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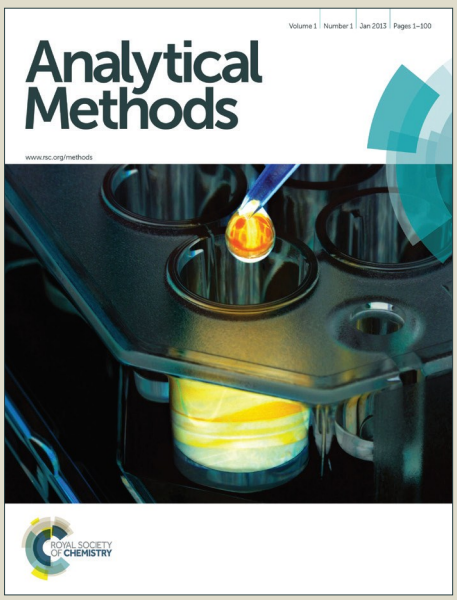

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ARTICLE

A Flexible Ion Chromatography Column-Switching System with a Switching Time Window (STW) Calibration Program for the Determination of Myo-Inositol in Infant Formula by Pulsed Amperometric Detection

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Shuifeng Zhang,[a] Liying Huang,[a] Hongyan Li,[a] Xiaozhen Chen,*[a] Fengli Wang,[b] Donglei Zhang[a] and Yan Zhu**[b]

This study introduced a flexible ion chromatographic column-switching technique for the direct analysis of myo-inositol in infant formula samples by pulsed amperometric detection. The system, based on two pumps, two columns and three six-pore valves, allowed for free automatic switching between the switching time window (STW) calibration mode and analysis mode. Chromatographic parameters, including the chromatographic columns, mobile phases, elution gradient, column-switching parameters, detector waveform parameters, *etc*., were optimized systematically for the analysis of myo-inositol in infant formula. In this method, both the pretreatment column and analytical column were arranged to activate and regenerate during their idle time by optimizing the switching program of the three valves, thus increasing the efficiency of the method. The detection potential, E1, of the quadruple-potential waveform was also optimized and the detection potential was set to 0.027 V, which offered both excellent sensitivity and a stable baseline. Applying this optimized method to real samples, the determination of myo-inositol in infant formula was achieved in only 20 minutes, with a linear dynamic range from 0.05 μg mL^{-1} to 120 μg mL^{-1}, and the limit of detection (LOD) (*S*/*N*=3) of 2.5 μg g^{-1}. The results demonstrate the feasibility for routine analysis of myo-inostiol in infant formula with the advantages of automation, simple pretreatment, low cost, good stability, and no use of organic reagents.

Introduction

Myo-inositol (Fig. 1), chemical name cyclohexanhexol, primarily found in the plasma, muscle, brain and myocardium of human body, promotes cell growth and prevents cell aging. Myo-inositol is also required to sustain cellular nutrition and physiological function, especially for liver and bone marrow cell growth. At present, myo-inositol is a daily essential nutrient additive in infant formula and is an important index of national infant food standards.

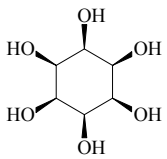

Fig. 1 Structure of myo-inositol.

Several analytical methods for analyzing myo-inositol have been reported, including high performance liquid chromatography (HPLC),[1] ion chromatography (IC),[2,3] microbial assay,[4] gas chromatography (GC)[4] and GC-mass spectrometry (GC-MS)[5,6] *etc*. The national standard of China provided two solutions that were analyzed via the microbial assay and gas chromatography method.[4] The microbial assay was a time-consuming, tedious and laborious operation. Both GC and GC-MS/MS were effective for analyzing myo-inositol, but these methods required a silylation pretreatment procedure involving the addition of derivatization reagents such as trimethylchlorosilane, hexamethyldisilazane, *N*,*O*-bis(trimethylsilyl)trifluoroacetamide, and so on.[4,5,6] A harsh anhydrous reaction condition was required to ensure the reproducibility and stability of the derivatization reaction.[4,5,6]

Because myo-inositol does not absorb in the UV region, the standard UV-Vis detector on the HPLC could not be used. A differential refractive index detector could potentially overcome

this challenge and be used for determination of sugars by HPLC. However, complex matrices result in poor chromatographic resolution and detector sensitivity.[1]

Ion chromatography coupled to a pulsed amperometric detector[7,8] has become one of the mostly used methods for carbohydrate analysis dues to its high separation capacity, powerful detection sensitivity and no requirement for sample derivatization. In this method, carbohydrates are electrocatalytically oxidized at high pH on the surface of a gold working electrode by applying the appropriate potential. Target compounds can also be selectively detected using this method by applying specific potentials. Therefore, combining ion chromatography with pulsed amperometric detection hold great potential for analyzing myo-inositol.[2,3]

However it can be extremely challenging to extract, purify and analyze analytes of interest in a complex sample matrix. Some matrices are strongly retained on analytical columns and it can be a time consuming task to re-activate and regenerate the column. Additionally, co-elution and baseline elevation from complex samples often cause serious chromatographic problems such as overlapping peaks. In addition, matrices with electrochemical activity can be oxidized at the working electrode and the oxidation products would gradually destroy the electrode surface causing decrease in detection signal. Matrix elimination is necessary to prevent signal loss and overloading.

Column-switching[9,10] is a widely used technique for the elimination of matrix and trace analyte enrichment in chromatographic system due to its good sensitivity, simple sample pretreatment and automated analysis. Column-switching offers unique advantages in the analysis of samples within a complex matrix and enhances the ion chromatography workflow.

There are several column-switching techniques used in IC. A two-pump system has been developed to analyze trace anions in weak acid matrices,[11] and cations matrices with high salt content.[12] Killgore *et al*. proposed a six-step procedure to optimize the "heart-cut" timing parameters for analysis of trace anions.[13] Additionally, some simple, low-cost single-pump systems have been developed. Shi *et al*.[14] reported a column-switching method for the determination trace bromate in high concentration matrices, where a ten-pore valve was used to simplify the system. Zhu *et al*.[15] proposed a method for fast determination of hexafluorophosphate and some inorganic anions by the column-switching technique. Zhong *et al*.[16] developed a single pump column-switching method to analyze trace anions in different organic matrices by ion chromatography using a homemade polystyrene-divinylbenzene-carbon nanotube column to eliminate the organic matrices. Wang *et al*.[17] reported a novel ion chromatography cycling-column-switching system for the determination of low-level anions in seawater, achieving very complete matrix elimination. Huang *et al*.[18] used a similar technique to analyze iodate in edible iodized salt using ion chromatography with UV detection. The previous work reported above were all based on a double-pump or a single-pump column-switching system and primarily focused on the analysis of inorganic ions. For column-switching systems, the retention time of the target analyte is the most important parameter and determines how the switching time window (STW) for the valves will be programmed.

Generally, measurement of this parameter requires the analytical column to be manually removed and the pretreatment column to be connected to the detector, which often makes the procedure rather inconvenient. In single-pump systems, a non-destructive conductivity detector in series seems to be a simple and clever design, allowing for accurate determination STW parameters; however, it is not feasible for the single-pump system to be combined with an electrochemical detector because it is destructive to the analytes.

Additionally, the single-pump system is less efficient, because it takes much longer to regenerate both the pretreatment and analytical columns since the analysis and regeneration would run sequentially, synchronously.

Herein, we expand upon previous work and present a flexible ion chromatography-column switching system for the determination of myo-inositol in infant formula. This system consists of two pumps, three six-pore valves and two ion exchange column. This system offers the flexibility and advantage of automatic switching between the STW calibration procedure and the analysis procedure, without needing to manually remove the analytical column and connect the pretreatment column to the detector. Another advantage is the high efficiency of fast regeneration and re-equilibrium for both the pretreatment column and analytical column during idle time in the analysis procedure, ascribing to the optimized elution program. Optimizing the detection parameters allowed for a fast and sensitive determination of myo-inositol in infant formula.

Experimental

Reagents and materials

Myo-inositol (≥99.0%) was obtained from Sigma-Aldrich (Japan). A 1.0 mg mL^{-1} stock solution of myo-inositol was prepared by dissolving the 100 mg standard in water and diluting to the mark line in a 100 mL volumetric flask. The working standard solutions were prepared by diluting the stock solution with water to the appropriate volume, daily. All infant formula samples were purchased from a local market. All the solutions and samples were stored in the tightly closed containers at 4 ℃. Before injection, all testing solutions were filtered through a 0.2 μm filter (Millipore Millex-LG Hydrophilic PTFE).

Sodium hydroxide solution (50% w/w) was offered by Merk KGaA (Darmstadt, Germany) and sodium acetate (≥99.0%) was purchased from Fisher Scientific (Sunnyval, California, USA). Glacial acetic acid was purchased from Hangzhou Chemical Reagent Co. Ltd. (Hangzhou, China). All the reagents were of analytical grade. The deionized water used was purified with a

Milli-Q Gradient system (Millipore, Molsheim, France) to 18.2 MΩ.

Eluent A-Deionized water. 1 L of deionized water was vigorously sparged with nitrogen for 20 minutes in the eluent bottle and pressurized with nitrogen (3~5 psi).

Eluent B-500 mM Sodium Hydroxide. 1 L of deionized water was vigorously sparged with nitrogen for 20 minutes in the eluent bottle. 40.0 g of 50% NaOH was transferred to the water, and the solution was sparged for 10 minutes and pressurized with nitrogen (3~5 psi).

Eluent C-300 mM Sodium Acetate. 1 L of deionized water was vigorously sparged with nitrogen for 20 minutes in a bottle. 40.8 g of sodium acetate was transferred to the water, stirred for 5 minutes and filtered through a 0.2 μm membrane under vacuum. The solution was transferred to the eluent bottle and pressurized with nitrogen (3~5 psi).

Sample preparation

Approximately 2.0 g (accurate to 0.1 mg) of sample was weighed out into a 50 mL beaker, mixed fully with 25 mL water, sonicated for 10 minutes, and transferred into a 100 mL volumetric flask. 300 μL glacial acetic acid was added to the flask and the sample solution was diluted to the 100 mL mark line, mixed it fully and incubated at 4 ℃ for 30 min. Approximately 1.5 mL of the solution was dispensed into a 2.0 mL polypropylene centrifugal tube and centrifuged for 5 min at 14000 r min^{-1}. The supernatant was removed and filtered through a disposable 0.2 μm filter into a sampler vial in preparation for injection.

Instrumental

Cyclic voltammetry (CV) was performed on a CHI 832 electrochemical work station (CH Instrument Co., Austin, TX, USA) with a conventional three electrode system, which comprised of a gold working electrode (2 mm i.d.), a Pt wire counter electrode and a KCl saturated Ag/AgCl reference electrode.

Ultrasonic extraction was performed using a DS-2510 DTH sonicator (Shanghai Sonxi Ultrasonic Instrument Co., Shanghai, China) and centrifugation was performed with a Biofuge Stratos centrifuge (Thermo Scientific, Osterode, Germany).

All chromatographic column-switching and analyses were performed on a Dionex ICS-5000 system (Sunnyvale, CA, USA) (Fig. 2), equipped with two quaternary pumps, a detector/chromatography module (DC), three six-pore valves and a AS 50 automatic sampler with a 10 μL sample loop. The detector was equipped with a triple-electrode system that includes a gold working electrode, an Ag/AgCl reference electrode and a titanium cell body as the counter electrode.

All columns used in this study were manufactured by Dionex. A CarboPac PA1 separation column (4 mm×250 mm, 10 μm) was used as the pretreatment column in this system in order to pre-eliminate the matrices and pre-concentrate the target analytes. A CarboPac MA1 (4 mm×250 mm, 7.5 μm) separation column with a CarboPac MA1 guard column (4 mm×50 mm, 7.5 μm) was employed for the analysis of myo-inositol.

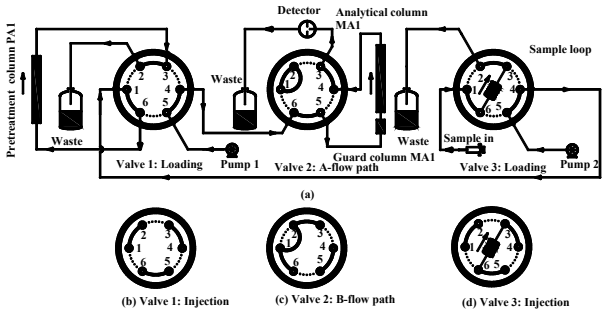

Fig. 2 Instrument configuration for the analysis of myo-inositol in milk powder sample: (a) filling the sample loop; (b-d) the positions of valves 1, 2 and 3; the flow path was shown as the bold line.

Polyether ether ketone (PEEK) tubes were employed to connect all chromatographic hardware. To reduce the void volume, the minimal tubing length was used to connect instrument parts.

Chromeleon 6.80 SR9 software (Dionex, Sunnyvale, CA, USA) was used to acquire the data and control the instrument. A schematic diagram of the chromatography system is presented in Fig. 2.

Table 1 The position of three valves in STW calibration mode

Timing sequence，min	Valve 1	Valve 2	Valve 3
Sampling	Loading	B-flow path	Loading
0.0	Loading		Injection
1.0	Injection		Injection
20.0	Injection		Injection

Table 2 The position of three valves in analysis mode

Timing sequence，min	Valve 1	Valve 2	Valve 3
Sampling	Loading	A-flow path	Loading
0.0	Loading		Injection
3.5	Injection		Injection
5.0	Loading		Injection
20.0	Loading		Injection

The column-switching parameters for myo-inositol

The entire analysis was comprised of two procedures, namely the switching time window (STW) calibration procedure and the analysis procedure. The detailed parameters and programs are listed in Tables 1 and 2, respectively.

Elution program

In the STW calibration procedure, a solution of 80% water (by volume) and 20% 500 mM NaOH was used as the eluent for both columns PA1 and MA1 isocratically, at the flow rate of 0.4 mL min^{-1} (Table 3). In the analysis procedure, the column MA1 was eluted isocratically with 500 mM sodium hydroxide, meanwhile, the column PA1 was eluted with a gradient elution program including three eluents with deionized water, 500 mM NaOH and 300 mM NaAc according to the given ratios in Table 4, with a flow rate of 0.4 mL min^{-1}.

Table 3 Elution program for STW calibration

Pumps	Timing sequence, min	Flow rate mL min^{-1}	Eluents		
			A/Water	B/500 mM NaOH	C/300 mM NaAc
Pump 1 Isocratic elution	0.0~20.0	0.4	80%	20%	0
Pump 2 Isocratic elution	0.0~20.0	0.4	80%	20%	0

Table 4 Elution program for analysis procedure

Pumps	Timing sequence, min	Flow rate, mL min^{-1}	Eluents		
			A/Water	B/500 mM NaOH	C/300 mM NaAc
Pump 1 Isocratic elution	0.0~20.0	0.4	0	100%	0
Pump 2 Gradient elution	0.0	0.4	80%	20%	0
	6.1	0.4	80%	20%	0
	6.2	0.4	0	20%	80%
	12.0	0.4	0	20%	80%
	12.2	0.4	80%	20%	0
	20.0	0.4	80%	20%	0

Detector parameters

The detector parameters for a typical triple-potential waveform[19] and the optimized quadruple-potential waveform are listed in Tables 5 and 6, respectively.

Table 5 Parameters of the triple-potential waveform

Timing sequence, second	Potential, V		Integration Evens
0.00	E1	0.050	Off
0.20		0.050	On
0.40		0.050	On
0.41	E2	0.750	Off
0.60		0.750	Off
0.61	E3	-0.150	Off
1.00		-0.150	Off

Table 6 Parameters of the optimized quadruple-potential waveform

Timing sequence, second	Potential, V		Integration Evens
0.00	E1	0.027	Off
0.20		0.027	On
0.40		0.027	On
0.41	E2	-2.000	Off
0.42		-2.000	Off
0.43	E3	0.600	Off
0.44	E4	-0.100	Off
0.50		-0.100	Off

Results and discussion

Design for the column-switching system

As mentioned above, the STW parameter is very important for setting the column-switching program. With traditional column-switching systems[3] to obtain the retention time parameters on the pretreatment column, the analytical column had to be manually removed and the pretreatment column was connected directly to the detector prior to analysis. By using non-destructive detector, such as the conductivity detector, it was possible to directly obtain the STW parameter online by optimizing the system.[17,20] However, compared to the traditional conductivity detector, it was not feasible for the electrochemical detector to monitor the retention time online for the column-switching system, because it causes analyte degradation. Therefore, obtaining an accurate STW parameter for the analytes of interest conveniently and automatically is of high priority for electrochemical detection method development.

An extra STW calibration program was designed to obtain the STW parameter. As shown in Fig. 2, the column-switching system consisted of two pumps and three valves, allowing the system to freely switch between the STW mode and analysis mode. Thus, the STW mode can be used to conveniently examine and calibrate the STW parameters.

In order to minimize the influence of the void volume, the flow path from the 6-position to the detector needs to be cut accurately to make sure its length not greater than the distance from the 6-position to the guard column MA1 (Fig. 2a). The eluents from the pumps 1 and 2 in the calibration mode were the same as the initial eluent (80% water and 20% 500 mM NaOH) from pump 2 in the analysis mode, and are listed in Tables 3 and 4 respectively, in order to minimize the influence of the eluents on analyte retention time.

The STW calibration procedure of method

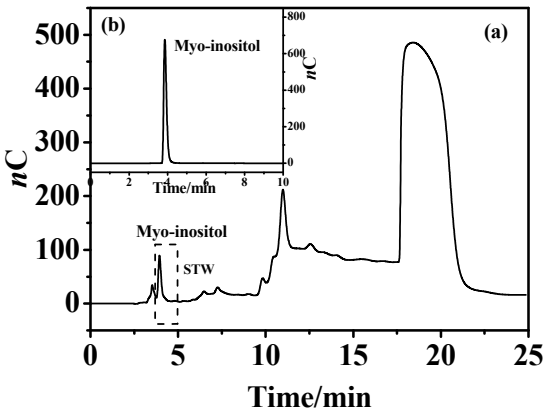

Fig. 3 Chromatograms of the myo-inositol in infant formula sample (a) and standard (b) by column PA1 without column-switching, eluted with 100 mM NaOH at 0.4 mL min^{-1} and the dashed line box shows the STW.

As shown in Fig. 2 and Table 1, the STW calibration procedure was as follows:

First, valve 2 switched to the B-flow path (Fig. 2c), and loaded the sample on the sample loop in the valve 3 prior to analysis, while valve 1 was waiting to receive the sample from the sample loop at the loading position (Fig. 2a).

Second, when the analysis began, valve 3 switched to the injection state (Fig. 2d) and the sample was delivered to the column PA1 by pump 2.

Third, after the sample had been completely loaded on column PA1, valve 1 switched to the injection state (Fig. 2b) until the end of the run.

Two analyses in total were required for standard and real sample to obtain two chromatograms, respectively. By comparing both chromatograms, the STW parameters were obtained (Fig. 3).

The analysis procedure of method

As shown in Fig. 2 and Table 2, after the STW parameters were obtained, the analysis procedure began as follows:

First, valve 2 switched to the A-flow path (Fig. 2a), and the sample was loaded on the 10 μL sample loop via the AS 50 automatic sampler.

Second, valve 3 switched to the injection state (Fig. 2d), and pump 2 delivered the sample from the sample loop to the pretreatment column, PA1. The myo-inositol was pre-separated from the matrices by column PA1. The first portion of the separation was sent to waste. Meanwhile, the analytical column, MA1, was equilibrated by the mobile phase delivered by pump 1.

Third, at the time the myo-inositol was eluting from column PA1, valve 1 switched to the injection state (Fig. 2b), to connect both ends of column PA1 to column MA1 and pump 1, respectively. Here, myo-inositol and its co-elution were eluted from column PA1 into column MA1 by the mobile phase from pump 1. When the analyte of interest had been transferred to column MA1 completely, column PA1 switched back to its original position (Fig. 2a), and continued to be flushed and regenerated by the mobile phase from pump 2.

Finally, the analyte was detected by the electrochemical detector.

It is worth mentioning that, once the target analyte has been eluted from column PA1, the column was switched back to the loading state for clean-up, which reduces the analysis time. Additionally, to increase the efficiency of the analysis, both columns PA1 and MA1 were arranged to be re-activated and regenerated in their idle time.

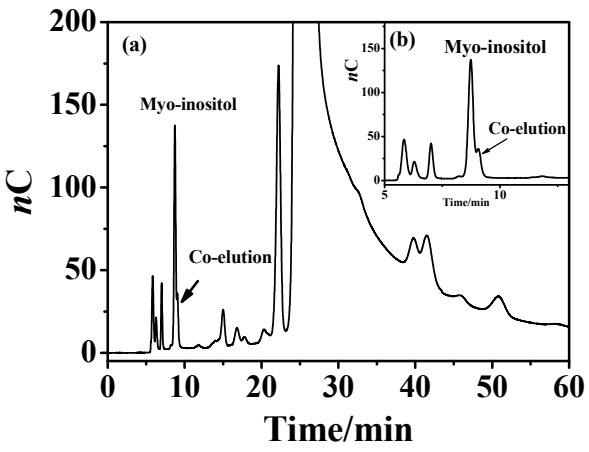

Fig. 4 Chromatograms of myo-inositol in infant formula sample (a) by MA1 without column-switching, eluted with 500 mM NaOH at 0.4 mL min^{-1} and its enlarged view (b).

Selection of chromatographic columns

The anion-exchange capacity of column MA1 was 1450 μeq/column, which is 14.5 times greater than that of column PA1. In terms of retention time of saccharides in column MA1, the retention time of myo-inositol was moderate, while it was too longer for most other components of the matrices, such as monosaccharides, disaccharides, oligosaccharides, *etc* (Fig. 4). Therefore, an extra flush procedure was required to eliminate the matrix compounds with strong retention. Column PA1 was chosen as a pretreatment column in order to quickly separate

myo-inositol from the matrices. Column MA1 with its guard column was chosen as the analytical column for its higher separation efficiency.

According to the manufacturer recommendations, the flow rate for column MA1 was 0.4 mL min^{-1} with an upper pressure limit of 2000 psi (14.0 MPa), and the flow rate recommended for column PA1 was 1.0 mL min^{-1} with an upper pressure limit of 4000 psi (27.9 MPa). For stabilization of the system, the flow rate was set to 0.4 mL min^{-1}.

Optimization of mobile phases

Sodium hydroxide was feasible eluent, providing a strong alkaline environment for the ionization of myo-inositol into the anion, which is required for detection by electrochemical detector.

Infant formula is a complex sample matrix. With pretreatment column PA1, the retention time of myo-inositol was shorter (Fig. 3); therefore, a gradient elution program was necessary to improve the reproducibility of the retention of myo-inositol. As shown in Table 4, during 0.0 min and 6.1 min, an eluent of 100 mM NaOH was used to preliminarily separate the myo-inositol from the matrices and obtain a suitable retention between 3.5 min and 5.0 min (Fig. 3). During 6.1 min and 12.0 min, a matrix clean-up was carried out using a strong eluent of 20% 500 mM NaOH and 80% 300 mM NaAc on column PA1, followed by an equilibration procedure for 8 minutes.

Because of the strong retention of saccharides on analytical column MA1 (Fig. 4), a higher concentration of 500 mM sodium hydroxide was employed as the eluent for the column, MA1. The optimized elution program is shown in Table 4.

Optimization of detector parameters

When the typical triple-potential waveform (Table 5)[19] was used, an obvious decrease in detection sensitivity and reproducibility, as well as a narrower linear dynamic range were observed, due to the use of a positive cleaning potential E2 which promoted electrochemical corrosion of the gold electrode. After one month an obvious recession of the gold working electrode was observed, which increased the detection cell volume and decreased the transport of analyte to the surface of the working electrode thus decreasing the detector response.[21]

In contrast, using the optimized quadruple-potential waveform resulted in great improvement. Compared to the triple-potential waveform, a high negative cleaning potential E2 was employed, which desorbed oxidation products of the carbohydrates from the gold working electrode completely, and also avoided electrode loss by using a positive cleaning potential E2 in the triple-potential waveform. Therefore, this work aimed to optimize the quadruple-potential waveform.

Electrochemical response of myo-inositol at the gold working electrode

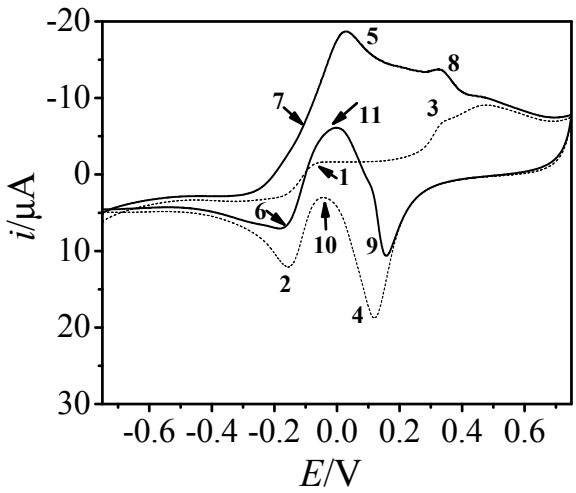

Fig. 5 Cyclic voltammogram of 50 μg mL^{-1} myo-inositol at an Au electrode in 0.1 M NaOH. The dashed line shows the residual response in the absence of myo-inositol. Potential scan rate, 100 mV s^{-1}.

The potential E1 of the detection waveform plays an important role in the sensitivity and lifetime of the working electrode, thus it was important to accurately measure the redox potential of the analytes in order to build the waveform.[22] Cyclic voltammetry was employed to study the electrochemical redox process of myo-inositol. Fig. 5 shows the voltammetric response at an Au electrode in a solution of 0.1 M NaOH with (solid line) and without (dashed line) 50 μg mL^{-1} myo-inositol. Both the oxidation peak 1 and reduction peak 2 show the redox of the dissolved oxygen.[23] Peaks 3 and 4, at about 0.300 V and 0.160 V (vs. Ag/AgCl, same as below), demonstrate the redox of the gold itself and the maximum current observed was 0.500 V. When 50 μg mL^{-1} myo-inositol was added, the current increased sharply, fully merging the oxygen oxidation peak 7, and the maximal oxidation current was observed at 0.027 V. With continued scanning to more positive potentials, the myo-inositol oxidation current decreased as the gold oxidation current continued to increase (peak 8), which showed that the gold oxidation caused the inhibition of the myo-inositol oxidation. On the reverse scan, the reduction peak of the gold working electrode appeared at 0.175 V (peak 9), which indicated the cathodic dissolution of the gold oxide.[23] Compared with the blank solution, a strong anodic wave [23] was observed (peak 11), which demonstrated that the reactivity of the gold working electrode returns for myo-inositol oxidation.

According to the discussion above, a maximal current response for myo-inositol was obtained at 0.027 V in 0.1 M NaOH, so the potential E1 for the waveform was obtained at approximately 0.027 V by further optimization.

Effects of potential E1 on response

For the quadruple-potential waveform shown in the Table 2, a series of tests were carried out by changing the potential E1 from -0.1 V to 0.25 V in order to examine the effect of E1 on the peak area (*A*) of myo-inositol.

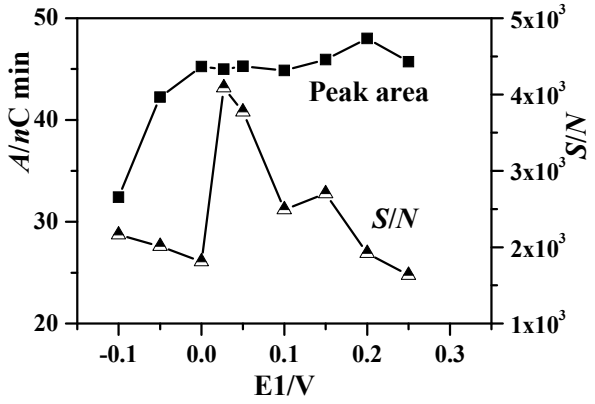

Fig. 6 Peak area responses at different E1 potential by the column MA1, eluted with 500 mM NaOH at 0.4 mL min^{-1}.

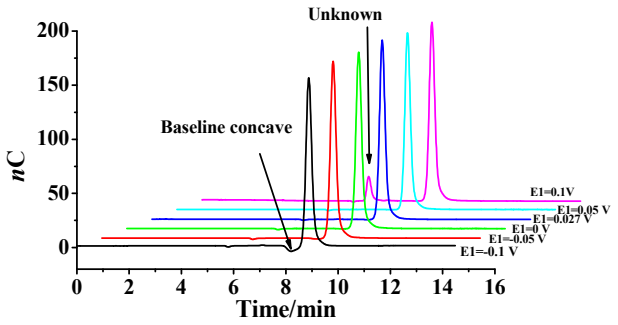

Fig. 7 Effects of different E1 on the peak shape of myo-ionositol by the column MA1, eluted with 500 mM NaOH at 0.4 mL min^{-1}.

As shown in Fig. 6, the peak area responses and the signal-to-noise ratios (*S/N*) of the myo-inositol show different trends when the potential E1 is changed from -0.1 V to 0.25 V. The peak area responses changed little in the range of -0.1~0.25 V; however, the noise clearly increased when the potential E1 was changed in the range of less than 0.027 V or greater than 0.027 V. The maximal signal-to-noise ratio was obtained at 0.027 V. When the potential becomes more negative, the reduction of dissolved oxygen will take place at the electrode surface; in all other conditions, oxidation occurs which accelerates the loss of the electrode material. Thus, as a result of an inappropriate detection potential, the increased background current from the electrochemical redox will greatly contribute to the noise observed.

The effect of the sodium hydroxide concentration on the potential E1 was examined by changing the concentration of sodium hydroxide in the range of 0.1 M~0.7 M. These results also indicated that myo-inositol has the optimum response signal-to-noise ratio at the potential E1 of 0.027 V.

A change in the myo-inositol peak shape was observed when the potential E1 was between -0.1 V and 0.1 V. When the potential E1 was set more negative, the baseline appeared to concave at the left of the peaks (Fig. 7), which can be attributed to the reduction of dissolved oxygen.[19,21] On the other hand, when the potential E1 was set more positive, for example, at 0.1 V, another unknown interfering peak was detected (Fig. 7). The results demonstrated that 0.027 V was the optimal detection potential for myo-inositol.

Determination of myo-inositol in the real sample using column-switching procedure

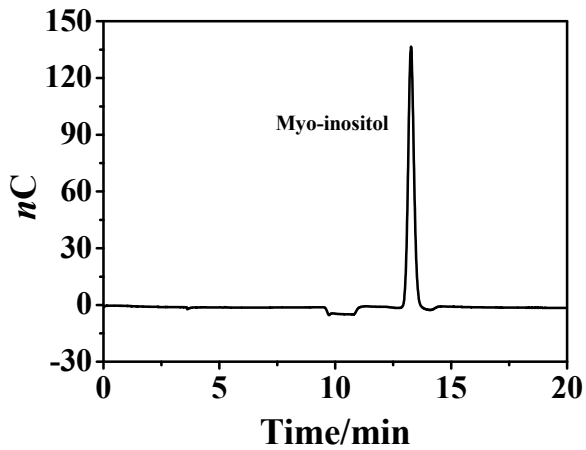

Fig. 8 Chromatogram of myo-inositol standard after matrix elimination with column-switching.

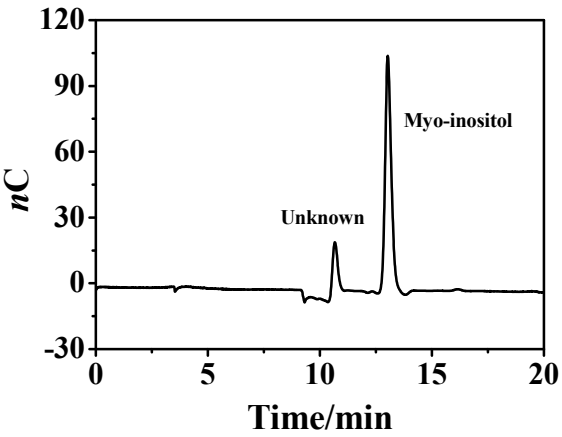

Fig. 9 Chromatogram of myo-inositol in infant formula sample after matrix elimination with column-switching.

The infant formula matrix was very complex as it contains a variety of carbohydrates, proteins, fats, free amino acids, vitamins and other nutrients, *etc*. The successful separation of myo-inositol from the matrix is challenging when using the traditional IC method. Fig. 3 and 4 show the chromatograms of

the sample by columns PA1 and MA1, respectively. As shown in Fig. 3 and 4, myo-inositol and its co-eluents were eluted earlier than most other matrix components. Some serious chromatographic phenomena were observed, including very complex components, severe peak overlapping and baseline instability, which directly led to a long analytical period (over 60 minutes) (Fig. 4). In addition, serious electrode fouling and interference were inevitable due to the complex matrix components, such as various saccharides (including fructose, sucrose, lactose, galactose, myo-inositol, maltol, glucose, oligose, *etc*.), free amino acids, polypeptides, proteins and so on, which severely reduced the detection sensitivity. The specific optimization used the STW calibration to obtain both the chromatograms of the real sample (Fig. 3a) and the standard (Fig. 3b) by the pretreatment column PA1. By comparing the chromatograms, the STW of myo-inositol was obtained between 3.50 min and 5.00 min as shown in Fig. 3a, marked with the dashed line box. Next, the free switching was achieved by assigning the optimized STW parameters to valve 2 by the analysis program.

Fig. 8 and 9 show the chromatrograms of myo-inositol in a standard and real sample afer matrix elimination, respectively. In comparing the results shown in Fig. 4 and 9, it can be observed that the matrices had been eliminatied completely, and excellent separation and detection were achieved using column-switching with IC.

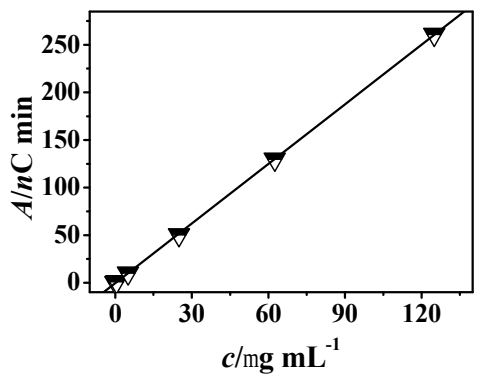

Fig. 10 The linearity fit of peak areas and concentrations of standards using the optimized quaternary-potential waveform.

Method validation

The method was validated prior to its application to real samples. As shown in Fig. 10, an excellent linearity was obtained between the peak areas (A) and the concentrations (c) in the range of 0.05 μg mL^{-1} and 120 μg mL^{-1}, and the linearity equation was A=2.1509c, with the correlation coefficient r=0.99993 (n=6). According to the sample preparation process and the signal-to-noise ratios of 3 (S/N=3) of 0.05 μg mL^{-1} myo-inositol, the limit of detection (LOD) was 2.5 μg g^{-1}, and the limit of quantitation (LOQ) (S/N=10) was 7.5 μg g^{-1}.

Three spiking levels (700 μg, 900 μg and 1100 μg) with five parallel measurements were carried out to validate the recoveries of the method. The external standard method was applied for quantitative determination of myo-inositol in infant formula samples. As shown in Table 7, the average recovery rate was 95.2%~102.0% and the relative standard deviation (*RSD*, n=5) was 1.11%~4.25%. The results indicate that by the proposed method, it was possible to achieve a fairly complete elimination of matrices without any loss of precision.

Table 7 Evaluation of recoveries for myo-inositol determination in the infant formula sample (n=5)

No.	Added ρ_A/(μg)	Found ρ_F/(μg)	Recovery R / %	*RSD* (%)
1	0	902.0	/	4.25
2	700	1585.2	97.6	1.11
3	900	1820.1	102.0	3.74
4	1100	1949.2	95.2	3.04

Conclusions

This work developed a flexible column-switching technique for the direct determination of free myo-inositol in infant formula samples by IC with pulsed amperometric detection. The column-switching system based on two pumps, two columns and three valves, allows for simple, automatic switching between the STW mode and analysis mode. Additionally, both the pretreatment column and analysis column were arranged so they could be activated and regenerated during their idle time, resulting in a highly efficient method. Optimizing both the chromatographic conditions and the detection parameters allowed for fast analysis of myo-inositol in infant formula with the advantages of simple pretreatment, low cost, good stability, and no use of organic reagents.

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[a] Department of Physical and Chemical Analysis, Zhejiang Institute of Quality Inspection Science, Xiasha, Hangzhou, 310018, China. E-mail: cxz730@163.com
[b] Department of Chemistry, Xixi Campus, Zhejiang University, Hangzhou 310028, China. Tel/Fax: +86-571-88273637. E-mail: zhuyan@zju.edu.cn

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