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Determination of Levoglucosan from Smoke Samples Using Microchip Capillary Electrophoresis with Pulsed Amperometric Detection

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Separation and detection of native anhydrous carbohydrates derived from the combustion of biomass using an electrophoretic microchip with pulsed amperometric detection (PAD) is described. Levoglucosan represents the largest single component of the water extractable organics in smoke particles and can be used to trace forest fires or discriminate urban air pollution sources. Detection of levoglucosan and other sugar anhydrides in both source and ambient aerosol samples is typically performed by gas chromatographic (GC) separation with mass spectrometric (MS) detection. This method is cost, time, and labor intensive, typically involving a multistep solvent extraction, chemical derivatization, and finally analysis by GC/MS. However, it provides a rich wealth of chemical information as the result of the combination of a separation method and MS and exhibits good sensitivity. In contrast, microchip capillary electrophoresis offers the possibility of performing simpler, less expensive, and faster analysis. In addition, integrated devices can be fabricated and incorporated with an aerosol collection system to perform semicontinuous, on-site analysis. In the present report, the effect of the separation potential, buffer pH and composition, injection time, and pulsed amperometric detection parameters were studied in an effort to optimize both the separation and detection of anhydrous sugars. Using the optimized conditions, the analysis can be performed in less than a minute, with detection limits ranging from 22 fmol (16.7μM) for levoglucosan to 336 fmol (258.7 μM) for galactosan. To demonstrate the capabilities of the device, a comparison was made between GC/MS and microchip electrophoresis using an aerosol source sample generated in a wood-burning chamber. A second example utilizing an ambient aerosol sample illustrates a matrix interference necessitating additional method development for application to samples not dominated by wood smoke.

Introduction

Emissions from residential combustion of wood are major contributors to air pollution during winter months in urban locations such as Portland, OR; Denver, CO; and Albuquerque, NM (*1*). The compounds produced during the combustion of pine or oak can be present in gas or particulate phase. Ethene, acetylene, phenol, substituted phenols, alkanes, aliphatic ketones, and dicarbonyls are only some of the compounds that have been identified in the gas phase (*1*). However, the largest single component of the water-extractable particle-phase organics mixture is 1,6-β-D-anhydroglucopyranose (Levoglucosan) and, as it was reported (*1*), may comprise up to 31% of the total fine particle (PM_{10}) organics mass. Levoglucosan has also been identified in liquid-smoke flavorings (*2*), caramel (*3*), as a subproduct of cellulose oxidation during incineration of municipal solid waste (*4*), and has also been proposed as a molecular marker for the long-range transport of biomass combustion aerosols (*5*−*7*).

Several studies have been performed to identify and quantify individual organic species in combustion source and ambient aerosol samples. Because of the complexity of the mixtures where these anhydrosugars are present, GC/MS has been primarily used to separate and detect them (*1*, *4*, *5*, *8*−*10*). However, GC/MS is cost, time, and labor intensive. Sample preparation typically involves a multistep solvent extraction and chemical derivatization to reduce the polarity of levoglucosan (and other compounds) and therefore increase its volatility (*11*).

Capillary electrophoresis (CE) is an alternative method for the separation of water-soluble compounds that can remove the derivatization step while also decreasing the separation time to only a few minutes (*12*, *13*). In CE, the separation takes place inside a capillary (i.d. < 100 μm) filled with an electrolyte solution. When a potential is applied at the extremes of the capillary, the analytes migrate depending on the mass/charge ratio, the magnitude of the applied potential, and the electrolytic solution conditions. Bulk flow is generated inside the capillaries by a process referred to as electro-osmotic flow (EOF). EOF occurs when a charged ionic double layer forms at the capillary/buffer interface and a potential is applied across the capillary. As a result, the double layer moves and carries the bulk solution through the capillary. This flow mechanism requires no moving parts and therefore can be easily miniaturized. Another advantage of CE is the possibility of using a wide range of pH values. This can be used not only to control the EOF but also to manipulate the charge of the analytes and, therefore, the separation resolution (*14*−*23*).

Microanalytical devices open up new possibilities for the miniaturization of conventional chemical and biochemical analyses. Since the introduction of the miniaturized total analysis system (μTAS) term less than 15 years ago (*24*), an enormous number of papers has been published showing the capabilities of these devices (*23*, *25*, *26*). Some of the advantages of the μTAS over conventional benchtop systems include custom design, reduced consumption of reagents and sample, lower waste generation, and increased analysis speed and portability (*27*). A wide variety of polymeric materials have been used more recently in an effort to reduce fabrication complexity and cost for electrophoresis chips (*28*). Poly(dimethylsiloxane) (PDMS) is an elastomeric material that has been used extensively because it is robust, optically transparent, nonpolar, and impermeable to aqueous solutions and allows the easy, fast, and inexpensive fabrication of devices using micromolding techniques (*29*) for a variety of applications (*21*, *23*,*26*, *30*−*37*).

Electrochemical detection (ECD) represents an attractive option for microchip devices (*25*, *36*−*40*). Since many

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compounds are electrochemically active, many applications can be found (*39*). However, when a constant potential is applied, the electrode can be fouled by the accumulation of adsorbed cabonaceus material, causing an unstable signal (*41*, *42*). This effect is particularly detrimental when carbohydrates (*43*), thiols, or phenols (*44*) are detected. To overcome problems associated with electrode fouling in conventional liquid chromatography, a potential waveform referred to as pulsed amperometric detection (PAD) is applied. In PAD, a high positive potential is applied to clean the electrode surface, followed by a negative potential step to reactivate the electrode surface. A third potential is applied for detection of the target analytes. PAD has proven to be effective for a large number of analytes including carbohydrates, amino acids, sulfur-containing species, and alcohols (*45*). PAD is particularly useful when the analyte lacks a strongly absorbing chromophore (i.e., UV/vis spectrometry) or where other electrochemical techniques are ineffective because of rapid electrode fouling (*46*).

In the present report, the use of a PDMS CE-microchip with PAD is described for the measurement of carbohydrates from wood-smoke aerosol particles. This is the first time that a determination of levoglucosan, glucose, and galactosan is achieved simultaneously using a microdevice. The effects of the separation potential, buffer pH and composition, injection time, and PAD parameters on the separation and detection are presented. A comparison study using GC/MS was also performed to demonstrate the capability of using the device to analyze extracts from wood-smoke source samples. Limitations of applying the method, in its current form, to ambient samples where wood smoke does not dominate the composition are also discussed.

Materials and Methods

Reagents and Solutions. SU-8 2035 photoresist and XP SU-8 developer were purchased from MicroChem Co. Sylgard 184 silicone elastomer and curing agent were obtained from Dow Corning. Aqueous solutions were prepared using analytical grade reagents and 18 MΩ·cm^{-1} resistance water (Milli-Q, Millipore). The running electrolytes were prepared by weighing the desired amount of sodium tetraborate ($Na_2B_4O_7$·$10H_2O$) (Fisher) and adjusting the pH with 2 M NaOH (Fisher). Sample stock solutions (10 mM) were prepared daily by dissolving the desired amount of each compound in 10 mL of water. Levoglucosan (1,6-anhydro-β-D-glucopyranose) (LV) was purchased from Fluka (>98% purity), while mannosan (1,6-anhydro-β-D-mannopyranose) (MS) and galactosan (1,6-anhydro-β-D-galactopyranose) (GS) were obtained from Sigma (98% purity). Glucose (GLU) was purchased from Sigma. Per-deuterated levoglucosan was used as internal standard and was purchased from Cambridge Isotope Laboratories (Andover, MA). Dichloromethane and methanol were obtained in Optima-grade from Fisher Scientific. All chemicals were used as received without any further purification. A 25 μm, 99.99% gold wire (Goodfellow, England) was used as the working electrode. For pH measurements, a combined glass electrode and a digital pH meter (Denver Instrument) were used and purchased from Fisher. Unless noted, the error bars on the plots represent the standard deviation obtained for at least three measurements. All experiments were performed at room temperature (22 ± 2 °C).

Instrumentation. A three-channel (two positives and one negative) laboratory-built high-voltage power supply, with an adjustable voltage range between 0 and ±4000 V, was used for all the experiments as previously reported (*47*). Electrical connections to the microfluidic device were made with platinum electrodes placed into reservoirs at the ends of each channel. Electrochemical detection by PAD was performed with a CHI660 Electrochemical Detector (CH Instruments) according to the potential scheme described below and as shown in Tables 1 and 2.

TABLE 1. Waveform Parameters Used for the Pulsed Amperometric Detection of Carbohydrates

	potential (V)	time (s)
clean	+1.6	0.05
reactivate	−0.5	0.025
detect	+0.9	0.15

TABLE 2. Potentials Applied and Solution in Each Reservoir on the Microchip (According to Figure 1) during Either the Injection or the Separation Step

reservoir	containing	injection/V	separation/V
A	buffer	+410	+1000
B	buffer	−160	+410
C	sample	+410	+410
D	waste	ground	ground

GC/MS Analysis and Procedure. Samples and standards were analyzed on an HP 6890 gas chromatograph (Hewlett-Packard) coupled with an HP 5973 mass selective detector (MSD). Separation was accomplished using a 30-m long × 250-μm i.d. HP-5MS (Hewlett-Packard) capillary column with a 0.25-μm 5% phenyl-methyl-siloxane film. The temperature ramp started with an isothermal step of 10 min at 65 °C, followed by a gradient of 10 °C/min up to a final temperature of 300 °C that was held constant for 20 min. The total run time was 54 min. Injections were done in splitless mode and helium was used as carrier gas. The MSD was operated in ion scan mode and ions were produced by electron impact ionization with a source temperature of 230 °C. Mass-to-charge ratios of 50−500 were scanned at 2.94 scans/s. To reduce the polarity, increase the volatility, and thus make the compounds more amenable for GC/MS analysis, a derivatization step was included. Hydroxy groups present in the anhydrous carbohydrates were converted into their trimethylsilyl esters using a previously reported method (*48*). Briefly, 50 μL of the sample containing the carbohydrates were mixed with 50 μL of bis(trimethylsilyl)trifluoroacetaminde (BSTFA) and 10 μL cholortrimethylsilane as catalyst. The mixture was then heated at 65 °C in a sealed vial for 5 h.

Fabrication of the PDMS Microchip. PDMS microchips were fabricated using a previously reported method (*47*, *49*, *50*). Briefly, a clean 76-mm silicon wafer (Silicon Valley Microelectronics Inc.) was coated with SU-8 2035 negative photoresist. A digitally produced mask containing the channel pattern was placed on the coated wafer, exposed to light via a near-UV flood source, and then baked. The positive relief was developed by placing the wafer in propylene glycol methyl ether acetate for 15 min, rinsing with methanol, and drying under a N_2 stream. The height of the positive pattern on the molding master, which is equal to the channel depth created on the PDMS layer, was 50 μm when measured with a profilometer. PDMS layers were fabricated by pouring a degassed mixture of Sylgard 184 silicone elastomer and curing agent (10:1) onto either a molding master or a blank wafer, followed by curing for at least 2 h at 65 °C. The cured PDMS was separated from the mold and reservoirs were made at the end of each channel using a 6-mm circular punch. For the detection, a 25-μm gold wire was aligned at the end of the separation channel in a perpendicular electrode channel (Figure 1). The electrode alignment channel was in the same PDMS layer as the separation channel. An additional flat PDMS layer was formed by curing Sylgard 184 on a blank Si Wafer. The two PDMS layers were placed in an air plasma cleaner (Harrick PDC-32G plasma cleaner/sterilizer), oxidized

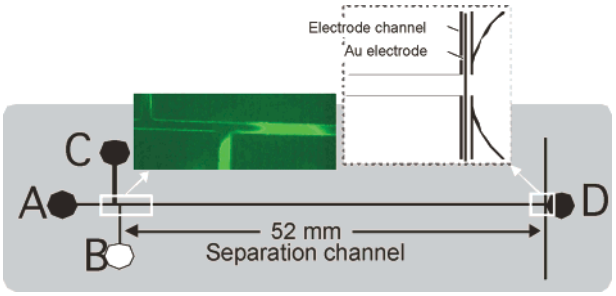

FIGURE 1. Capillary electrophoresis chip with integrated pulsed amperometric detection. Channels: 50 μm width, 50 μm deep. Sample loop: 580 μm long, separation channel: 52 mm long. The diagram shows also the electrode position at the end of the separation channel and the connections to the electrochemical analyzer.

for 20 s, and immediately brought into conformal contact to form an irreversible seal. Finally, the extremities of the electrode channel were sealed with superglue, and electrical connection to the working electrode was made using silver paint and a copper wire. A double-T injector with a 580-μm gap between side channels (1.3 nL) was used for all experiments. During the injection, +410 V, −160 V, and +410 were applied to the reservoirs A, B, and C, respectively (Table 2). During the separation, the potential applied to the reservoir A was raised to +1000 V while the potential applied to the reservoir B was changed to +410 V. The waste reservoir (D) was always grounded. A 1 MΩ resistor was included in series with the chip to avoid Joule heating in the A−B channel during the injection procedure.

Electrochemical Detection. Electrochemical detection was performed using a three-electrode setup. As mentioned previously, a gold wire was used as the working electrode and the corresponding detection potential was optimized for each compound. The auxiliary electrode was a platinum wire placed in the waste reservoir (D). A Ag/AgCl wire, also placed in the waste reservoir, was used as reference electrode while 5 mM NaCl was added to the electrolyte to complete the reference cell.

Aerosol Collection and Extraction Procedures. Atmospheric aerosol particles were collected using Thermo Andersen (Smyrna, GA) high-volume collectors. The samplers were equipped with an impactor to give a nominal cutoff of 2.5-μm aerodynamic particle diameter. Samples were collected on prefired Whatman (Maidstone, England) QM-A quartz fiber filters at a flow rate of 1130 L/min. Ambient sample collection times were 12 h; much shorter sample times were used for collection of wood-smoke source samples. Exposed filters were stored in a freezer until analysis. Prior to the extraction for the GC/MS analysis, the collection filters were spiked with deuterated levoglucosan to control the extraction and to account for losses during the sample preparation procedure. The particles present in a portion of the filter (typically one-quarter) were extracted with 3 aliquots of 25 mL of dichloromethane (DCM) under ultrasonic agitation during 15 min. The combined extracts were filtered through a prefired quartz filter and subsequently reduced in volume to 250 μL. For the CE-PAD analysis, a portion of the quartz fiber filter (typically one-eighth) was extracted with 2 aliquots of 20 mL of DI water under ultrasonic agitation during 30 min. The combined extracts were then filtered through prefired quartz fiber filters before analysis.

Results and Discussion

Effect of the Detection Potential. It has been proposed by LaCourse that Au electrodes are better suited for the oxidation of carbohydrates when oxide-free (*45*). These surfaces stabilize free-radical oxidation products by adsorption and,

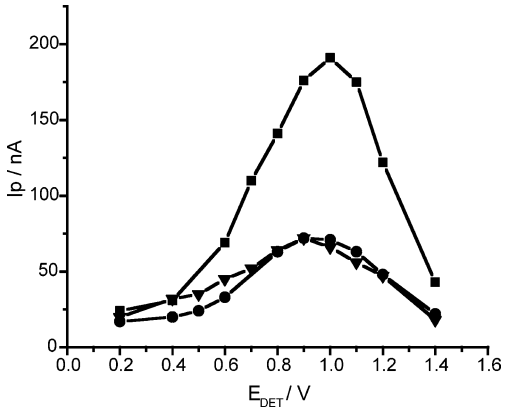

FIGURE 2. Hydrodynamic voltammograms for levoglucosan, glucose, and mannosan. Conditions: 5 mM $Na_2B_4O_7$ buffer pH = 12.30, separation potential = +1000 V (A−D), 10-s injection.

thereby, can promote faradaic reactions. A clean electrode is achieved by the use of both a highly positive potential (+1.6 V) to remove the adsorbed material by oxidation and a negative potential (−0.5 V) to reconstruct the surface. Once a clean surface is obtained, a potential should be chosen to maximize the electrode response. The effect of the detection potential on the signal was analyzed between +0.2 V to +1.4 V for levoglucosan, glucose, and mannosan (Figure 2), as no such data are available for anhydrosugars. As can be seen, the peak current (I_p) increases as the detection potential increases until a maximum is obtained at +0.9 V (for glucose and mannosan) or +1.0 V (for levoglucosan). The current decrease observed at potentials higher than +0.9 V can be explained as the result of the formation of oxide on the working electrode surface during the measurement step. The formation of this oxide prevents adsorption of the compounds on the electrode surface, reducing the signal intensity. Since similar profiles were found for all carbohydrates, +0.9 V was chosen as the optimum detection potential. These results are comparable to those seen with normal carbohydrates (glucose, galactose, lactose, mannose, sucrose, maltose, maltotriose, and glucosamine) using the same method (*50*−*52*).

Effect of Solution pH. Alkaline conditions have been used for the analysis of carbohydrates not only to achieve the separation by CE (*53*) (on the basis of their degree of dissociation) but also to enhance PAD (*50,51*). Because of its unique properties and common use as a marker for tracking fires, the most important compound to target in the particulate extracts is levoglucosan. The electrophoretic separation of a mixture of 0.6 mM levoglucosan, 2.8 mM glucose, and 0.6 mM galactosan using 5 mM $Na_2B_4O_7$ (pH = 12.30), +1000 V as the separation potential, and +0.9 V as the detection potential is shown in Figure 3. As can be observed, the combination of the formation of negatively charged borate complex and the solution pH allows the separation of levoglucosan from the other carbohydrates (glucose and galactosan). The corresponding migration times were 40.7 ± 0.2 s (n = 3), 48.3 ± 0.2 s (n = 3), and 61 ± 0.2 s (n = 3) for levoglucosan, glucose, and galactosan, respectively. This is a significant point because of the possibility of using alkaline pH values to achieve separation while also favoring the electrochemical detection of carbohydrates. It was also observed that mannosan comigrates with galactosan under these conditions (data not shown) and therefore will not interfere with the measurement of levoglucosan.

Effect of the Separation Potential. Applied potential can be used to control EOF and separation resolution in CE. Applied potential will also control the amount of time that the analytes spend in the detection zone. To evaluate the effect of separation potential, the migration time and peak

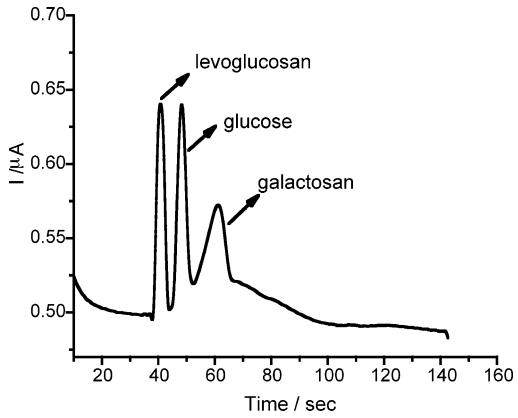

FIGURE 3. Electropherogram showing the separation of levoglucosan, glucose, and galactosan using microchip−CE−PAD. Conditions: pH = 12.30, separation potential = +1000 V (A−D), 10-s injection, detection potential: +0.9 V.

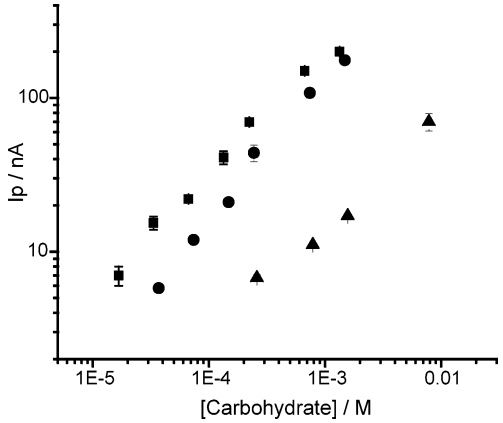

FIGURE 4. Calibration curves for levoglucosan (■), glucose (●), and galactosan (▲). Conditions: 10 mM $Na_2B_4O_7$ buffer pH = 12.30, detection potential: +0.9 V, 10-s injection.

current of glucose were measured as a function of the separation potential between +600 and +1500 V (data not shown). If lower separation potentials are applied, lower baseline noise is achieved but run time increases (110 s at 700 V vs 45 s at 1200 V). In addition, higher peak currents were obtained with lower separation potentials because of the increase in the residence time at the electrode surface. As a compromise between the analysis time and the signal/noise ratio, +1000 V was chosen as the separation potential for the present study.

Calibration Curves and Limits of Detection. Using 5 mM $Na_2B_4O_7$ (pH = 12.30), +1000 V as the separation potential, and +0.9 V as the detection potential, a linear relationship between the concentration and the peak current was obtained for the three analyzed carbohydrates (Figure 4). The corresponding slopes for the calibration curves were 0.238 ± 0.01 nA/μM (R = 0.99) for levoglucosan, 0.126 ± 0.006 nA/μM (R = 0.99) for glucose, and 9.07 ± 0.48 (R = 0.9999) for galactosan. The detection limit (signal/noise ratio = 3) was established for levoglucosan, glucose, and galactosan to be 16.7 μM, 36.9 μM, and 258.7 μM, respectively. As can be observed, levoglucosan is the most electroactive compound of the series (even compared to glucose which is the corresponding hydro carbohydrate), and the least active compound is galactosan. Mannosan showed similar responses to galactosan (data not shown). This is a potential advantage because the response of possible interfering compounds is significantly lower than the response for levoglucosan. The mechanism that gives rise to differences in response is not known at this time.

Analysis of Aerosol Extracts by Microchip−CE−PAD and GC/MS. The aqueous extracts of the collected aerosol particles were analyzed by CE-PAD using the optimum conditions and the results were compared for the same sample analyzed by GC/MS. The analysis time for the microchip is less than a minute, while the GC/MS run takes approximately 1 h. Another advantage of the microchip is the possibility to perform direct analysis of the extracts, avoiding the derivatization procedure (>5 h) and contributing to an even faster and simpler overall methodology. The small amount of material required for the microchip analysis and the possibility of coupling the microchip directly to a particle impaction system also combine to make eventual incorporation of this analytical approach into an inexpensive online aerosol measurement system quite promising.

For the present study, samples generated at the Cloud Simulation Laboratory of the Atmospheric Science Department were selected to obtain a higher concentration of levoglucosan in the proof-of-principle experiments. Commercial fireplace wood (pine, aspen, and spruce) was burned in a flaming fire for 60 min and the corresponding smoke

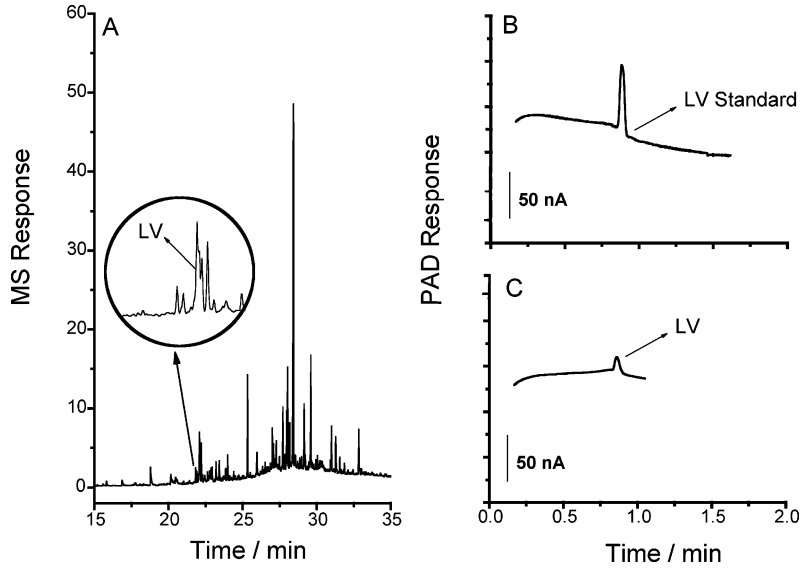

FIGURE 5. Comparison of the analysis of smoke particulate extracts by the two methodologies. (A) GC/MS analysis. Insert showing the peak corresponding to levoglucosan at 22.1 min. (B) Microchip−CE−PAD analysis of a levoglucosan standard. (C) Microchip−CE−PAD analysis of the smoke extract.

particles were collected according to the previously described procedure. Successful application of the microchip−CE−PAD method to analysis of this type of source sample would provide a significant advance over current methods since many more samples could be analyzed with much less effort and expense than by GC/MS. In addition, if the method were employed in an online sampling and analysis scheme, it could provide highly time-resolved information about changes in the composition of wood smoke as burn conditions evolve.

In Figure 5, the analysis of the extract by microchip−CE−PAD and a comparison with analysis by GC/MS is shown. It can be observed that only one peak is obtained by microchip−CE−PAD, matching with the migration time for the levoglucosan standard. The calculated concentration by microchip−CE−PAD was 126 ± 5 μg/mL while by GC/MS it was 129 ± 9 μg/mL, demonstrating the performance similarities of the two methods. Samples collected at the Turtleback Dome Site in Yosemite National Park (August 2002) were also analyzed. However, because of the presence of a much lower concentration of carbohydrates and a higher content of ions such as NH_4^+, SO_4^{2-}, NO_3^-, Cl^-, and Na^+, the peak corresponding to levoglucosan is hidden inside a negative peak (data not shown). These results were attributed to a conductivity difference between the running buffer and the sample plug. At the dilution necessary to avoid the dip, the concentration of levoglucosan falls below the detection limit. Work is currently under way to both improve the extraction protocol and develop a strategy for equalizing the conductivity of the samples with the running electrolyte to avoid this problem and extend the methodology to ambient aerosol sample analysis.

Conclusions. The possibility of performing fast and inexpensive analysis of anhydrous carbohydrates using a PDMS microchip and pulsed amperometric detection was demonstrated for the first time. The concentration of levoglucosan in smoke particles was analyzed, and separation from other carbohydrates (glucose and galactosan) was achieved. Optimal conditions were obtained using +1000 V as the separation potential, 5 mM $Na_2B_4O_7$ as the buffer (pH = 12.30), and +0.9 V for the detection potential. In comparison with GC/MS, the microdevice can perform faster measurements (1 min vs 54 min for GC/MS) with a similar analytical performance without the need of tedious extraction and derivatization procedures. This capability makes the system a promising new alternative for offline analysis of levoglucosan concentrations in wood-smoke source samples. In addition, the presented system uses a much simpler and less expensive instrument and has the potential to eventually perform onsite automated determinations of smoke particle extracts. Differences in conductivity between ambient aerosol samples and the CE buffer solution presently pose a limitation for analysis of levoglucosan at low concentrations in ambient samples. Changes in separation conditions (to move the levoglucosan peak) and conductivity matching between buffer and sample (e.g., by extracting with buffer solution rather than deionized water) may permit analysis of ambient aerosol extracts as well.

Acknowledgments

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