fluorescence detection is applicable after post column reactions [9]. For the separation of saccharides reversed-phase liquid chromatography typically in combination with pre column derivatization can also be employed [10].

In addition to these HPLC methods nowadays available for saccharide analysis, capillary electrophoresis (CE) presents an attractive technique providing a separation selectivity different to chromatography [11,12]. Saccharides with pK_a values typically around or above 12 can be turned into charged species either by complexation with borate anions [13,14] or by using a high-pH background electrolyte (BGE) [12,15,16]. Some detection techniques used in HPLC of saccharides also work for CE such as MS detection or pulsed amperometric detection, but their robustness may be poorer than in HPLC. Also a variety of derivatization methods has been developed to introduce chromophoric or fluorophoric groups [12]. Direct UV detection of underivatized saccharides at 195 nm is applicable if saccharides are separated as borate complexes, but does not work for the analytes being separated as such under strongly alkaline conditions. In the latter case indirect UV detection with sorbate as UV absorbing carrier electrolyte [15,16] or contactless conductivity detection [17] was reported.

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As non-derivatized saccharides do not possess chromophoric groups, detection strategies based on direct UV detection may be considered as less promising. Nevertheless, Rovio et al. [18] followed by others [19–25] reported the application of direct UV detection in CE due to the unexpected fact that under high pH conditions saccharides exhibit UV absorption around 270 nm. The reason for this spectroscopic behavior was first assumed to be related to a complexation with alkali-ions of the BGE [18], but this was afterwards shown to be inconsistent [26]. Instead, a base-catalyzed photooxidation reaction in the detection window of the separation capillary initiated by the light of the lamp of the diode array UV detector (DAD) is supposed to take place that leads to the formation of UV absorbing substances [26,27]. This phenomenon of generating UV absorbing species from saccharides upon UV irradiation under alkaline conditions had already been observed earlier, although not in CE [28,29]. Products suggested in that context included ascorbic acid, 2,3-dihydroxyacrylaldehyde, 2-keto-gluconic acid, 4-desoxy-5-keto-3,6-mannosaccharolactone [28] and later malondialdehyde (MDA) [30]. Results from electron spin resonance investigations of free radicals formed from saccharides after addition of hydroxyl radicals [31,32] were adapted for explanations of the possible reaction mechanisms taking place during direct UV detection in CE, but none of the compounds mentioned above was detected by nuclear magnetic resonance (NMR) spectroscopy [27]. Also in another study based on NMR spectroscopy [33] various compounds proposed earlier as products from UV irradiation of glucose [34] were not observed. In short, although a number of studies have been carried out, the actual reaction mechanism as well as the identity of the involved UV absorbing compounds is far from being entirely resolved.

The present study contributes to further elucidating the underlying reaction mechanisms that allow direct UV detection of saccharides in CE by a more detailed investigation of the reaction products. For this purpose, an additional UV lamp used for online irradiation during CE separations is introduced. Products formed during irradiation are separated in the following part of the capillary before they reach the detector. The use of a UV detector reveals the peaks that contribute most to the UV absorption while identification can be achieved by coupling the set-up with an MS. In addition, the stability of the compound that contributes most to the UV absorption regarding possible degradation in highly alkaline solutions and under UV light is investigated by specific CE experiments as well as by NMR spectroscopy.

2. Materials and methods

2.1. Chemicals and reagents

Formic acid (LC–MS grade), ammonium formate (≥99.995%), sucrose, ribose, glucose (≥99.5%) and 1,1,3,3-tetramethoxypropane (99%) were obtained from Sigma–Aldrich (Steinheim, Germany). 2-Propanol, acetonitrile and methanol, all analytical grade, were purchased from VWR (Vienna, Austria). Mesityl oxide (99%), sodium hydroxide (1 M, Titripur®) and potassium hydroxide (85.8%) were obtained from Merck (Darmstadt, Germany). For NMR measurements formic acid (>99%) was obtained from Univar (Ingleburn, Australia), sodium hydroxide pellets (>98%) and 1,1,3,3-tetramethoxypropane (99%) were purchased from Sigma–Aldrich (Castle Hill, Australia) and deuterium oxide (D, 99.9%) was sourced from Cambridge Isotope Laboratories (Andover, MA, USA). Throughout the study purified water from a Milli-Q system from Millipore (Bedford, MA, USA) was used.

As malondialdehyde (MDA) is an unstable substance it is usually generated by *in situ* hydrolysis of its bis-acetal derivatives [35,36]. In this study the procedure of Syslová et al. [37] was followed with slight modifications: a 1 g L^{-1} solution of 1,1,3,3-tetramethoxypropane was prepared in 1 M formic acid and kept at 40 °C. To monitor the progress of hydrolysis samples were taken every 10–30 min and diluted to 10 mg L^{-1}. KOH was added to a final concentration of 30 mM to increase the pH, since the absorption maximum is different for acidic and basic conditions [38]. Progress of hydrolysis was observed by UV spectroscopy at 266 nm using an Agilent 8453 UV–vis spectrophotometer (Agilent Technologies, Waldbronn, Germany). As after 75 min an absorption plateau was reached, the hydrolysis reaction was considered as complete. In order to keep the concentration of formic acid low, hydrolysis of 1 g L^{-1} 1,1,3,3-tetramethoxypropane was repeated with 0.1 M formic acid. Under these conditions the reaction proceeded slower. After 120 min absorption amounted to 82.5% of previous hydrolysis' plateau, from which the concentration of MDA was calculated. Until use the solution was kept at −20 °C and then diluted with H_2O (unless stated differently) to the desired concentration.

2.2. Instrumentation

2.2.1. CE–UV and CE–MS experiments

CE separations were carried out using an Agilent 3D CE system. Fused silica capillaries (50 μm ID × 360 μm OD) were purchased from Polymicro Technologies Inc. (Phoenix, AZ, USA). New capillaries were flushed with 1 M NaOH, H_2O and BGE for 10 min each. Preconditioning was performed by flushing with BGE for 3 min.

For CE–UV experiments the capillaries were cut to a length of 50 cm. Detection was done using a DAD equipped with a deuterium lamp. In addition, a second UV lamp of the same type was installed at a distance of 12.1 cm before the detector, where the polyimide coating of the capillary was removed (Fig. S1 (A)). The length of this irradiation window was 1 cm and the distance between lamp and capillary about 2 cm.

CE–MS experiments were carried out by coupling the CE to an Agilent 6510 Q-TOF/MS. Detection was done in the negative ion mode using an ESI source and an Agilent G1607A coaxial sprayer. The sheath liquid was supplied by an HPLC pump (Agilent 1100 Series G1311A) using a 1:100 splitter (also from Agilent Technologies). For CE–MS experiments the capillaries were cut to a length of 65 cm. A UV lamp was installed 15.0 cm before the end of the capillary, again with 1 cm of irradiation path length and a distance of 2 cm from the capillary (Fig. S1·(B)). Based on investigations of the sheath liquid composition (see Section 3.3.1) 90% (v/v) 2-propanol in H_2O with 0.01% (v/v) NH_3 was selected. MS related parameters were optimized using the experimental design software Design-Expert® (version 9) from Stat-Ease (Minneapolis, USA) and resulted in a nebulizer pressure of 23 psi, sheath liquid flow of 7 μL min^{-1}, drying gas flow (nitrogen) of 7 L min^{-1} and drying gas temperature of 200 °C. Capillary voltage and fragmentor voltage were set to 3750 V and 75 V, respectively. A mass range from m/z 50 to 1000 was selected at a scan rate of 4 spec s^{-1}.

2.2.2. HPLC–UV–MS experiments

An Agilent Series 1100 HPLC instrument equipped with UV detector coupled to the Agilent 6510 Q-TOF/MS was employed for HPLC measurements. For some experiments, the UV detector of the HPLC was bypassed when MS detection was performed. For the separation of the analytes an ODS-AQ column (250 mm × 3.0 mm ID, 3 μm particle size) from YMC (Dinslaken, Germany) was used. The mobile phase consisted of 40% (v/v) acetonitrile in H_2O with 0.02% (v/v) NH_3 and was pumped at a flow rate of 0.4 mL min^{-1} in the isocratic mode. An ESI source was used, employing the following parameters: nebulizer pressure 50 psi, drying gas flow (nitrogen) 8 L min^{-1}, drying gas temperature 250 °C, capillary voltage 3750 V and fragmentor voltage 75 V. A sample volume of 5 μL was injected.