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Amperometric Detection of Carbohydrates with a Portable Silicone/Quartz Capillary Microchip by Designed Fracture Sampling

Chun Zhai, Chen Li, Wei Qiang, Jianping Lei, Xiaodong Yu, and Huangxian Ju*

Key Laboratory of Analytical Chemistry for Life Science, Ministry of Education of China, Department of Chemistry, Nanjing University, Nanjing 210093, China

A silicone/quartz capillary microchip (SQCM) coupled with an ultranarrow sampling fracture was for the first time constructed without any micromachining. The SQCM could be used for direct determination of carbohydrates at a detection potential of +0.8 V (vs Ag/AgCl) with a copper microdisk electrode. The ultranarrow sampling fracture could be conveniently formed on a quartz capillary, which was fixed by a frame of poly(dimethylsiloxane) (PDMS). The designed fracture sampling suppressed the leakage of sample, thus simplifying the power supply. Furthermore, it thinned the sample plug for enhancing the resolution. The quartz capillary reduced the adsorption of analytes on the separation channel wall compared with a general PDMS microchip, thus enhanced the separation efficiency up to 239 000 plates/m for carbohydrates. This proposed system could satisfactorily separate eight carbohydrates within 180 s with good reproducibility and sensitively detect them in the linear ranges from 1 μM to 0.5 mM for trehalose and sucrose, 2.5 μM to 0.5 mM for lactose, galactose, glucose, and mannose, and 2.5 μM to 1.5 mM for fructose and xylose with the detection limit down to 90 amol. The designed microchip was successfully applied to detect carbohydrates in a practical acacia honey sample.

Carbohydrates are extensively distributed in nature and related to many physiological disorders such as diabetes and galactosemia.[1,2] Thus, their detection has attracted considerable attention in clinical, food, industrial, and environmental fields and biological studies. However, the detection of carbohydrates is extremely difficult due to lack of a sensitively detectable signal by the conventional methods based on photometry and fluorimetry and the structural diversity in unit monosaccharide species.[1] Although enzyme-modified electrodes have been used to detect specific carbohydrates, it is impossible to use them for analysis of a mixture of carbohydrates.[3] Fortunately, the electrocatalytic mechanisms toward oxidation of carbohydrates under alkaline conditions have been observed at the surface of noble[4] and transition metals,[5−7] which provides considerably promising methods for their amperometric detection coupled with electrophoresis separation. The direct amperometric detection with transition metals such as copper electrodes[7] shows remarkable sensitivity, portability, independence of optical path length or sample turbidity, and low cost and power requirements.[8] Thus these electrodes have been used as detectors of capillary zone electrophoresis (CZE)[7] and microchip capillary electrophoresis (MCE) for carbohydrates analysis.[9]

In comparison with CZE, a capillary microchip allows high separation speed, low analysis cost, and good portability and is thus a rapidly growing analytical technology in carbohydrates analysis.[9−11] It was initially constructed using glass substrates,[12,13] which provided excellent optical properties, high chemical and mechanical stability, and abundant groups for surface modification. But the fabrication of a glass-based capillary microchip is relatively difficult and expensive due to the need of expensive clean-room facilities, corrosive etchants (HF), and time-consuming bonding.[10] Moreover, the devices are prone to clogging, difficult to clean, and very often have to be discarded after failure.[14] Thus, much effort has been devoted to development of polymer MCE devices such as poly(dimethylsiloxane) (PDMS),[15] poly(methylmethacrylate),[10] and polycarbonate.[16] Especially, PDMS is chemically inert, nontoxic, easily handled, and commercially available.[15] However, the shortcomings of plastic microchips are also obvious, i.e.,

* Corresponding author. Phone and Fax: +86-25-83593593. E-mail: hxju@nju.edu.cn.

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relatively unstable microscopic channel, low electroosmotic flow (EOF), and a highly hydrophobic surface.[17] The latter is not compatible with aqueous separation media, thereby causing poor separation performance due to serious analyte adsorption on the channel surface.[10] This work combined the advantages of both a glass (quartz) capillary and PDMS for the first time to design a novel capillary microchip. This device could be conveniently fabricated and integrated with a copper microdisk electrode for separation and detection of a carbohydrate mixture.

Quartz or fused-silica capillary is of ideal optical characteristics, highly uniform inner surface, and good stability and has been universally employed for conventional CZE.[18] Its use in the proposed capillary microchip can greatly simplify its fabrication, reduce the adsorption of analytes on the wall of the microchannel, improve the separation efficiency of the microchip by coupling with the good hydrophilicity, and particularly, produce a novel sampling strategy, a fracture sampling method, by easily forming an ultranarrow fracture at one end of the capillary.

The on-column fracture of the fused-silica capillary has been used in CZE for isolation of separation voltage from the voltage applied in electrochemical detection[19,20] or forming an electroosmotic syringe for sample introduction from the end of the column.[21,22] Different from these methods, the designed sampling method introduced sample from the ultranarrow fracture directly and thus allowed very narrow sample plugs to be injected into the separation channel, leading to high separation efficiency. Moreover, the ultranarrow sampling channel could suppress sample leakage in MCE,[13,23,24] therefore avoiding additional equipment and programs usually applied in MCE for leakage control. The proposed MCE showed excellent performance such as fast separation speed for high-throughput detection, high separation efficiency or resolution, low cost, good reproducibility, high sensitivity, and extremely low detection limit for the determination of carbohydrates and could be successfully applied to detect carbohydrates in practical samples without special pretreatment.

EXPERIMENTAL SECTION

Reagents. The 95% trehalose (Tre), sucrose (Suc), and lactose (Lac), and 98% galactose (Gal), glucose (Glu), mannose (Man), fructose (Fru), and xylose (Xyl) were obtained from Shanghai Chemical Reagent Co. (Shanghai, China). Acacia honey (Jiufengtang, Nanjing, China) was commercially available. Sylgard 184 silicone elastomer and curing agent were purchased from Dow Corning (Midland, MI). All aqueous solutions were prepared using ≥18 MΩ ultrapure water (Milli-Q, Millipore). The carrier and supporting electrolyte for MCE separation and amperometric detection were 100 mM NaOH (Nanjing Chemicals, Nanjing, China), which was passed through a membrane filter (0.2 μm pore size) prior to use. Stock solutions of carbohydrates were prepared daily. Fused-silica capillaries (360 μm o.d., 25 μm i.d.) were obtained from Yongnian Optical Fiber Factory (Hebei, China).

Equipment. Two positive laboratory-built high-voltage power supplies controlled automatically by computer during experiments had a voltage range between 0 and +3000 V. Electrochemical measurements were performed on a CHI 812 electrochemical station (CH Instruments Co., U.S.A.). An inverted fluorescence microscope (Nikon Eclipse TE2000-U) was used to observe the fracture on the silicone/quartz capillary microchip (SQCM). A 40 multiple light microscope (Nanjing Optics Instruments Factory, Nanjing, China) was employed to monitor the position of the copper microdisk working electrode (WE) and detect the distance between the WE and the end of the separation channel.

Fabrication of SQCM. The fused-silica capillary (360 μm o.d., 25 μm i.d., 110 mm length) was successively flushed with 0.2 M NaOH, water, absolute alcohol, and water. After being dried by air, one small scratch and two deep scratches were made at positions 20, 12, and 92 mm from one end of the capillary. Then the capillary was horizontally placed in the center of a mold (30 mm width, 98 mm length, 6 mm depth), which had two holes at the centers of two ends of the mold with 2 mm distance from the bottom for holding the capillary. The mixture of silicone and curing agent at the quality ratio of 10:1 was prepared, degassed, and poured into the mold until the capillary was completely covered in the mold. After being cured at 70 °C for 2 h and cooled at room temperature, the capillary was broken in three segments at the positions of 12 and 92 mm by pushing up the PDMS matrix above the deep scratches. The two outer segments of the capillary were pulled out from the PDMS matrix to leave an 80 mm capillary and one straight PDMS guide channel for fixing the WE in the matrix, which was then carefully peeled off from the mold. One cooling cavity and three reservoirs of 2−4 mm in diameter for buffer (BR), sample (SR), and detection (DR) were prepared on the cured polymer as shown in Figure 1A. The sampling fracture was finally formed by pushing up gently the PDMS matrix above the small scratch. Its fluorescence photograph obtained by filling 0.4 mM fluorescein in 1 mM NaOH solution in the capillary is shown in Figure 1B.

The WE was prepared by inserting a 250 μm diameter Cu wire (Wuxi Cable Factory, Wuxi, China) into a glass capillary (400 μm o.d., 300 μm i.d.), which was then sealed with 705 glue (Nanjing Chemicals, Nanjing, China). The electrode was successively polished with 2000-mesh and 4000-mesh sandpapers (Wuxi, China) until a mirror-like appearance was observed, followed with washing and sonication in a water bath. The prepared WE was oxidized in an air oven at 100 °C for more than 4 h and then stored in a desiccator before use. It could be easily mounted in the PDMS guide channel, exactly opposite to the end of the separation channel due to the flexibility of PDMS matrix (Figure 1C).

Electrophoretic Procedure. The capillary in the SQCM was first washed with 100 mM NaOH and ultrapure water followed with electrophoresis medium, which was filled in the BR and DR. The SQCM with sample solution in the SR was then placed in a Faraday cage. The fracture sampling was performed by applying an optimum injection voltage of 100 V between the SR and BR. The corresponding separation voltage was applied to the BR with the DR grounded and the SR floating by automatically switching the high-voltage contacts, and the electropherogram was recorded

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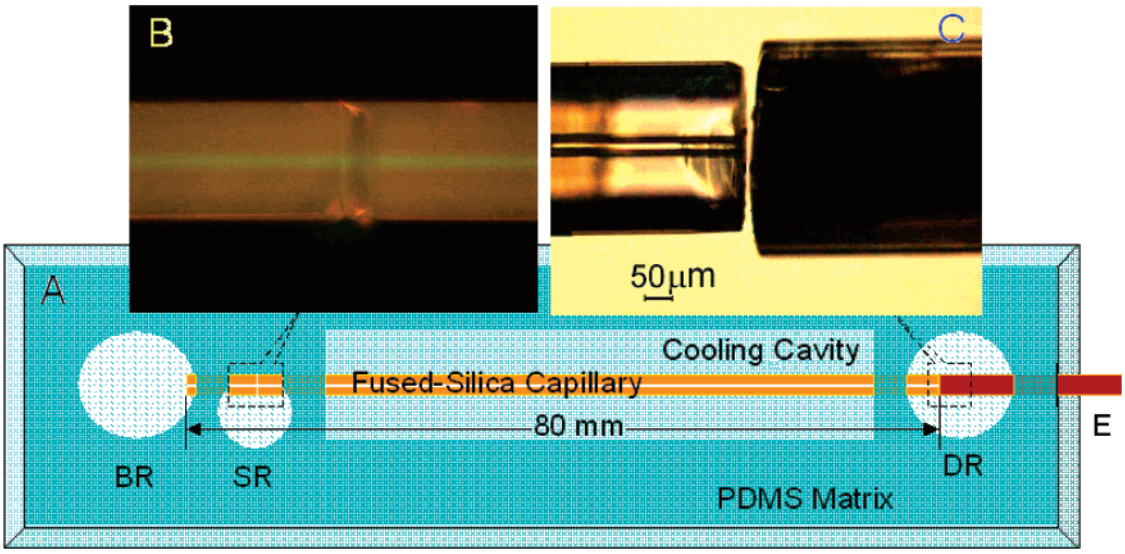

Figure 1. Scheme of SQCM with integrated end column amperometric detection (A), fluorescence photograph of the fractured capillary (B), and working electrode opposite to the exit of the separation channel (C). BR, buffer reservoir; SR, sample reservoir; DR, detection reservoir; E, working electrode.

on a CHI 812 using the "amperometric $i-t$ curve" mode at +0.8 V with a three-electrode system composed of the copper microdisk electrode as WE, Ag/AgCl wire as reference electrode, and a Pt wire as counter electrode. All experiments were performed at room temperature.

RESULTS AND DISCUSSION

Properties of SQCM and Fracture Sampling. The separation channel was prepared with a short commercial fused-silica (quartz) capillary, which produced a strong capillary force due to its good hydrophilicity. Thus the buffer and sampling solution as aqueous separation media could permeate into the channel in several seconds. Furthermore, the bubbles in the channel could be easily removed along the very slippery wall of the capillary by driving up one end of the SQCM to more than 30° against horizon for a few minutes. These advantages greatly simplified the operation for buffer perfusion and bubble removal, which often needs pressure or vacuum equipment.[12,14] In addition, the quartz capillary reduced the adsorption of analytes on the separation channel wall compared with a general PDMS microchip,[10] thus enhanced the separation efficiency.

The width of the fracture as a sampling channel was about 1 μm, close to $^1/_{20}$ of the width of the separation channel. The ultranarrow sampling width produced a very narrow sample plug, thus improved greatly the separation efficiency[23] and reduced the sample consumption. Furthermore, such a sampling fracture could effectively suppress sample leakage in the MCE;[23] thus, the power supply was unnecessary for controlling the leakage, which was usually used in MCE operation.[13,23,24]

On the other hand, the procedure to fabricate the SQCM formed one straight PDMS guide channel opposite to the end of the separation channel for fixing the WE in the matrix. Thus, the WE could be conveniently equipped in the channel without need of an *XYZ* positioner.[25] Moreover, after being used the copper microdisk WE could be conveniently substituted or taken out for improving its response to analytes by a polishing step followed with an oxidation treatment at 100 °C. The oxidation treatment in air formed an even layer of copper oxide to produce an electrocatalytic action toward oxidation of carbohydrates in alkaline medium[7] and suppress the background for obtaining a good baseline,[26] which would be responsible for the high sensitivity. Contrarily, if the WE was not treated by oxidation, the baseline was unstable and the response to carbohydrates was also very weak.

Detection Potential. The electrochemical oxidation of carbohydrates at the copper microdisk electrode produced detectable amperometric signals. When the applied potential was less than +0.2 V, no signal could be observed for all of these eight carbohydrates. With the increasing applied potential from +0.2 to +0.8 V the amperometric responses increased, and the sharp increases occurred at the potentials more positive than +0.6 V (Figure 2). When the applied potential was higher than +0.8 V the oxidation current decreased quickly, which was due to the excessively superficial oxidation to form a thick layer of copper oxide and, thus, prevent the electron transfer between the electrode and the carbohydrates. The similar phenomena were also observed in pulsed amperometric detection of carbohydrates.[9] At the same time high oxidation also resulted in high noisy and unstable baseline. Since the similar profiles were observed for all carbohydrates, +0.8 V was used as the optimum detection potential.

At the optimum detection potential the distance between the WE and the outlet of the separation channel showed an effect on the electropherogram. Too small a distance would cause a large noise, whereas a far distance would cause band broadening.[27] In this work the distance of 15 ± 5 μm could give excellent performance, which was used for all measurements.

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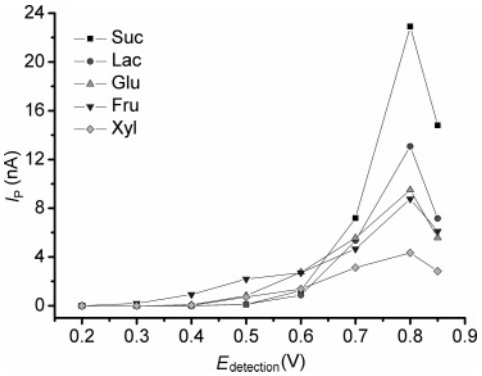

Figure 2. Effects of detection potential on signals for 100 μM Suc, Lac, Glu, Fru, and Xyl in 100 mM NaOH electrophoresis medium at 1500 V separation voltage.

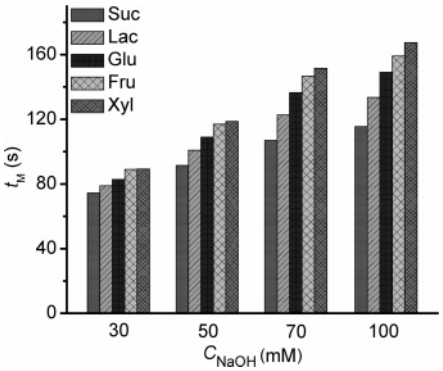

Figure 3. Effects of NaOH concentration on migration times for Suc, Lac, Glu, Fru, and Xyl at +0.8 V detection potential and 1500 V separation voltage.

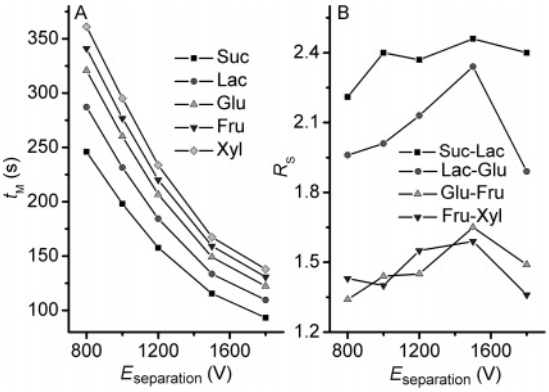

Figure 4. Effects of separation voltage on (A) migration times and (B) resolution for 200 μM Suc, Lac, Glu, Fru, and Xyl in 100 mM NaOH electrophoresis medium at +0.8 V detection potential.

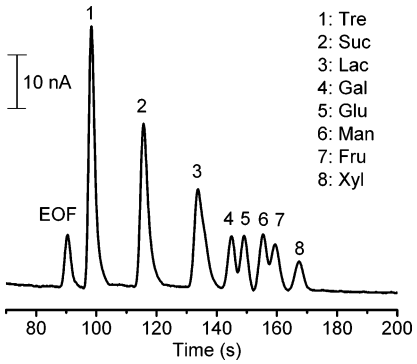

Figure 5. Electropherogram for 125 μM carbohydrates under optimal conditions.

Effects of NaOH Concentration and Separation Voltage. The majority of the works to separate carbohydrates by CZE are based on their different degrees of hydroxyl dissociation in alkaline medium (pH > 12).[9] Thus, the NaOH concentration would greatly affect their resolution. The dependence of migration times (t_M) for Suc, Lac, Glu, Fru, and Xyl on NaOH concentration is shown in Figure 3. When the NaOH concentration was less than 70 mM, it was difficult to distinguish these carbohydrates, especially between Fru and Xyl. Good resolution could be achieved at the NaOH concentration of 100 mM. On the other hand, with the increasing concentration of NaOH, the t_M increased, which would result in a longer analysis time. When the NaOH concentration was larger than 100 mM, the remarkably rising current passing the separation channel, produced from the high electric field needed for electrophoresis, led to a high noisy and unstable baseline (not shown). Thus, 100 mM NaOH was selected as the optimum concentration for carbohydrates separation, which was similar to the condition applied in conventional CZE for carbohydrates separation.[7]

The separation voltage decides the EOF, which is directly related to the t_M and separation efficiency. To evaluate the effect of separation voltage, the t_M of Suc, Lac, Glu, Fru, and Xyl and the resolution (R_s) between two adjacent carbohydrates were measured as a function of the separation voltage between 800 and 1800 V (Figure 4). Here R_s is defined as $2((t_M)_B - (t_M)_A)/(W_B + W_A)$, W is the full peak width.[28] With the increasing separation voltage, the t_M decreased due to the increasing EOF. At the separation voltage of 1500 V, five analytes could be completely separated with the R_s values more than 1.5, at which the separation time was less than 180 s and the maximum R_s value of 2.45 occurred between Suc and Lac.

Sampling Conditions. The fracture sampling was driven by an injection voltage. The design of the SQCM could inject various sample plug lengths by changing the sampling time or injection voltage. Although high injection voltage can produce large EOF and increase the sampling speed, which have been used for MCE analysis, e.g., 420,[1] 1000,[3] 1080,[23] and 1500 V,[29] it may usually result in high injection current to induce an unstable baseline and low separation efficiency. The ultranarrow sampling fracture could produce a fast sampling speed at relatively low injection voltage, which allowed a short sampling time. The short time favored depressing the diffusion of the analytes during the sampling process for avoiding the broadening of the sample zone. Furthermore, the ultranarrow sampling fracture could suppress sample leakage[13,23,24] to further reduce the sample zone broadening. Thus, this technique could form an ultranarrow sample plug to be introduced into the separation channel, which led to the high separation efficiency.[23] However, when the injection voltage was lower than 50 V, one long sampling time was needed to obtain a detectable sample plug, resulting in serious sample broadening and low separation efficiency. This work examined the effect of injection voltage on R_s; an optimum injection voltage of 100 V at

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Table 1. Separation Efficiency and Reproducibility of MCE-EC for Carbohydrates at 125 μM (n = 3)

		RSD (%) of t_M			RSD (%) of I_P		
analytes	N (plates/m)	run-to-run	day-to-day	chip-to-chip	run-to-run	day-to-day	chip-to-chip
Tre	185500	0.3	0.4	1.6	0.9	0.6	1.4
Suc	178200	0.2	0.9	1.0	2.8	2.4	3.3
Lac	134300	0.2	1.3	1.2	1.2	1.8	3.9
Gal	221300	0.2	1.5	0.9	3.7	5.1	7.1
Glu	217900	0.2	1.7	1.1	2.5	3.2	7.2
Man	193900	0.3	1.7	1.6	2.2	2.2	5.7
Fru	190200	0.3	2.0	1.2	3.3	7.6	6.7
Xyl	239600	0.3	2.0	1.0	1.3	3.4	5.1

the injection time of 1 s was selected for obtaining good separation efficiency and appropriate detection sensitivity, at which the sampling volume was approximately calculated to be less than 0.39 nL.

Separation of Carbohydrates. A series of carbohydrates solutions with different concentrations were prepared by gradual dilution with 100 mM NaOH. Figure 5 shows the electropherogram for 125 μM Tre, Suc, Lac, Gal, Glu, Man, Fru, and Xyl. The R_s values for Tre−Suc, Suc−Lac, Lac−Gal, Glu−Man, and Fru−Xyl were larger than 1.5, showing baseline separation within 180 s. The R_s values for Gal−Glu and Man−Fru were 0.89 and 0.70, respectively, indicating their part separation. The similar results were also observed in CZE of carbohydrates in 100 mM NaOH with a running time of about 40 min.[7]

The theoretical plate numbers (N) and separation reproducibility of these carbohydrates are illustrated in Table 1. Here, N is defined as $5.54(t_M/W_{1/2})^2$, $W_{1/2}$ is the full peak width at the half-maximum points.[30] The N values ranging from 134 300 to 239 600 plates/m were much greater than those of 100 000 plates/m for Suc and Glu,[3] 8600 plates/m for Glu,[31] and 43 500 and 51 300 plates/m for Glu and Suc[32] with MCE, showing much better separation efficiency of the proposed method. The relative standard deviation (RDS) (n = 3) of t_M was less than 0.3% for run-to-run, 2.0% for day-to-day, and 1.6% for chip-to-chip; thus, the proposed method and separation channel had good stability and preparation reproducibility. The RSD (n = 3) of peak currents (I_p) measured at the concentration of 125 μM was from 0.9% to 3.7% for run-to-run, from 0.6% to 7.6% for day-to-day, and from 1.4% to 7.2% for chip-to-chip; thus, the SQCM including the used copper microdisk electrode and the fracture sampling had good fabrication reproducibility.

The amperometric detection of these carbohydrates showed the linear ranges from 1 μM to 0.5 mM for Tre and Suc, 2.5 μM to 0.5 mM for Lac, Gal, Glu, and Man, and 2.5 μM to 1.5 mM for Fru and Xyl with the relative coefficients ranging from 0.9988 to 0.9998 and the slopes ranging from 0.041 to 0.344 nA/μM. The detection limits at the ratio of S/N of 3 were 0.23, 0.38, 0.67, 0.86, 0.75, 0.85, 0.67, and 1.45 μM, respectively. Considering the sampling volume of 0.39 nL, the mass detection limits were between 90 and 567 amol. To our best knowledge, these results were the lowest detection limits obtained by electrochemical detection of corresponding carbohydrates on the MCE[3,29,31,33,34] and CZE.[7,35] Moreover, it was also the first work to simultaneously separate and detect directly eight carbohydrates on the MCE without derivation.

The high sensitivity of the end column amperometric detection of carbohydrates described above was attributed to the stable baseline obtained and high separation efficiency resulting from the fracture sampling, as well as the short injection time and quartz separation channel to provide a highly hydrophilic environment, the large surface area of the WE relative to the channel diameter,[36] and the electrocatalysis of the copper oxide toward oxidation of carbohydrates.[7,26,33] In addition, no obvious assurgency of baseline could be observed in the electropherograms for carbohydrates, which clearly resulted from the ultranarrow sample injection channel to effectively suppress the leakage. The sensitivity of the pretreated electrode to carbohydrates detection was also stable, and no obvious decrease of current was observed after tens of analysis at +0.8 V.

This portable SQCM can be used in some specific fields such as medical, agricultural, or environmental analysis. These samples generally contain uric acid, phenols, amino acids, or alcohols, which can be oxidized on copper electrodes at +0.8 V. However, these interfering species showed much different migration times from the eight analyzed carbohydrates (data not shown); thus, they do not interfere with the separation and detection of these carbohydrates.

Analysis of Carbohydrates in Acacia Honey. The portable SQCM and the fracture sampling method were used for analysis of carbohydrates in honey. The extremely viscous honey sample was first diluted at 1:6000 (w/v) with 100 mM NaOH solution. The electropherograms for reduplicate analyses of the sample are illustrated in Figure 6. The two components observed in the electropherograms corresponded to Glu and Fru, which were confirmed by adding standard Glu and Fru solutions to the sample. The other six carbohydrates including Suc were not seen in the sample. The electropherograms showed good reproducibility, and the concentrations of Glu and Fru in the sample were measured to be 26.30 wt % and 32.15 wt % with the RSD (n = 3) of 6.2% and 3.5%, respectively. The recoveries of these carbohydrates added

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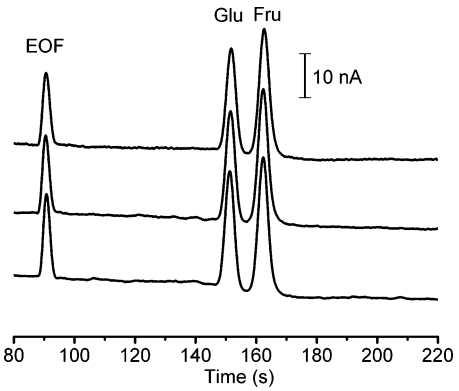

Figure 6. Electropherograms for three continuous samplings of a diluted acacia honey.

to the honey sample were from 95.4% to 109.1%, indicating acceptable accuracy.

CONCLUSIONS

A portable SQCM and a fracture sampling method are developed. They have been used for highly efficient separation and highly sensitive detection of carbohydrates by integrating a pretreated copper microdisk electrode opposite to the end of the separation channel. This microchip combines the advantages of quartz and PDMS and shows low cost, convenient operation, high separation efficiency, and good fabrication and separation reproducibility. The fracture sampling technique further increases the resolution, reduces the injection voltage and sampling time, and suppressed sample leakage, with which additional equipment and programs usually applied in MCE for leakage control are unnecessary. The proposed method for analysis of carbohydrates shows wide linear range, extremely low detection limit, and acceptable accuracy and could be successfully applied to quickly detect carbohydrates in practical samples without special pretreatment. The developed techniques of SQCM preparation and sampling have great potential for fabrication of portable and user-friendly microchips in different analytical applications.

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