Analyst

Cite this: *Analyst*, 2011, **136**, 1753

www.rsc.org/analyst

PAPER

Glucose in human serum determined by capillary electrophoresis with glucose micro-biosensor

Xiaolei Wang,*[ab] Yan Zhang,[a] Cuicui Cheng,[a] Ranran Dong[a] and Jingcheng Hao[c]

Received 25th May 2010, Accepted 8th February 2011
DOI: 10.1039/c0an00348d

A glucose micro-biosensor was employed as detector in capillary electrophoresis (CE) for determining the concentration of glucose in human serum. The micro-biosensor was based on the immobilization of the SWNTs–glucose oxidase–chitosan biocomposite at a platinized Au electrode by electrodeposition. The influencing factors including separation voltage, detection potential, pH value, and the concentration of the buffer were studied. Suitable conditions were obtained for the determination of glucose: running buffer, 25 mM PBS (pH 8.0); separation field strength, 250 V cm^{-1}; detection potential, 0.80 V *vs.* saturated calomel electrode. Under optimized detection conditions, glucose responded linearly from the range of 5 μM to 1 mM with a correlation coefficient of 0.9986 for the injection voltage of 5.0 kV and injection time of 10 s. The concentration limit of detection of the method was 1 μM (S/N = 3). The micro-biosensor exhibited good stability and durability in the analytical procedures. The relative standard deviation of the migration time and peak current were 1.7% and 2.6%, respectively. Glucose in human serum from two healthy individuals and two diabetics was successfully determined, giving a good prospect for a new clinical diagnostic instrument.

Introduction

Glucose, one of the indispensable substances in an organism, can directly participate in metabolic processes in which energy is needed in life processes. Glucose plays a very important role in the life cycle and forms the base of many foodstuffs.[1] An abnormal glucose concentration in blood is linked with some diseases. The well-known diabetes mellitus is a major health problem characterized by increased sugar levels in blood and urine. Sugar diabetes is the most familiar chronic disease with high overall mortality. The incidence of diabetes shows an upward trend year by year combined with the improvement of people's living standard. So it is very important to monitor blood glucose for diagnosis and effective treatment of diabetes.

Several analytical techniques have been used over the years to detect glucose in real samples, which include chemiluminescence (CL),[2] electrochemiluminescence (ECL),[3] and electrochemical detection (ECD).[4,5] Among the techniques, the amperometric biosensors, based on glucose oxidase (GOx), have played a leading role in the move to simple easy-to-use blood sugar testing and are expected to play a similar role in the move toward continuous glucose monitoring. Two different kinds of glucose biosensors are most popular in blood sugar determination. The first is an indirect method by using mediators to shuttle the electrons. The mediators used in these sensors include ferrocene derivatives,[6] quinones[7] and poly-2-aminoaniline polymer[8] *etc.* Another is based on direct electron transfer between GOx and an electrode. Various nanomaterials, including gold nanoparticles[9] or carbon nanotubes (CNTs),[10] have thus been used as electrical connectors between the electrode and the redox center of GOx. Owing to the inherent high sensitivity and simplicity of the instrument needed, these biosensors have been studied popularly. The amperometric measurement of hydrogen peroxide at biosensors requires application of a higher potential at which endogenous reducing species, such as ascorbic and uric acids and some drugs (*e.g.*, acetaminophen) in blood, are also electroactive. Although a permselective coating can minimize the access of these constituents toward the biosensor surface, the current contributions of these and other oxidizable constituents (especially the metabolites of some drugs) of biological fluids will compromise the selectivity sometimes and hence the overall accuracy of measurement. ECD with amperometric biosensors is time-consuming, reagent-consuming, and difficult to be automated in clinical detection for the necessity of changing solution after every experiment. Capillary electrophoresis (CE) has been used to detect glucose in real samples due to its undisputable advantages of fast analysis time, high sensitivity, separation efficiency, ultra-small sample volume, and little consumption of solvent. Several detection methods of CE have been reported to

[a]*College of Chemistry, Chemical Engineering and Materials Science, Key Lab of Molecular and Nano Probes, Engineering Research Center of Pesticide and Medicine Intermediate Clean Production, Ministry of Education, Shandong Provincial Key Laboratory of Clean Production of Fine Chemicals, Shandong Normal University, Jinan 250014, P. R. China*
[b]*Institute of Crystal Materials, Shandong University, Jinan 250100, P. R. China*
[c]*Key Lab of Colloid and Interface Chemistry of Ministry of Education, Shandong University, Jinan 250100, P. R. China*

determine glucose such as *via* spectrophotometer,[11] chemiluminescence,[12] and electrochemical detection.[13–17] For the determination of glucose in biological samples by CE, derivatization with a suitable reagent is necessary to facilitate UV or laser-induced fluorescence (LIF) detection.[11] Despite the higher sensitivity of spectrophotometry and chemiluminescence, the cost of optical instrumentation, the need for analytic derivatization, and the limited portability of LIF have led to the investigation of electrochemical detection as an attractive alternative. It has been used to glucose analysis with both conventional[13] and microchip CE[14] in recent years. It is less sensitive than LIF but much more versatile, economical, and easy to scale down in the micrometric range. Most of the available methods by CE-ECD were based on the pre-column enzymatic oxidation of glucose by glucose oxidase (GOx).[14,15] The glucose concentration is proportional to the formed product (hydrogen peroxide) which can be detected by electrochemical methods. Because a Cu electrode can catalyze the oxidation of the carbohydrates in basic media, Wang *et al.*[16] have shown great interest in the detection of glucose with Cu electrodes on microfabricated electrophoresis separation systems. Lee and Chen[17] detected several carbohydrates by microchip capillary electrophoresis with amperometric detection.

When a glucose biosensor is used to be the detector of CE, the sensitivity of determination will be increased over using Cu electrodes, and the time of experiment needed will be shortened remarkably. In the present article, we describe a new strategy to prepare a micro-disk biosensor (micro-biosensor) as the electrochemical detector of CE. It is based on the immobilization of the SWNTs–glucose oxidase–chitosan biocomposite at a platinized Au electrode through a simple electrodeposition method as reported in our previous work,[18] in which a new kind of glucose biosensor was fabricated. The presence of Nafion and chitosan as solubilizing agents for single-walled carbon nanotubes (SWNTs) served as an effective barrier to decrease the loss of enzyme. Compared with the other simple fabrication method of the Au/Pt/PPD/GOx micro-biosensor, experimental results showed that this new kind of micro-biosensor used in CE-ECD had the properties of good reproducibility, stability, and anti-interference to the high voltage of CE. Glucose in human serum from both healthy and diabetic individuals was successfully determined. The results showed that this method is a simple, sensitive and reliable technique.

Experimental

Apparatus and equipment

All electropherograms were obtained on a self-assembled CE system. Briefly, a reversible high-voltage power supply (Instrument Company, Shandong Normal University, China) provided a variable voltage of 0.0–30.0 kV across the capillary, with the outlet of the capillary at ground potential. A 32 cm fused-silica capillary (25 μm I.D., 375 μm O.D. Yongnian Optical Conductive Fiber Plant, China) was placed between the two buffer reservoirs. The positive pole of high voltage was applied at the injection end, while the negative pole was held at ground potential by a coiled Pt wire placed at the bottom of the reservoir containing the ECD cell. Separations were carried out at an applied voltage of 8.0 kV. The detection cell was shielded in a metal box to reduce external disturbance. A CHI802b Electrochemical Analyzer (Shanghai Chenhua Instrument Company, China) was used to determine the concentration of glucose with electrochemical detection (ECD). ECD was carried out with a three-electrode system consisting of a glucose micro-biosensor as the detector, a saturated calomel electrode (SCE) as the reference electrode, and a coiled Pt wire placed at the bottom of the cell as the auxiliary electrode. The schematic of the CE-ECD detection cell was shown in Fig. 1. The cell volume was about 12 nL. The surface of the micro-biosensor was characterized by using a S-4800 ultra-high resolution scanning electron microscopy (operated at a 1–5 kV accelerating voltage).

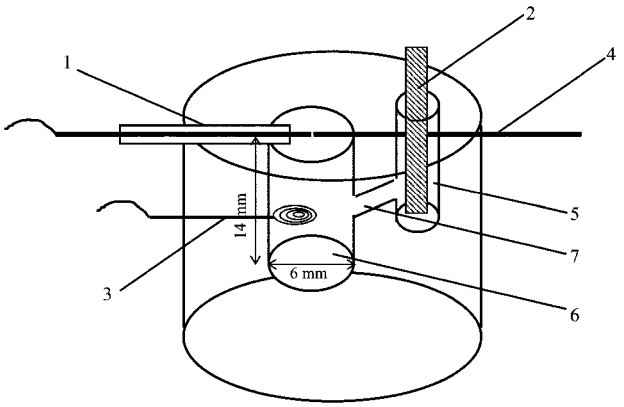

Fig. 1 Schematic of the CE-ECD detection cell: (1) glucose micro-biosensor; (2) reference electrode; (3) auxiliary electrode; (4) capillary; (5) reference electrode port; (6) buffer reservoir; (7) connecting passage between (5) and (6).

Reagents and solutions

Glucose oxidase (GOx, 109 U mg^{-1}) and D-glucose (>99.8%) were received from Amreco. Single-Walled Carbon Nanotubes (purity of CNTs ≥ 90%, SWNTs ≥ 50%, 5–15 μm in length) were obtained from Shenzhen Nanotech. Port. Co. Ltd. (China) and were treated with nitric acid during the purification process followed by filtering, rinsing with water and drying.[19] Chitosan from crab shells (85% deacetylated) was purchased from Sigma-Aldrich (St. Louis, Mo., USA). Chitosan solution (1.0 wt%) was prepared by adding chitosan flakes to water and gradually adding 0.1 M HCl to the solution to maintain the pH near 3.0. After undissolved material was filtered off, the pH was adjusted to about 5.0 using 1.0 M NaOH. Nafion (5%) was obtained from Fluka (Buchs, Switzerland). Glucose stock solutions were allowed to mutarotate overnight at room temperature before use and were stored at 4 °C in a refrigerator and freshly prepared weekly. Human serum samples were obtained from the Hospital of Shandong Normal University. Fresh human serums from four persons were analyzed within a day. The study was approved by Human Research Ethics Committee of Shandong Normal University, and informed consent was obtained from each participant who provided plasma for this experiment. As glucose in a diabetic serum was at a higher concentration (>7 mM), the serums from two healthy individuals and two diabetics were diluted 20-fold and 30-fold with 25 mM PBS (pH 8.0) before

analysis, respectively. The resulting solution was filtered through a membrane and directly injected into the capillary without further preparation. Stock buffer solutions were 25 mM PBS (pH 8.0) before analysis. Au wire and Ag electric adhesive were purchased from Sino-platinum Metals Co., Ltd. (China). All other chemicals such as H_2PtCl_6, ascorbic acid (AA), and uric acid (UA) *etc.* were of analytical grade. Double-distilled water was used throughout. All the solutions for the electrophoresis procedure were filtered through a 0.45 μm nylon membrane syringe filter (Supelco, Bellefonte, PA, USA) before use.

Fabrication of Au/Pt/SWNTs/chitosan/Nafion micro-biosensor

The fabrication of the novel micro-biosensor was performed as reported for the conventional-size biosensor,[18] but with slight changes for its micro-size. Briefly, Au wire (200 μm diameter, 4 cm in length) was carefully inserted into a fused-silica capillary (250 μm I.D., 3 cm in length), then the fused-silica capillary was filled with ethyl α-cyanoacrylate adhesive. The other end of the Au wire was circled on a copper wire and smeared with Ag electric adhesive. This was dried in the oven at 80 °C for 1 h, and the joint of the fused silica capillary and the Au wire was inserted and fixed into a glass tube (400 μm I.D., 700 μm O.D., 4 cm in length) by using ethyl α-cyanoacrylate adhesive. The Au electrode was well prepared.

Prior to being modified, all electrodes were polished first with diamond paper, and then washed in turn with water, alcohol and water for 5 min by using a supersonic wave cleaner. According to the method previously reported by Deng and co-workers,[20] the platinization of Au electrodes was performed in 0.1 M HCl solution containing 2 mM H_2PtCl_6 by controlling the potential at −0.3 V for 5 min. The electrode was then removed from the plating cell and washed thoroughly with water.

For electrochemical deposition, the cleaned Au electrode was connected to a direct current power supply (−3.0 V) and dipped into the deposition solution for 15 s. The deposition solution was chitosan solution (1.0%) containing 0.5 mg mL^{-1} SWNTs and 800 U mL^{-1} GOx. At this potential, H^+ was reduced to H_2 at the cathode and released, and the pH near the cathode surface gradually increased. When the pH was higher than 6.3, chitosan became insoluble[21] and chitosan hydrogel incorporated with GOx and SWNTs was electrodeposited onto the cathode surface as a result. The electrode was rinsed with water. The electrode was dried in air and dipped into Nafion solution (1.0%, diluted by water) twice. For comparison, a micro-biosensor based on the Au electrode without being platinized was prepared in a similar manner. All the micro-biosensors were dried in air at room temperature for about 2 h, and then stored in a refrigerator (4 °C) for use.

Fabrication of Au/Pt/PPD/GOx micro-biosensor

In the experiments, we prepared another kind of micro-biosensor with an electrochemical polymer membrane according to ref. 22. Briefly, the above-mentioned platinized Au electrode was electrochemically pretreated by potential cycling between −0.21 and +1.19 V in 0.5 M H_2SO_4 solution until a steady state was reached. Poly(*o*-phenylenediamine) (PPD) films were electrochemically grown from a fresh solution containing 5 mM of *o*-phenylenediamine (*o*-PD) in an acetate buffer (pH 5.2, 0.20 mM). Enzyme immobilization was performed by adding 600 U mL^{-1} of GOx to the *o*-PD solution prior to immobilization. Films were grown for 15 min at a constant applied potential of +0.65 V from deaerated unstirred solution. Then the Au/Pt/PPD/GOx micro-biosensor based on the electrochemically synthesized PPD film for GOx immobilization was stored in phosphate buffer pH 7.0 (0.2 mM) at +4 °C when not in use.

Procedure of glucose analysis and data treatment

CE was performed on the self-assembled capillary electrophoresis system. The CE separations were conducted in a 32 cm fused-silica capillary (25 μm I.D., 375 μm O.D.) at room temperature by applying a separation voltage of 8.0 kV. The injection end of the capillary was immersed in the electrophoresis buffer reservoir. The other end of the capillary (*ca.* 5 mm of the polyimide-coating capillary was removed by burning) was immersed in the other buffer reservoir and adjusted to the same horizontal line as the micro-biosensor under the guidance of a three-dimensional micromanipulator. In order to see the collimation and space between the micro-biosensor and the end of the capillary, a microscope with a magnification of 40× was used. When the equipment was fixed well, a separation field strength of 250 V cm^{-1} was applied across the capillary and the detection potential of 0.80 V was applied at the micro-biosensor and the electropherogram was recorded. After the electroosmotic current reached a constant value, the electromigration injection was carried out. An injection voltage of 5.0 kV was applied for 10 s to transport the glucose solution into the capillary tip. Then the capillary was put carefully to the CE electrophoresis buffer and the separation voltage was turned back to 8.0 kV. The capillaries were rinsed with 0.1 M NaOH solution, double distilled water and the corresponding buffer solutions for 5 min respectively by means of a syringe before each run. Amperometric measurements in three-electrode mode were done using an Electrochemical Analyzer. Peak currents and the concentration standard calibration method were used for glucose quantitation in human serum.

Results and discussion

SEM images of the platinized Au micro-disk electrode and Au/Pt/SWNTs/chitosan micro-biosensor

The morphologies of the platinized Au micro-disk electrode and Au/Pt/SWNTs/chitosan micro-biosensor were characterized by scanning electron microscopy (SEM). Fig. 2A shows the SEM image of the platinized Au electrode. The aggregates of the electrodeposited platinum on the Au electrode surface are fairly uniform and have a porous structure which provides a greatly enhanced effective electrode surface for high enzyme loading.[20] From the SEM image of the micro-biosensor (Fig. 2B), its surface becomes smoother because of the covering of the chitosan film. Many wire-like substances with diameters of about 1.5∼2 nm and 5∼14 μm in length were homogeneously distributed within the film. This can be attributed to the wrapping of the SWNTs with chitosan chains.[23]

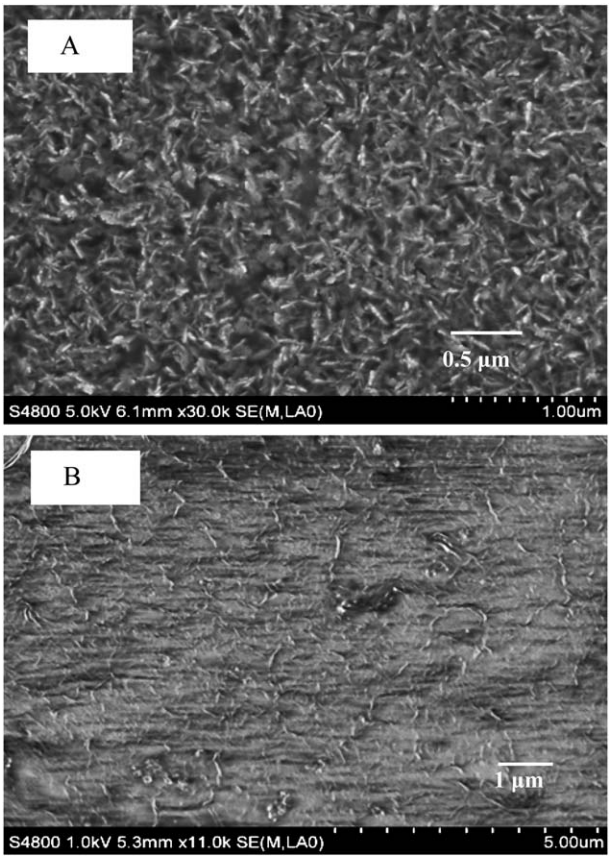

Fig. 2 SEM images of the platinized Au micro-disk electrode (A) and Au/Pt/SWNTs/chitosan micro-biosensor (B). Accelerating voltage: 5000 V (A) and 1000 V (B).

Effect of electrochemical deposition time

The thickness of the enzyme layer influenced the response time of the micro-biosensor and hence the sensitivity and the separation ability. The effect of electrochemical deposition time was investigated by varying the deposition time (5, 15, 30, 60 s, not shown). It was found that the peak width increased with the increase of the time of deposition. When 15 s was applied, the peak current became largest. In this experiment, 15 s was selected as the optimum electrochemical deposition time.

Effect of detection potentials

In amperometric detection, the potential applied to the working electrode directly affects the sensitivity, detection limit, and stability of this method. The effect of detection potential (E_d) on the current of peak (i_p) was studied in the range from 0.5 V to 1.0 V and the corresponding result was shown in Fig. 3. When the detection potential was lower than 0.4 V, nearly no evident oxidation currents appeared. The peak current of glucose began to increase sharply after 0.5 V. With the detection potential >0.9 V, the amperometric response decreased. However, the current of micro-biosensor showed bad reproducibility and larger baseline noise at a voltage of 0.90 V. Therefore, the applied potential of +0.80 V (*vs.* SCE) was selected, where the background current is not too high, the stability and reproducibility are better and the S/N ratio is the highest.

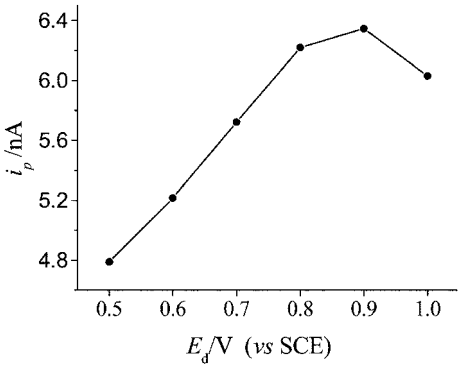

Fig. 3 Hydrodynamic voltammogram for 1 mM glucose. Fused-silica capillary: 25 μm I.D./32 cm; running buffer: 25 mM PBS (pH 8.0) buffer; separation voltage: 8 kV; injection time: 5.0 kV/10 s.

Effect of separation voltage and injection time

The applied separation voltage controls the electro-osmotic flow and the separation process. So it affects the migration time, the peak height and the width at the peak half-height ($W_{1/2}$). To evaluate the effect of the separation voltage, we compared the migration time, the peak height and $W_{1/2}$ of glucose over the range of 6–16 kV (Fig. 4). As can be observed, if a higher separation voltage was applied, larger noisy baselines, shorter runs and weaker peak currents were achieved. In addition, when a lower separation voltage was applied, the peak height became larger, which probably resulted from the increased residence time at the electrode. When a voltage of 6 kV was applied, the peak current became the largest but $W_{1/2}$ turned broader and the migration time became longer. As a compromise between the analysis time, electrochemical response and $W_{1/2}$, 8.0 kV (250 V cm^{-1}, field strength) was chosen as the optimum separation voltage.

The effect of injection time on CE separation was investigated by varying the injection time (5, 7, 10, 15, 20 s with a injection voltage of 5 kV, not shown). It was found that both the peak current and the peak width increased when the time of injection was increased. When a shorter injection time was applied the

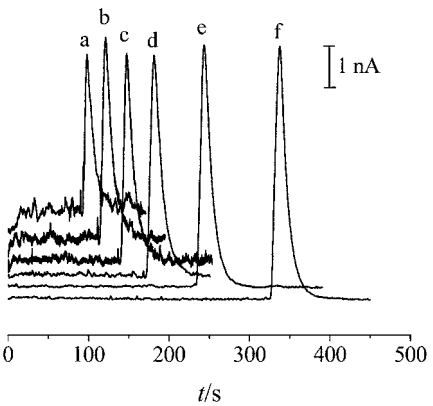

Fig. 4 Effect of separation voltage on 1 mM glucose. Separation voltage: (a) 16 kV; (b) 14 kV; (c) 12 kV; (d) 10 kV; (e) 8 kV; (f) 6 kV; detection potential: 0.80 V. Other analysis conditions as in Fig. 3.

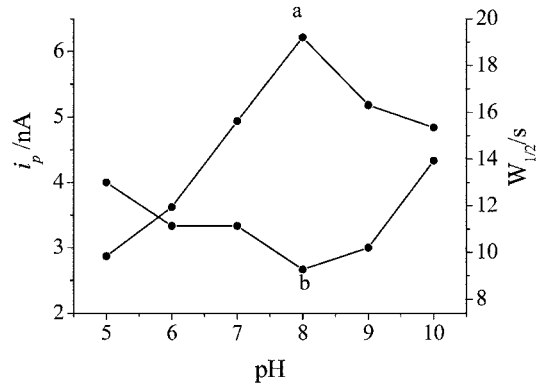

Fig. 5 Effect of acidity on peak current (a) and peak half-height (b). Buffer: 25 mM PBS; other conditions as in Fig. 4.

Table 1 Influence of buffer concentration on the detection of 1 mM glucose ($n = 3$)[a]

C_b/mM	i_p/nA	t_m/s	$W_{1/2}$/s	N
25	6.611	318	7.9	8753
50	6.261	265	8.0	6078
75	6.157	253	7.0	7237

[a] Other analysis conditions as in Fig. 4.

peak current was weaker, whereas a longer injection time would result in severe peak broadening. In this experiment, 10 s (at an injection voltage of 5 kV) was selected as the optimum injection time.

Influence of the pH and concentration of the buffer

The concentration and pH of the running buffer have an important effect on the surface characteristics of the fused-silica capillary and the effective electric charge of the ion. The effect of the pH of the 25 mM PBS buffer on i_p (curve a) and $W_{1/2}$ (curve b) was shown in Fig. 5. The peak height gradually increased as the pH was increased from 5.0 to 8.0. The maximum peak current could be observed at pH 8.0 after which the peak current decreased. Moreover, $W_{1/2}$ reached a minimum value at this pH. The result is close to the previous study of Luo *et al.*,[5] where the GOD-chitosan-based biosensor has an optimal pH value at 9.0. The deposited chitosan provides a biocompatible microenvironment for GOx to withstand outside conditions. So pH 8.0 was chosen for the CE separation.

The effect of the running buffer concentration (C_b) on migration time (t_m), i_p, $W_{1/2}$, and theoretical plate number (N), was studied in the range from 25 mM to 75 mM and the corresponding results were shown in Table 1. It shows that when the concentration of PBS increases from 25 mM to 75 mM, the electrochemical response decreases gradually. This indicates that, at a constant separation voltage, a higher buffer concentration results in lower resistance through the channel and thus in a higher electrophoretic current and Joule heating.[24] For a satisfactory detection the optimum buffer concentration was fixed at 25 mM.

Detection of standard glucose

On the basis of our above-described investigations, 25 mM PBS (pH 8.0) for running buffer, 250 V cm^{-1} for the field strength, 0.80 V for the detection potential were adopted as appropriate analytical conditions for the quantitative assessment of our CE-ECD system. Under the optimized detection conditions, the concentration linear range was from 5 μM to 1 mM with the peak current regressive equation y(nA) = −0.0608 + 5.74x(mM) and a correlation coefficient of 0.9988 for the injection voltage of 5.0 kV and an injection time of 10 s. The number of replications of each concentration level is five. The mean values were used to get the regression equation. The relative standard deviation (RSD) of the slope and intercept were 4.3% and 3.5%, respectively. As glucose in blood serum was at a high concentration (>3 mM), the serum should be diluted before analysis. The concentration limit of detection (LOD_c) of glucose was 1 μM (S/N = 3) which was lower than the calculated 6 μM of glucose by the CE-ECD microchip at a copper electrode in the paper of Wang's group[16] and it was also lower than the LOD_c of 0.10 mM of glucose by CE-ECD at a copper wire electrode (diameter, 130 μm) reported in the paper of Chen and co-workers.[21] The most important feature is its reproducibility, which influences greatly the reliability and hence the viability of the method. When seven consecutive injections of 0.1 mM glucose were performed in the analytical procedures, the RSDs of the migration time and peak current were 1.7% and 2.6%, respectively. The peak current response of the micro-biosensor remained at 87% of the initial current after a month's storage in a refrigerator (4 °C).

The reason for the good reproducibility and durability is probably that the immobilized enzyme was firmly retained on the electrode surface after repeated measurements as a result of the presence of chitosan and Nafion. While the other kind of micro-biosensor (Au/Pt/PPD/GOx) showed bad reproducibility and its peak current decreased seriously with the increase of determination (RSD > 64%, $n = 7$). This may be because a gradual loss of enzyme with the increase of determination[25] and the influence of high voltage. Therefore, the Au/Pt/PPD/GOx micro-biosensor is incapable of being used as the detector in CE-ECD despite the fact that this method is more simple and more popular.[22,26]

The Au/SWNTs/chitosan micro-biosensor showed lower sensitivity (about 34.6%) according to the experiment results (not shown) compared with the Au/Pt/SWNTs/chitosan/Nafion micro-biosensor. This is mainly because that the electrodeposited platinum on the Au electrode surface has a porous structure which provides a greatly enhanced effective electrode surface for high enzyme loading. According to our previous work,[18] the micro-biosensor based on the Pt electrode (prepared similarly) was also less sensitive than the Au/Pt/SWNTs/chitosan/Nafion micro-biosensor. Compared with the micro-biosensors mentioned above, the Au/Pt/SWNTs/chitosan/Nafion micro-biosensor used in this study had the properties of good reproducibility and stability because the biocomposite provided a biocompatible environment for the existence of enzyme.

Determination of glucose in human serum

Human serum is composed of water, proteins, salts as well as various ions (Na^+, Ca^{2+}, HCO_3^-, *etc.*), glucose and traces of

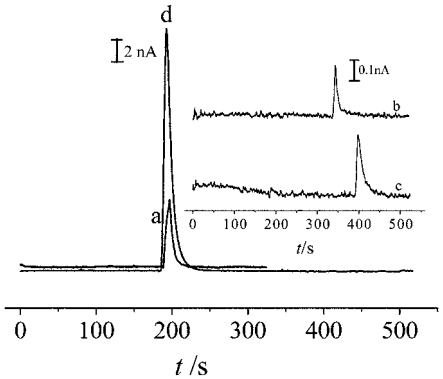

Fig. 6 Electropherogram of glucose, AA, UA and their mixture: (a) 1 mM glucose; (b) 0.33 mM AA; (c) 0.125 mM UA; (d) 5 mM glucose + 0.33 mM AA + 0.125 mM UA. Other conditions as in Fig. 4.

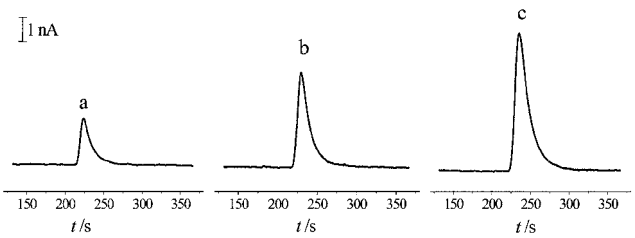

Fig. 7 Electropherogram of glucose in serum from a healthy person: (a) 20-fold diluted human serum sample; (b) plus 0.25 mM glucose standard solution; (c) plus 0.50 mM glucose standard solution. Other conditions as in Fig. 4.

other sugars, other organic acids, lipids, urea, and other wastes. Among them, the main interferences for CE-ECD are ascorbic acid (AA) and uric acid (UA) which can also be oxidized on the micro-biosensor.[27] The level of endogenous ascorbic acid and uric acid is about 0.125 mM and 0.33 mM respectively.[28] To investigate the feasibility of the detection system for analysis of a biological sample, Fig. 6 showed the electropherograms of standard glucose (curve a), AA (curve b), UA (curve c) and the mixture of them (curve d) whose concentration was close to that of human serum samples. According to the electropherograms, the peaks of AA and UA were too small to be identified in the mixture. Other substances like cysteine and acetaminophen cannot give interference effects, because their peak currents are too small to be detected at the concentration of diluted human serum, too. Because of the usage of the glucose biosensor as the detector of CE, the sensitivity of determination was improved and continuous blood sugar monitoring was realized. The time and amount of reagent could be saved greatly.

Under the optimized detection conditions, the concentration of glucose in serum was determinated by capillary electrophoresis with electrochemical detection by standard addition. Before experiment, serum from the healthy and diabetics individuals was diluted 20-fold and 30-fold with PBS (pH 8.0) respectively. The electropherogram of human serum from healthy persons was shown in Fig. 7. The height of the corresponding peak current was enhanced after standard glucose was added. Once the glucose oxidation peak was identified, the concentration of glucose was determined (Table 2). The detected concentration values of glucose in serum were smaller but close to the reference value. Experiments were done to identify the validity of the standard addition method. Three standard solutions were added into one standard solution. The mixture solution was determined by our method. There was not too much inaccuracy (the recovery was 98–105%). Because the blood samples we used were supplied from a hospital, serum was not separated from erythrocyte and leukocytes immediately. It took 4–5 h when we extracted and determined the serum. Therefore, the reason for the smaller detected glucose concentrations compared to the reference values was because of the degradation of glycolysis enzymes from erythrocytes and leukocytes in the blood.[29] Through the analysis of clinical samples, we demonstrated that the new kind of micro-biosensor could be applied in CE-ECD for the rapid, sensitive determination of glucose in human serum, and showed the application prospects and attraction for clinical bioassays.

Table 2 Concentration of glucose in human serum[a]

Sample	Glucose concentration in serum/mM	Reference value/mM[b]
1	4.9	5.1
2	4.9	5.0
3	6.9	7.3
4	7.0	7.4

[a] Sample 1 and 2: serum from healthy individuals; sample 3 and 4: serum from the diabetics. [b] Reference values are cited from those obtained with a Hitachi 7020 Automatic Analyzer in Shandong Normal University Hospital.

Conclusions

In the present work, a new type of micro-biosensor applied in CE-ECD had been developed for the determination of glucose in human serum. Compared with the copper electrode which has been commonly used to determine the carbohydrate, the micro-biosensor reported in this paper has higher sensitivity, anti-interference ability to high voltage, and reproducibility. This method had been successfully applied to the determination of glucose in human serum. It was proposed that this method could be developed into a useful method for the analysis of blood sugar. In order to increase the kinds of substances being detected and to improve the sensitivity of determination, the study of novel chemical modified electrodes and micro-biosensors will be one of hot fields in CE-ECD.

Acknowledgements

Financial support from Natural Science Foundation of Shandong Province (No. Y2007B28) and Shandong Postdoctoral Science Foundation (200801007) are gratefully acknowledged.

References

1 N. P. Evmiridis, N. K. Thanasoulias and A. G. Vlessidis, *Anal. Chim. Acta*, 1999, **398**, 191.
2 Y. Lv, Z. Zhang and F. Chen, *Talanta*, 2003, **59**, 571.
3 X. Liu, W. Niu, H. Lia, S. Han, L. Hua and G. Xu, *Electrochem. Commun.*, 2008, **10**, 1250.
4 S. A. G. Evans, J. M. Elliott, L. M. Andrews, P. N. Bartlett, P. J. Doyle and G. Denault, *Anal. Chem.*, 2002, **74**, 1322.
5 X. L. Luo., J. J. Xu, Y. Du and H. Y. Chen, *Anal. Biochem.*, 2004, **334**, 284.

6 A. E. G. Cass, G. Davis, G. D. Francis, H. A. O. Hill, W. Aston, I. J. Higgins, E. V. Plotkin, L. D. L. Scott and A. B. Turner, *Anal. Chem.*, 1984, **56**, 667.
7 T. Ikeda, H. Hamada, K. Miki and M. Senda, *Agric. Biol. Chem.*, 1985, **49**, 541.
8 J. Losada and M. R. G. Armada, *Electroanalysis*, 1997, **9**, 1416.
9 Y. Xiao, F. Patolsky, E. Katz, J. F. Hainfeld and I. Willner, *Science*, 2003, **299**, 1877.
10 F. Patolsky, Y. Weizmann and I. Willner, *Angew. Chem., Int. Ed.*, 2004, **43**, 2113.
11 Z. Jin, R. Chen and L. A. Colon, *Anal. Chem.*, 1997, **69**, 1326.
12 Y. Lv, Z. Zhang and F. Chen, *Talanta*, 2003, **59**, 571.
13 Y. H. Cao, Y. W, X. L. Chen and J. N. Ye, *Food Chem.*, 2004, **86**, 131.
14 H. L. Lee and S. C. Chen, *Talanta*, 2004, **64**, 750.
15 R. Wilke and S. Büttgenbach, *Biosens. Bioelectron.*, 2003, **19**, 149.
16 Y. Du, J. L. Yan, W. H. Zhou, X. Y. Yang and E. K. Wang, *Electrophoresis*, 2004, **25**, 3853.
17 H. L. Lee and S. C. Chen, *Talanta*, 2004, **64**, 210.
18 Y. Zhang, H. B. Zhong, C. C. Cui and X. L. Wang, *Chem. Bull.*, 2009, **72**, 1116.
19 C. Niu, E. K. Sichel, R. Hoch, D. Moy and H. Tennent, *Appl. Phys. Lett.*, 1997, **70**, 1480.
20 Z. E Zhang, H. Y. Liu and J. Q. Deng, *Anal. Chem.*, 1996, **68**, 1632.
21 R. Fernandes, L. Q. Wu, T. H. Chen, H. Yi, G. W. Rubloff, R. Ghodssi, W. E. Bentley and G. F. Payne, *Langmuir*, 2003, **19**, 4058.
22 C. Malitesta, F. Palmisano, L. Torsi and P. G. Zambonin, *Anal. Chem.*, 1990, **62**, 2735.
23 M. J. O'Connell, P. Boul, L. M. Ericson, C. Huffman, Y. Wang, E. Haroz, C. Kuper, J. Tour, K. D. Ausman and R. E. Smalley, *Chem. Phys. Lett.*, 2001, **342**, 265.
24 J. Wang, M. P. Chatrathi and G. E. Collins, *Anal. Chim. Acta*, 2007, **585**, 11.
25 A. Guerrieri, G. E. Benedetto, F. Palmisano and P. G. Zambonin, *Biosens. Bioelectron.*, 1998, **13**, 103–112.
26 A. Guerrieri, V. Lattanzio, F. Palmisano and P. G. Zambonina, *Biosens. Bioelectron.*, 2006, **21**, 1710–1718.
27 S. Hrapovic and J. H. T. Luong, *Anal. Chem.*, 2003, **75**, 3308.
28 S. Hrspovic, Y. L. Liu, K. B. Male and J. H. T. Luong, *Anal. Chem.*, 2004, **76**, 1083.
29 J. T. Irving, *Biochem. J.*, 1926, **20**, 1320.