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Short communication

Use of ion-pairing reagent for improving iodine speciation analysis in seaweed by pressure-driven capillary electrophoresis and ultraviolet detection

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ABSTRACT

This study achieved resolution improvement for iodine speciation in the presence of an ion-pairing reagent by a pressure-driven capillary electrophoresis (CE) system. Addition of 0.01 mM tetrabutyl ammonium hydroxide (TBAH) as the ion-pairing reagent into the electrophoretic buffer resulted in the complete separation of four iodine species (I^-, IO_3^-, mono-iodothyrosine-MIT and di-iodothyrosine-DIT), because of the electrostatic interaction between TBAH and the negatively charged analytes. A +16 kV separation voltage was applied along the separation capillary (50 μm i.d., 80 cm total and 60 cm effective) with the inlet grounded. The detection wavelength was fixed at 210 nm, and the pressure-driven flow rate was set at 0.12 mL min^{-1} with an injected volume of 2 μL. The optimal electrolyte consisted of 2 mM borate, 2 mM TBAH and 80% methanol with pH adjusted to 8.5. Baseline separation of iodine species was achieved within 7 min. The detection limits for I^-, IO_3^-, MIT and DIT were 0.052, 0.040, 0.032 and 0.025 mg L^{-1}, respectively. The relative standard deviations of peak heights and areas were all below 3% for 5 mg L^{-1} and 5% for 1 mg L^{-1}. Application of the proposed method was demonstrated by speciation analysis of iodine in two seaweed samples. The developed method offered satisfactory recoveries in the 91–99% range and good precisions (<5%). Good agreement between the determined values by the proposed CE method and the HPLC-ICP-MS method was also obtained. All results proved its great potential in routine analysis of iodine speciation in environmental, food and biological samples.

1. Introduction

As a well-known essential element for human, iodine is dominantly utilized for the in vivo synthesis of thyroid hormones. The latter compounds play an important role in many life processes, such as cell development and differentiation as well as metabolic balance [1]. As a result, the United States National Research Council recommends a daily dietary allowance level of 150 μg iodine for adult [2]. Lack of iodine is sometimes encountered, which is related to several adverse effects (known as iodine deficiency disorders) [2]. To prevent iodine deficiency, iodized edible salt has been taken for many years in most countries in the world. On the contrary, excessive intake of iodine also leads to many diseases, for instance, iodine-induced hyperthyroidism and autoimmune thyroid diseases [3,4].

Dietary intake is the most important source of iodine for the general population. Seafood, mainly seaweed, is a good dietary source of iodine aside from edible salt because seaweed can accumulate trace iodine species from seawater. However, the bioavailability of iodine is mainly dependent on the chemical form. The bioavailability of iodo amino acids such as mono-iodotyrosine (MIT) and di-iodotyrosine (DIT) has been reported to be less than that of mineral iodide [3]. The absorption and metabolism of iodine are slightly different for each chemical form. Iodine and iodate ingested are reduced to iodide in the gut and then completely absorbed [5]. MIT and DIT are the precursors

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in the biosynthesis of thyroid hormones, which are characteristic products of iodinated casein with high activity in animal growth [6]. Greater than 97% of iodine in the body is excreted through urine [7]. In addition, the toxicity of iodine is species-specific. Inorganic forms of iodine such as iodide and iodate are less toxic than molecular iodine and some organically bound iodine [5]. Moreover, iodine species in seaweed indirectly offer important clues of marine environment, such as geochemically active processes and hazardous contaminants. Hence, a highly efficient method for iodine speciation analysis in seaweed is of great significance.

The analysis of different iodine species is predominantly performed by the hyphenation between a separation technique and a selective detector [7]. Gas chromatography (GC) has been employed for iodine determination with electron capture detector and mass spectrometric detection in seaweed [8], atmosphere [9,10] and lake water [11]. However, only volatile iodinated species (molecular iodine and iodine alkanes) could be directly analyzed by GC. A time-consuming derivatization procedure must be performed to transform non-volatile iodine species (e.g., iodide, iodate, MIT and DIT) into volatile compounds. High performance liquid chromatography (HPLC) is one of the most preferred separation techniques for non-volatile and thermolabile compounds and iodine speciation has been analyzed by many researchers with various HPLC modes (reversed phase, ion exchange and size exclusion modes) [7]. For example, Romarís-Hortas et al. [12–14] presented an HPLC method with inductively coupled plasma mass spectrometry (ICP-MS) detection for the speciation of bio-available iodine and iodinated amino acids in seaweed. However, the drawbacks of HPLC methods rely on long analysis time and high consumption of organic solvents, which is in violation of the principles of green analytical chemistry [15].

Capillary electrophoresis (CE) has attracted intense interest for the determination of iodine species owing to unique features including high separation efficiency and short separation time. Coupled with ICP-MS and ultraviolet (UV) detectors, CE has been utilized to separate various iodine species (iodide, iodate, 3,3′,5-triiodothyronine – T_3 and 3,3′,5,5′-tetraiodothyronine – T_4) in human serum and urine [16,17], marine [18–21] and underground [22] water, antiseptics [23], foodstuff [24] and table salt [25]. Although ICP-MS supplies superior analytical performance over UV, the purchasing cost and operating cost of the former far exceed that of the latter. Moreover, the coupling of CE to ICP-MS is a difficult issue. Therefore, UV is the preferred detector in the CE analysis of iodine speciation. Established CE methods have focused on molecular and inorganic iodine compounds. The target analytes were extended to organic iodinated compounds (T_3 and T_4) only by Michalke and Schramel [17]. To the best of our knowledge, literature on the determination of iodinated amino acids (MIT and DIT) by CE is very minimal.

Herein, we first reported the simultaneous determination of inorganic iodine species and iodinated amino acids (I^-, IO_3^-, MIT and DIT). Considering the similar mobilities of MIT and DIT, an ion-pairing reagent, namely, tetrabutylammonium hydroxide (TBAH) was added into the background electrolyte to achieve baseline separation based on the interaction between TBAH and iodinated amino acids under alkaline conditions. The effects of the type of the ion-pairing reagent and its concentration on the separation were studied. Experimental conditions including the electrophoretic buffer (type, concentration and pH), the separation voltage and pressure-driven flow rate were also optimized to obtain best separation. Finally, the method was applied to the analyses of two seaweed samples.

2. Materials and methods

2.1. Chemicals and reagents

All the chemicals and solvents used in this work were of analytical or chromatographic grade. Ultrapure water with a resistivity of 18.2 MΩ cm, obtained from a Milli-Q Plus water purification system (Millipore, Bedford, MA, USA) was used throughout the experiment. Potassium iodide (≥99.99%) and potassium iodate (≥99.99%) were purchased from Sinopharm Chemical Reagent Co., Ltd (Huangpu District, Shanghai, China) to prepare standard stock solutions of 1000 mg L^{-1} for iodide and iodate in 1 mM KOH. Standard stock solutions of MIT and DIT (1000 mg L^{-1} as iodine) were prepared by individually dissolving appropriate amounts of 3-iodo-L-tyrosine (≥98%) and 3,5-diiodo-L-tyrosine dihydrate (≥98%) in 1 mM KOH, which were supplied by Suzhou Chemland Pharmaceutical & Technologies Co., Ltd (High-tech District, Suzhou, China). All standard stock solutions were stored in the dark at 4 °C. A series of standard mixture solutions containing 0.2, 0.5, 1, 2, 5, 20 and 100 mg L^{-1} of the above iodine species (as iodine) were diluted from their stock standard solutions in the ultrapure water. Ammonium chloride (99.999%), tetramethylammonium hydroxide (TMAH), tetraethylammonium hydroxide (TEAH) and tetrabutylammonium hydroxide (25% v/v in water) as ion-pairing reagents were obtained from Sinopharm. Sodium tetraborate ($Na_2B_4O_7{\cdot}10H_2O$) was also purchased from Sinopharm for the preparation of 100 mM borate solution. The background electrolyte used for iodine separation was a mixture solution of 2 mM borate and 2 mM TBAH (pH 9.0), which was diluted from 100 mM borate solution and 25% v/v TBAH with pH adjustment by boric acid and sodium hydroxide (99.99% for metal analysis) from Sinopharm. All solutions were filtered through 0.22 μm membrane filters before analysis and all the utensils were soaked in 10% HNO_3 for 24 h and then rinsed thoroughly with ultrapure water before use.

2.2. Instrumentation

The pressure-driven capillary electrophoresis system was based on a TriSepTM-2100 pressurized capillary electrochromatography unit (Unimicro (Shanghai) Technologies Co., Ltd., Pudong District, Shanghai, China), similar to our previous work [26]. In brief, it consisted of two micro-volume double plunger pumps with a 0.0001–9.9999 mL min^{-1} flow rate range, a mixer with two inlets and one outlet, an electric six-port injection valve with a 2 μL sample loop, a PEEK cross, an 190 ~ 700 nm variable wavelength ultraviolet-visible detector and a high voltage power supply with an output voltage range of 0 ± 30 kV and an output current range of 0–100 μA. The four openings of the PEEK cross were separately connected to the injection valve, the splitting capillary, the separation capillary and the ground electrode. The splitting capillary was a 60-cm fused silica capillary (50 μm i.d. and 365 μm o.d.) from Yongnian Ruifeng Optical Fabric Factory (Yongnian, Hebei, China). The separation capillary was a bare fused silica capillary (75 μm i.d. and 365 μm o.d., 80 cm in length) from the same company with a 5-mm open window at 60-cm from the inlet terminal for UV detection. A separation voltage of +16 kV was applied at the exit terminal of the separation capillary while the detection wavelength was constant at 210 nm. The two high-pressure pumps individually fed the electrolyte and methanol through the PEEK cross at a total flow rate of 0.12 mL min^{-1} and a composition ratio of 1:4.

For method validation, contents of iodine species in seaweed extract solutions were also determined by an HPLC method [27]. In brief, the homemade HPLC system consisted of a reversed phase C_{18} column (Diamonsil C_{18} (2), 5 μm × 4.6 mm i.d. × 150 mm, Dikma