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New Avenue for Mid-UV-Range Detection of Underivatized Carbohydrates and Amino Acids in Capillary Electrophoresis

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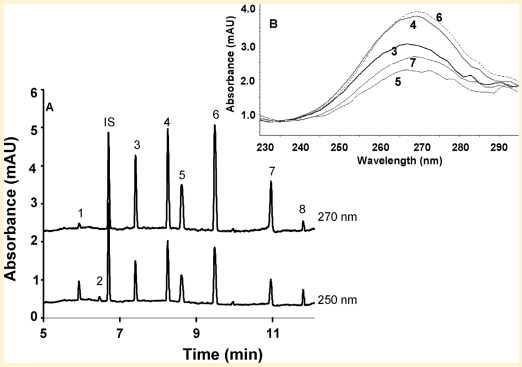
Supporting Information

ABSTRACT: Capillary electrophoresis (CE) appeared as an interesting alternative to chromatographic methods for carbohydrate analysis, but it can be difficult to implement, because of the lack of easily ionizable functions and chromophore groups. Recently, a promising method was proposed by Rovio et al. for the CE separation under extremely high alkaline conditions of neutral carbohydrates under their alcoholate form and their direct UV detection [Rovio et al. *Electrophoresis* **2007**, *28*, 3129−3135; and Rovio et al. *J. Chromatogr. A* **2008**, *1185*, 139−144], which is claimed to be due to the absorption of enediolate at 270 nm. Even so, most of the detected compounds in Rovio's paper (for example, sucrose) cannot give such enediolate, lacking a carbonyl group. In this work, a deeper insight was paid to the understanding of detection mechanism. In effect, unusual detection phenomena were observed in comparing reducing and nonreducing carbohydrate behaviors, which pointed to the existence of photochemical reactions in the detection window. A more systematic study of the influence of many parameters (carbohydrate nature, electrolyte pH, residence time in the detection window, and capillary diameter) was undertaken. In addition to this, most of this work was performed under cathodic (reversed) electro-osmotic flow conditions (using Polybrene-modified capillaries), to obtain much faster separations than under Rovio's conditions. This study also opens up new avenues for the detection in mid-UV range of non-UV-absorbing compounds bearing reducing moieties, such as amino acids.



Carbohydrate analysis remains challenging, because of the great molecular diversity, the presence of several structural isomers, and the lack of easy-to-implement and sensitive detection methods. Currently, methods used for the separation of carbohydrates include thin-layer chromatography,[1] gas chromatography,[2,3] and liquid chromatography (LC).[4−6] Most often, LC methods used normal phase (HILIC),[7] reverse-phase,[8] or anion-exchange modes[8,9] with direct low-UV-range or refractive index detections[4] and most often mass spectrometry, ultraviolet (UV), or fluorescence detection after precolumn derivatization.[10−12] More recently, important developments were carried out with the coupling between high performance anion-exchange chromatography and pulsed-amperometric detection.[4,9]

Currently, capillary electrophoresis (CE), with its high separation efficiency, low reagent consumption, and speed, appears to be an interesting alternative to chromatographic methods, especially for sulfated and carboxylated carbohydrates,[4,12−16] but the analysis of so-called neutral carbohydrates can be more difficult to implement, because of the absence of easily ionizable functions (other than anomeric hydroxyl group) and chromophore groups (see Table S-1 in the Supporting Information), and the high hydrophilic character of these compounds, which makes the use of micelles inoperable. Different strategies are available to circumvent these different drawbacks. Complexation with borate anions at pH comprised between 8.0 and 10.0 has long been used in carbohydrate analysis and exploited in CE with direct UV detection at 195 nm;[17,18] however, this low wavelength does not favor selective detection, generates interferences, and suffers from poor sensitivity. As of today, the most widely used CE

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methods have involved the labeling of carbohydrates by a chromophore or fluorophore through a precolumn derivatization step.[19−25] Limits of detection in the micromolar range were obtained; however, these methods suffer from complex and time-consuming derivatization steps. Alternatively, indirect UV detection of underivatized carbohydrates can be carried out at extremely high pH (12−14) with the use of an anionic chromophore, such as 2,6-pyridine dicarboxylate[26−28] (detection wavelength, 275 nm), 1-naphtyl acetate[29] (detection wavelength, 220 nm), riboflavin[30] (detection wavelength, 254 nm), tryptophan[31] (detection wavelength, 280 nm), or sorbate[32−34] (detection wavelength, 254 nm). Nevertheless, the limits of detections were observed to be within the range between 20 and 1000 μM, because of the deleterious presence of hydroxyl anion at high concentration.

More recently, a new method described by Rovio et al. has allowed direct UV detection under extremely high alkaline conditions (130 mM NaOH, 36 mM Na_2HPO_4).[35,36] According to the authors, carbohydrates were detected under their enediolate forms at 270 nm; however, this explanation cannot be valid for most of the detected compounds (for example, sucrose), which lack a carbonyl group. In this work, we revisited the experimental conditions of this approach, with respect to detection performances. In effect, unusual detection phenomena were observed in comparing reducing and nonreducing carbohydrate behaviors, which pointed to the existence of photochemical reactions in the detection window. A more systematic study of the influence of several parameters, including carbohydrate nature, background electrolyte (BGE) pH, residence time in the detection window, and capillary diameter, was undertaken. This study brings a deeper insight into the understanding of the physicochemical mechanisms involved in this new direct UV detection mode and opens up new avenues for detection in the mid-UV range of non-UV-absorbing compounds bearing reductive moieties, such as amino acids and peptides. In addition to this, most of this work was performed under cathodic (reversed) electro-osmotic flow conditions (using Polybrene-modified capillaries), to obtain much faster separation than under Rovio's conditions.

EXPERIMENTAL SECTION

Standards and Solutions. Polyols (glycerol, erythritol, xylitol, mannitol, sorbitol, and myo-inositol), cyclodextrins (α-CD, β-CD, γ-CD, HP-α-CD, and heptakis(2,3,6-tri-*O*-methyl)-β-CD), amino acids (arginine, proline, glycine), naphthalene sulfonic acid (internal reference), and δ-gluconolactone were purchased from Sigma−Aldrich (L'Isle-d'Abeau, France). Glucose, lactose, sucrose, fructose, and *N*-dimethylformamide (DMF) were obtained from VWR (Fontenay-sous-Bois, France). Individual solutions of each compounds were prepared weekly at concentrations of 1000 mg L^{-1} by dissolution in ultrapure water delivered by a Direct-Q3 UV system (Millipore, Molsheim, France). BGEs for CE were prepared from 1 M NaOH solution (Carlo Erba, Val-de-Reuil, France) and Na_2HPO_4 $2H_2O$ (Sigma−Aldrich). The final BGE was composed of 130 mM NaOH (pH 13.1). Hexadimethrine bromide (HDMB), which was used as a coating agent, was purchased from Sigma−Aldrich. HDMB at 0.1 g/100 mL was prepared by dissolving the appropriate amount in ultrapure water.

Apparatus. Detection studies and separations were carried out with a Beckman Coulter P/ACE MDQ system (Villepinte, France) equipped with a diode array detector (DAD) set at 270 nm (measurement wavelength) and 350 nm (reference wavelength) and with bandwidths set at ±10 nm and ±40 nm, respectively. Instrument control and data acquisition were performed using Beckman 32 Karat software. Corrected areas were calculated as the peak-area-to-migration-time ratio (A_i/t_{Mi}). For comparison purposes, CE systems (Agilent Technologies, Models HP^{3D}CE and HP 7100), equipped with DADs (Massy, France) were also used. A PHM210 Meterlab pH-meter, (Metrohm, Villebon Courtaboeuf, France), equipped with a high-alkalinity electrode was used to measure BGE pH. A Varian Cary 100 UV spectrometer, purchased from Bioserv (Thiais, France), was used to monitor the UV spectra.

Electrophoretic Conditions. Electrophoretic separations were performed using 60-cm Polymicro bare fused-silica capillaries with internal diameters of 50, 75, or 100 μm purchased from Photonlines (Marly-Le-Roi, France). A window was created for the UV detection on the capillary at 10 cm from the anodic end, and detection windows of 200 and 800 μm aperture lengths were compared. Before first use, capillaries were conditioned by successive flushing with 1 M NaOH, 0.1 M NaOH, ultrapure water, HDMB solution, and finally BGE, each under 2.8 bar for 3 min (12 capillary volumes). Between each run, because of the high BGE pH, the HDMB coating was regenerated by percolating the solution under 2.8 bar for 1 min (4 capillary volumes) and, afterward, the capillary was re-equilibrated with BGE under 2.8 bar for 3 min (12 capillary volumes). Injections were performed hydrodynamically under 50 mbar for 5 s (0.7% of the capillary volume). Unless specified, separations were run at 20 °C under −16 kV. BGE was renewed between each run.

RESULTS AND DISCUSSION

Rovio et al. demonstrated that neutral carbohydrates can be separated in bare fused-silica capillaries within less than 30 min and detected in a new mode by direct UV absorbance at 270 nm, applying extremely high pH conditions. Better resolutions were obtained with a BGE composed of 130 mM sodium hydroxide and 36 mM Na_2HPO_4, as compared with a BGE composed of 130 mM sodium hydroxide only.[35,36] We compared this (NaOH + phosphate)-based BGE with a BGE only composed of 130 mM NaOH and similar analytical performances were obtained in our case, even for resolutions (cf. the Supporting Information). Therefore, this NaOH-based BGE, which is simpler to prepare and provides slightly faster separations, was selected for this study. Interestingly, a decrease in peak corrected areas was observed with a decrease in separation voltage, which suggested the existence of a photochemical reaction in the detection window (see Figure S1-A in the Supporting Information). This assumption was corroborated by the fact that the sucrose UV spectrum in 130 mM NaOH, which was acquired with a classical grate spectrophotometer, did not exhibit any absorbance band at ∼270 nm (see Figure S1-B in the Supporting Information). No absorbance band (and no peak, either) was observed in our separation conditions (carbohydrate concentrations, capillary length, etc.) either with Agilent HP^{3D}CE or HP 7100, which are equipped with a deuterium-lamp-based DAD detector. Moreover, when the Beckman Coulter P/ACE MDQ system was equipped with a fixed-wavelength UV detector (mercury lamp) at 280 nm, no signal was detected either. A particular feature of the Beckman DAD is that the incident UV light (from a deuterium lamp with a 160−400 nm emission continuum) does not go past a filter before reaching the sample, which means that

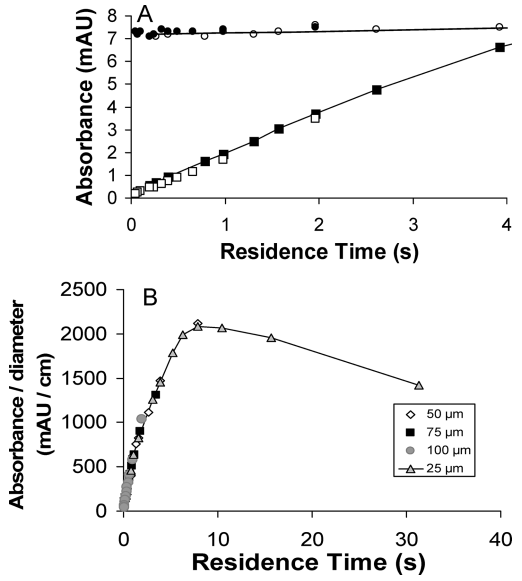

Figure 1. (A) Variation of absorbance front intensities as a function of residence time in the separation window for sucrose ((■) 800 μm aperture and (□) 200 μm aperture) and for DMF ((●) 800 μm aperture and (○) 200 μm aperture). (B) Variation of sucrose absorbance front intensities normalized by capillary inner diameter (25, 50, 75, and 100 μm) as a function of residence time in separation window. A bare fused-silica capillary, 50 μm id (A), 25, 50, 75, and 100 μm (B) × 60 cm (UV detection at 10 cm). Capillary initially filled with 130 mM NaOH, then hydrodynamic short-end introduction of 0.05 mM sucrose + 0.01% (v/v) DMF in 130 mM NaOH solution. Direct UV detection at 270 nm (sucrose) and 210 nm (DMF).

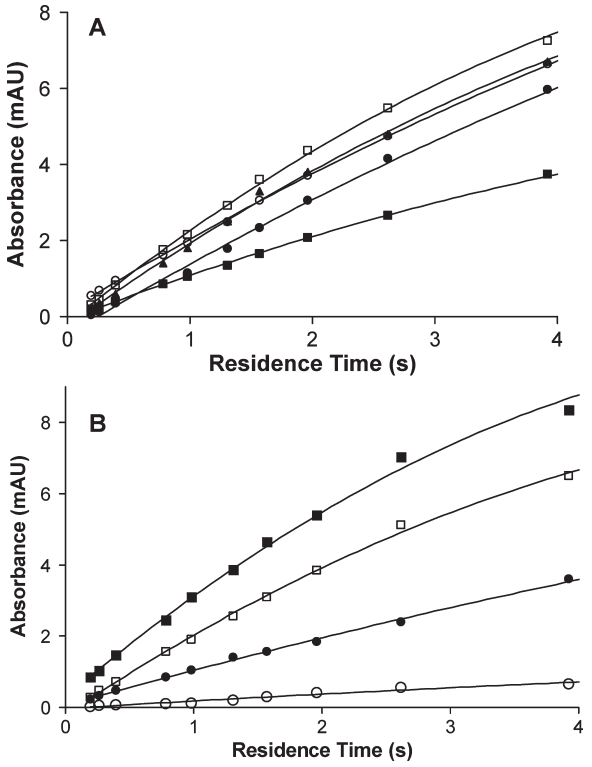

Figure 2. Absorbance front intensities as a function of residence time in the separation windows for (A) various reducing and nonreducing carbohydrates at 0.05 mM in 130 mM NaOH (pH 13.1) solution and (B) for 0.05 mM mannitol in NaOH solutions of various concentrations. Bare fused-silica capillary, 50 μm id × 60 cm (UV detection at 10 cm). Capillary initially filled with (A) 130 mM NaOH, (B) NaOH solutions of various concentrations, then hydrodynamic short-end introduction of (A) 0.050 mM carbohydrate in 130 mM NaOH and (B) 0.050 mM mannitol in NaOH solution of the same concentration as that used for initial filling. Direct UV detection at 270 nm. Identification: (A) (□) mannitol, (▲) glucose, (●) lactose, (○) sucrose, and (■) fructose; (B) (○) 10 mM NaOH (pH 12.0), (●) 32 mM NaOH (pH 12.5), (□) 63 mM NaOH (pH 12.8), and (■) 130 mM NaOH (pH 13.1).

the sample is irradiated by very short wavelength UV light (<200 nm, called VUV for Vacuum UV), which actually may initiate a photochemical reaction.

Highlight of the Photochemical Reaction. The hypothesis of a photochemical reaction in the separation window was tested by varying carbohydrate residence time in the detection window more systematically. For this purpose, a 50-μm inner diameter (id) (365 μm outer diameter (od)) × 60 cm total length (detection window, 50 cm) capillary was first filled with the 130 mM NaOH BGE, and, next, 0.05 mM sucrose in 130 mM NaOH was continuously introduced hydrodynamically under various successive applied pressures. The resulting hydrodynamic fronts were recorded at 270 nm. The carbohydrate residence time was thus modulated by the applied pressure value (from 280 to 7 mbar) and the length of the separation window (800 and 200 μm). DMF was added to the sucrose solution, at a concentration of 0.01% (v/v), as an internal reference and detected at 210 nm. The analyte residence times were then calculated from the lengths of the detection window and the flow velocities obtained by Hagen−Poiseuille law. The plots of the measured plateau absorbance for sucrose and DMF, as a function of residence time, are represented in Figure 1A. As expected, DMF plateau absorbance was almost constant upon increasing the residence time, whereas sucrose absorbance increased almost linearly over the range of residence times experienced (0−4 s). Interestingly, identical absorbances were obtained with the two different aperture lengths (i.e., detection window length along the capillary length) of 200 and 800 μm for a given residence time. Linear absorbance variations were also obtained for glucose, fructose, lactose, and mannitol. These results clearly identified residence time as the parameter influencing plateau absorbance and demonstrated that a photochemical reaction was taking place as the carbohydrate went past the detection window. Similar experiments were then resumed with capillaries of different inner diameters (25, 75, and 100 μm) and identical outer diameter (365 μm). Figure 1B shows that, for all four capillary id values (25, 50, 75, and 100 μm), the curves that represent the variation in the absorbance plateau, as a function of residence time, could be quite superimposed, provided that absorbances were normalized by the capillary id. This additional result shows that, for a given residence time, absorbance plateau values were mainly dependent on optical path length solely, and that varying the capillary id had no peculiar effect on the photochemical reaction that was occurring. For residence time longer than ca. 8 s, a decrease in absorbance was noticed, which suggests the existence of successive reactions, involving the transient formation of an intermediary compound that would be responsible for the absorbance at 270 nm. It is worthy to note that the derivative of the plot of absorbance versus residence time is proportional to the speed of the reaction giving the absorbing intermediate.

Photo-oxidation of Carbohydrates. The photochemical reaction that was previously set forth for sucrose was subsequently

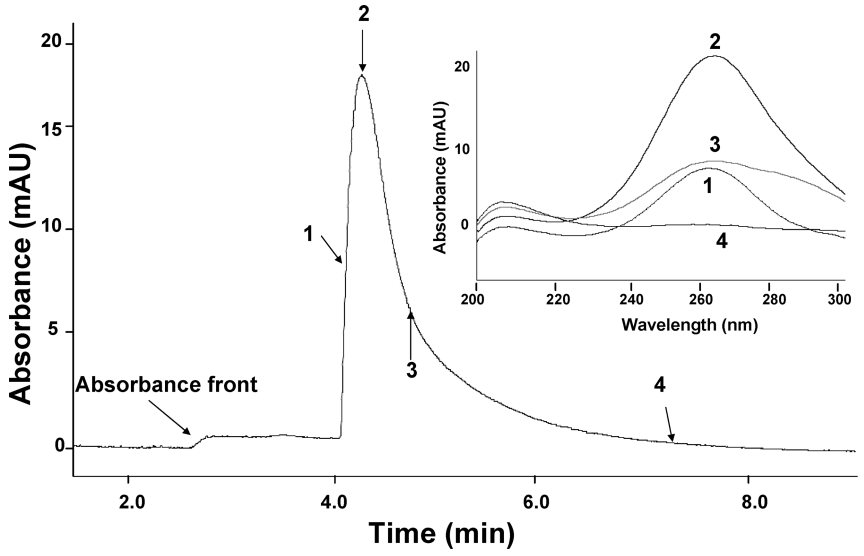

Figure 3. Evolution of the absorbance as a function of time after stopping the hydrodynamic flow 4 min after sucrose introduction. Bare fused-silica capillary, 50 μm id × 60 cm (UV detection at 50 cm). Capillary initially filled with 130 mM NaOH, then hydrodynamic introduction of 0.05 mM sucrose in 130 mM NaOH. Direct UV detection at 270 nm.

exemplified for other carbohydrates. Figure 2A presents the variation of absorbance front intensities as a function of the residence time in the separation window for various reducing and nonreducing carbohydrates at 0.05 mM in 130 mM NaOH solution. It appeared that all the carbohydrates studied displayed the same behavior as sucrose. The reducing or nonreducing character of the carbohydrate solely does not explain the observed absorbance, which seems to be also influenced by the pK_a value of the carbohydrate[37,38] (see Table S-1 in the Supporting Information).

In effect, as shown for mannitol (pK_a = 13.5) in Figure 2B, the higher the pH (in the range from 12.0 to 13.1), the higher the absorbance front intensity (which indicates that the formation of the absorbing intermediate is favored by the presence of mannitol in its deprotonated form and/or OH^- concentration).

An important amount of work was then carried out to identify the nature of the detected species. A stop flow method, consisting of stopping solutions of carbohydrates in the detection window and recording their UV traces and their associated UV spectra were implemented. The capillary was first filled with 130 mM NaOH solution, then percolated with 0.05 mM carbohydrate in 130 mM NaOH under 207 mbar. At a time of ~2.5 min, the absorbance front was detected and at a time of 4.0 min, the hydrodynamic flow was stopped. The resulting UV traces for sucrose are presented in Figure 3 with UV spectra acquired at 4.2, 4.4, 4.7, and 7.3 min.

As soon as the hydrodynamic flow was stopped, a steep increase in absorbance was observed, corresponding to the formation of an intermediate species having a maximum absorbance at 265 nm (UV spectrum at time 2), which is probably the same as that detected in the CE analyses. This intermediate species, which is obviously unstable, was quickly transformed to a more stable species having a maximum absorbance at 210 nm. It is worth noting that similar UV traces were obtained for lactose, mannitol, glucose, and fructose; however, for the case of monosaccharides, the intermediate species vanished faster. The intermediate cannot be an enediolate that originated from sugar deprotonation: an enediolate comes from the deprotonation of an alpha hydroxy ketone or aldehyde. Enediolates are known as reactive intermediates in the aldolization reaction, prone to retroaldolization in carbohydrate series, and thus are not expected to be present at high concentration. Aldehydes, ketones, or carboxylates show weak absorption at these wavelengths, because of their very small molar absorptivity. Dihydroxy acetone in water (pH 7.0) absorbed with a molar absorptivity similar to isolated ketone.[39] Even more critically, most of the detected compounds in Rovio's paper and in this paper (for example, sucrose) cannot give such an enediolate, lacking a carbonyl group. In basic media, no hydrolysis of the glycosidic bond can occur; therefore, an unsaturated group cannot originate for hydrolysis, but rather must come from an oxidative process.

Irradiation of carbohydrates in basic water giving 265-nm absorbing compounds is an already known phenomenon, and the formation of UV absorbing species (λ_{max} 268 nm) by UV irradiation of alkalinized solution of sugars (Glc, Frc, Suc, Lac) was already used as an analytic tool for carbohydrates in juices and wines,[40,41] although the nature of absorbing compounds was not defined at this point: these products have been identified as carbonyl compounds by dinitrophenylhydrazine colorimetry. A plateau was reached after 10 min of irradiation (low-pressure Hg lamp (184 nm, 253 nm), pH 9.5) then the absorbance decreased. As shown in Figure 3, a faster decrease was obtained with a deuterium lamp. In the absence of any filter, before reaching the sample, oxygen and water absorbed UV light (at 200−250 nm and <200 nm, respectively) and gave rise to a complex system of reactive interconverting oxygen species (OH° hydroxyl radical, $O^{\circ-}$, ozone, ...) with high quantum yield.[42] Such species react with all the present organic compounds, leading to their oxidation (by hydrogen atom abstraction or heteroatom oxidation) to ketones, aldehydes, acids, and after intensive cleavage of H−C/C−C bonds to unsaturated C3 or C2 fragments, and eventually to CO_2 and water. Thus, the oxidation reaction equally proceeds on reducing and not reducing carbohydrates. Putative intermediates and mechanisms were proposed, but without clear experimental evidence as formed compounds are further transformed. These reactions have found uses in environmental remediation and water sterilization. As an example, pinene was

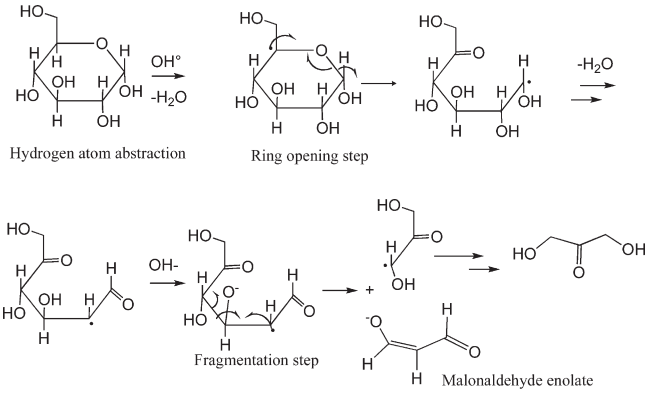

Figure 4. Example of the glucose fragmentation pathway (adapted from Bucknall et al.[44]).

completely photo-oxidized into C3, C2 acids, and aldehydes.[43] Similar phenomena were observed with irradiations with γ-rays of basic solutions of carbohydrates, and the mechanism was investigated in detail only with gamma irradiation (not with photo-oxidation).[44] The conditions of irradiation, which were similar to those found in the present study (OH°, pH 13.0), produced UV-absorbing species and the major chromophoric compound identified was malondialdehyde. In a basic medium, malondialdehyde is deprotonated to give a conjugated enolate (λ_{max} = 265 nm, molar absorptivity = 30 000 L mol^{-1} cm^{-1}). It is important to note that the rate of formation of absorbing compounds increased dramatically above pH 12.0. This value coincides with the pK_a of $OH^{\circ}/O^{-\circ}$ (11.8) and may suggest a stronger reactivity of $O^{-\circ}$. As the reaction proceeds, many compounds (absorbing at 265 nm or not) are formed[45] (polymer, aromatic aldehydes, etc.). According to these literature data, we propose that similar photo-oxidation of carbohydrates occurs in the detector cell, leading to the formation of UV-absorbing unsaturated compounds (probably malonaldehyde, or related conjugated carbonylated derivatives). The mechanism of the malonaldehyde formation (Figure 4) was previously proposed by Buknall et al. after carbohydrate irradiation in basic media.[44]

The following experiments were carried out to confirm this hypothesis. In order to determine if there is an influence of the number of hydroxyl functions present in the carbohydrate on the photochemical reaction taking place, a series of polyols containing three (glycerol), four (erythritol), five (xylitol), and six (mannitol, sorbitol, myo-inositol) C atoms—and, hence, three to six hydroxyl functions—was investigated (see Table S-1 in the Supporting Information for their structures, pK_a values, and molecular weights). Figure 5A shows that the absorbance front intensity at 270 nm increased with the number of hydroxyl groups in the carbohydrate. This is consistent with an increase in the number of chromophoric fragments. But obviously, other factors than merely the hydroxyl group number play a part in the photo-oxidation mechanism and absorptivity. Indeed, sorbitol, mannitol, and myo-inositol, all three of which have six OH groups but different structures (especially myo-inositol, which is a cyclic molecule), behave quite differently, while xylitol (5 OH groups) absorbs less than erythritol (four OH groups). UV spectra acquired while these compounds were in the detection window showed similar absorbance maxima at 265 nm, except myo-inositol, which has a cyclic conformation, and for which the

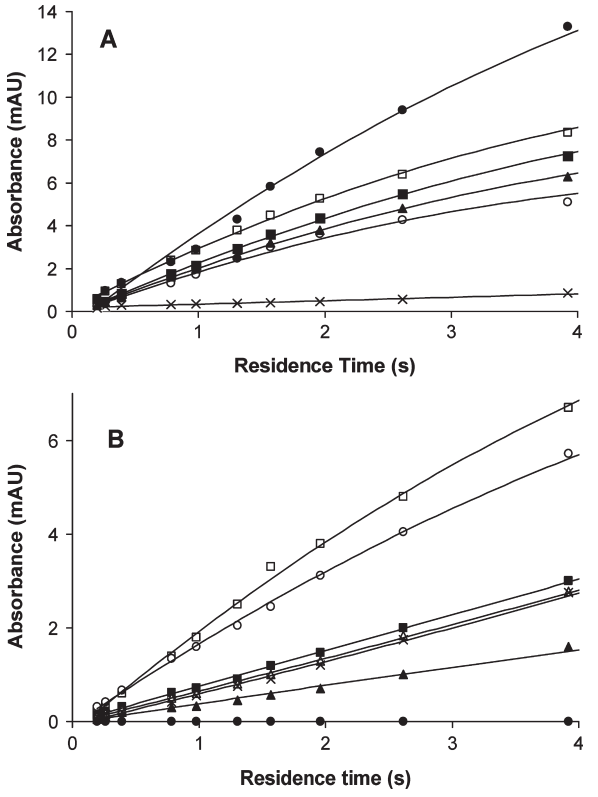

Figure 5. Variation of absorbance front intensities as a function of residence time in the detection window for (A) polyols and (B) glucose-based compounds, all at a concentration of 0.05 mM. For the experimental conditions, see Figure 3. Legend for panel A: (×) glycerol, (○) erythritol, (▲) xylitol, (■) mannitol, (□) sorbitol, and (●) myo-inositol. Legend for panel B: (●) heptakis(2,3,6-tri-*O*-methyl)-β-CD, (▲) HP-α-CD, (×) α-CD, (△) β-CD, (■) γ-CD, (○) δ-gluconolactone, and (□) glucose.

absorbance maximum was shifted to 270 nm. The same experiments were performed with a series of cyclodextrins (α-CD, β-CD, γ-CD, HP-α-CD, and heptakis(2,3,6-tri-*O*-methyl)-β-CD), and the results were compared with those for glucose. Figure 5B shows that, contrary to what was observed for polyols, absorbance did not increase, in terms of the number of glucose units. In effect, glucose exhibited the maximum absorbance whereas α-CD, β-CD, and γ-CD, which are constituted of six, seven, and eight glucose units, respectively, almost displayed the same absorbance variations (but slightly increasing in this order, nevertheless). Interestingly, a partially derivatized CD such as HP-α-CD showed less sensitivity, while a CD that is totally devoid of hydroxyl groups, such as heptakis-(2,3,6-tri-*O*-methyl)-β-CD, presented no signal at all. As a final assessment of the obtained results, δ-gluconolactone, which, in basic media, is quickly transformed to gluconate, was also tested, and its behavior was very similar to that of glucose (see Figure 5B). This point is consistent with a chain oxidation. These results established that hydroxyl groups are involved in a photochemical transformation in the detection window, which is favored by the number of hydroxyl groups and their ionization degree, but is also dependent on structural issues. This photochemical reaction should first lead to a carbonylated species, having a maximum absorbance at ~265−270 nm. The fact that nonreducing carbohydrates and polyols are

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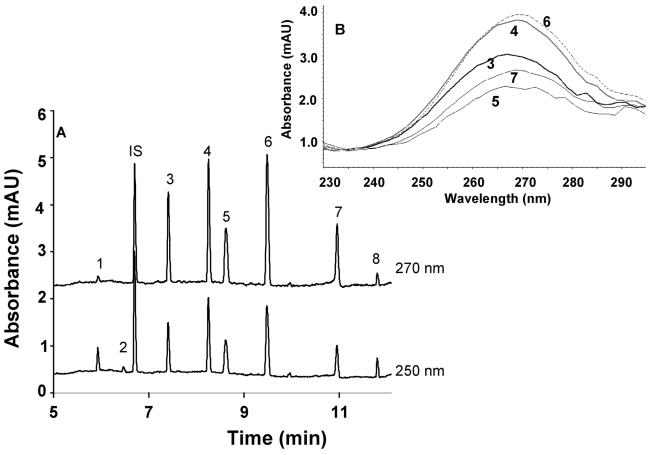

Figure 6. CE separation of a mixture of carbohydrates and amino acids in water. Bare fused-silica capillary, 50 μm id × 60 cm (UV detection at 50 cm) modified with HDMB (0.10 g in 100 mL ultrapure water). BGE: 130 mM NaOH (pH 13.1). Conditions: temperature, 20 °C; applied voltage, −16 kV; hydrodynamic injection, 5 s, 50 mbar. Direct UV detection at 250 and 270 nm. Analyte concentration = 0.05 mM each in ultrapure water. Legend: (1) glycine, (2) proline, (3) gluconolactone, (4) glucose, (5) β-CD, (6) sucrose, (7) mannitol, (8) arginine, and (IS) naphthalene sulfonic acid. The UV spectra for glycine, proline, and arginine were not overlaid to make the figure more easily readable and because their absorbance intensities were low.

detected under the same conditions as reducing carbohydrates lends support to considering a preliminary oxidative step. Afterward, as UV absorbance at 265−270 nm vanished, oxidation in the detection window should be completed to the carboxylate degree, giving rise to absorbance at 210 nm.

Moreover, a small increase in signal intensity with the presence of oxygen was found. Because the effect was small and difficult to investigate (nonanaerobic conditions with the nonhermetic CE vials, even if the electrolyte was previously degassed), the influence of oxygen impact was not studied further.

All the described phenomena (signal suppression by a filter intercalated between light source and sample, correlation with UV spectra, effectiveness of the reaction with reducing and non reducing carbohydrates, UV signal increasing with the number of OH groups, and dependence of signal intensity on the presence of oxygen) were consistent with the malonaldehyde detection or related compounds as intermediate species in the detection window.

Photo-oxidation of Amino Acids and Peptides. The photo-oxidation detection mode was thoroughly studied for reducing and nonreducing carbohydrates and for polyols, and was then contemplated for another reducing-group-containing family of utmost importance, such as amino acids. Only some representative UV-transparent amino acids were tested in this preliminary work: glycine, proline, and arginine. These amino acids, injected at a concentration of 0.05 mM, were also detected at 270 nm, under the same electrophoretic conditions as the carbohydrates, but with a lower sensitivity. As for carbohydrates and polyols, the influence of pH on absorbance front intensities was considered for glycine between pH 11.0 and pH 13.0 (see Figure S-2 in the Supporting Information). Although the degree of ionization for glycine (pK_a = 9.6) was almost unaffected in this pH range, a marked increase in absorbance and a higher rate for the formation of the absorbing intermediate was observed. This behavior lend support to considering OH^- concentration as a key parameter in yielding a series of UV-absorbing photo-oxidation products, probably including imine or aldehyde functions and eventually comprising formol, according to the study of the radiolysis of amino acids.[46−48]

To give an idea of possible application of this method in various areas and especially such as food analysis, the CE separation of a standard mixture containing glucose, sucrose, δ-gluconolactone, mannitol, β-CD, glycine, proline, and arginine, all at 0.05 mM, and naphthalene sulfonic acid as an internal standard is given in Figure 6. The UV spectrum of each compound was also recorded. Operation under reversed (cathodic) electro-osmotic flow conditions accelerated the separation and allowed the detection of all the injected compounds within <12 min. Gluconolactone, which is quickly transformed to gluconate, migrated faster than glucose. Amino acids were better detected at ∼250 nm.

The repeatability of the method was evaluated and details are present in the Supporting Information. It appeared that the method was reproducible and the time of use of the deuterium lamp had no influence between 350 h and 1200 h. Obtained limits of detection (LODs) varied from 0.04 mM for glucose to 0.06 mM for fructose and varied from 0.20 mM for glycine and arginine to 0.05 mM for proline. For underivatized amino acids, this method did not bring any improvement in the LODs obtained via mass spectrometry[49] but did require much simpler instrumentation.

CONCLUSIONS

This work highlights that a photo-oxidation reaction of carbohydrates and polyols can occur under high alkaline conditions in the detection window of some UV detectors, according to their optical design. Substantial insight into the complex molecular mechanisms involved was provided, which is consistent with literature data, and malonaldehyde or related compounds were proposed as intermediate chromophoric structures. The residence time in detection window appeared as the key parameter governing signal intensity. Preliminary experiments on the use of this detection mode for other reducing compounds demonstrated direct UV detection capabilities of underivatized amino acids at 250 nm, but with a lower sensitivity than that for carbohydrates and polyols. Optimization of the instrumental and optical design of the detector in order to take the best part from this detection mode was not the purpose of the present work and is still remains to be done. At least a 10-fold improvement in the present limits of detection could reasonably be reached.

ASSOCIATED CONTENT

Supporting Information. This material is available free of charge via the Internet at http://pubs.acs.org.

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