Available online at www.sciencedirect.com
SCIENCE DIRECT®
ELSEVIER
Journal of Chromatography A, 992 (2003) 181–191
JOURNAL OF CHROMATOGRAPHY A
www.elsevier.com/locate/chroma

Determination of carbohydrates as their 3-aminophthalhydrazide derivatives by capillary zone electrophoresis with on-line chemiluminescence detection

Xiaoyan Wang*, Qiang Wang, Yi Chen*, Huiwan Han
Center for Molecular Science, Institute of Chemistry, Chinese Academy of Sciences, P.O. Box 2709, Beijing 100080, China

Received 2 October 2002; received in revised form 19 December 2002; accepted 29 January 2003

Abstract

A method based on pre-capillary derivatization with luminol (3-aminophthalhydrazide) for carbohydrate analysis using capillary electrophoresis with on-line chemiluminescence (CL) detection was developed. The derivatives of seven monosaccharides were separated and detected by using 200 m*M* borate buffer containing 100 m*M* hydrogen peroxide at pH 10.0 as separation electrolyte and 25 m*M* hexacyanoferrate in 3 *M* sodium hydroxide solution as post-capillary chemiluminescence reagent with separation efficiencies ranging from 160,000 to 231,000 plates per metre. The minimum amount of carbohydrate derivatized was 2 pmol (corresponding to the concentration of 2 μ*M*). The method also provided a linear response for glucose in the concentration range of 0.1–250 μ*M* with a mass detection limit of 420 amol or a concentration detection limit of 0.1 μ*M*. Preliminary work using the CE–CL format to determine glucose in a rat brain microdialysis sample is presented as a typical case.
© 2003 Elsevier Science B.V. All rights reserved.

Keywords: Derivatization, electrophoresis; Detection, electrophoresis; Chemiluminescence detection; Carbohydrates; Aminophthalhydrazide; Luminol

1. Introduction

Capillary electrophoresis (CE) has become one of the most powerful techniques for carbohydrate analysis because of its high efficiency, high speed and low sample consumption. However, the poor chromophore properties of carbohydrate and the low detection length of the capillary present significant challenges to carbohydrate detection. Incorporation of a UV-absorbing or fluorescing group into carbohydrate molecules by a labeling process is the most commonly used means to improve sensitivity for CE detection. However, conventional UV absorption detection produces poor concentration detection limits, which hindered the use of CE in the analysis of trace carbohydrates. Laser-induced fluorescence (LIF) detection has thus been developed and shown to be a powerful detection approach for trace components, but costly apparatus limited its wide utilization.

Chemiluminescence (CL) detection has been

*Corresponding author. Present address: Department of Chemistry, Fudan University, Shanghai 200433, China. Tel.: +86-21-6564-2009; fax: +86-21-6564-1740.
*Corresponding author. Tel.: +86-10-6261-8240; fax: +86-10-6255-9373.
E-mail addresses: xiaoyanwang@fudan.edu.cn (X. Wang), chenyi@infoc3.icas.ac.cn (Y. Chen).

0021-9673/03/$ – see front matter © 2003 Elsevier Science B.V. All rights reserved.
doi:10.1016/S0021-9673(03)00273-5

proven to be another sensitive detection method for CE. It features a simple, cheap optical system and an excellent sensitivity comparable to LIF [1]. Glucose could be determined by CL detection as glucose oxidase. The mechanism is based on utilizing existing oxidase enzymes to react with glucose to produce hydrogen peroxide then measure the radiation via a reaction of hydrogen peroxide with luminol in the presence of a metal ion as catalyst. Although the method can provide perfect detection sensitivity, its utilization is limited only to glucose [2–4] or those sugars which can be converted into glucose, e.g. sucrose [5]. Besides, carbohydrates also take part in other CL reactions. Agater et al. [6] determined carbohydrates by CL with permanganate oxidation in acidic solution. The applications of this system are much wider than the glucose oxidase system. However, the system suffers from poor sensitivity (detection limit at 10^{-4} *M*) and relatively slow reaction rate (taking ~2.5 min to reach the maximum signal) which would cause extra zone broadening of the post-capillary reaction system [7]. Another post-column CL system developed for determining carbohydrates is based on the reaction of ruthenium complex with diketone, which is produced by the oxidizing reaction of sugar with vanadate [8]. This system is quite sensitive with a detection limit of 5 pmol (corresponding to 2.5×10^{-7} *M*) for sorbitol in FIA. However, the reaction takes place only in the case of UV irradiation. To eliminate the interruption of the irradiated light, some accessories such as filters were needed which would greatly complicate the optical design and result in a decrease of detection sensitivity. Moreover, carbohydrates can directly react with luminol to emit radiation in alkaline solution in the presence of hexacyanoferrate(III) as catalyst in terms of the detection limit for glucose and fructose, 6×10^{-5} *M* and 75×10^{-5} *M*, respectively [9]. Lin et al. [10] discovered that polyhydroxyl compounds containing *cis*-hydroxyl groups including carbohydrates could evidently enhance the CL signal in the KIO_4–K_2CO_3–KOH system. The *S*/*N* ratio of glucose at 10^{-5} *M* by flow injection analysis is 12. We tried to explore those systems for CE detection, but their detection sensitivity proved even poorer than UV detection in the case of the capillary employed.

To conduct CL detection of carbohydrates in CE, more sensitive CL approaches have to be explored. A simple way seems to be tagging the carbohydrates with a reagent bearing strong CL ability. CL reagents such as luminol containing an amino-group can react with an aldehyde group of carbohydrate through reductive amination. In this paper, we have firstly explored the reactability of luminol with carbohydrate. As expected, our experiment showed that this was quite effective; sugars even as low as 2 pmol can be labeled, which is a significant advancement for biological samples with only small quantities available. In this paper, the reaction of luminol with carbohydrates, CL and separation characteristics of luminol–sugar derivatives will be reported in detail.

2. Experimental

2.1. Reagents

3-Aminophthalhydrazide was purchased from Fluka (Buchs, Switzerland). Sodium cyanoborohydride was obtained from Acros Organics (NJ, USA). Potassium hexacyanoferrate(III) and dimethyl sulfoxide (DMSO) were the products of Beijing Chemical Factory (Beijing, China). All monosaccharides were purchased from Sigma (St. Louis, MO, USA). The chemicals were used directly without further purification.

2.2. CE–CL

2.2.1. CE–CL apparatus

Separation was performed on a laboratory-built CE–CL system (as shown in Fig. 1). The CE was driven by a high voltage power supply (Beijing New Technology Institute, Beijing, China) which provided a separation voltage up to +30 kV. The sizes of the capillaries (Yongnian Optical Fiber Factory, Hebei, China) for separation, post-column reaction and CL reagent delivering were 50 cm×50 μm I.D.×365 μm O.D., 20 cm×520 μm I.D. and 40 cm×320 μm I.D., respectively. After scraping a 4 mm section of polyimide over-coating on the reaction capillary to make a detection window, the separation capillary was inserted up to the front of the detection window. The CL reagent was driven into the reaction capillary through the reagent capillary by air pressure, which was provided by a low-pressure air pump. The flow-

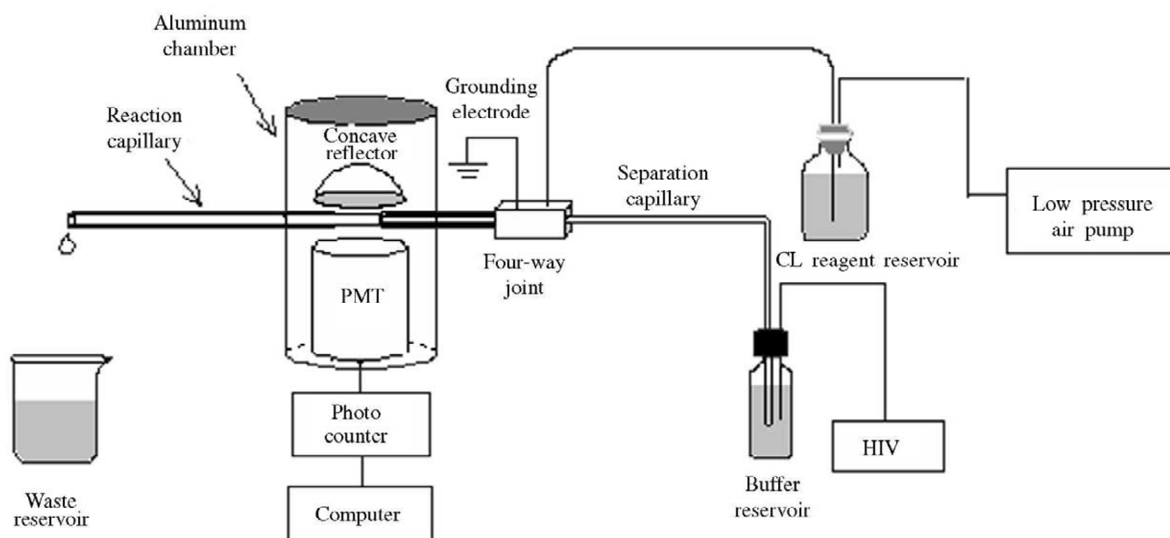

Fig. 1. Schematic diagram of CE–CL.

rate was kept at ~1 ml per 30 min. All the capillaries and the grounding electrode (cathode electrode) were fixed in a four-way PTFE connector and sealed by silicon rubber. To prevent the CL reagent from flowing back into the separation capillary due to siphonage, the injection end of the separation capillary was kept at a position 5 cm higher than the PTFE connector. The photomultiplier tube (PMT) (MPT; GDB44DK, Beijing Nuclear Instrument Factory, Beijing, China) was directly situated in front of the detection window; an additional concave reflector was put in the back of the detection window to collect the light more effectively. The reflector and PMT were enclosed in a light-tight aluminum chamber. The output current of the PMT was further amplified by a γ-radiation analyzer (FH 1936, Beijing Nuclear Instrument Factory) and the final voltage signal was displayed and stored in an IBM compatible computer via an A/D interfacing board (Beijing Kangzi Electron Technology Institute, Beijing, China). All the equipment was installed in a dark room.

2.2.2. CE–CL procedures

Electrophoresis buffer was composed of boric acid and hydrogen peroxide solution, adjusted to the desired pH value with KOH powder. The post-column CL reagent was hexacyanoferrate(III) in sodium hydroxide solution. All the electrophoresis buffer and CL reaction solutions were prepared with double distilled water and filtered through a 0.22 μm pore-size membrane just before use. New electrophoresis capillaries were sequentially treated by flushing with 0.1 *M* NaOH solution, 0.1 *M* HCl solution, water and the separation buffer for 15 min, respectively. Before each injection, the electrophoresis capillary was flushed by separation buffer for 1 min. After four runs, the separation buffer was renewed and the separation capillary was treated with 0.1 *M* NaOH solution, water and electrophoresis buffer for 2 min each. After each working day, the separation capillary was rinsed by 0.1 *M* NaOH solution and water; while the reaction and reagent capillaries were washed thoroughly with water. All the capillaries were filled with water for overnight storage. Sample was introduced to the anode end of the separation capillary by gravity injection for 20 s at 10 cm height (~4.2 nl). Before insertion into the buffer reservoir, the injection end was quickly washed with water to avoid contamination of the separation buffer by sample. A 15 kV voltage was

applied for CE separation and the working voltage of the PMT was set at −900 V.

2.3. Derivatization of sugar with luminol

Carbohydrate derivatization was performed according to the following procedures: the labeling reagent was freshly prepared just before derivatization by dissolving 0.08 mmol of luminol and 0.5 mmol of sodium cyanoborohydride in 0.8 ml of DMSO and then carefully adding 0.2 ml of acetic acid. An aliquot of 5 μl of 2×10^{-6}–5×10^{-3} *M* monosaccharide solution was placed in an Eppendorf tube and mixed with 20 μl of reagent solution. The sample volume can be reduced to 1 μl but the label reagent should be reduced proportionally. The reaction mixture was allowed to react at 30 °C for 3 h, then diluted by water at 2–100-fold to discontinue the reaction and precipitate most of the excess luminol. The suspension was centrifuged at 1000 rev./min for 2 min. The supernatant was used for separation directly or stored at −20 °C for later use.

2.4. Microdialysis sample

The microdialysis procedure was adopted from the literature [11]. Briefly, adult male Sprague–Dawley rats weighing 250–300 g were anesthetized with ketamine hydrochloride (100 mg/kg, i.p) throughout the surgery to maintain stable anesthesia. The microdialysis probe was stereotactically implanted into the striatum. The probe was perfused with an artificial cerebrospinal fluid (120 m*M* NaCl, 3.0 m*M* KCl, 20 m*M* $NaHCO_3$, 1.2 m*M* $CaCl_2$, 1.0 m*M* $MgCl_2$, 0.25 m*M* Na_2HPO_4, pH 7.4) via polyethylene tubing connected to a microinjection pump, at a flow-rate of 1.5 μl/min. After a 48-h stabilization period, the perfused fraction was collected every 15 min for 60 min. An aliquot of 2 μl microdialysis sample was mixed with 8 μl of label reagent and derivatized as described above; the labeling solution was diluted to 20-fold for CE–CL analysis.

3. Results and discussion

3.1. Derivatization

In all labeling experiments, the concentration of sodium cyanoborohydride was kept constant at 0.5 *M* because it was sufficiently high for reduction. Since the reductive amination is acid catalyzed, acetic acid in the reaction solution affects the kinetics and yields of the derivatization. The effect of concentration of acetic acid on derivatization was examined with the selected carbohydrates (fucose and galactose). As shown in Fig. 2A, the peak areas obtained by CL detection for fucose and galactose increased continuously with increasing concentration of acetic acid, and reached a maximum at 20% (w/v), and then decreased remarkably with the decrease of acetic acid concentration.

The concentration of labeling reagent is another key factor. Fig. 2B shows the dependence of peak area on luminol concentration; with increase of luminol concentration the peak areas show almost linear increases for both fucose and galactose and the concentration reaches even 0.1 *M*. Since the luminol is insoluble in water, too high a concentration of luminol would cause precipitation when the labeling reagent was mixed with the aqueous sample solution, so the concentration of luminol at 0.08 *M* was thus selected for further experiments.

The effect of reaction temperature on the yield of derivatives is shown in Fig. 2C. Both fucose and galactose give convex curves with the maximum point at 30 °C. Fig. 2D plots the relationship between peak area and reaction time. Under a reaction temperature of 30 °C, the optimal reaction time was proved to be 3 h.

The results indicate that mild reaction conditions, namely 20% acetic acid, 30 °C/3 h, are beneficial for the derivatization, while in other reports [12–14] an increase in the acetic acid, temperature and time was considered to give a higher yield for reductive amination of sugar. The possible reason for the abnormal reactivity of the luminol labeling reaction might be attributed to those factors (higher acetic acid concentration, temperature and prolonged reaction time) which would promote the hydrolysis of the luminol molecule and finally result in the loss of chemiluminescence.

Before analysis by CE–CL, the reaction mixture should be pretreated with water to precipitate the excess luminol utilizing the poor solubility of luminol in water. This dilution step has proved to be a simple, effective way to remove most of the excessive reagent and decrease interference on the

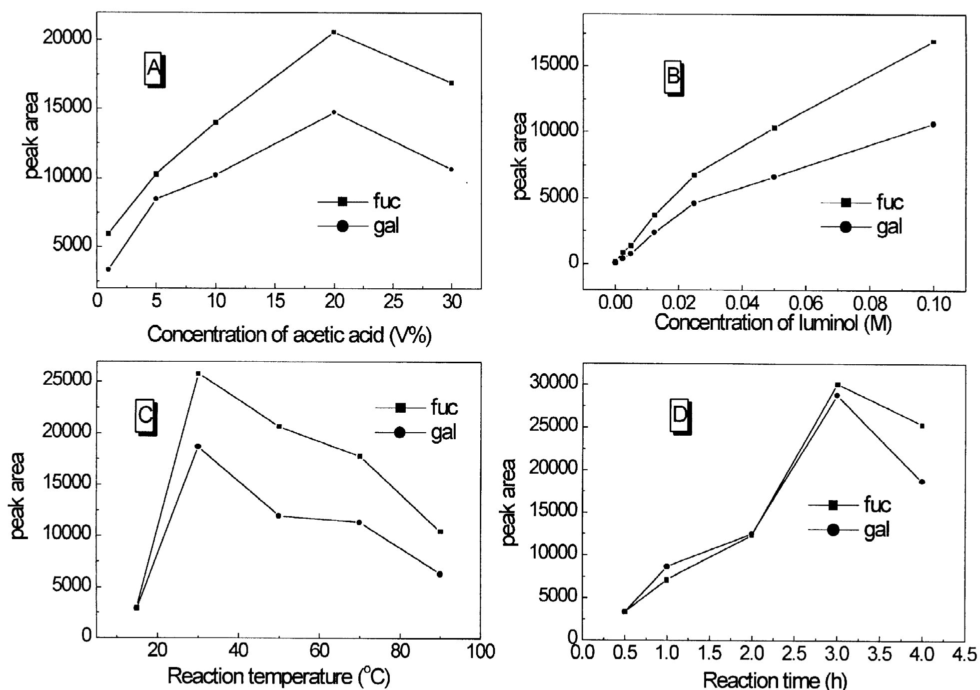

Fig. 2. Effects of various factors on the derivatization of selecting carbohydrates to luminol. (A) Concentration of acetic acid. The concentration of luminol was 0.1 *M*. Reaction temperature, 70 °C; reaction time, 4 h. (B) Concentration of luminol. The concentration of acetic acid was 20% (v/v). Reaction temperature, 70 °C; reaction time, 4 h. (C) Reaction temperature. The concentration of acetic acid and luminol were 20% (v/v) and 0.08 *M*, respectively. Reaction time, 4 h. (D) Reaction time. The concentration of acetic acid and luminol were 20% (v/v) and 0.08 *M*, respectively. Reaction temperature 30 °C. The concentration of carbohydrates in the sample solution was 0.5 m*M*. In all experiments the reaction mixture was diluted by water 20 times, then separated and detected by using 200 m*M* borate + 100 m*M* hydrogen peroxide (pH 9.2) as separation buffer and 25 m*M* potassium hexacyanoferrate in 0.1 *M* sodium hydroxide solution as post-capillary CL reaction reagent.

separation. The diluted reaction solution can be reconcentrated by flushing with nitrogen gas.

3.2. Chemiluminescence detection

An off-column coaxial interface design of Zhang et al. for CL detection was adopted, which allowed more stable working current and background [15]. The improvement of the interface is to set a high voltage ground electrode in front of the reaction capillary in one arm of the four-way connector, instead of the outflow reservoir of the reaction capillary, which prevents disturbance from the bubble formed by hydrogen peroxide hydrolysis both in separation and detection. When luminol reacted with hydrogen peroxide in alkaline medium in the presence of metal ions as catalyst, radiation was observed. The parameters affecting the CL signals including catalyst, pH and hydrogen peroxide have been investigated. Potassium hexacyanoferrate was selected as catalyst because it gives a linear CL signal, good sensitivity and a soluble reaction product [16]. As shown in Fig. 3A, the CL intensity of luminol and sugar derivatives increases with the concentration of hexacyanoferrate from 10 m*M* to 20 m*M*. Above 20 m*M*, the signal of luminol decreases while fucose and galactose remain almost constant at the range of ferricyanide concentration from 20 to 50 m*M*.

The influence of sodium hydroxide concentration is shown in Fig. 3B. Sodium hydroxide was used to maintain the pH of the CL reaction. Since the sample volume flowing into the electrophoretic capillary was small compared to the volume of the CL reagent in the reaction capillary, the pH medium of the CL reaction was dominated by the reagent pH. As

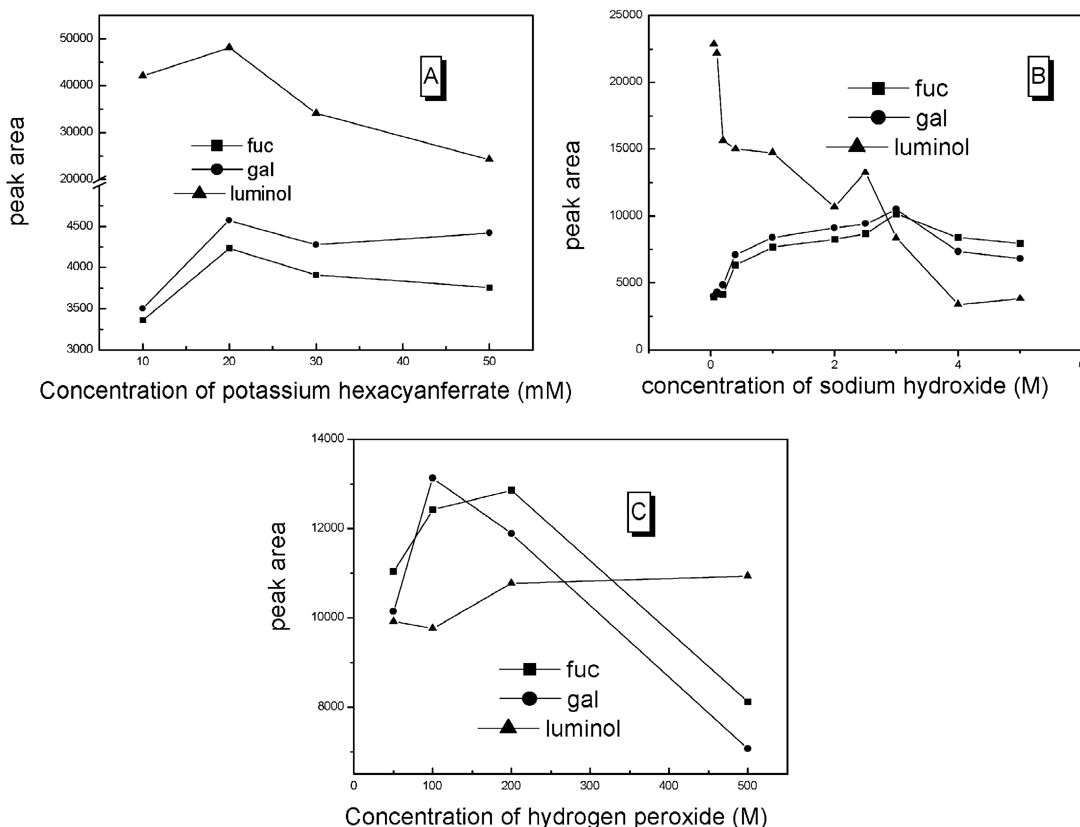

Fig. 3. Effects of various factors on CL intensity of selecting carbohydrate derivatives and luminol. (A) Concentration of potassium hexacyanoferrate. The concentration of sodium hydroxide in CL reagent and hydrogen peroxide in separation buffer were 0.1 *M* and 100 m*M*, respectively. (B) Concentration of sodium hydroxide. The concentration of potassium hexacyanoferrate and hydrogen peroxide were 25 m*M* and 100 m*M*, respectively. (C) Concentration of hydrogen peroxide. The concentrations of potassium hexacyanoferrate and sodium hydroxide were 25 m*M* and 3 *M*, respectively. The concentrations of luminol and derivatized carbohydrates were 2 μ*M* each. Other conditions as in Fig. 2.

reported [17], the ideal pH is 11.8 for the reaction of luminol with hydrogen peroxide using metal ions as catalyst, however, the optimum sodium hydroxide concentration for fucose and galactose derivatives are found to be 3 *M*. With 3 *M* sodium hydroxide, the peak areas of the labeled sugar start larger than that of luminol. No current fluctuation of CE was observed as sodium hydroxide concentration was increased even up to 5 *M*.

The effect of hydrogen peroxide on CL intensity is shown in Fig. 3C. The peak areas of sugar reached their maximum at 100 m*M* hydrogen peroxide, and then decreased with the increase of hydrogen peroxide concentration; on the contrary, the CL intensity of luminol was slightly increased. Since a significant advantage of choosing a particular condition of detection is the ability both to improve detection limit of analytes and to eliminate the interferences of labeling reagent in separation, 25 m*M* potassium hexacyanoferrate in 3 *M* sodium hydroxide solution as post-capillary flowing reagent and 100 m*M* hydrogen peroxide in the separation electrolyte were thus selected for the following experiments.

3.3. Capillary zone electrophoresis (CZE) separation of luminol-labeled carbohydrates

Although a number of separation modes similar to 1-phenyl-3-methyl-5-pyrazolone (PMP) derivatives may be selected for luminol labeled sugars [18–22],

a suitable separation system should have no negative effects on post-capillary CL detection and be favored for electrophoretic separation of derivatives. We choose the alkaline borate buffer for carbohydrate analysis by zone electrophoresis because sugar derivatives are negatively charged through complex with the borate and the buffer does not interfere with the CL reaction. Fig. 4A depicts the relationship between the resolution, R_s, of four pairs of sugar derivatives and the concentration of separation buffer in the medium of pH 10.0. To eliminate the derivation between different CE runs, the resolution of each pair of sugars was calculated from the value obtained by the same run. As shown in Fig. 4A, the R_s values continuously increased for all pairs of sugars with increasing boric acid concentration. In order to avoid Joule heating and to shorten the separation time, a lower concentration of borate was found to be beneficial; therefore, 200 m*M* was the optimal concentration. Fig. 4B shows the effect of pH on the resolution of sugars at a constant borate concentration of 200 m*M*. The maximum R_s appeared at pH 10.0.

Fig. 5 is an electropherogram illustrating the separation of a mixture of seven monosaccharides derivatized with luminol under optimized conditions. Separation was complete with relatively large numbers of theoretical plates ranging from 160,000 to 231,000 per metre, which was comparable to those in on-column UV detection [23] and much better than

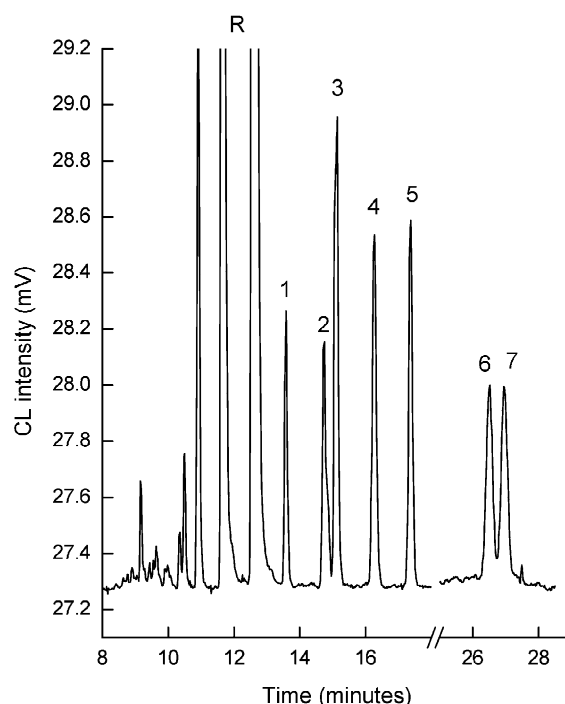

Fig. 5. Electropherogram of seven luminol derivatized monosaccharides at 2 μ*M* each. Peak identification: 1, rhamnose; 2, glucose; 3, arabinose; 4, fucose; 5, galactose; 6, glucuronic acid; 7, galacturonic acid. Conditions: fused-silica capillary, 50 cm×50 μm I.D.×375 μm O.D.; applied voltage, 15 kV; gravity injection, 10 cm 20 s; separation buffer, 100 m*M* hydrogen peroxide in 200 m*M* borate buffer, pH 10.0; CL reagent, 25 m*M* potassium hexacyanoferrate in 3 *M* sodium hydroxide solution.

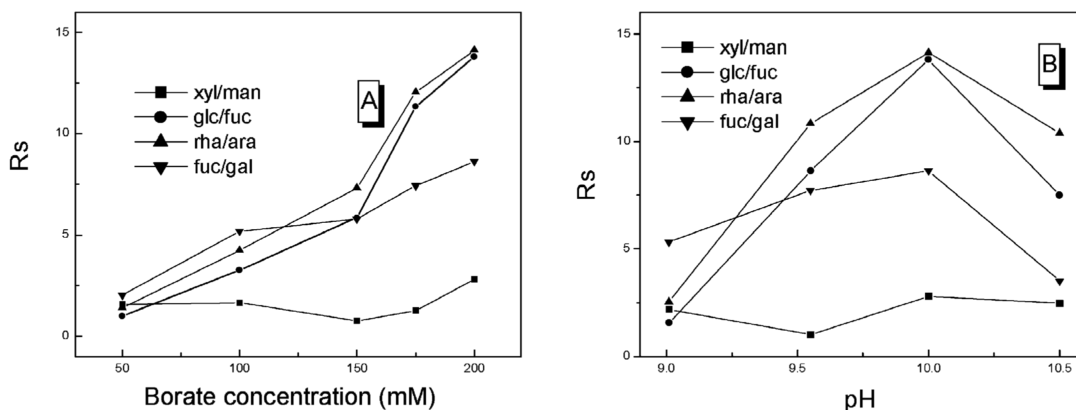

Fig. 4. Effects of borate concentration and pH of separation buffers on the separation. The pH in (A) and borate concentration in (B) were fixed at 10.0 and 200 m*M*, respectively. The other conditions were as in Fig. 3.

those obtained in most post-capillary CL detections [24,25]. The increased efficiency can be explained in terms of a ‘‘chemical bond narrowing’’ effect which related to the chemiluminescence kinetics and diffusion of analytes in the flow cell [26–28]. In the chemiluminescence process, radiation is emitted only when the analyte reacts with CL reagents. If the kinetics of the CL reaction is fast enough, light would be decayed before there is significant diffusion of the analyte to unreacted reagent. Acceleration of the CL reaction or depressing the analyte diffusion would enhance this effect. At the high concentration of sodium hydroxide (3 *M*) employed as CL reaction medium, the increase of viscosity of the reaction solution (as shown in Table 1) leading to the analyte diffusion in the CL reagent decreased which is conducive to avoid excessive broadening of the analyte bond. The assumption was verified with experimental results, as shown in Fig. 6; the separation efficiency was remarkably improved with the increase of concentration of sodium hydroxide. The high separation efficiency illustrated that the luminol tagged sugars were quite suitable for the CE–CL system.

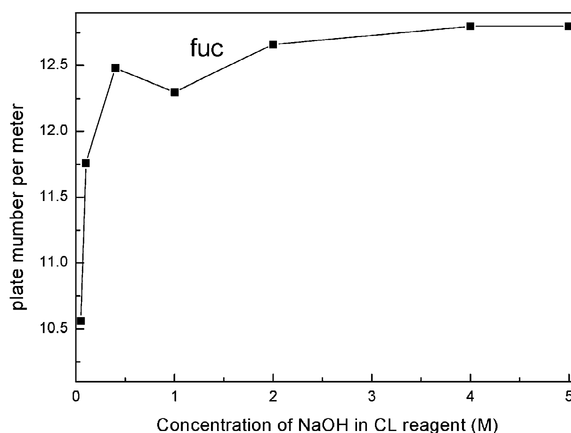

Fig. 6. Effect of concentration of sodium hydroxide in CL reagent on the separation efficiency of luminol–fucose. Separation and detection conditions: 100 m*M* hydrogen peroxide in 200 m*M* borate buffer (pH 9.2) as separation buffer; the concentration of potassium hexacyanoferrate in CL reagent was 25 m*M*.

3.4. *Derivatizable concentration, sensitivity and quantification features*

Another significant advantage for the luminol derivative protocol is that as low as 2 μ*M* of sugar could be derivatized and detected by CE–CL. If only a little of the sample solution was available, its volume can reduce to 1 μl for the labeling process. Considering the relatively low reaction temperature (30 °C) and 80% DMSO as reaction solute, the effect of reaction mixture evaporation on the analysis precision could be ignored. Fig. 7 shows electropherograms of background, 2 μ*M* (2 pmol), 5 μ*M* (5 pmol) and 50 μ*M* (50 pmol) reacted glucose with the background substrate from the electropherogram, the signal-to-noise ratio is 4 for the 2 μ*M* (2 pmol) derivatized glucose sample.

Quantitative investigation of luminol tagged sugar was performed by derivatizing different amounts of carbohydrates with a constant excess amount of luminol and sodium cyanoborohydride to calibrate the relationship between the peak area and sugar concentration, and derivatizing the glucose at constant concentration to evaluate the reproducibility of the reaction. A linear response was obtained over the range of 0.1–250 μ*M* of glucose ($R=0.9998$) and the relative standard derivation (RSD) of the peak area for derivatized glucose at 0.5 m*M* was 5.3% ($n=3$), which indicated the efficiency of the derivatization is close to 100% or remaining at a constant rate and they were reproducible enough to allow reliable quantification. In addition, the RSD of peak area for five different CE runs was 4.3% and the mass detection limit of glucose was 420 amol or 0.1 μ*M* in terms of concentration, at a signal-to-noise ratio of 3.

Table 1
The relative viscosity of sodium hydroxide aqueous solution[a]

Concentration (*M*)	0.125	0.252	0.510	1.043	1.597	2.027	3.079	4.034	5.040
η/η_o (20 °*C*)	1.025	1.052	1.11	1.246	1.413	1.558	2.035	2.563	3.337

[a] Data from Ref. [29]. η/η_o, relative viscosity ratio of the absolute viscosity of a solution at 20 °C to the absolute viscosity of water at 20 °C.

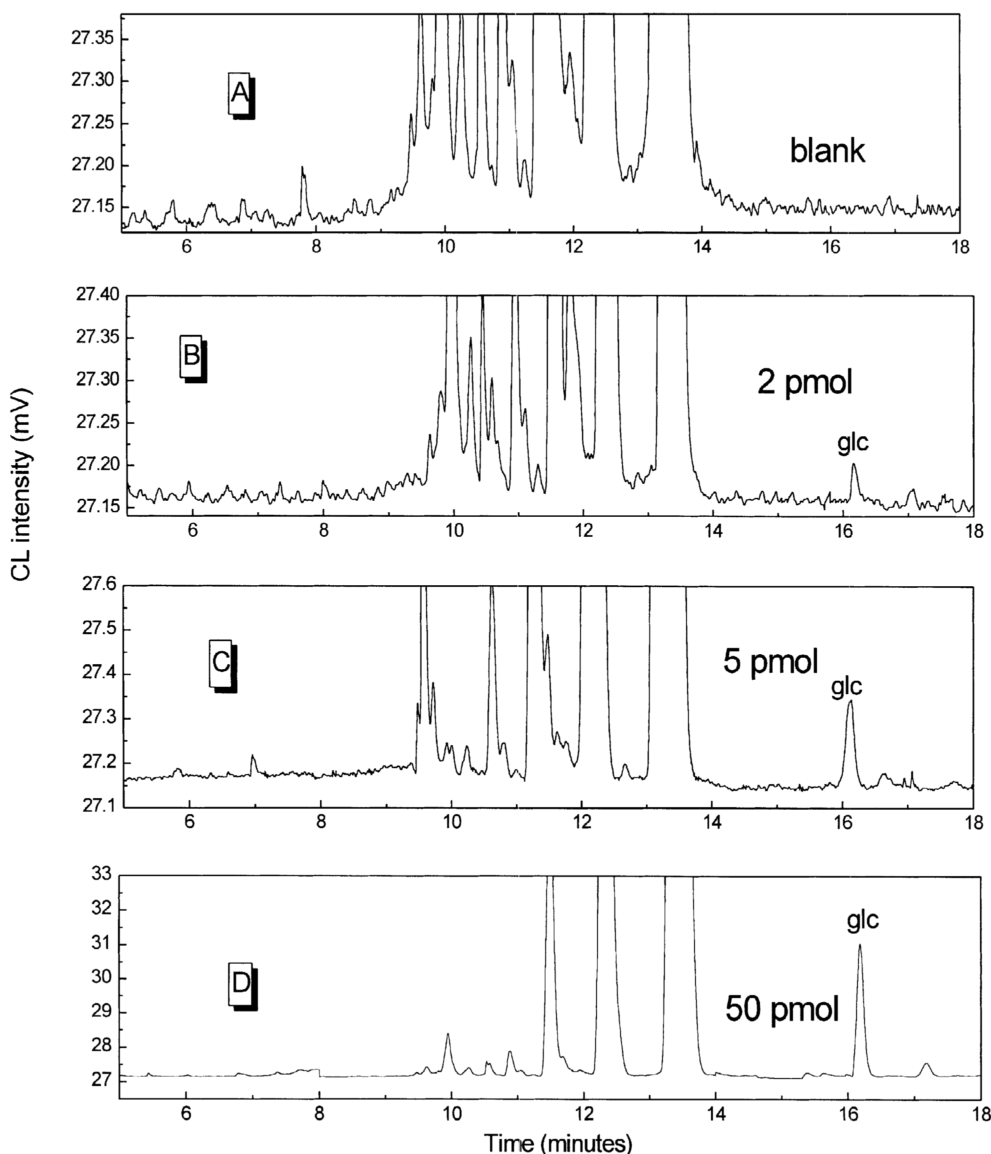

Fig. 7. Electropherograms of two-fold diluted reaction mixture from derivatization of various amounts of glucose. (A) Blank; (B) 2 pmol; (C) 5 pmol; (D) 50 pmol reacted glucose. Derivatization condition: an aliquot of sample 1 μl mixed with 4 μl of labeling reagent and reacted followed the procedure described in Section 2.3. Separation and detection conditions as in Fig. 5.

In comparison with other commonly used sugar derivatization reagents (as listed in Table 2), the detection limit and linear range of this method was much superior to those obtained by UV detection [12–14,23] and was close to those of DHZ [31] and ANTS derivatives [32] detected by LIF. However, the detection capability of the present method was still inferior to most cases of the LIF method which usually are below the 100 amol level. It is remarkable that the lowest amount of sugar that can be derivatized and analyzed by the present method is substantially lower than the other sugar labeling reagents except APTS [30] and DHZ [31]. In most cases, for example, in glycobiological research, the

Table 2
The characteristics of some commonly used reagents for sugar labeling

Reagent	Derivatization limit	Magnitude of linear range	Detection limit	Detection mode	Ref.
APTS	2×10^{-7} mol/l or 2×10^{-12} mol	4–5	4×10^{-10} mol/l 8×10^{-19}mol	LIF	[30]
DHZ	10^{-7} mol/l or 10^{-11} mol	2–3	5×10^{-8} mol/l 10^{-16} mol	LIF	[31]
ANTS	10^{-6} mol/l	ND[a]	5×10^{-8} mol/l or 4×10^{-16} mol	LIF	[32]
CBQCA	500 μg	ND	10^{-17} mole	LIF	[33]
TRSE	2×10^{-2} mol/l	ND	4.8×10^{-11} mol/l or 4.3×10^{-22} mol	LIF	[34]
NBD-F	1×10^{-10} mol/l	2.70	1×10^{-17} mol	LIF	[35]
AP	10^{-2} mol/l	1	10^{-11} mole	UV	[14]
ABN	2 mg/ml	1.6	3×10^{-7} mol/l or 10^{-15} mol	UV	[13]
ABA	2 mg/ml	2	4×10^{-6} mol/l 1.5×10^{-14} mol	UV	[12]
BHZ	3×10^{-4} mol/l	1.96	3.6×10^{-6} mol/l 1.75×10^{-14} mol	UV	[23]

[a] No related data reported. ABA, *p*-aminobenzoic acid; ABN, 4-aminobenzonitrile; AP, 2-aminopyridine; ANTS, 8-aminonaphthalene-1,3,6-trisulfonic acid; APTS, 9-aminopyrene-1,4,6-trisulfonate; BHZ, *p*-hydrazinobenzenesulfonic acid; CBQCA, 3-(4-carboxybenzoyl)-2-quinolinecarboxaldehyde; DHZ, dansylhydrazine; NBD-F, 7-nitro-2,1,3-benzoxadiazole; TRSE, 5-carboxytetramethylrhodamine succinimidyl ester.

lowest amount of sugar analyte that can be derivatized and detected is of greater importance than the lowest detection concentration obtained by diluting a sample that was derivatized at a higher concentration. Taking into account that the results were obtained through a simple-design and low-cost CE–CL system, this approach was much more meaningful.

3.5. Preliminary application

Microdialysis is a new approach to the continuous monitoring of small extracellular molecules. Glucose is the primary energy source for the brain and its content is related to cerebral function. Up to now, glucose in brain microdialysis samples was usually determined by a glucose biosensor based on glucose oxidase coupled with electrochemical detection [36,37]. Some components in the brain microdialysis such as ascorbic acid and glutathione that have electrochemical responses would interrupt the analysis if the sensor does not combine with separation techniques. We have applied our procedure to determination of glucose in brain microdialysis samples. The microdialysis sample was directly derivatized and analyzed as described above with only 2 μl of volume. The electropherogram of a luminol derivatized microdialysis sample is illustrated in Fig. 8. The glucose was identified by spiking of the standard glucose–luminol derivatized sample. Other components in the brain microdialysis sample, such as amino acid, peptide, ascorbic acid and amine etc., have not shown any disturbance to glucose analysis. The concentration of glucose in a rat brain microdialysis sample was 310 μ*M*. The analytical recovery of glucose spiked in a microdialysis sample was 91%.

In conclusion, sensitive sugar probing was achieved by CE–CL with luminol as the chemiluminescence reagent. The labeling reaction allows derivatization of sugars even at the pmol level. High efficiency separation of a sugar derivatives mixture was obtained in alkaline borate buffer. Our preliminary work shows that the present method has fairly good selectivity and allows determination of glucose in brain microdialysis samples, which suggests to us that the method has potential for analyzing sugars in other complex biological samples.

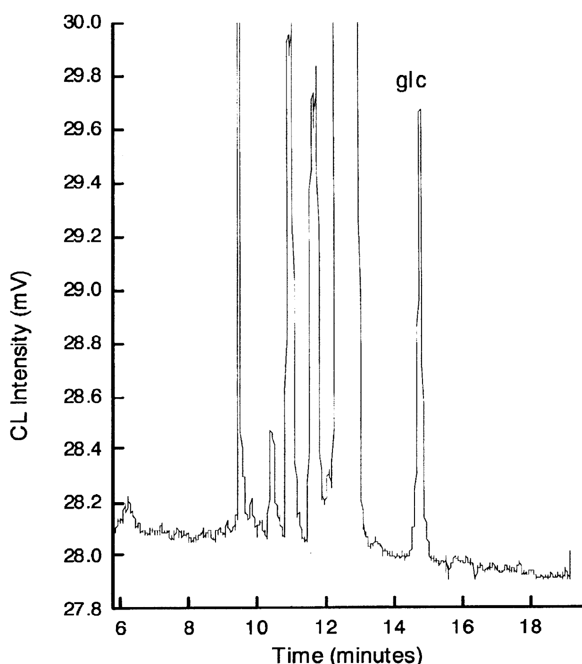

Fig. 8. Electropherogram of a rat brain microdialysis sample derivatized with luminol. The reaction mixture was diluted by water 20 times before CE–CL analysis. Separation and detection conditions as in Fig. 5.

Acknowledgements

This work was financially supported by the National Science Foundation of China (no. 29825112) and The Chinese Academy of Sciences (no. KJ951-A1-507). We also thank Dr. Caihong Xu (Center for Molecular Science, Institute of Chemistry, CAS) for her kind donation of silicon rubber.

References

[1] X.J. Huang, Z.L. Fang, Anal. Chim. Acta 414 (2000) 1.
[2] S. Igarashi, W. Linze, Anal. Chim. Acta 225 (1989) 147.
[3] B.A. Petersson, Anal. Lett. 22 (1989) 83.
[4] P.J. Worsford, Anal. Chim. Acta 164 (1984) 103.
[5] C.A. Koerner, T.A. Nieman, Anal. Chem. 58 (1985) 116.
[6] I.B. Agater, R. Jewsbury, A.K. Williams, Anal. Commun. 33 (1996) 367.
[7] R.H. Zhu, W.T. Tok, J. Pharm. Biomed. Anal. 17 (1998) 985.
[8] I. Takashi, H. Katsuo, Y. Kuriko, K. Daisuke, Y. Shigeo, Chromatography 18 (1997) 114.
[9] H. Kubo, A. Toriba, Anal. Chim. Acta 353 (1997) 345.
[10] J.M. Lin, M. Yamada, Anal. Chem. 71 (1999) 1760.
[11] D.M. Zhang, J.M. Zhang, W.Y. Ma, D.Y. Chen, H.W. Han, H.J. Shu, G.Q. Liu, J. Chromatogr. B 758 (2001) 277.
[12] E. Grill, C. Huber, P. Oefner, A. Vorndran, G. Bonn, Electrophoresis 14 (1993) 1004.
[13] H. Schwaiger, P. Oefner, C. Huber, E. Grill, G.K. Bonn, Electrophoresis 15 (1994) 941.
[14] S. Honda, S. Iwase, A. Makino, S. Fujiwara, Anal. Biochem. 176 (1989) 72.
[15] Y. Zhang, Z.L. Gong, H. Zhang, J.K. Cheng, Anal. Commun. 35 (1998) 293.
[16] D.T. Bostick, D.M. Hercules, Anal. Chem. 47 (1975) 447.
[17] W.L. Hinze, T.E. Riehl, N.H. Singh, Y. Baba, Anal. Chem. 18 (1995) 667.
[18] S. Honda, K. Togashi, A. Taga, J. Chromatogr. A 791 (1997) 307.
[19] S. Honda, S. Suzuki, A. Nose, K. Yamamoto, K. Kakahi, Carbohydr. Res. 215 (1991) 193.
[20] C. Chiesa, P.J. Oefner, L.R. Zieske, R.A. O'Neill, J. Capillary Electrophor. 4 (1995) 175.
[21] S. Honda, A. Taga, M. Kotani, E.R. Grover, J. Chromatogr. A 792 (1997) 385.
[22] S. Honda, K. Togashi, K. Uegaki, A. Taga, J. Chromatogr. A 805 (1998) 277.
[23] X.Y. Wang, Y. Chen, Carbohydr. Res. 332 (2001) 191.
[24] J.Y. Zhao, J. Labbe, N.J. Dovichi, J. Microcol. Sep. 5 (1993) 331.
[25] R. Dadoo, L.A. Colón, R.N. Zare, J. High Resolut. Chromatogr. 15 (1992) 133.
[26] B. Huang, J.J. Li, L. Zhang, J.K. Cheng, Anal. Chem. 68 (1996) 2366.
[27] G.J. Jong, N. De Lammers, F.J. Spruit, U.A.Th. Brinkman, R.W. Fei, Chromatographia 18 (1984) 129.
[28] M.L. Grayeshi, Anal. Lett. 17 (1984) 1339.
[29] A.V. Wolf, M.G. Brown, P.G. Prentiss, in: 63rd edn, CRC Handbook of Chemistry and Physics, CRC Press, Boca Raton, FL, 1982, p. D265.
[30] R.A. Evangelista, M.S. Liu, F.T. Chen, Anal. Chem. 67 (1995) 2239.
[31] S.A. Perz, L.A. Colon, Electrophoresis 17 (1996) 352.
[32] A. Klockow, H.M. Widmer, R. Amado, A. Paulus, Fresenius J. Anal. Chem. 350 (1994) 415.
[33] J. Liu, O. Shirota, M. Novotny, Anal. Chem. 64 (1992) 973.
[34] J.Y. Zhao, P. Diedrich, Y. Zhang, O. Hindsgaul, N.J. Dovichi, J. Chromatogr. B 657 (1994) 307.
[35] S. Honda, J. Okeda, H. Iwanaga, S. Kawakami, A. Taga, S. Suzuki, K. Imai, Anal. Biochem. 286 (2000) 99.
[36] S. Bohm, D. Pijanowska, W. Olthuis, P. Bergveld, Biosens. Bioelectron. 16 (2001) 391.
[37] J.P. Lowry, M. Miele, R.D. O'Neill, M.G. Boutelle, M. Fillenz, J. Neurosci. Methods 79 (1998) 65.