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Analytical Methods

Determination of iodate in table salt by transient isotachophoresis–capillary zone electrophoresis

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ABSTRACT

A transient isotachophoresis–capillary zone electrophoresis (tITP–CZE) method was first time developed for determining iodate in table salt. Sensitivity enhancement was accomplished by coupling on-capillary tITP with CZE. The interference of sample matrix was overcome by using electrophoretic buffer containing high concentration of sodium chloride. The optimal terminating electrolyte for tITP was 1500 mmol/L phosphate and the separation buffer of capillary zone electrophoresis was 10 g/L sodium chloride (pH 8.0) containing 20 mmol/L cetyltrimethylammonium chloride (CTAC). Calibration graphs based on peak height and peak area showed good linearity. Use of chromate as the internal standard significantly improved the precision of the quantitative results. The wavelength of UV detection for iodate was set at 218 nm with the detection limits of 3.5 μg/L for iodate. The quantitative results of iodate in table salt measured by the tITP–CZE method were compared with those measured by the redox titration and there was no significant difference between the two means. The method developed was sensitive, fast and simple.

1. Introduction

Iodine deficiency is a major public health problem for populations throughout the world, particularly for pregnant women and young children. It is a threat to the social and economic development of countries. The most devastating outcomes of iodine deficiency are increased perinatal mortality and mental retardation – iodine deficiency is the greatest cause of preventable brain damage in childhood which is the primary motivation behind the current worldwide drive to eliminate it (World Health Organization, 2004).

Salt has been chosen as a vehicle for iodine fortification in many countries (World Health Organization, 1994). Iodised salt is therefore, expected to be an important source of iodine for people in many areas of the world. Potassium iodide (KI) and potassium iodate (KIO_3) are normally used for salt fortification due to their higher iodine availability and lower cost. However, potassium iodate is recommended by international organizations i.e., World Health Organization (WHO) and United Nations Children's Fund (UNICEF) especially for salt that is stored under warm and humid conditions (Sinawat, 1997). According to the National Standard of the People's Republic of China – Edible Salt. (GB5461) (China State Bureau of Quality, 2000), iodine in salt used for human consumption must be in the range of 35 ± 15 mg/1000 g. Today, iodated salt is an important source of iodine and cooking with iodated salt is highly promoted in China and many Asian countries.

Simple, reliable and cost-effective analytical methods are required for quality control in the production of iodated salt and quality monitoring of iodated salt on the market. Several analytical techniques have been applied to determining iodate in table salt. Titration demanded of high skill of operators and was a time-consuming process, especially in the processes of preparing and standardising the titrant of thiosulfate solution (China State Bureau of Quality & Technical Supervision, 2000). Generally, special chemical regents and complicating reactions were involved in development of the colouring products in spectrophotometry (Afkhami & Zarei, 2003; Ghasemi, Saaidpour, & Ensafi, 2004; Sun, Chen, & Hu, 1997; Xie & Zhao, 2004). Modification of detection system (Bichsel & Gunten, 1999) or special sample treatment (Kumar, Maiti, & Mathur, 2001; Xu, Li, Gu, & Paeng, 2004) was required in ion chromatographic methods with relatively high operation cost. To our knowledge, no one has reported the determination of iodate in table salt by capillary zone electrophoresis.

Capillary zone electrophoresis (CZE) is a powerful tool for separation and quantification of inorganic ions with a broad spectrum of application areas (Paull & King, 2003; Timerbaev, 2002). The application of CZE in determining inorganic ions in food samples has been the subject of several reviews (Boyce, 2001; Frazier & Papadopoulou, 2003; Lindeberg, 1996; Sádecká & Polonsky, 1999). Using CZE for determining inorganic ions in foodstuffs offers advantages of high resolution with short analysis time, low cost of

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disposables and minimal reagent usage. Shortcomings of CZE are low concentration sensitivity and marginal reproducibility in terms of migration time and peak area in quantification. It was expected that improvements on the sensitivity of CZE analysis would undoubtedly pave the way for more routine applications of the technique, particularly for the analysis of trace components.

Determining iodate in table salt by CZE is challenging because concentration of iodate is relatively low but concentration of sodium chloride very high. In general, CZE of minor ionic components in the presence of high concentration of matrix ions leads to peak broadening, poor resolution and low sensitivity (Boden & Bächmann, 1996; Song, Ou, Yu, & Xu, 1995). A useful approach to addressing the above problems was to couple transient isotachophoresis (tITP) with CZE, so called tITP–CZE (Foret, Szoko, & Karger, 1992; Foret, Szoko, & Karger, 1993). ITP is a well-known process of an intrinsic function of concentration for minor ionic components (Timerbaev & Hirokawa, 2006). In ITP, discontinuous electrolyte systems are employed. Ions of similar charge quality are separated into consecutive zones of regulated concentration behind the leading ion of the highest effective mobility and in front of the terminating ion of the lowest effective mobility. During the isotachophoretic migration process, ionic components of low concentration in the sample form concentrated zones defined by the Kohlrausch regulating function (Holloway & Trautschold, 1982). tITP–CZE can be implemented by the use of discontinuous electrolyte systems such that only at the beginning isotachophoretic conditions hold for the sample ions. At this initial stage, minor ionic components in the sample are therefore concentrated. After a certain time, however, the conditions change so that the sample ions are separated zone electrophoretically. The main advantage of tITP–CZE is that it can be carried out easily in a single-capillary arrangement with a commercially available instrument. Several successful examples of using tITP–CZE in determining minor ionic components have been reported (Boden, Bächmann, Kotz, Fabry, & Pahlke, 1995; Ding, Thornton, & Fritz, 1998; Timerbaev, Takayanagi, & Motomizu, 1999; Yokota, Fukushi, Takeda, & Wakida, 2004; Yokota et al., 2003; Huang, Ito, Timerbaev, & Hirokawa, 2004). Among these examples, determining iodate in seawater provided us useful information and good expectation of developing the tITP–CZE method for the determination of iodate in table salt. Yokota et al. (2003, 2004) employed artificial seawater as the background when determining iodate in seawater. However, highly conductive artificial seawater used as the background might result in excessive Joule heating and deteriorate the separation. In addition, the preparation of artificial seawater was tedious. So, the use of artificial seawater as the background was not adopted in our work.

This paper is the first report on the tITP–CZE determination of iodate in table salt. Chromate was chosen as the internal standard to improve the precision of the quantitative results. The results obtained using the method developed and the titration were in good agreement.

2. Experimental

2.1. Apparatus

Experiments were performed on a CE-L1 instrument (CE Resources Pte, Singapore) equipped with a Linear UVIS 200 detector (Alltech, Deerfield, IL, USA). Electropherograms were recorded with the CSW (Chromatography Station for Windows) (DataApex, Prague, Czech Republic). Fused-silica capillaries of 60 cm total length and 50 cm effective length (from the injection end to the detection window), and of 375 μm OD and 75 μm ID used in the experiments were purchased from Yongnian Optical Fiber Factory (Hebei, China). Nylon filters with pore size of 0.45 μm were obtained from Quandao Technical Company (Shanghai, China). The pH values of the running buffers were measured with a pHS-3C Meter from Mettler Toledo Instruments (Shanghai, China).

2.2. Reagents and solutions

Table salt samples were obtained from a local department store. Cetyltrimethylammonium chloride (CTAC) of chemical pure grade was purchased from Shanghai Haoshen Chemical Reagent (Shanghai, China). Sodium chloride, potassium iodate, potassium chromate, sodium dihydrogen phosphate dihydrate, sodium chlorate, and borax were all analytical grade and were purchased from Sinopharm Chemical Reagent Co. (Shanghai, China). Stock solutions containing potassium iodate (500 mg/L) and potassium chromate (500 mg/L) were prepared in 10 g/L NaCl solution and the standard solutions of lower concentrations were prepared by appropriately diluting the stock solutions with 10 g/L NaCl solution. Stock solutions of sodium dihydrogen phosphate dihydrate (2 mol/L), sodium chlorate (1 mol/L), borax (120 mmol/L) were prepared as terminating ion solutions and the solutions of lower concentrations were prepared by appropriately diluting the stock solutions with deionized water. The running buffer for the CZE was prepared by dissolving 20 mmol/L CTAC in 10 g/L NaCl buffer (pH 8.0). Deionized water was used throughout the experiments. All solutions were filtered through 0.45-μm membrane filters before use.

2.3. tITP–CZE procedure for the determination of iodate in table salt

Sample solution of table salt of 10 g/L was prepared with deionized water and filtered through a 0.45-μm membrane filter before sample injection. Buffers for the CZE were ultrasonicated for 10 min before use. Prior to running a sample, a new capillary was washed with 1 mol/L sodium hydroxide for 40 min, deionized water for 10 min and the running buffer for 3 min in order. The detection wavelength was set at 218 nm. The temperature was maintained at 25 °C. After sample solution was introduced into the capillary by applying pressure of 2.07 kPa for 30 s, the terminating ion solution of 1500 mmol/L phosphate was introduced under the same pressure for 10 s. The injection period of 1 s corresponds to the sample volume of 20 nL. Between runs, the capillary was flushed with the running buffer for 3 min. A voltage of 8 kV was applied with the sample inlet side as the cathode.

3. Results and discussion

3.1. Selection of separation voltage and detection wavelength

With the increment of voltage from 4 to 9 kV (the sample inlet side as the cathode), migration time of iodate became short continuously. But when the voltage was higher than 8 kV, the current was greater than 160 μA and it led to noisy baseline as a result of Joule heating. Therefore, 8 kV was adopted as the separation voltage.

The effect of wavelength on peak height for iodate was examined over the range of 210–225 nm to obtain the detector response as high as possible. As wavelength increased, the peak height for iodate decreased. However, when wavelength was shorter than 215 nm, the baseline became worse as the result of the influence of the electrodispersion in sample zone caused by chloride ion and its absorption (Woodland & Lucy, 2001). Therefore, detection wavelength was set at 218 nm in this work.

3.2. Optimization of terminating ion solutions

It was found that iodate in table salt could not be determined by CZE procedure with homogenous electrophoretic buffers using

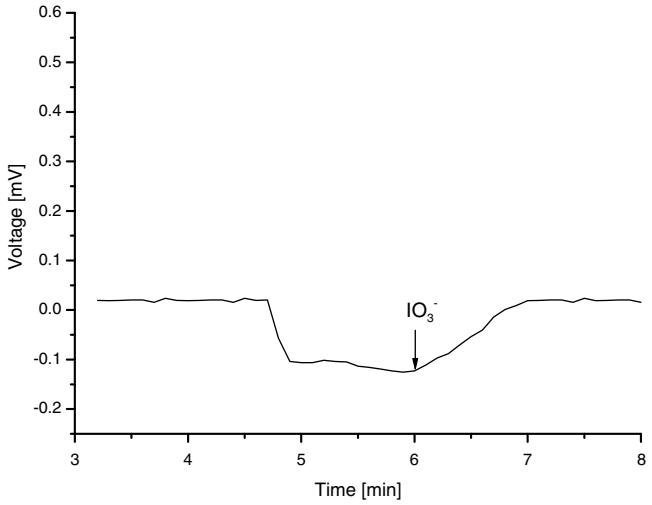

Fig. 1. Capillary zone electrophoresis of iodate in table salt. Capillaries: $L_{tot.}$ = 60 cm, $L_{det.}$ = 50 cm, 75 μm ID × 375 μm OD. Electrophoretic buffer: sodium chloride solution (pH = 8.0) containing 20 mM CTAC. Voltage: −8 kV. Wavelength: 218 nm. Sample: table salt in deionized water (200 g/L). Sample injection: 2.07 kPa for 30 s.

CTAC as the electroosmotic flow (EOF) modifier (the concentration of CTAC varying in a range of 1–25 mmol/L). Fig. 1 was a typical electropherogram obtained with the CZE. Abnormal response of the detector in the time scale range of about 4.5–7.0 min was likely resulted from the electrodispersion and UV absorption of sodium chloride of high concentration in the sample zone.

To improve detectability of iodate and eliminate the interference of high concentration of sodium chloride, we decided to use tITP–CZE technique. The leading electrolyte in the transient ITP at the initial stage of the experiments was 10 g/L of sodium chloride solution (pH 8.0) containing 20 mmol/L CTAC. Generally, selection of right terminating ion plays a key role of obtaining the best enrichment for tITP experiment. The effective mobility, charge and concentration of the terminating ion all have influences on the results of enrichment of minor ionic components in ITP (Holloway & Trautschold, 1982). Borate, chlorate, and phosphate were examined as the terminating ion for sensitivity improvement of iodate. The sample used in these experiments was 0.5 mg/L iodate and 5.0 mg/L chromate in 10 g/L sodium chloride. Sample injections were made by applying pressure of 2.07 kPa for 30 s. After the sample injection, the terminating ion of 120 mmol/L borate, 500 mmol/L chlorate or 500 mmol/L phosphate was injected into the capillary by applying pressure of 2.07 kPa for 10 s. The corresponding electropherograms are shown in Fig. 2a–d. When borate of 120 mmol/L was used as the terminating ion, the peak height of iodate in Fig. 2b was 3.0 times higher than that obtained in the CZE without transient ITP (Fig. 2a). When chlorate of 500 mmol/L was used as the terminating ion, iodate was comigrating with chlorate and the iodate peak was on the tailing part of the chlorate peak (Fig. 2c). Similar results were obtained when concentration of chlorate was varied in the range of 100–800 mmol/L and injection time in the range of 1–10 s. Surprisingly, when phosphate was used as the terminating ion, the peak height of iodate (Fig. 2d) was 35 times higher than that obtained in the CZE without transient ITP. Phosphate was the best among the terminating ions tested for the sensitivity improvement of iodate.

The effect of concentration of phosphate on the sensitivity improvement of iodate was also investigated. Fig. 3a shows the results of peak height ratio of iodate obtained using the tITP–CZE to the CZE. H represents the peak height of iodate obtained using the tITP–CZE and H_0 obtained using the CZE without the tITP. Concentration of phosphate was examined in the range of 250–2000 mmol/L. The terminating phosphate solution was introduced into the capillary by applying pressure of 2.07 kPa for 10 s after the sample injection. It can be seen in Fig. 3a that the peak height of iodate increased significantly with concentration of the terminating ion of phosphate up to 800 mmol/L and then slightly when concentration of the terminating ion of phosphate was higher than 800 mmol/L. But when concentration of phosphate was higher than 1500 mmol/L, the iodate peak became far deviated from Gaussian shape. The zone length of the terminating ion was another factor influencing the results of enrichment in tITP since it had an effect on the period of time available for the isotachophoretic migration process. The effect of injection time (in the range of 2–15 s) of the terminating ion of phosphate on the sensitivity improvement of iodate is shown in Fig. 3b. Peak height of iodate increased first and then decreased with the injection time. The optimum was located at about 10 s. Therefore, phosphate of concentration of 1500 mmol/L and injection time of 10 s were used for tITP in later experiments. The good combination of the effective mobility, charge, concentration and zone length of the terminating ion of phosphate resulted in the significant enrichment of iodate.

3.3. Optimization of the concentration of CTAC

The effect of cetyltrimethylammonium chloride (CTAC) on the electrophoretic behaviour of iodate has been investigated in depth (Hirokawa, Ichihara, Ito, & Timerbaev, 2003; Yokota et al., 2003), it showed that iodate had no appreciable interaction with CTAC, so that the migration time of iodate was almost constant as concentration of CTAC alerted. However, concentration of CTAC affected the enrichment of iodate. With sample (0.5 mg/L iodate and 5.0 mg/L chromate in 10 g/L sodium chloride) injection by applying pressure of 2.07 kPa for 30 s and introduction of the terminating ion of 1500 mmol/L phosphate by applying pressure of 2.07 kPa for 10 s, the peak height of iodate increased with increasing concentration of CTAC up to 22 mmol/L and then almost remaining constant (see Fig. 4) when concentration of CTAC in the sodium chloride solution (pH 8.0) was varied in the range of 5–25 mmol/L. But the baseline noise started to increase when concentration of CTAC was higher than 20 mmol/L. Therefore, CTAC of 20 mmol/L was used for quantitative measurements.

3.4. Optimization of sample injection

Time of sample injection needs to be optimised to obtain best results in tITP–CZE. Time duration of 10–60 s was examined. Both responses of peak height and peak area for iodate increased with increasing injection time up to 40 s. However, the peak of iodate became far deviated from Gaussian shape when sample injection time was longer than 35 s. Consequently, 30 s was set for sample injection in quantitative measurements.

3.5. Calibration curve and analytical performance

The proposed tITP–CZE method was applied to determination of iodate in table salt and a typical electropherogram is shown as trace A in Fig. 5. For comparison, a typical electropherogram obtained in the CZE is shown as trace B in the same figure. It was satisfactory to determine iodate in table salt by the tITP–CZE while it was difficult to determine iodate in table salt by the CZE without tITP.

Standard solutions of iodate were prepared using 10 g/L sodium chloride solution. Calibration graphs were linear up to 5.00 mg/L of iodate, using both peak area ratio and peak height ratio with chromate of as the internal standard at 5.00 mg/L versus concentration of iodate. Regression equations relating area ratio and height ratio to concentration of iodate were $y = 0.1241x + 0.0019$ (correlation

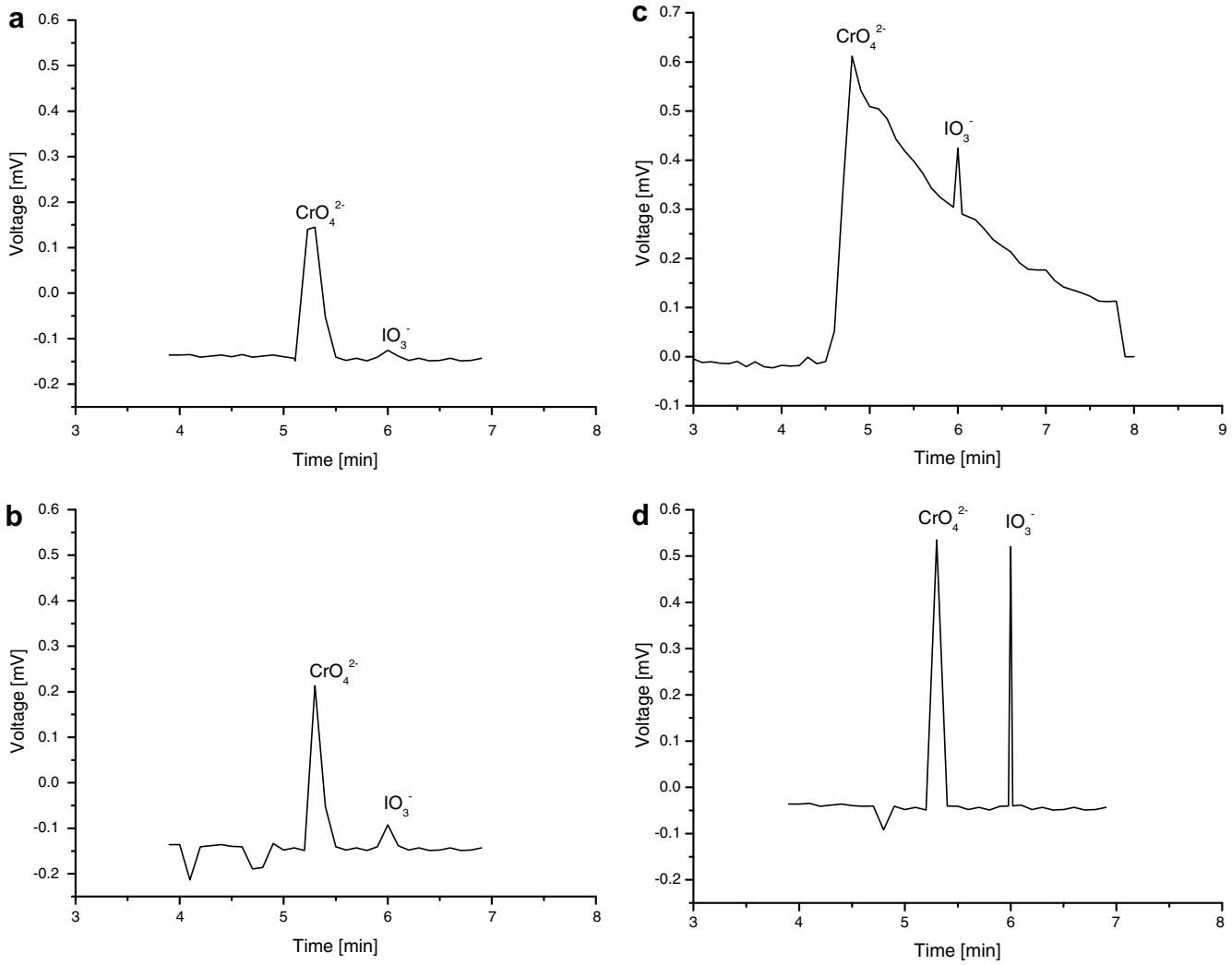

Fig. 2. Effect of terminating ions on the tITP–CZE of iodate. Capillaries: $L_{tot.}$ = 60 cm, $L_{det.}$ = 50 cm, 75 μm ID × 375 μm OD. Electrophoretic buffer: sodium chloride solution (pH = 8.0) containing 20 mM CTAC. Voltage: −8 kV. Wavelength: 218 nm. Sample: 0.5 mg/L iodate and 5.0 mg/L chromate in 10 g/L NaCl solution (pH = 8.0). Sample injection: 2.07 kPa for 30 s. (a) CZE without tITP. (b) tITP–CZE. Terminating ion of 120 mmol/L borate was introduced into the capillary by applying pressure of 2.07 kPa for 10 s. (c) tITP–CZE. Terminating ion of 500 mmol/L chlorate was introduced into the capillary by applying pressure of 2.07 kPa for 10 s. (d) tITP–CZE. Terminating ion of 500 mmol/L phosphate was introduced into the capillary by applying pressure of 2.07 kPa for 10 s.

coefficient, 0.9939) and $y = 2.3109x - 0.0088$ (correlation coefficient, 0.9993), respectively.

Precision was evaluated based on five replicate measurements. The relative standard deviation (RSD) were 2.25% and 1.08% in terms of peak area ratio and peak height ratio, respectively. Linearity of calibration curve in terms of correlation coefficient and precision in terms of RSD for peak height ratio were better than those for peak area ratio. The reason might be narrowness of the peak width and the parameters set for the detection and integration. Hence, the quantitative results of the tITP–CZE were calculated by using data of the peak height ratio with chromate as the internal standard. RSD ($n = 5$) was 0.85% in terms of migration time. The detection limit of iodate ($S/N = 3$) was determined to be 3.5 μg/L in table salt solution.

The recovery of potassium iodate in table salt was determined by five measurements to be in a range from 101 to 103%.

3.6. Improvement on the precision of quantitative results of the tITP–CZE by using chromate as the internal standard

Commonly, using an internal standard improves the precision of quantitative results. The quantitative results of potassium iodate in table salt were obtained by using the tITP–CZE with and without the use of chromate as the internal standard. It was observed that the RSD ($n = 5$) of the results was 2.5 times smaller when using the internal standard of chromate than that without the use of the internal standard.

3.7. Comparison of the results of potassium iodate in table salt determined by the tITP–CZE and by the redox titration

For method comparison and validation, potassium iodate in three different samples of table salt was determined by both the tITP–CZE and the redox titration method (China State Bureau of Quality & Technical Supervision, 2000). The respective averages were 32.5 (1.08%), 33.5 (1.01%), and 33.9 (1.00%) mg/kg, and 33.0 (2.09%), 33.8 (1.45%), and 34.3 (1.52%) mg/kg. Figures in parentheses indicate the relative standard deviation (RSD) of five replicate measurements.

For the two set of the results of each sample, the *F*-test method (Kaiser, 1971) was employed to evaluate whether there was significant difference between the two standard deviations, i.e., to determine whether the two variances were from the same population. It was found that there was no significant difference between the

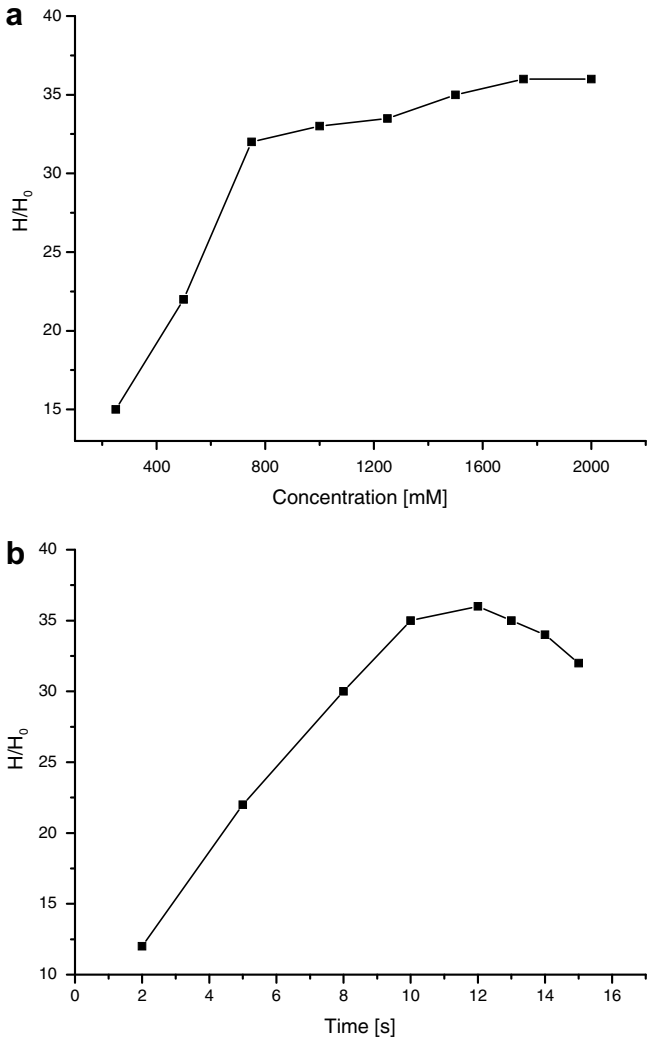

Fig. 3. (a) Effect of concentration of the terminating ion of phosphate on the sensitivity improvement of iodate. *H* represents the peak height of iodate obtained using the tITP–CZE and H_0 obtained using the CZE without the tITP. (b) Effect of injection time of the terminating ion of phosphate on the sensitivity improvement of iodate. Other conditions were the same as given in Fig. 2d.

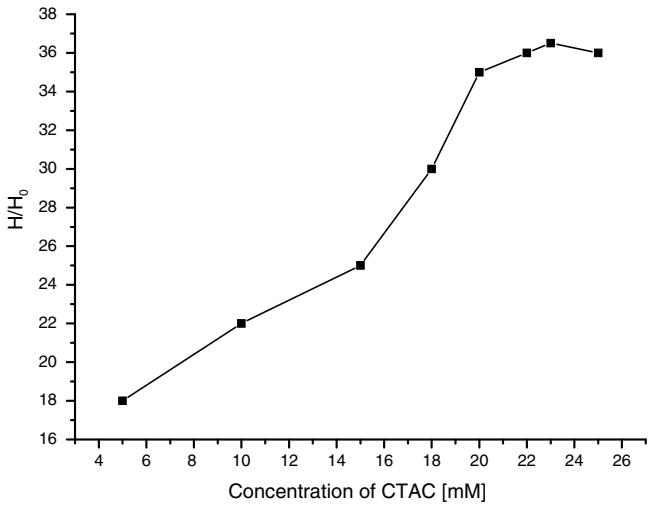

Fig. 4. Effect of concentration of cetyltrimethylammonium chloride (CTAC) on the enhancement of iodate. Other conditions were as described in Fig. 2d.

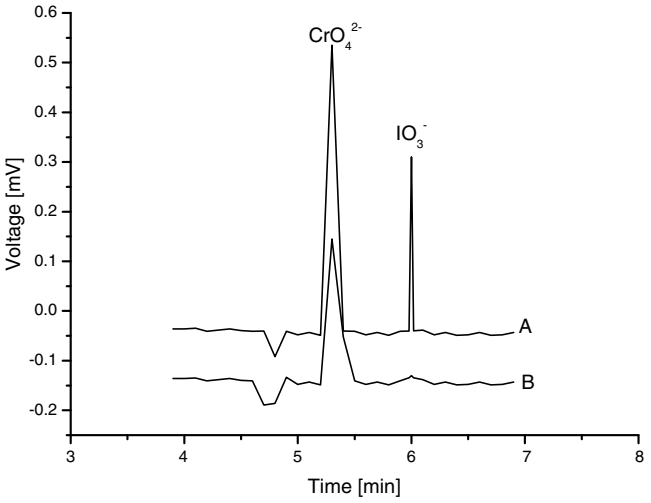

Fig. 5. Typical electropherograms of iodate in table salt obtained by using the tITP–CZE and the CZE. A: tITP–CZE. B: CZE. Chromate was added in the sample solutions as the internal standard. For electrophoretic conditions and peak identification, see the legends for Fig. 2a and d, respectively.

standard deviations of the two sets of data, or there was no significant difference between the two sets of measurements of the redox titration and the tITP–CZE in terms of precision. Then, the *t*-test was performed to compare the two means. The calculated *t*-value was less than the statistical *t*(90). Therefore, it could be concluded that there was no significant difference between the two means of the respective results of potassium iodate in table salt obtained by the redox titration and the tITP–CZE, and the tITP–CZE method developed could be an alternative means for the determination of iodate in table salt.

4. Conclusions

A transient isotachophoresis–capillary zone electrophoresis method for determining iodate in table salt with UV detection has been first time developed. The sensitivity of the proposed method was enhanced by coupling an on-capillary preconcentration technique using the principle of transient isotachophoresis with capillary zone electrophoresis. The interference of sample matrix was overcome by using electrophoretic buffer containing high concentration of sodium chloride. Use of chromate as the internal standard could significantly improve the precision of the quantitative results. The quantitative results of iodate in table salt measured by the transient isotachophoresis–capillary zone electrophoresis method were well agreed with those measured by the redox titration. There was no significant difference between the two means of the results obtained using the two methods. The method developed was sensitive, fast and simple. It was agreeable to the determination of iodate in real samples of table salt with sufficient precision.

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