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Capillary electrophoretic determination of the component monosaccharides in hemicelluloses

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Abstract. This study describes the precolumn derivatization of carbohydrates with p-aminobenzoic acid and their efficient separation as borate complexes by means of capillary zone electrophoresis, using a capillary tube of fused silica containing 150 mmol/L borate buffer, pH 10.0, as carrier. On-column UV-monitoring at 285 nm allowed the detection of aldoses and uronic acids in the lower femtomole range. Reproducible quantification of carbohydrates was achieved at least in the concentration range of 0.1–10 mmol/L by the relative peak area method, using cinnamic acid as internal standard. The method was successfully applied to the determination of the monosaccharide composition of both commercially obtained xylans as well as of hemicelluloses obtained by hydrothermal degradation of biomass.

Introduction

Besides cellulose and lignin, hemicelluloses are the most important constituents of terrestrial plant cell walls [1–5]. They are built up of a relatively limited number of sugar residues, mainly D-xylose, D-mannose, D-glucose, D-galactose, L-arabinose, 4-O-methyl-D-glucuronic acid, D-galacturonic acid, and glucuronic acid. Less common sugar units are L-rhamnose, L-galactose, L-fucose, and O-methylated neutral sugars. Heteroglycans containing two to four, or rarely five or six, different sugar types are encountered more frequently than homoglycans. There are considerable differences in the hemicellulose content and composition between plants as well as between the different parts of a plant. Isolated hemicelluloses have many potentially useful properties including their use as food additives, thickeners, emulsifiers, gelling agents, adhesives, and adsorbants [6]. They can also be utilized as substrates for the production of microbial enzymes [7]. Further applications are the acidic hydrolysis of xylan to yield furfural and the reduction of xylose to xylitol, which has attracted attention as a non-carcinogenic sweetener. Finally, hemicelluloses can be converted by microorganisms to various products, such as methane, monosaccharides, organic acids, sugar alcohols, solvents, and ethanol [8–11].

The most common method of isolating hemicelluloses from plant biomass is alkaline extraction [12]. An alternative method is the hydrothermal degradation of biomass in pure water at elevated temperature and pressure [13–17]. It allows the gradual and temperature-dependent degradation of biomass. Specifically, at approximately 200 °C hemicellulose and a considerable part of the lignin components are dissolved, at 280 °C the cellulose part is degraded to a large extent with formation of monomeric carbohydrates, and above 300 °C the residual lignin can be transformed into soluble compounds, such as phenol. By this method, > 90% of the plant matter can be solubilized, with further degradation of the solutes being effectively reduced by continuous elution to a cooling unit.

Determination of the kind and quantity of glycosyl units present in a hemicellulose is usually the first step in its characterization. Hemicelluloses are commonly hydrolyzed by mineral acids, formic acid, or trifluoroacetic acid, with the latter being advantageous in that it can be removed by evaporation, hence neutralization is not necessary. Although progress has been made in the enzymic hydrolysis of hemicellulose, it is still expensive and mostly incomplete, even when a battery of enzymes is used [18]. The subsequent determination of the component monosaccharides has been based mainly on thin-layer chromatography [19, 20] and thin-layer electrophoresis [21] on silanized silica gel. Both methods are cheap and allow the identification of carbohydrates based on the specific colour they yield upon visualization with various reagents [22]. But the preparation of the plates is labour intensive and the whole procedure cannot readily be automated. Furthermore, resolution is not always sufficient to separate all monosaccharides of interest, and the quantitative evaluation is limited. Gas chromatography, which has also been used for the determination of the sugar components of hemicellulose [23, 24], offers the advantage that it can easily be coupled to mass spectroscopy, thus facilitating the identification of biomass degradation products. It suffers, however, from the shortcoming that carbohydrates need to be converted to volatile derivatives

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prior to analysis. Derivatization may be incomplete and can lead to the formation of multiple peaks per analyte. Finally, a number of high-performance liquid chromatographic techniques have been used to analyze the carbohydrates present in hemicellulose [25–27]. However, both partition chromatography on a μ-Bondapak carbohydrate column [25] as well as ion moderated partition chromatography on metal loaded cation exchange resins [26, 27] did not provide the selectivity required to separate the major component monosaccharides in hemicellulose. Although higher resolution can be realized by means of the high-performance liquid chromatographic separation of carbohydrates on a strong anion-exchange resin using a sodium hydroxide gradient, the method has the shortcomings that neutral saccharides and uronic acids cannot be analyzed under the same conditions and that elaborate sample pretreatment is needed in order to remove contaminants usually encountered in biomass degradation samples,such as lignins, tannic acids, trifluoroacetic acid, and various hydroxylated components, which otherwise interfere either with the retention or with the pulsed amperometric detection of carbohydrates [28, 29].

More recently, capillary zone electrophoresis has been shown to be a valuable tool in analyzing carbohydrates allowing high-resolution separation and reproducible microquantification by on-column detection [30]. However, detection of the nanoliter sample volumes injected in capillary electrophoresis is a challenging problem, especially in the case of carbohydrates which exhibit a very low UV-absorbance because of the small proportion of the carbonyl or open-chain form in aqueous solutions. Recently, a 2–20 fold increase in UV-absorbance at 195 nm has been observed by adding borate to aqueous solutions of carbohydrates [31]. Their resultant transformation to negatively charged complexes also constituted the basis for their migration in an electric field [31, 32]. But since the observed increase in absorptivity has been comparably small, sensitivity of detection is restricted to the nanomole range. A more sensitive method, which permits the analysis of underivatized saccharides in the lower picomole range, is indirect UV-detection, using sorbic acid as both carrier electrolyte and chromophore [33]. This approach utilizes high pH for the proper ionization of the sugars. However, the sensitivity is limited because at a pH higher than approximately 12 the competition of hydroxide ions is no longer negligible relative to the concentration of the chromophore. This also limits the degree of resolution obtainable, which could be significantly enhanced at higher pH values. Moreover, the linear dynamic range is limited to approximately one order of magnitude. The same drawbacks are also true for indirect fluorescence detection with visible laser detection, which permitted the analysis of native carbohydrates in the femtomole range using coumarin as background electrolyte [34]. Moreover, the high cost of a laser and its non-availability in most commercial capillary electrophoretic systems precludes the use of indirect fluorescence detection in many laboratories. This also applies to on-column laser-based refractive index detection, which has been reported to give detection limits in the lower femtomole range, with the linear dynamic range extending to more than three orders of magnitude [35]. Another alternative, which has been shown to be both sensitive and selective, is the electrochemical monitoring of native carbohydrates using amperometric detection at a copper electrode [36]. Detection limits were reported to be below 50 femtomole. Hence, detectability is clearly superior to that obtained by UV approaches [31, 33], and similar to that of indirect fluorescence detection [34], without the ion displacement problems encountered in indirect methods. But the highly alkaline conditions required for dissociating the carbohydrates are obvious drawbacks because of the lack of reliable capillary column technology and the chance of sugar decomposition. Moreover, reproducibility of measurement may suffer from fouling of the electrode surface, although it has been reported that copper electrodes, in contrast to gold and platinum electrodes, which require for their proper performance the application of a multistep potential waveform to clean them of products from carbohydrate oxidation, allow the electrocatalytic detection of sugars at a constant applied potential without significant losses in sensitivity [36]. Nevertheless, lifetime of the electrode is restricted to a maximum number of 100 runs and it seems to be prone to breakage.

Some of the aforementioned problems can be avoided by precolumn derivatization with reagents that contain suitable chromophoric or fluorogenic groups. Using on-column UV monitoring at 240 nm, carbohydrates, which had been derivatized with 2-aminopyridine by means of reductive amination in the presence of sodium cyanoborohydride, were detected at the lower pmol level [37]. Furthermore, depending on the composition of the background electrolyte, heterooligosaccharides could be separated either on the basis of their degree of polymerization in a fused silica capillary coated with polyacrylamide and filled with a phosphate buffer, pH 2.5, or on the basis of structural difference by means of borate complexation [38]. However, successful coupling of carbohydrates with 2-aminopyridine by reductive amination requires the presence of a free aldehyde group. Therefore only aldoses and uronic acids can be determined. This is also true for the reductive amination of carbohydrates with 3-methyl-1-phenyl-2-pyrazolin-5-one [39] and the fluorophore 3-(4-carboxybenzoyl)-2-quinolinecarboxaldehyde [40, 41], with the latter enabling the determination of mono- and oligosaccharides by means of laser-induced fluorescence detection at a low to medium attomole level depending on the mode of injection employed.

Recently, we introduced a set of derivatizing agents, namely p-aminobenzoic acid, ethyl p-aminobenzoate, and p-aminobenzonitrile, which did not only enable the successful derivatization of ketoses in addition to aldoses and uronic acids, but also their sensitive determination at the low femtomol level with readily available UV detection at 285, 305, and 285 nm, respectively [42–45]. Separation was achieved by both borate complexation and micellar electrokinetic capillary chromatography [46]. The latter technique resolved neutral carbohydrates, which had been derivatized with p-aminobenzonitrile, through their

differential distribution between the aqueous buffer phase and the micellar pseudo-stationary phase [44].

In the present study, we applied p-aminobenzoic acid to the precolumn derivatization of the component monosaccharides liberated both from commercially obtained xylans as well as from various plants degraded by means of hydrothermolysis. Separation of the derivatives was then achieved by means of borate complexation in an uncoated fused silica capillary.

Experimental

Chemicals

A reagent grade sample of p-aminobenzoic acid was purchased from Sigma (St. Louis, MO, USA). Trifluoroacetic acid, acetic acid, cinnamic acid, ethanol and sodium cyanoborohydride were obtained from Sigma and Merck (Darmstadt, Germany). Standard solutions were prepared from chromatographic or analytical reagent grade chemicals (Merck, Sigma) in deionized water (NANOpure, Barnstead Co., Newton, MA, USA). Xylans isolated from oat spelts and birchwood were obtained from Sigma, Roth (Carl Roth, Karlsruhe, Germany) and Lenzing AG (Lenzing, Austria). The background electrolyte was prepared by dissolving an appropriate amount of boric acid (Sigma) in doubly distilled water to yield a final concentration of 150 mmol/L. The pH was adjusted to 10.0 by the addition of 2 mol/L sodium hydroxide (Merck).

Isolation of hemicellulose

Hemicellulose was solubilized by means of hydrothermal degradation. Wheat straw and sugar cane bagasse were percolated with 5 ml of deionized water per minute at a pressure of 35 bar and a temperature of 190 °C. Rice straw and elephant grass were percolated at the same pressure with a flow-rate of 9 ml/min at a temperature of 200 °C. Subsequently, the hemicellulose was precipitated by the addition of 5 volumes of ethanol.

Hydrolysis of hemicellulose

To obtain the component monosaccharides, the extracted hemicelluloses were hydrolyzed by adding 8 ml of 2 mol/L trifluoroacetic acid to 40 mg of hemicellulose contained in a small round-bottom flask. The sample was dissolved by gentle swirling, and the solution was refluxed for 2 h at 120 °C. Then the hydrolysis solution was lyophilized to dryness.

Precolumn derivatization of carbohydrates with p-aminobenzoic acid

This was performed under the optimized conditions described previously [43, 45]. The reagent solution was freshly prepared before derivatization by dissolving 10 mg of sodium cyanoborohydride in 1 ml of a methanolic solution containing p-aminobenzoic acid and acetic acid at concentrations of 7 and 10% (w/v), respectively. A saccharide or a mixture of saccharides was taken into a small sample tube, and the reagent solution was added to make the final concentration(s) of the saccharide(s) 0.15–2.0 mg/ml. The tube was capped with a rubber stopper. Dissolution of the saccharide(s) was facilitated by gentle vortexing. The resultant solution was heated for 2 h at 50 °C and, after cooling to room temperature, diluted 1:10 with methanol prior to capillary zone electrophoretic analysis. By using Teflon-lined screw-caps, which effectively prevented evaporation of the solvent, the time needed for derivatization could even be reduced to 15 min by carrying out the reaction at a temperature of 90 °C instead of 50 °C [44].

Capillary zone electrophoresis

Analyses were performed on an Applied Biosystems (ABI, San Jose, CA, USA) Model 270A capillary electrophoresis system, which was equipped with a 72-cm fused silica capillary of I.D. 50 μm. Detection was carried out by on-column measurement of UV absorption at 285 nm at a position of 22 cm from the cathode. Pherograms were recorded on a Shimadzu Chromatopac C-R6A integrator (Kyoto, Japan). Samples were loaded by applying a vacuum at a pressure of 16.9 kPa for 1 s.

Every new fused silica capillary was flushed with 1 mol/L NaOH for 1 h followed with 0.001 mol/L NaOH for 5 min. Between runs, the capillary was washed first with 1 mol/L NAOH for 3 min, and then with 0.001 mol/L NaOH for 2 min. Subsequently, it was equilibrated with running buffer for 3 min. The capillary was stored overnight in 0.001 mol/L NaOH.

Results and discussion

Capillary zone electrophoresis of carbohydrates

Figure 1 depicts the electropherogram of a reaction mixture of 7 p-aminobenzoic acid derivatives of various aldoses and uronic acids commonly found in hemicellulose. Mesityl oxide served as an electroosmotic flow-marker, while cinnamic acid was included as an internal standard in order to allow the quantitative determination of the carbohydrate derivatives by the relative peak area method.

At the selected pH value, the p-aminobenzoic acid derivatives are negatively charged both due to their inherent charges as well as due to their complexation with tetrahydroxyborate, which rather than boric acid is complexed by polyols [47]. Therefore, the derivatives will migrate away from the detector located near the cathode toward the anode. However, due to the large electroosmotic flow in the system, which is faster than electrophoretic migration, analytes are propelled together with the bulk solution toward the cathode, but at a much lower rate. The electrophoretic mobilities of the

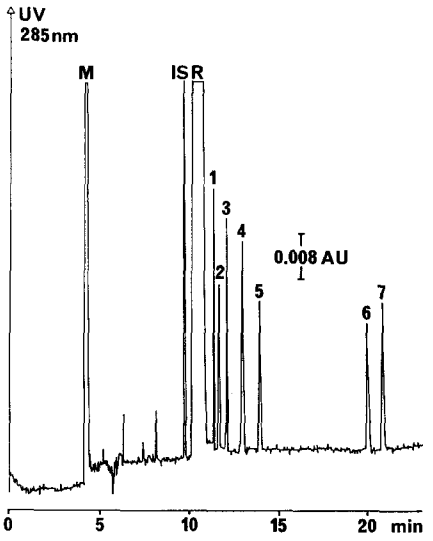

Fig. 1. Capillary zone electrophoretic separation of a standard mixture of carbohydrates (0.15–1.73 mg/ml) derivatized with p-aminobenzoic acid. Capillary: fused silica, L = 72 cm, 1 = 50 cm, ∅ = 50 μm; carrier: 150 mmol/L borate; pH 10.0; voltage: 28 kV; current: 79 μA; detection: UV, 285 nm; temperature: 30 °C; injection: vacuum, 1 s. Zone identification: *M* mesityl oxide, *IS* internal standard (cinnamic acid), *R* reagent (p-aminobenzoic acid), *1* xylose, *2* glucose, *3* arabinose, *4* fucose, *5* galactose, *6* glucuronic acid, and *7* galacturonic acid

carbohydrates are determined by the stability of the sugar-borate complexes, which depends primarily on the configuration of the three vicinal hydroxyl groups at the carbons C2 to C4, and to a lesser degree extent at the carbons C3 to C5 [43, 45]. Corroborating a previous finding that *threo*-diols are energetically more favourable than *erythro*-diols for complex formation with borate [48], complex stability and, hence, electrophoretic mobilities increased in the following order: e-e (adjacent *erythro* pairs), e-t (an *erythro*-diol adjacent to a *threo*-diol), t-t (adjacent *threo* pairs), and t-e (a *threo*-diol adjacent to an *erythro*-diol). Accordingly, arabinose, fucose and galactose formed the strongest complexes with borate and, hence, exhibited the highest electrophoretic mobilities of the investigated monosaccharides. Uronic acids are detected even later because of their additional carboxyl group which is completely dissociated under the electrophoretic conditions used. For this reason, glucuronic acid and galacturonic acid exhibit higher electrophoretic mobilities as caused by borate complexation only.

Besides carbohydrate structure, the stability of the sugar-borate complexes and, hence, the magnitude of their negative charge is mainly determined by the pH and the borate concentration of the background electrolyte. Given a constant amount of carbohydrate, the complex concentration increases with rising borate concentration according to the law of mass action as well as with rising pH due the higher concentration of alkaline borate ions. Therefore, the electrophoretic mobility is increased in the same way, which leads to an increase in the migration rates of the p-aminobenzoic acid derivatives against the electroosmotic flow, resulting in retardation of their elution. Finally, a pH-value of 10.0 and a borate concentration of 150 mmol/L were chosen for all analyses, because this offered sufficient resolution of the carbohydrates of interest, namely xylose, glucose, arabinose, fucose, galactose, glucuronic acid, and galacturonic acid, within a relatively short time of analysis.

In order to obtain baseline resolution under the aforementioned conditions, the difference in electrophoretic mobility between two carbohydrates has to be approx. 0.02×10^{-4} $cm^2V^{-1}s^{-1}$. The excellent resolving power of the system and the selectivity obtained by derivatization with p-aminobenzoic acid allowed the simultaneous determination of almost all sugar units encountered in hemicelluloses. Exceptions are rhamnose, which is masked by the reagent peak and mannose, which has been found to comigrate with glucose. Although precolumn derivatization with ethyl p-aminobenzoate or p-aminobenzonitrile would allow the determination of rhamnose and the successful separation of glucose and mannose by borate complexation, it would be impossible, on the other side, to resolve mannose and arabinose efficiently [42, 44]. Only micellar electrokinetic capillary chromatography allows the complete separation of all neutral monosaccharide residues found in hemicellulose [44]. However, it is not possible to detect the uronic acid residues in the same run. In addition, migration times become irreproducible after a few runs in an uncoated fused silica capillary due to a gradual decrease in electroosmotic flow. Most probably, this is caused by the adsorption of sodium dodecyl sulfate to the inner capillary surface, which leads to a decrease in ζ-potential.

Quantification

Although the extent of derivatization differed among the carbohydrates investigated, yields were reproducible enough (RSD = 5.4%, n = 6) to obtain quantitative information from capillary zone electrophoretic analyses by dividing peak area with migration time and relating it to that of cinnamic acid, which was used as an internal standard. The calibration curves for the aldoses and uronic acids most commonly found in hemicelluloses showed excellent linearity at least over a concentration range of 0.1–10 mmol/L and negligible Y intercepts. In least-square linear regression analysis, the respective equations of the line and the regression coefficients for xylose, glucose, arabinose, fucose, galactose, glucuronic acid and galacturonic acid were $Y = 1.668X - 0.01$ ($R^2 = 0.998$), $Y = 1.628X - 0.0004$ ($R^2 = 0.991$), $Y = 1.602X + 0.162$ ($R^2 = 0.998$), $Y = 1.428X + 0.087$ ($R^2 = 0.999$), $Y = 1.425X - 0.170$ ($R^2 = 0.989$), $Y = 1.146X + 0.184$ ($R^2 = 0.963$), and $Y = 0.965X - 0.091$ ($R^2 = 0.961$), respectively. The reproducibility of the measurements of relative peak area was checked by six repeated runs. A mean coefficient of variation of 4.7% was obtained.

The mass detection limits for the investigated carbohydrates were approximately 15 to 30 femtomole at a signal-to-noise ratio of 3. Concentration sensitivity was about 4 to 8 μmol/L. While the mass detection limits are about one to two orders of magnitude higher than those

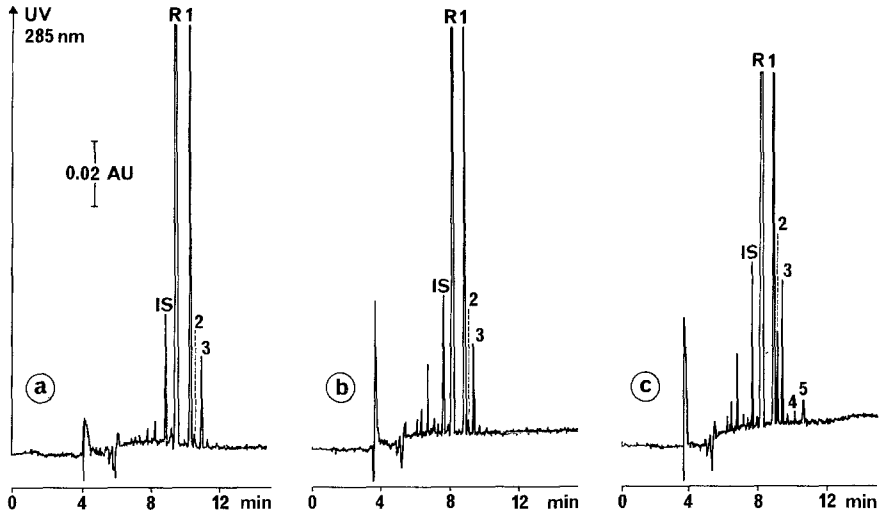

Fig. 2 a–c. Capillary zone electrophoretic analyses of the component monosaccharides in commercially obtained xylanes after hydrolysis with 2 mol/L trifluoroacetic acid at 120 °C for 2 h and precolumn derivatization with p-aminobenzoic acid: **a** oat spelts, Sigma, **b** oat spelts, Roth, and **c** birchwood, Roth. Capillary: fused silica, L = 72 cm, l = 50 cm, ∅ = 50 µm; carrier: 150 mmol/L borate; pH 10.0; voltage: 28 kV; current: 79 µA; detection: UV, 285 nm; temperature: 30 °C; injection: vacuum, 1 s. Zone identification: *IS* internal standard (cinnamic acid), *R* reagent (p-aminobenzoic acid), *1* xylose, *2* glucose, *3* arabinose, *4* fucose, and *5* galactose

Table 1. Relative yields of monosaccharides in percent of the initial weight of commercial xylan subjected to acidic hydrolysis

Source of xylan	Birchwood (Roth)	Hemicellulose (Lenzing)	Oat spelts (Sigma)	Oat spelts (Roth)
Total sugar	77.93	76.98	85.79	77.46
Xylose	61.67	76.16	77.84	70.26
Glucose	6.08	0.82	0.99	0.81
Arabinose	7.93	n.d.	6.95	6.39
Fucose	0.65	n.d.	n.d.	n.d.
Galactose	1.58	n.d.	n.d.	n.d.

n.d. = not detected

reported for capillary electrophoretically separated mono- and oligosaccharides derivatized with the fluorophore 3-(4-carboxybenzoyl)-2-quinolinecarboxaldehyde and, subsequently, detected by means of laser-induced fluorescence with an argon ion laser [40], they are slightly better than those obtained for underivatized carbohydrates, which were determined by means of capillary zone electrophoresis under alkaline conditions and amperometric detection at a copper microelectrode [36]. In this context, it is also interesting to note that the detection limits obtained in the present study were about four orders of magnitude better than those reported for carbohydrates which had been separated by anion-exchange chromatography and monitored with triple-pulsed amperometric detection [28, 29]. With regard to concentration sensitivity, pulsed amperometric detection for a 200-µl injection equals that of direct UV-detection for a 8-nl injection of glucose derivatized with p-aminobenzoic acid.

Capillary zone electrophoretic evaluation of the quality of commercially obtained xylans

Xylan, by definition, is a polymer of D-xylosyl residues. A variety of other sugar components, however, could be determined in the commercial xylan preparations investigated by capillary zone electrophoresis (Fig. 2). While the xylan sample obtained from Lenzing contained xylose residues greater than 98.5%, various amounts of glucose, arabinose, fucose and galactose were found in a xylan sample from birchwood (Table 1). The relative content of xylose in the latter sample was the lowest of all investigated xylans and amounted to 79.1%. On the other side, the two xylan samples from oat spelts, which had been purchased from two different companies, did not differ significantly in monosaccharide composition from each other. Both contained approximately 90.7% of xylose, as well as 8.1% and 1.1% of arabinose and glucose, respectively. The total yield of carbohydrate obtained after hydrolysis with 2 mol/L trifluoroacetic acid for 2 h at 120 °C varied from 77 to 86%. The loss of carbohydrate is most probably due to the partial conversion of carbohydrates to various degradation products, including furfural and organic acids, during hydrolysis [49].

Monosaccharide composition of hemicelluloses obtained from plant biomass by means of hydrothermal degradation

Figure 3 shows the capillary zone electrophoretic separation of the p-aminobenzoic acid derivatives of the component monosaccharides found in the hemicellulose

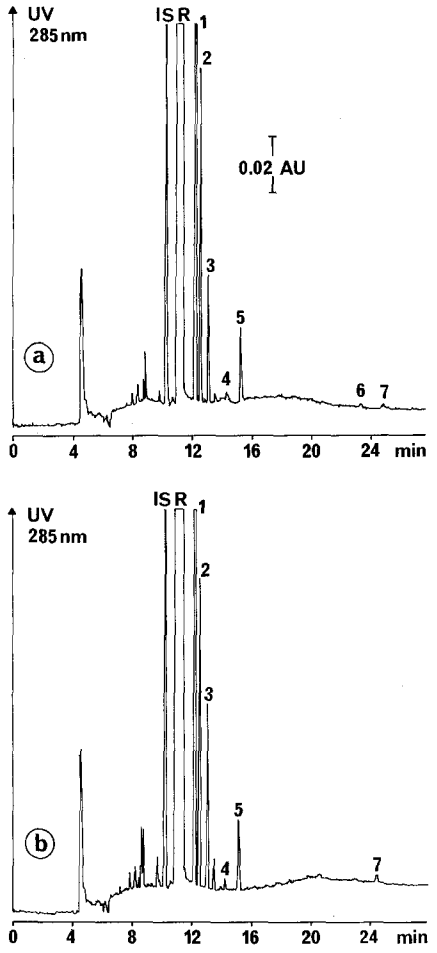

Fig. 3 a,b. Capillary zone electrophoretic determination of the monosaccharide composition of hemicelluloses obtained from **a** rice straw and **b** elephant grass by means of hydrothermal degradation at 190 °C and 200 °C, respectively. Capillary: fused silica, L = 72 cm, l = 50 cm, ∅ = 50 µm; carrier: 150 mmol/L borate; pH 10.0; voltage: 28 kV; current: 79 µA; detection: UV, 285 nm; temperature: 30 °C; injection: vacuum, 1 s. Zone identification: *IS* internal standard (cinnamic acid), *R* reagent (p-aminobenzoic acid), *1* xylose, *2* glucose, *3* arabinose, *4* fucose, *5* galactose, *6* glucuronic acid, and *7* galacturonic acid

hydrolyzates of rice straw (Fig. 3a) and elephant grass (Fig. 3b), respectively. The relative yields of sugars and sugar acids of the investigated samples of hemicellulose, which had been solubilized from four different sources of plant biomass by means of hydrothermal degradation, are summarized in Table 2. The major component of the investigated hemicelluloses was xylose, the relative amount of which ranged from 64.5% to almost 80% in sugar cane bagasse and elephant grass, respectively. In addition to other neutral monosaccharides already identified in commercially obtained xylans, small amounts of glucuronic acid and galacturonic acid were found in rice straw and elephant grass, but not in wheat straw and sugar cane bagasse. The observed loss in total yield of carbohydrates has several reasons. Firstly, the solubilized hemicelluloses are already partly converted to furfural, organic acids and other polyhydroxy compounds during hydrothermal degradation [21, 26]. Secondly, acetyl groups that are common constituents of hemicellulose are lost during hydrothermal degradation and are converted to acetic acid. Furthermore, the loss of the acetyl groups may cause hemicelluloses to become insoluble in water. Thirdly, uronic acid units are not readily hydrolyzed by trifluoroacetic acid and are usually linked to another sugar as an aldobiouronic acid. The stability of the glycosidic bond of aldobiouronic acids to acid hydrolysis is so great that extensive destruction of the uronic acid and of other sugar units occurs on attempts to completely hydrolyze polysaccharides. The aldobiouronic acids present in the samples most probably account for some of the small peaks migrating in front of the reagent peak.

Conclusions

Precolumn derivatization of carbohydrates with p-aminobenzoic acid and their subsequent separation as borate complexes in an uncoated fused silica capillary with on-column UV monitoring permits the quantitative determination of the monosaccharide composition of hemicellulose in the lower femtomole range. In spite of the need of derivatization, which can be carried out reliably within a few minutes, and the need of an internal standard for accurate quantification, the method lacks most of the shortcomings of the analytical techniques used up to now, hence representing a very sensitive and selective tool for determining the kind and quantity of glycosyl units present in hemicellulose.

Table 2. Yields of monosaccharides in percent of the initial weight of hemicellulose solubilized from plant biomass by hydrothermal degradation and subjected to acidic hydrolysis

Source of hemicellulose	Rice straw	Elephant grass	Wheat straw	Bagasse
Total sugar	68.87	69.23	81.47	65.36
Xylose	47.60	55.31	55.36	42.16
Glucose	11.76	7.23	15.62	14.92
Arabinose	4.25	4.01	5.80	4.10
Galactose	4.07	2.04	4.69	4.10
Fucose	0.44	0.12	n.d.	n.d.
Galacturonic acid	0.70	0.85	n.d.	n.d.
Glucuronic acid	0.87	n.d.	n.d.	n.d.

n.d. = not detected

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