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Sensitive measurement of polyols in urine by capillary zone electrophoresis coupled with amperometric detection using on-column complexation with borate

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ABSTRACT

Little is known about human polyol metabolism, but recent studies indicate that abnormal polyol concentrations in body fluids are related to several diseases. In this study, a rapid and sensitive method for the determination of seven major polyols in urine including two groups of polyol isomers, C5-polyols (Rib + Arb + Xyl) and C6-polyols (Sor + Gal + Man), was developed using capillary zone electrophoresis coupled with amperometric detection (CZE-AD). The effects of the working electrode potential, pH, running buffer components and concentrations, separation voltage and injection times were investigated. Under the optimised conditions, seven types of polyols could be perfectly separated via the formation of anionic polyol–borate complexes in a borate buffer solution. Highly linear current responses to the polyol concentrations were obtained with good correlation ($0.9984 < R^2 < 0.9997$), and the limits of detection (LODs) ranged from 1.33×10^{-6} to 5.8×10^{-7} mol L^{-1} (S/N = 3). The proposed method has been successfully used to detect polyols in urinary samples from healthy subjects and diabetes patients, demonstrating accurate and reliable results. This method has potential applications in the recognition of inborn errors affecting polyol metabolism.

1. Introduction

Polyols, commonly termed sugar alcohols, are formed when a sugar carbonyl (aldehyde or ketone) is reduced to the corresponding primary or secondary hydroxyl group. They have characteristics similar to the corresponding reducing sugars but metabolise much more slowly. Polyols are widely used to improve the nutritional profile of food products due to their health-promoting properties, such as lower caloric content, noncarcinogenicity, low glycemic index (GI) and low insulin response [1,2]. Polyols are also found many applications in pharmaceuticals, chemicals production, oral or personal care and animal nutrition [3].

Polyols are also present in almost every living organism; however, knowledge of their metabolism and function is very limited and their potential importance in human disease has only been investigated in the last decade [4]. Currently, diabetes mellitus (DM) is a potentially global problem. Sorbitol (Sor) has previously been suggested as a biomarker for chronic diabetic complications, such as retinopathy, neuropathy, nephropathy, and microvascular damage, and even renal failure [5,6]. Aldose reductase (AR) catalyses the initial reaction of the polyol pathway, increasing the conversion of glucose to Sor [7]. The accumulation of Sor causes osmotic stress, which allows the influx of excessive sodium and water, causing nerve degeneration. Several inborn errors of metabolism (IEMs) with abnormal polyol concentrations in body fluids are known to date. In the neonatal period, when the neonate suffered from transaldolase (TALDO) deficiency in the pentose phosphate pathway (PPP), its ribitol (Rib), arabitol (Arb) and erythritol (Ery) concentrations in urine were elevated [8,9]. This defect is associated with liver disease symptoms, whereas other organs are affected to various degrees [10,11]. Classic galactosaemia is caused by the defective functioning of galactose-1-phosphate uridyltransferase (GALT) in galactose metabolism [12]. Thus, the reduction of galactose to galactitol (Gal) has emerged as an important alternative metabolic pathway for galactose disposition, which may severely affect the central nervous system (CNS), liver, kidneys and eyes. Other polyol metabolites that can also represent important clinical target(s) for the early detection of in-born errors of metabolism (IEMs) associated with hyperglycaemia [13] and ribose-5-phosphate isomerise (RPI) deficiency [14,15], among other disorders. Thus, their simultaneous determination is desirable when investigating the biochemical basis and aetiology of neurological disorders.

The quantification of polyols in body fluids is difficult because of the wide variety of carbohydrate constituents and their isomeric structures. Therefore, the simultaneous separation and detection

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of polyols is an interesting and challenging topic in analytical biochemistry. At present, several analytical methods have been proposed to quantify polyols, including enzymatic assays [16], GC–MS [17–19], LC–MS [20,21], CE-UV [22,23] and HPLC-PAD [5,24]. GC–MS was the most widely used method for the analysis of polyols to diagnose inborn errors in biological fluids. In 2004, Schadewaldt et al. developed a stable isotope dilution GC–MS method for the diagnosis of galactosemia, and it revealed that, compared with healthy controls, galactosemic patients had elevated levels of urinary galactitol [25]. Meanwhile, Wamelink et al. have reported an LC–MS–MS method to detect polyols and established age-related reference ranges for polyols in urine [20]. Recently, Britz-McKibbin group have also introduced a method for the direct analysis of sugar alcohols using 3-nitrophenylboronic acid (NPBA) as an electrokinetic probe in CE-UV detection [23,26]. The designed boronic acids probes can be applied for early detection of galactosemia, as well as other disorders of polyol metabolism. However, most determination methods are only able to detect one individual polyol or two polyols which are structural isomers. What's more, due to the lack of suitable chromophores or fluorophores in the polyol molecules for photometric and fluorometric detection, a pre- or post-column derivatisation is sometimes employed prior to analysis for enhancing the sensitivity [21]. Apart from this, most of the above-mentioned analytical methods, such as MS, require expensive instruments and complicated derivatisation procedures prior to analysis. UV detection entails a simple operation but suffers from a high detection limit.

On the contrary, capillary zone electrophoresis (CZE) has been increasingly recognised as an important analytical separation technique due to its easy operation, ultra-small sample volume, good reproducibility and high separation efficiency. As mentioned above, several reports have focused on polyols analysis using CZE [22,23]. In addition, amperometric detection (AD) has the advantages of low cost, free derivatisation, good selectivity and high sensitivity. AD allows the direct detection of sugar alcohols and polyols with strongly alkaline electrolytes. The pKa values of neutral sugar alcohols are high (between 12 and 14); thus, strongly alkaline electrolyte solutions (pH > 12) were usually used to negatively charge carbohydrates and promote their migration towards the anode. Thus, CZE-AD is a promising technique for the separation and determination of polyols.

Borate buffer, which exhibits high electroosmotic flow in capillary, is better than other media for the separation of sugar molecules. Boric acid/borate is known to react with neutral polyol compounds containing a 1,2-diol group, generating anionic complexes at high pH [27]. In addition, the stability of the complexes depends on the structure of the carbohydrates, the number of hydroxyl groups, and the presence of substituents. Recently, borate complexation to 1,2-diol biomolecules has received attention for such applications as sensing, separation, and self-assembly [28,29]. Many studies show that borate buffers can be used to induce the separation of neutral polyols at moderate pH in background electrolyte (BGE), enabling the effective stereoselective resolution of sugar alcohols and sugars [23,30,31].

In this study, we aimed to develop a CZE-AD method for the simultaneous separation and quantitative profiling detection of seven types of major polyols in urinary sample matrices. Ideally, this method would be simple, sensitive, non-invasive and applicable for the diagnosis of inborn errors affecting polyol metabolism. Borate buffer was used to alter the selectivity for the formation of anionic polyol–borate complexes, enabling the separation of seven polyols, including two groups of different isomers, C5-polyols (Rib + Arb + Xyl) and C6-polyols (Sor + Gal + Man). Using optimised conditions, the proposed method was successfully applied to the analysis of polyols in urine samples from healthy subjects and DM patients. The results proved that this method was simple and economical for the analysis of polyols.

2. Materials and methods

2.1. Apparatus and chemicals

Our CZE-AD system, built in-house, was similar to an earlier system [32]. A high-voltage power supply (0 to ±30 kV, Shanghai Institute of Nuclear Research, Shanghai, China) was used to provide the required voltage. All electrochemistry experiments were performed using a CHI 660C electrochemical workstation (Shanghai Chenhua Instrument Co., Ltd., Shanghai, China). The fused-silica capillaries used for separations had a 25 μm inner diameter (i.d.), 360 μm outer diameter (o.d.), and 70 cm length (Yongnian Optical Fiber Factory, Hebei, China). In the CZE-AD system, the three-electrode system consisted of a copper-disk (300 μm i.d.) working electrode, a saturated calomel reference electrode (SCE), and a platinum wire counter electrode. The pH was monitored using a PHS-3C Acidometer (Shanghai Jicheng Instrument Factory, China). The creatinine was detected via Automatic biochemical analyser (Hitachi 7100, Japan). The copper-disk electrode was prepared exactly the same in our former work [33].

Analytical reagent-grade chemicals (at least 99% purity) were used unless otherwise stated. The polyols, including xylitol (Xyl), Sor, Arb, Rib, mannitol (Man), were obtained from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). Ery and Gal were purchased from Alfa Aesar (Johnson Matthey, Tianjin, China). The structures of the seven types of polyols were shown in Fig. 1. The organic additives sodium dodecyl sulfonate (SDS), poly(ethylene oxide) (PEO) and poly(sodium-p-styrenesulfonate) (PSS) were obtained from Aldrich (Milwaukee, WI, USA). Sodium tetraborate ($Na_2B_4O_7{\cdot}10H_2O$), sodium hydroxide (NaOH), hydrochloric acid (HCl), methanol, ethanol, acetonitrile and all other chemicals were purchased from Shanghai First Reagent Factory (Shanghai, China). Deionised water was prepared using a Milli-Q water purification system (Millipore, Milford, MA).

2.2. Preparation of buffer solutions and sample stock solutions for CZE separation

A borate buffer stock solution (0.2 mol L^{-1}, pH = 9.2) was prepared by dissolving sodium tetraborate in deionised water. Different concentrations of the borate buffer were diluted with deionised water and then titrated with 1.0 mol L^{-1} NaOH solutions to obtain the diluted borate buffer with different pH values. Individual polyol stock solutions (5×10^{-2} mol L^{-1}) were prepared in deionised water. In the CZE-AD experiments, these stock solutions were diluted by running buffer. Before the experiments, all solutions were filtered through a 0.22 μm polypropylene Acrodisc syringe filter (Xinya Purification Instrument Factory, Shanghai, China) and sonicated for 5 min to remove bubbles.

2.3. Preparation of urine samples

Following an overnight fasted, 12 control samples from apparently healthy persons and 16 samples from DM patients with diet therapy or drug therapy of different ages and sexes were collected from Hospital (Shanghai Sixth People's Hospital and Putuo District Center Hospital, Shanghai, China). Next, urine samples were centrifuged at 2000 rpm for 15 min to separate the deposits and obtain the supernatant. These samples were stored at −20 °C until analysis. A 200 μL aliquot of the supernatant was diluted with 400 μL of acetonitrile and vortexed thoroughly for 5 min to precipitate out the proteins [34]. After centrifuging at 8000 rpm for 15 min, the supernatant was transferred into a 1.5 mL vial and the acetonitrile

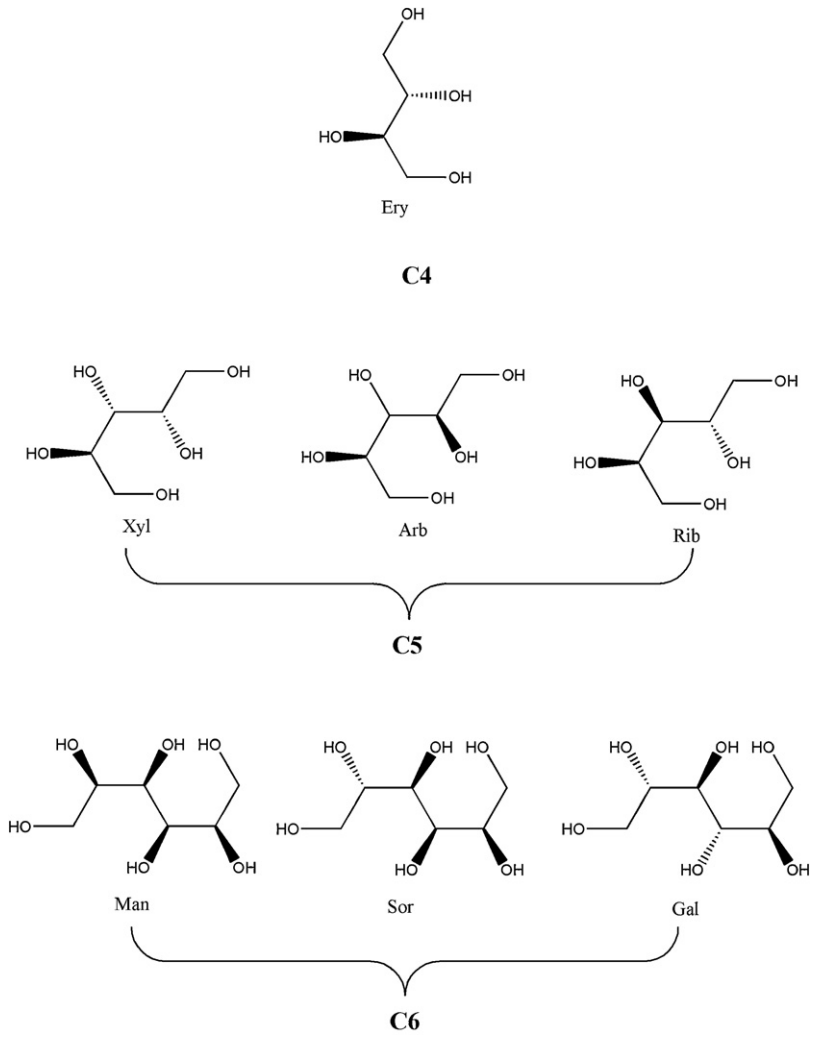

Fig. 1. Molecular structures of the seven types of polyols: C4: Erythritol (Ery); C5: Xylitol (Xyl), Arbaitol (Arb), Ribitol (Rib); C6: Mannitol (Man), Sorbitol (Sor), Galactitol (Gal).

was dried with a N_2 stream. Given the trace levels of polyols in the real samples, the remaining solution could be directly injected into the CZE-AD analysis system without dilution. The supernatant was stored at −20 °C until injection.

2.4. Capillary zone electrophoresis conditions

Prior to the CZE experiments, the components of the three-electrode system were placed in the corresponding holes of the electrochemical reservoir and the copper-disk electrode was positioned directly opposite the capillary outlet, as close as possible, using a three-dimensional locator. The surface of the copper-disk electrode had first been polished with emery sand paper and then sonicated in deionised water for 3–5 min. Each new capillary was first conditioned by flushing with deionised water, 0.1 M HCl, deionised water, 0.1 M NaOH, deionised water successively for 10 min, followed by 15 min of flushing with the BGE. Before each injection, the capillary was washed for 2 min with NaOH, 2 min with deionised water and 5 min with CZE running buffer to obtain a reproducible electroosmotic flow (EOF). All experiments were carried out at ambient temperature. Currents were allowed to reach a stable baseline prior to amperometric monitoring.

3. Results and discussion

3.1. Optimisation of the CZE-AD conditions

3.1.1. Effect of the potentials applied to the working electrode

Cyclic voltammetry (CV) was employed to investigate the electrochemical characteristics of sugar alcohols and polyols in different buffer systems, including NaOH, $Na_2B_4O_7$ and $Na_2B_4O_7$–NaOH, at a copper-disk electrode to identify the best electrochemical response. The potential applied to the working electrode directly affected the method LOD, sensitivity and stability. The results show anodic peaks at approximately +0.60 V (vs. SCE) for all of the polyols in NaOH and $Na_2B_4O_7$–NaOH (pH > 12) buffer solution. As polyols can be oxidised at relatively moderate potentials, AD was employed in the subsequent CZE separation. Hydrodynamic voltammograms (HDVs) were used to measure the electroactive compounds, Ery, Xyl, Sor, Arb, Rib, Man and Gal, under the optimised conditions. As illustrated in Fig. 2, when the applied potential exceeded +0.55 V (vs. SCE), the peak currents of all of the analytes increased rapidly. When the potential applied to the working electrode exceeded +0.80 V (vs. SCE), the baseline noise and background current increased dramatically, reducing the sensitivity and stability of the detection. To obtain the highest S/N ratio and maintain the stability of the baseline, +0.75 V (vs. SCE)

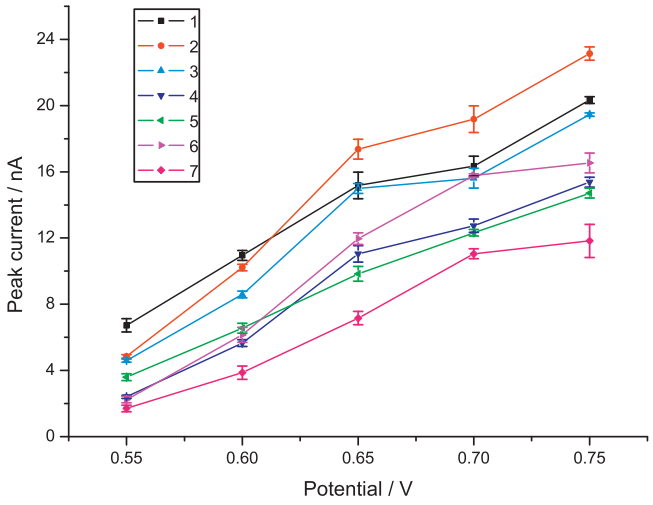

Fig. 2. Hydrodynamic voltammograms for the polyols. Sample concentrations were 5×10^{-4} mol L^{-1} (1) Ery, (2) Xyl, (3) Sor, (4) Arb, (5) Rib, (6) Man and (7) Gal. Experimental conditions: 25 μm i.d. × 70 cm fused-silica capillary, running buffer of 70 mmol L^{-1} borate buffer (pH = 9.2), 17 kV separation voltage and 10 s electrokinetic injection at 17 kV. Error bars: ±SD, $n = 3$.

was selected as the optimum detection potential for the following experiments.

3.1.2. Effect of the running buffer components, concentrations and pH

In CZE, the optimisation of the buffer composition is crucial in the method development. Meanwhile, strongly alkaline electrolytes (pH > 12) are often used to partially ionise native sugar alcohols and sugars that are weakly acidic (e.g., D-sorbitol, $pKa_1 \approx 13.6$) with electrochemical methods [35]. Thus, NaOH solution was investigated as a potential running buffer in the CZE separation. But the resolutions were very poor, as shown in Fig. 3A. As the concentration of the NaOH solution increased from 30 to 120 mmol L^{-1}, there were no evident improvements for the resolution. However, high concentrations of NaOH (>100 mmol L^{-1}) increased the baseline noise and background current due to Joule heating. We also tried using additives in the running buffer to improve the resolution, including organic solvents (methanol, ethanol, and acetonitrile), surfactant SDS and polymer molecules (PEO, PSS). The results showed that both acetonitrile and SDS improved the resolution of the isomers but greatly reduced the detection sensitivity due to the adsorption of the additives onto the working electrode.

The borate buffer system has previously been recognised as a suitable medium for sugar alcohol separation. By adding borate to the BGE, polyols could be transformed into negatively charged polyol–borate complexes in alkaline media, improving the selectivity and resolution of polyol stereoisomers with different vicinal polyol chain lengths, even those with similar binding affinities, due to the differences in the mobility of their complexes in CZE. Polyol–borate complexes migrate toward the anode because they are negatively charged under the specific conditions utilised in this experiment.

The BGE pH affects the peak current and migration time of the analytes. The pH of the borate buffer was investigated in the range of 9.2–12.7. Fig. 3B–D shows the electrophoresis behaviours of the seven polyols at different pH values. When the BGE pH exceeded 12, the baseline was stable but baseline separation of the isomers, C5-polyols (Rib + Arb + Xyl) and C6-polyols (Sor + Gal + Man), was not achieved, as shown in Fig. 3B and C. In addition, when the pH of the running buffer solution was below 12, the same concentration of NaOH solution was loaded in the detection reservoir to keep the polyols at ionised states, as mentioned above. However, baseline noise was too strong to ignore and the electroosmotic flow (EOF) was too high to control. This phenomenon may be caused by the NaOH solution in the detection reservoir flowing back into the capillary based on the "like dissolves like" concept. Therefore, borate solution systems were used directly, without pH adjustment in the following experiments.

As the borate concentration increased from 30 to 120 mmol L^{-1}, the resolution improved and the EOF decreased, this prolonged the migration time (cf. Figs. 3D and 4A). Fortunately, these results have also shown that all of the polyols achieved baseline separation under mild conditions using 70 mmol L^{-1} borate buffers without any additives. In contrast, higher buffer concentrations (>100 mM) improved the resolution by increasing the background current and the generation of Joule heat.

Based on a comprehensive consideration of the effects of the peak current, resolution, buffer capacity and analysis time, 70 mmol L^{-1} borate buffer (pH = 9.2) was chosen as the running buffer and was also suitable for achieving a stable EOF with the same concentration of the NaOH solution (pH = 13.3) in the detection reservoir for electrochemical detection. Our chosen conditions were fairly mild in comparison to those used in the studies of Chen and Huang [36] and Pospíšilová and Liu et al. [22,30], who used higher concentrations of running buffer (250 mmol L^{-1} NaOH and 200 mmol L^{-1} borate, respectively) for the separation of all isomers, which could cause significant baseline noise due to the strong Joule heating.

3.1.3. Effect of separation voltage and sampling time

For a given capillary length, the separation voltage determines the electric field strength, which affects both the velocity of EOF and the migration velocity of the analytes. The effect of the separation voltage on the migration time of the analytes was investigated for voltages of 12, 15, 17 and 20 kV. As expected, higher separation voltages led to shorter migration times for all analytes. However, when the separation voltage exceeded 20 kV, the baseline noise increased. Based on these results, 17 kV was selected as the optimum separation voltage, providing good separation for all analytes within approximately 30 min.

Samples were injected into the capillary in electrokinetic mode. The effect of injection time on peak current of the analytes was also studied by varying injection time from 2 to 14 s at a voltage of 17 kV. The experimental results show that the peak current increased with increasing sampling time. When the injection time was longer than 12 s, the peak currents of the analytes nearly levelled off and peak broadening became more severe. Based on these results, 10 s (at 17 kV) was selected as the optimum injection time.

Based on the results described above, the optimal conditions for the CZE-AD determination the seven polyols were as follows: detection potential +0.75 V (vs. SCE), separation voltage 17 kV, electrokinetic injection time 10 s (at 17 kV), 70 mmol L^{-1} borate (pH = 9.2) as the running buffer and the same concentration of NaOH solution (pH = 13.3) in the detection reservoir. Fig. 4A shows the electropherograms of a standard mixture solution of the seven targets polyols (Ery, Rib, Arb, Xyl, Sor, Gal and Man: 5×10^{-4} mol L^{-1}) obtained using the optimised conditions at a copper-disk electrode. A very good baseline separation was achieved for all of the analytes within approximately 30 min.

3.2. Method validation

To determine the linearity of the Sor, Ery, Xyl, Gal, Arb, Man and Rib detection using CZE-AD, a series of mixed standard solutions of the analytes over the concentration range of 2.0×10^{-6}–5.0×10^{-4} mol L^{-1} were tested. The correlation between the peak current and analyte concentration was

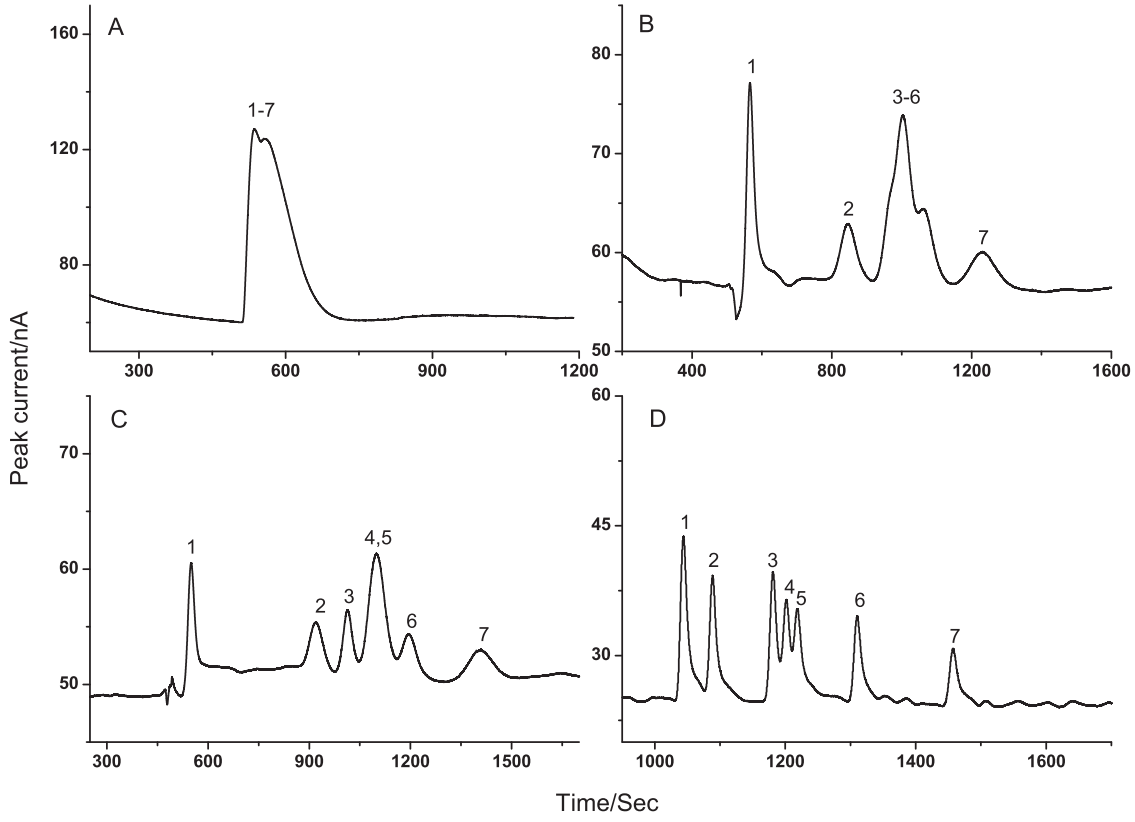

Fig. 3. CZE-AD electropherograms of polyols under different BGE conditions: (A) 70 mmol L^{-1} NaOH (pH = 13.3), (B) 60 mmol L^{-1} $Na_2B_4O_7$–NaOH (pH = 12.7), (C) 75 mmol L^{-1} $Na_2B_4O_7$–NaOH (pH = 12.4), (D) 60 mmol L^{-1} $Na_2B_4O_7$ (pH = 9.2). Sample concentrations were 5×10^{-4} mol L^{-1} (1) Ery, (2) Xyl, (3) Sor, (4) Arb, (5) Rib, (6) Man and (7) Gal. Experimental conditions: Detection potential of +0.75 V (vs. SCE), separation voltage of 17 kV and 10 s electrokinetic injection.

subjected to regression analysis to obtain the calibration equations and correlation coefficients (R^2). Table 1 summarises the linearity ranges, regression equations, R^2, LODs and relative standard deviations (RSDs) of the peak currents value. Good linearity relationships of the polyols ($0.9984 < R^2 < 0.9997$) were obtained. The regression analysis revealed that the CZE-AD results exhibited an excellent correlation for concentrations ranging from 2.0×10^{-6} to 5×10^{-4} mol L^{-1}. The LODs were calculated according to the $3\,sb/m$ criterion, where m is the slope of the calibration curve and sb is the standard deviation [37]. As listed in Table 1, the LODs of Ery, Xyl, Sor, Arb, Rib, Man and Gal were 8.0×10^{-7}, 6.6×10^{-7}, 8.3×10^{-7}, 1.33×10^{-6}, 7.3×10^{-7}, 5.8×10^{-7} and 8.9×10^{-7} mol L^{-1} (S/N = 3), respectively, and the sensitivity of the polyol determination was comparable with that of GC–MS [19] and higher than that of CE-UV [22,23]. After three repetitions of the analysis under the same conditions, the peak signals decreased only slightly. The RSDs of both the migration time and peak current were also used as measures of precision and reproducibility. The RSDs of the migration time and peak current were in the range of 3.9–6.2% and 3.5–6.0%, as listed in Table 1, indicating good reproducibility and high precision. To determine the accuracy of the method, its recovery for urinary samples was also assessed by spiking standard solutions with two concentrations: 300 (or 250) and 500 μM ($n = 3$) and the data listed in Table S1 (supplementary information). As shown in Table S1, the recovery and RSD range of polyols were 82.9–123.4% and 1.5–12.6%, it is indicated that this method was sufficiently accurate for the simultaneous determination of polyols in urine.

3.3. Application to practical sample matrices

Under the optimised experimental conditions, the proposed method was successfully used to analyse the polyols in the urine

Table 1
Regression equations and the limits of detection for polyols.

Polyols	Concentration range (μM)	Regression equation[a]	Correlation coefficient (R^2)	LOD[b] (μM)	RSD (%)[c]	
					Migration time	Peak current
Ery	2–500	$Y = 42.5X$	0.9989	0.80	4.52	3.71
Xyl	2–500	$Y = 49.6X - 0.105$	0.9990	0.66	6.16	4.20
Sor	2–500	$Y = 41.9X + 0.007$	0.9984	0.83	3.94	3.49
Arb	2–500	$Y = 33.0X - 0.127$	0.9985	1.33	5.72	5.97
Rib	2–500	$Y = 28.2X + 0.507$	0.9996	0.73	4.30	2.66
Man	2–500	$Y = 30.1X + 0.260$	0.9992	0.58	3.87	3.76
Gal	2–500	$Y = 19.4X + 0.287$	0.9997	0.89	5.22	3.94

[a] In the regression equation, the X value was the concentration of analytes ($\times 10^3$ mol L^{-1}), the Y value was the peak current (nA).
[b] Detection limit according to the $3\,sb/m$ criterion, m is slope of the calibration curve and sb the standard deviation.
[c] Three injections in the same capillary (50 μM of each polyols), and CZE/AD conditions were the same as those in Fig. 4.

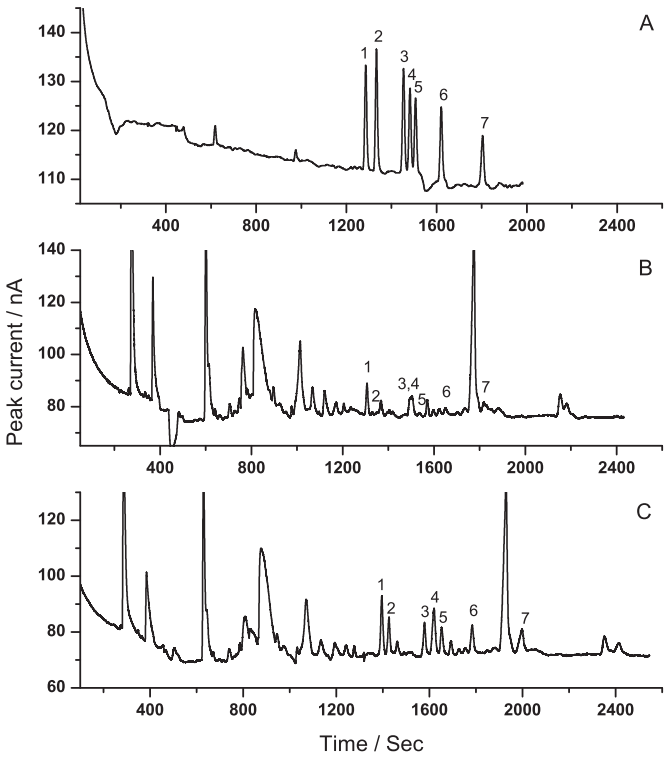

Fig. 4. Electropherogram of a standard solution A containing 5×10^{-4} mol L^{-1} of (1) Ery, (2) Xyl, (3) Sor, (4) Arb, (5) Rib, (6) Man and (7) Gal. Typical electropherograms for urine B and spiked C samples from healthy volunteer No. 1 obtained using CZE-AD. Experimental conditions: Detection potential of +0.75 V (vs. SCE), 25 μm i.d. × 70 cm fused-silica capillary, running buffer of 70 mmol L^{-1} borate buffer (pH = 9.2), separation voltage 17 kV and 10 s electrokinetic injection.

of healthy subjects and DM patients. Compared to traditional techniques, the sample preparation for this method was considerably shorter, less laborious and more cost-efficient and required 200 μL of urine at most, as mentioned in Section 2.3.

Fingerprint electropherogram of urine sample from a healthy subject was shown in Fig. 4B and C and the electropherogram of the DM patient urine sample shown in Fig. S1 (supplementary information). The seven polyols were well separated from the other components in the urine. Because polyols do not contain functional groups, they cannot be selectively isolated from many substrates, such as organic acids, amino acids, amines and carbohydrates, which also have electrochemical activity on copper electrode under strongly alkaline electrolyte solutions. Hence, possible interference of the corresponding reducing sugars was also measured, indicating that their migration times are distinctly different from those of polyols (data not shown).

Fig. 4B and C, respectively, displayed typical electropherograms for the urine and spiked samples. By comparing the migration time of analytes with the electropherogram of the standard solution (Fig. 4A), it can be seen that the migration time of some analytes is varied, so as to ensure polyols in the urinary samples, standard addition method is used for further detection. Using a standard addition method, seven polyols could be determined in practical sample matrices, indicating that the LODs of this method were sufficiently sensitive to determine the low concentrations of polyols found in urine. The contents of polyols in healthy subjects and DM patients were summarized and two tailed test (*t*-test) was also applied for further data processing in Table S2 (supplementary information). There was no great difference in Sor content between the healthy subjects and DM patients via diet therapy or drug therapy. With regard to the average contents of other polyols, such as Arb, Man and Gal (mean ± SD: 27.3 ± 10.0, 28.8 ± 11.8, 20.1 ± 7.2 μmol/mmol creatinine), they have distinctly elevated compared with DM patients (mean ± SD: 69.3 ± 51.1, 85.6 ± 46.7, 111.8 ± 102.7 μmol/mmol creatinine, $p < 0.05$), while for other polyols, they have no significant difference with healthy subjects. $p < 0.05$ was considered statistically significant. Therefore, this method can provide an effective way for diagnosis of IEMs with abnormal polyols concentrations in the future.

4. Conclusions

A rapid, sensitive and non-invasive method has been developed for the simultaneous quantisation of seven major polyols in human urine using CZE-AD and can therefore provide important information for the screening, quantification and diagnosis of inborn errors affecting polyol metabolism. Because the BGE used for the examined polyols do not contain any derivatisation or additives, borate complexation must occur with the native form of the polyols. As all the isomers can be well separated under mild conditions for the polyol–borate complex formation, more detailed information about the concentration of different polyols can be obtained. Although some chromatographic methods can obtain lower LODs, they require sophisticated and expensive instrumentation or complicated sample pre-treatment and/or pre-column derivatisation. In contrast, our proposed method is superior in terms of the number and type of polyols examined simultaneously. Given the finite number of random samples, the obtained statistical data were insufficient for diagnosing metabolic diseases. Further work in progress will optimise the sample size and extend the analytical application of this method to human plasma and CSF samples and, eventually, the quantitative determination of numerous other sugar alcohols and polyols.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.jchromb.2012.12.017.

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