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Short communication

Isolation of cycloamylose by iodine affinity capillary electrophoresis

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ABSTRACT

In contrast to α-, β- and γ-cyclodextrins, little information is available on the isolation and separation of cycloamylose (CA) with degree of polymerization (DP) larger than 22. The objective of the current study was to develop a new iodine affinity capillary electrophoresis (CE) for separation of CA with DP of 22–42, which was based on the formation of CA-iodine inclusion complexes, CA with twisted conformations made complicated mobility behaviors on CE instead of merely size dependent. The influences of iodide/iodine ratio, iodine concentration, pH, ion strength of running phosphate buffer, voltage, and temperature on the peak resolution and electrophoretic mobility were further investigated. Our results suggest that iodine affinity capillary electrophoresis provides a versatile and selective tool for the isolation and analysis of CA with DP from 22 to 42.

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1. Introduction

Cycloamylose (CA), also called large-ring cyclodextrins (LR-CDs), is composed of nine to more than several hundreds of glucopyranose units and only has been fully proven during the past two decades. CA with DP larger than 9 exhibit a distorted shape, such as CD9 was a boat-like shape, CD10 was an elliptical macrocyclic ring folded in a saddle-like shape, while CD26 has channel like cavities composed of two short V-amylose helices in antiparallel orientations, and structures of CA with DP more than 21 would be similar to the structure of CD26 [1–3]. These distorted cyclic structures can provide special properties for CA. For example, CA with DP larger than 20 has properties of high water solubility, low viscosity, and resistant to retrograde [4]. In addition, CA mixture with mean molecular weight of 7720 Da has been made a wide range of application, it was studied as stabilization and solubilization for about 20 drugs in pharmaceuticals [5,6], and also it was used as artificial chaperones in biotechnology [7]. Furthermore, CA was claimed as additive in food and drink products to improve texture, mouth feeling and retain flavour, and also, it was suggested as paper coating material in paper industry [8].

Due to the difficulties in isolation and separation of CA, there have relatively few reports on the separation of CA. For instance, one of the methods is the use of thin layer chromatography (TLC) to separate CA with (degree of polymerization) DP up to 17 [8]. However, to the best of our knowledge, no reports were available for separation of CA with DP more than 17 by TLC. HPLC for separation of CA with DP from 6 to 25 has been reported with the combination of octadecyl silica (ODS) and amino-bonded (NH_2) columns [9–12]. However, this separation procedure was too much complicated and the special column such as ODS-AQ was expensive, which makes difficult for most of laboratories to carry out similar research. High-performance anion-exchange chromatography (HPAEC) has been introduced for the analysis of CA with a DP over 80 using pulsed amperometric detection (PAD) detector under gradient elutions [13], but the sample separation time was too long (about 140 min) to run many samples in a day. Therefore, it would be of vital importance and interesting to develop a new method for quick analysis of CA with large DP.

It is well known that capillary electrophoresis (CE) is a very powerful tool for characterization of biomolecules. Since cyclodextrins (CDs) are colourless, without any chromophore group, and only electrified at a very high pH, a direct separation and detection of CDs on CE using detectors of UV and laser induced fluorescence (LIF) is impossible. Merely, with special inclusion capabilities, CDs could form inclusion complexes with a large range of aromatic ions, which would provide UV absorption, and was able to facilitate the analysis of CDs using CE. CD with DP of 6–13 had been analyzed and characterized using CE [14,15]. But no studies were reported on the separation of CA (DP of 22–50) using CE, it maybe because previous studies thought that the inclusion complex forming properties of LR-CD might be too similar to separation by CE, in contrast to the distinct inclusion complex forming properties of the smaller

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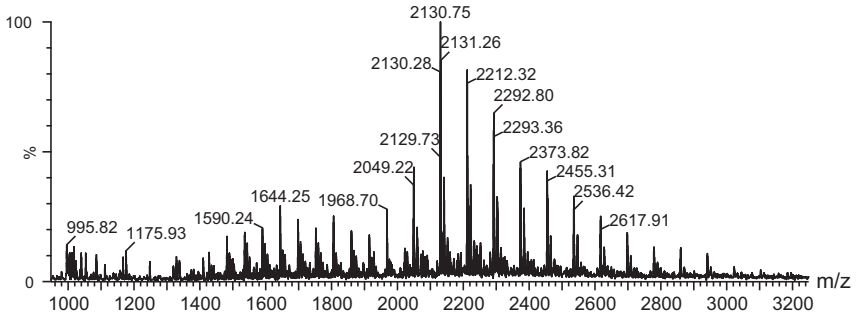

Fig. 1. Molecular mass diagram of cycloamylose.

CDs [16]. However, what surprisingly is, our preliminary study on analysis of CA (DP of 22–50) by forming complexes with most common molecular such as benzoate, 4-methyl benzoate, salicylate, 4-tert-butyl benzoate and ibuprofen anion did not provide satisfy separation and isolation results, but iodine affinity capillary electrophoresis was highly efficient in CA separation. The discovery of this accident is very interesting and worthy of further study.

The objective of the current study was to develop a new method of iodine-affinity CE to separate CA. The influence factors including running buffer system and operation conditions on the peak resolution and mobility behaviors of CA were investigated.

2. Experimental

2.1. Apparatus

CE was conducted with a Beckman P/ACE MDQ instrument equipped with a diode array spectrophotometric detector. The instrument equipped with an autosampler, column temperature control, and the fused silica capillaries were 75 μm i.d. × 375 μm o.d. with overall length of 37 cm and injector to detector of 28 cm.

2.2. Reagents and samples

CA mixture standard was purchased from Ezaki Glico Co. Ltd. (Japan). All reagents were of analytical-reagent grade. Solutions were prepared with ultrapurewater obtained from a water-purification system (Milli-Q, USA).

2.3. Separation procedure

CA solutions were prepared at room temperature with the final concentration of 10 mg/mL. Running buffers were prepared by mixing iodine stock solution and phosphate buffer before use. The running buffer concentration and pH were adjusted according to the separation results. Before running, a new capillary was alternately rinsed by 0.5 M HCl, water, and 1 M NaOH for 10 min, and then rinsed by running buffer for 5 min. In order to obtain good reproducibility, after running one sample, the running buffer was refilled, and capillary was alternately rinsed by water for 10 min, and 1 M NaOH for 10 min. Samples injection was performed at 8 s at 0.8 psi and the separation was carried out at adjusted temperature and voltage. Detection wavelength of iodine-CA complexes was at 496 nm although the entire spectrum in the range 200–600 nm was recorded.

2.4. Determination of CA mixture with TOF-MS

The molecular mass of CA was detected by Time of Flight (TOF) mass using ESI spectrometry (WATERS SYNAPT Q-TOF MS, USA), the accelerating voltage in the ion source was 30 kV, and spray voltage was 2.5 kV. All samples were measured in positive modes.

3. Results and discussion

3.1. Molecular mass of CA detected by TOF-MS

TOF-MS with ESI spectrometry gave CA multiply charge for each molecular as shown in Fig. 1, thus the data was deconvolution by MaxEnt (data not shown). With detection mass interval of 162 Da, CA was found with degree of polymerization (DP) from 22 to 42, but DP23 was not found. Thus we deduce 20 main components existed in CA mixture.

3.2. Selection of separation conditions

Iodine affinity capillary electrophoresis was previously mentioned by Brewster for detection starch using iodine-containing buffers in unmodified capillaries [17], inspired the authors for CA separation by iodine affinity CE due to the “starch-like” properties of CA. The initial conditions for CA separation were based on the published iodine affinity CE for amylose and amylopectin determination, which was reported dependent on iodide/iodine ratio, iodine concentrations, and oligomer chain length of polysaccharides [18]. Considering that iodine was colourless under alkali condition, acidity condition was therefore selected for buffer preparation. With iodine concentration of 0.6 mM, Fig. 2(A) shows the log–log plot of electrophoretic mobility for CA at iodide/iodine ratio varied from 2 to 8. We observed that large iodide/iodine ratio resulted in much more mobility time. For a further discussion of the CA-polyiodide complexes, we shall arbitrarily divide the mobility into five groups: group 1 with DP of 22–26, group 2 with DP of 27–30, group 3 with DP of 31–34, group 4 with DP of 35–39, group 5 with DP of 40–42 (Fig. 2(A)). Peak resolution between groups was much higher than that inside a group. From the slope of each group, peak resolution of groups 2, 3, and 4 (with DP of 27–39) were improved with the increasing of iodide/iodine ratio. For CE separation, the components were separated by charge-mass ratio. In relation to the current study, with polyiodides present, the separation of CA by CE was presumably based on charge-mass ratio of CA/polyiodide complex. Considering increasing I^- concentrations in I_2/I^- solution would favor the formation of shorter polyiodides [19], and X-ray analyses showed I_3^- units are located in the central channels of the V-helices of CA26 [20], we suppose that the entrapped polyiodide chain was $(I_3^-)_n$ for CA. Furthermore, previous studies points out that the conformational flexibility of CA chain would be decreased when mixed with iodine solution [21], and iodine solution as running buffer contain the same charge for all the

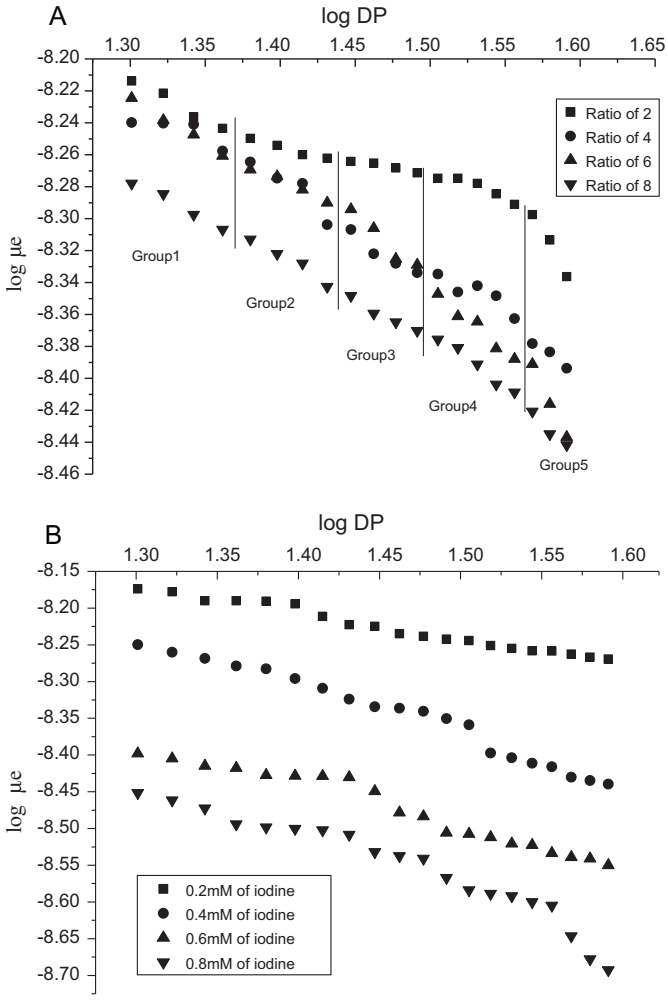

Fig. 2. (A) Log–log plot between DP and mobility with different iodine/iodide ratio. Lower log μ_e means much more mobility time and larger slope means much better resolution. The other separation concentrations were as follows: iodine concentration 0.6 mM, PBS concentration 20 mM, pH 6.4, voltage 20 kV, temperature 25 °C, pressure injection, 0.8 psi for 8 s, the effective length of column was 28 cm. (B) Log–log plot between DP and mobility with different iodine concentration. Lower log μ_e means much more mobility time and larger slope means much better resolution. The other separation concentrations were as follows: iodine/iodide ratio of 6, PBS concentration 20 mM, pH 6.4, voltage 20 kV, temperature 25 °C, pressure injection, 0.8 psi for 8 s, the effective length of column was 28 cm.

components of CA, thus the migration was probably mainly based on the molecular weight. But, conformational flexibility of CA was probably related to separation resolution. In turn, a similar conformation for CA in each group could be deduced from the separation results.

At iodide/iodine ratio of 6, iodine concentration from 0.2 to 0.8 mM also showed significant effect on log–log plot (Fig. 2(B)). The mobile time prolonged with increasing of iodine concentration, further, the slope of groups 3 and 5 (DP 31–34 and DP 40–42) increased with increasing of iodine concentration, suggesting that the better peak resolution of CA were achieved with higher iodine concentration. From Fig. 2(B), we found that the peak mobility was not simple linearly increased with iodine concentration increasing, it was probably indicated that distorted conformations of CA with variable size resulted in complicated charge to mass and surface charge for CA/polyiodides complexes, which in reverse affect the mobility of CA with different DP when separating by CE. During detection, we found that increasing of iodine concentration caused the increase of Joule energy in capillary, and resulting in poor separation reproducibility. Thus, 0.6 mM I_2 and 3.6 mM KI was selected for further analysis, which gave a proper resolution as shown in Fig. 3(A).

3.3. Optimization conditions for CA separation

Electrophoretic mobility can be induced by a dynamic formation of charged complexes between CA and polyiodide presented in the PBS electrolyte. Further, in an uncoated fused silica capillary, the mobility due to electroosmotic flow is much higher than the electrophoretic mobility of most sugar conjugates [22]. Thus, effluences related to electroosmosis could severely affect the resolution of separation results. pH has been widely chosen for adjusting electroosmosis [23,24], presumably due to changing charge and hydrogen of Si–OH on the silicon wall of capillary at various pH. With various pH, we interestingly observed that peak resolution of group 1 (DP of 22–26) and group 3 (DP of 31–34) were increased with the decrease in pH as shown in Fig. 4(A), but for group 5 (DP of 40–42), peak resolution were decreased. In addition, the mobility time was prolonged with pH decrease. A proper pH of 5.1 was suitable for CA separation and the separation results are shown in Fig. 3(B).

It has been previous demonstrated that increase in ion strength of the buffer also affects electroosmosis mobility [25,26]. The log–log graphs at varies ionic strength in Fig. 4(B) showed that the peak resolutions and mobility time both increased with increas-

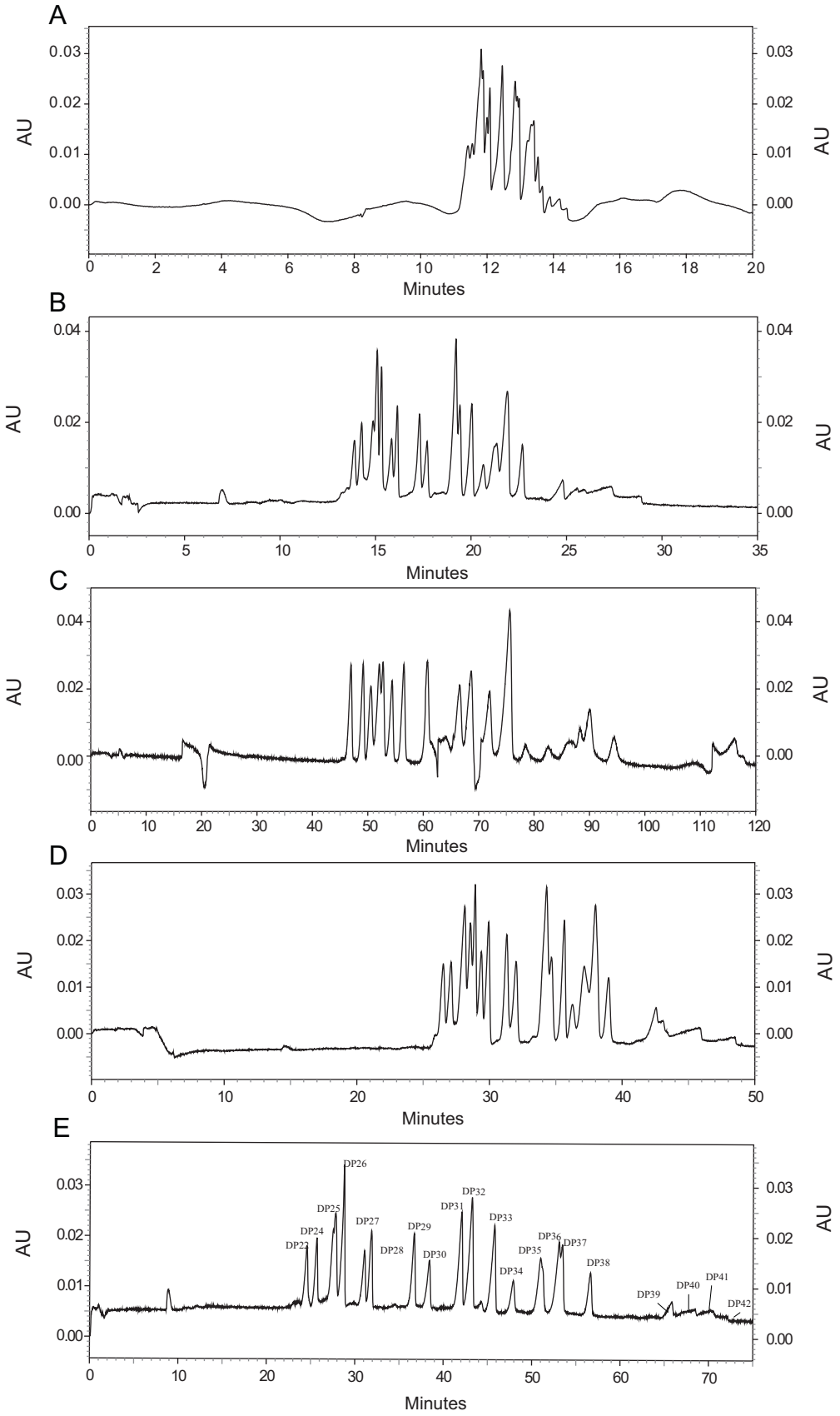
A
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Fig. 3.

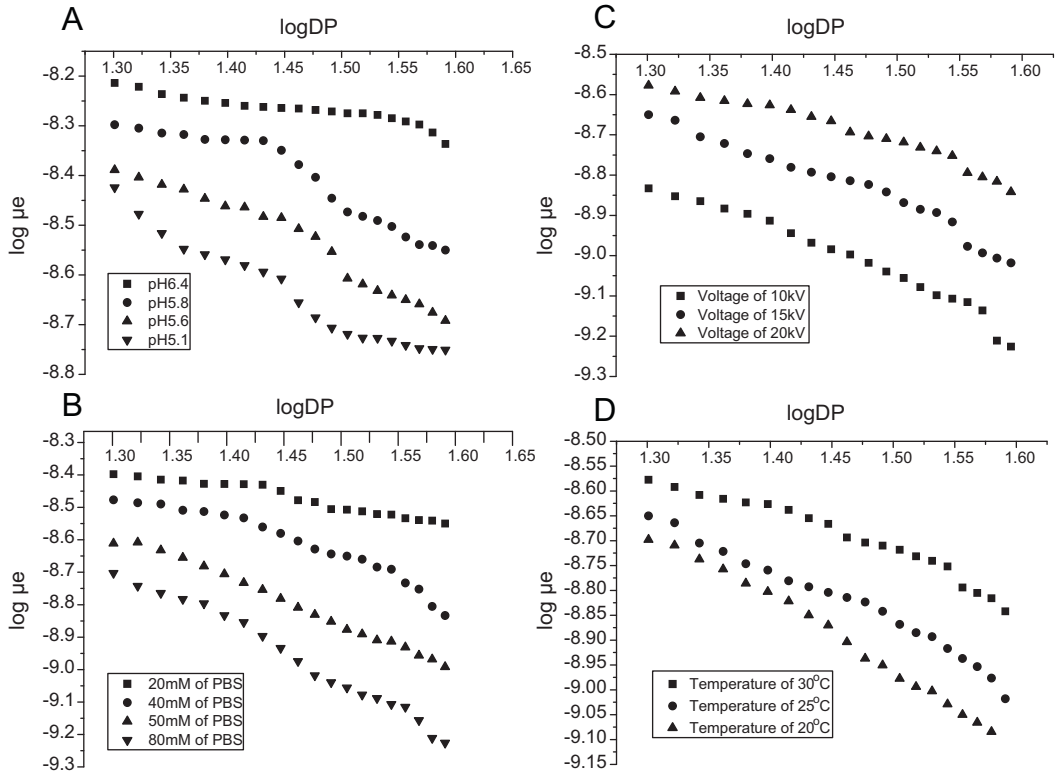

Fig. 4. (A) Log–log plot between DP and mobility at different pH. The other separations were as follows: PBS concentration 20 mM, voltage 20 kV, temperature 25 °C, pressure injection, 0.8 psi for 8 s, the effective length of column was 28 cm, iodide/iodine ratio of 6 and iodine concentration of 0.6 mM. Lower log μ_e means much more mobility time and larger slope means much better resolution. (B) Log–log plot between DP and mobility at different PBS concentration. The other separations were as follows: pH 5.1, voltage 20 kV, temperature 25 °C, pressure injection, 0.8 psi for 8 s, the effective length of column was 28 cm, iodide/iodine ratio of 6 and iodine concentration of 0.6 mM. Lower log μ_e means much more mobility time and larger slope means much better resolution. (C) Log–log plot between DP and mobility at different voltage. The other separations were as follows: PBS concentration 20 mM, pH 5.1, temperature 25 °C, pressure injection, 0.8 psi for 8 s, the effective length of column was 28 cm, iodide/iodine ratio of 6 and iodine concentration of 0.6 mM. Lower log μ_e means much more mobility time and larger slope means much better resolution. (D) Log–log plot between DP and mobility at different temperature. The other separations were as follows: PBS concentration 20 mM, voltage 20 kV, pH 5.1, pressure injection, 0.8 psi for 8 s, the effective length of column was 28 cm, iodide/iodine ratio ratio of 6 and iodine concentration of 0.6 mM. Lower log μ_e means much more mobility time and larger slope means much better resolution.

ing of ionic strength, and reached the maximum at 80 mM of PBS (Fig. 3(C)). It was probably caused by the following events: strength in ion decreased the zeta electric potential and attenuated the electric double layer on the capillary wall, which resulting in a weakened electroosmosis and a proper separation [26]. Also, the increase in ionic strength could decrease the "charge to mass" ratio of CA and low down electrostatic interactions between CA and capillary wall [26], which improve the separation of CA basing on CA/polyiodide complexes. But the obvious disadvantage for increasing PBS concentration was that a great amount of Joule energy was produced under electric field, and the produced heat would cause the temperature gradient and viscosity gradient between the running buffer centre and the edge, which would result in poor separation reproducibility.

In order to study the effect of buffer composition on peak resolutions and mobility, Tris–HCl and acetic acid–sodium acetate buffer at the same pH and ion concentration were mixed with 0.6 mM I_2 and 3.6 mM KI, and the compounds were utilized as running buffer for CA separation by CE. To our surprise, no peak resolution for CA in these two buffer systems was found. We suppose that ionic strength and buffering capability of Tris–HCl and acetic acid–sodium acetate were lower than PBS even at the same concentration, thus the captured CA on the capillary wall can not be separated.

For CE separation, electric field is one of the most important factors for mobility and peak resolution. Various electric field strengths were explored to observe the separation results. Fig. 4(C) shows the log–log plots between electrophoretic mobility and the DP of CA at different voltage. The mobility time was increased with the increasing of voltage, which comes at the cost of decreasing peak resolution. To avoid of too much heat under high electric field, 10 kV was the proper separation voltage and separation results are shown in Fig. 3(D).

Due to influence sample viscosity, electroomotic and final separation results, energy generated during the separation cannot be ignored. Thus temperature control was utilized to minimize Joule energy. The log–log plots at different temperature are present in Fig. 4(D). Results showed that the peak resolution decreased and

Fig. 3. (A) Spearation diagram of CA at iodide/iodine ratio of 6 and iodine concentration of 0.6 mM. The other separations were as follows: PBS concentration 20 mM, pH 6.4, voltage 20 kV, temperature 25 °C, pressure injection, 0.8 psi for 8 s, the effective length of column was 28 cm. (B) Spearation diagram of CA at pH 5.1. The other separations were as follows: PBS concentration 20 mM, voltage 20 kV, temperature 25 °C, pressure injection, 0.8 psi for 8 s, the effective length of column was 28 cm, iodide/iodine ratio of 6 and iodine concentration of 0.6 mM. (C) Spearation diagram of CA at PBS concentration of 80 mM. The other separations were as follows: pH 5.1, voltage 20 kV, temperature 25 °C, pressure injection, 0.8 psi for 8 s, the effective length of column was 28 cm, iodide/iodine ratio of 6 and iodine concentration of 0.6 mM. (D) Spearation diagram of CA at 10 kV. The other separations were as follows: PBS concentration 80 mM, pH 5.1, temperature 25 °C, pressure injection, 0.8 psi for 8 s, the effective length of column was 28 cm, iodide/iodine ratio of 6 and iodine concentration of 0.6 mM. (E) Spearation diagram of CA at temperature of 20 °C. The other separations were as follows: PBS concentration 80 mM, pH 5.1, voltage 10 kV, pressure injection, 0.8 psi for 8 s, the effective length of column was 28 cm, iodide/iodine ratio of 6 and iodine concentration of 0.6 mM.

mobility time was reduced with increasing of temperature. For proper peak resolution of CA, 20 °C was the ideal operation temperature and the separation results at 20 °C are shown in Fig. 3(E). Under these optimum conditions, the peak almost reached baseline separation, and the separation was repeatable.

4. Conclusions

It has been shown that iodine-affinity CE could be successfully used to separate CA with DP of 22–42 and this separation does not require complicated derivatization steps. The analysis time is much shorter than the most frequently used CA analysis method, e.g. HPAEC. This successful separation may provide a novel idea for complicated CA detection.

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