Analytica Chimica Acta 787 (2013) 254–259

Contents lists available at SciVerse ScienceDirect

Analytica Chimica Acta

journal homepage: www.elsevier.com/locate/aca

Determination of artificial sweeteners by capillary electrophoresis with contactless conductivity detection optimized by hydrodynamic pumping

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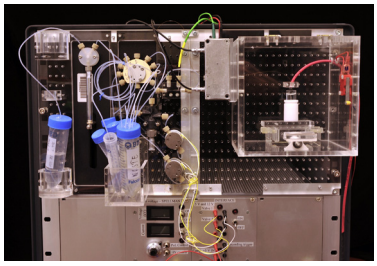
HIGHLIGHTS

- Capillaries of 10 μm ID were employed.
- Superimposed hydrodynamic pumping is possible without penalty in band broadening with such narrow capillaries.
- Analysis times of less than 3 min were possible.
- The use of a computer controlled sequential analysis system allows flexible optimization of injection volumes and pumping rates.
- Detection limits in the low μM range were achieved.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 10 April 2013
Received in revised form 16 May 2013
Accepted 23 May 2013
Available online 3 June 2013

Keywords:
Artificial sweeteners
Capillary electrophoresis
Contactless conductivity detection
Hydrodynamic flow
Sequential injection analysis

ABSTRACT

The common sweeteners aspartame, cyclamate, saccharin and acesulfame K were determined by capillary electrophoresis with contactless conductivity detection. In order to obtain the best compromise between separation efficiency and analysis time hydrodynamic pumping was imposed during the electrophoresis run employing a sequential injection manifold based on a syringe pump. Band broadening was avoided by using capillaries of a narrow 10 μm internal diameter. The analyses were carried out in an aqueous running buffer consisting of 150 mM 2-(cyclohexylamino)ethanesulfonic acid and 400 mM tris(hydroxymethyl)aminomethane at pH 9.1 in order to render all analytes in the fully deprotonated anionic form. The use of surface modification to eliminate or reverse the electroosmotic flow was not necessary due to the superimposed bulk flow. The use of hydrodynamic pumping allowed easy optimization, either for fast separations (80 s) or low detection limits (6.5 μmol L^{-1}, 5.0 μmol L^{-1}, 4.0 μmol L^{-1} and 3.8 μmol L^{-1} for aspartame, cyclamate, saccharin and acesulfame K respectively, at a separation time of 190 s). The conditions for fast separations not only led to higher limits of detection but also to a narrower dynamic range. However, the settings can be changed readily between separations if needed. The four compounds were determined successfully in food samples.

1. Introduction

Artificial sweeteners are widely used as additives in food, beverages and pharmaceutical products as a low calorie alternative to natural sugar. Diabetes patients, who have to control blood sugar, make use of them, as well as persons who wish to control their body weight or are concerned about dental caries. Since these

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http://dx.doi.org/10.1016/j.aca.2013.05.039

sweeteners are prepared by chemical synthesis, their presence in food is however the cause of extensive consumer mistrust [1]. Frequently used are aspartame, cyclamate, saccharin and acesulfame K. These compounds are often employed in combination as this leads to the masking of undesired aftertastes, such as bitterness [2]. The exact composition used in these mixtures is important in order to correctly balance the tastes.

The most common method for the determination of the artificial sweeteners is HPLC [2]. However, cyclamate requires chemical derivatization to make it detectable by the most commonly employed UV-absorbance method due to a lack of a chromophore. For this and other reasons few HPLC methods for the concurrent determination of the sweeteners exist and usually have been based on detection by mass-spectrometry [2]. An attractive alternative is capillary electrophoresis (CE) due to its simplicity, high separation power, relatively short analysis times and low consumption of consumables. Pesek and Matuska [3], Walker et al. [4] and Sabah and Scriba [5] reported methods for aspartame based on capillary zone electrophoresis with UV-detection. Boyce [6] and Frazier et al. [7] later extended the method for the simultaneous determination of aspartame, saccharin and acesulfame K. More difficult again is the determination of cyclamate, but methods for this species alone, based on its detection by indirect UV measurements, have also been developed [8,9]. The concurrent determination of a range of sweeteners which includes cyclamate is best carried out using a more universal detector. Schnierle et al. [10] and Kappes et al. [11] demonstrated the possibility of using potentiometric detection in zone electrophoresis for cyclamate and other sweeteners and Herrmannová et al. [12] have described an isotachophoretic method covering a total of 8 sweeteners employing conductivity detection. The use of contactless conductivity detection (C^4D) in zone electrophoresis is an other attractive and simple alternative and Tanayanyiwa et al. [13] in 2004 demonstrated the detection of acesulfame K and cyclamate on a lab-on-chip device using this technique. More recently Bergamo et al. [14] described the determination of the 4 species, aspartame, cyclamate, saccharin and acesulfame K, by CE-C^4D. A difficulty encountered by Bergamo et al. was related to the fact that a relatively high pH value of 9.4 had to be used in order to render all species in the anionic charged form required for separation and detection. In CE a high electroosmotic flow is then present, which means that for the determination of anions usually a surface modification is carried out in order to reverse the electroosmotic flow, so that it is the same direction as the migration of the anions. This was, however, not found possible, as the most often used approach, dynamic coating of the capillary wall by addition of cetyltrimethylammonium bromide (CTAB) to the running buffer, was found to interfere with the separation of the sweeteners [14]. Separation of the anionic sweeteners was therefore carried out by sweeping them against their mobility with the electroosmotic flow, which is not ideal because of the resulting relatively long separation times.

In a separate development, it has very recently been shown that it is possible to superimpose a bulk flow when carrying out electrophoretic separations in narrow capillaries with inner diameters of 10 μm [15–17]. This was found to be useful for the optimization of separation time and efficiency, and in the determination of anions the compensation of the electroosmotic flow is possible without requiring chemical additives [16]. This operating parameter can be changed readily between measurements, or even instantly during a separation. Despite its utility, the employment of pressure assistance during electrophoretic separations has hitherto largely been considered impossible due to potential band broadening caused by the laminarity of the hydrodynamic flow. However, for the narrow capillaries this is not an issue. Note, that these pressure assisted CE methods are enabled by C^4D, as the detection technique, in contrast to UV-detection, can be readily implemented on the narrow capillaries and allows measurements without degradation of the detection limits [15–17]. In addition, the use of narrow capillaries is preferable as the separation efficiency is generally better, *i.e.* also in the absence of pumping [15].

Herein a more detailed study of the benefit of the superimposition of a bulk flow in the determination of anions by CE-C^4D is presented. The artificial sweeteners are a worthwhile application of this technique due to the call for conductivity detection when determining cyclamate and the difficulties imposed by the electroosmotic flow when including aspartame.

2. Experimental

2.1. Solutions

Ultrapure water of 18 MΩ cm resistivity, obtained from a Milli-Q 185 system (Millipore, Saint-Quentin-en-Yvelines, France), was used to prepare all solutions. All reagents were of analytical grade. Cyclamic acid (cyclohexanesulfamic acid) sodium salt, saccharin sodium salt hydrate, 2-(cyclohexylamino)-ethanesulfonic acid (CHES) and tris(hydroxymethyl)aminomethane (Tris) were purchased from Sigma (Buchs, Switzerland); histidine (His), aspartame, acesulfame-K, and sodium hydroxide from Fluka (Buchs, Switzerland). Standards were prepared by dilution of aqueous stock solutions of 10 mM of the sweeteners, which were stored in the refrigerator at 4 °C. The solid samples were dissolved in deionized water and the soft drink samples were diluted with water as required. All sample solutions were degassed in an ultrasonic bath and filtered through 0.45 μm membrane filters (Macherey-Nagel, Oensingen, Switzerland) before injection. The background electrolyte for the separations consisted of a Tris/CHES buffer at pH 9.1 which was prepared fresh daily to minimize the uptake of carbon dioxide from air.

2.2. Instrumentation

Preliminary experiments and buffer optimization were carried out on an instrument built in-house similar to the one previously described [18] but using a dual polarity high voltage power supply with a range of ±30 kV (Spellman CZE2000, Pulborough, UK). The system used for pressure assisted separations was also constructed in-house and employed a sequential-injection analysis (SIA) manifold for fluid manipulation and pressurization and is based on earlier designs reported by us [15–17,19,20]. A schematic drawing of the SIA-CE-C^4D system is given in Fig. 1. The syringe pump (Cavro XLP 6000) fitted with a 1 mL syringe and the 9-port channel selection valve (Cavro Smart Valve) were obtained from Tecan (Crailsheim, Germany) and used for flushing of the capillary, for aspiration and injection of the sample, and for creating the bulk flow through the capillary during separation. The different required backpressures were achieved with the help of a micro-graduated needle valve (P-470, Upchurch Scientific, Oak Harbor, WA, USA) and solenoid isolation valves from NResearch (HP225T021, Gümligen, Switzerland). Split injection was accomplished by pumping the sample plug past the capillary inlet under partial pressurization. Note that a similar system for hydrodynamic injection, albeit using a pressurized gas for liquid propulsion, had been reported previously [21]. The two different effective injection volumes employed were achieved by changing the volume of the dispensed plug (90 μL and 2.5 μL) at a fixed pumping rate (13 pulses s^{-1}) and the two flow rates through the capillary during the separation were created by adjusting the pump rate (6 pulses s^{-1} and 156 pulses s^{-1}). The injection end of the capillary was electrically grounded while the separation voltage was applied at the detector end using a dual polarity high voltage power supply with a range of ±30 kV