Food Chemistry 187 (2015) 130–134

Contents lists available at ScienceDirect

Food Chemistry

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

Analytical Methods

Chiral ligand-exchange separation and determination of malic acid enantiomers in apple juice by open-tubular capillary electrochromatography

Cemil Aydoğan [a,*], Veyis Karakoç [b], Adil Denizli [b]

[a] Department of Food Engineering, Bingöl University, Bingöl, Turkey
[b] Department of Chemistry, Biochemistry Division, Hacettepe University, Ankara, Turkey

ARTICLE INFO

Article history:
Received 17 January 2014
Received in revised form 15 April 2015
Accepted 17 April 2015
Available online 22 April 2015

Keywords:
Ligand-exchange
Open-tubular capillary electrochromatography
Malic acid
Apple juice
Cosmetics
Chiral separation
Food analysis

ABSTRACT

This study describes the application of an open tubular capillary column for chiral ligand-exchange separation and determination of malic acid enantiomers in apple juice by open-tubular capillary electrochromatography (OT-CEC). The open tubular column was prepared by *in-situ* grafting polymerization of 3-chloro-2-hydroxypropyl methacrylate (HPMA-Cl) and followed by L-Histidine (L-His) modification. L-His was used as a chiral ligand-exchange selector and copper (II) as a central ion. The electrochromatographic characterization of the open tubular column was performed with the use of thiourea as an electroosmotic flow (EOF) marker. Factors affecting electrochromatographic enantioseparation of malic acid were also studied. The running buffer conditions for optimum enantioseparation of malic acid were found to be ACN/5.0 mM $CuSO_4$, 20.0 mM $(NH_4)_2SO_4$ (60/40%, v/v) adjusted to pH 3.0. The separation and determination of the enantiomers of malic acid in the apple juice solution diluted 10- to 40-folds were successfully achieved.

1. Introduction

Malic acid, a kind of carboxylic diacid, was first isolated from apple juice a number of years ago by Scheele (Dawson, 1959). It has two stereoisomeric forms: D- and L- enantiomers. Natural malic acid is the L- enantiomer. The synthetic malic acid is a racemic mixture of the D- and L- enantiomers which is used as a food additive (Kodama et al., 2001) to prevent alteration and degradation by microorganisms during its storage period (Castro-Puyana, Garcia-Canas, Simo, & Cifuentes, 2012). However, excessive concentrations of malic acid may result in allergic contact dermatitis, convulsion and hives.

Malate is known as the salts and esters of malic acid and occurs all metabolizing cells as a key intermediate in the major energy-producing biochemical pathway, which is known as the citric acid cycle or Krebs cycle (Ali Khan et al., 2013). In evaluating the acceptance of malic acid, emphasis is placed on its well-established metabolic pathways and the daily consumption of malic acid-containing food. However, there is some doubt concerning the utilization in the body of the D- enantiomer of malic acid (WHO, 1967). Malic acid is also included in cosmetic products to improve skin texture and reduce pigmentation and wrinkles (Liu, Lin, Feng, & Chen, 2012). Therefore, the development of convenient and inexpensive malic acid analysis methods is of great importance for food safety and the other related issues. A number of investigations on malic acid in different food samples have been performed with the use of different systems (Doner & Cavender, 1988; Eisele & Heuser, 1990; Yamamoto et al., 2001).

Open-tubular capillary electrochromatography (OT-CEC) is a promising bioanalytical separation system for microscale bioseparations and has gained increased attention in recent years (Aydoğan, 2015; Aydoğan & Denizli, 2014; Cheong, Ali, Kim, & Lee, 2013; Xu & Sun, 2008). In open-tubular CEC, the stationary phase is coated to the capillary inner wall. There are several methods for coating the capillary column. These include polymer coatings, porous silica layers, etching and sol gel techniques (Christodoulou, Zhu, & Warner, 2003). Among other types, polymer coating method with long-term stability and different ligand modifications provides a better alternative for the preparation of the convenience stationary phase onto the inner walls of the capillary. Open tubular column with a polymer stationary phase can confer high efficiency due to a flat flow induced by electroosmotic flow

* Corresponding author. Tel./fax: +90 426 216 17 81.
E-mail addresses: aydoganc@hacettepe.edu.tr, caydogan@bingol.edu.tr (C. Aydoğan).

http://dx.doi.org/10.1016/j.foodchem.2015.04.062
0308-8146/

(EOF). Recently, the use of these materials has been playing an important role in the rapid separation of chiral compounds and different biomolecules (Schmid & Gübitz, 2011).

The study of chirality of food compounds seems to play a significant role in food science and technology. Now that chiral separation has much importance for food science, a variety of recent investigations are focused on the use of enantiomeric separation applying capillary electromigration techniques such as capillary electrophoresis (CE), capillary electrochromatography (CEC) and open tubular-CEC (Chankvetadze, 2007; Kofink, Papagiannopoulos, & Galensa, 2007; Sanchez-Hernandez, Castro-Puyana, Garcia-Ruiz, Crego, & Marina, 2010; Sanchez-Hernandez, Sierras-Serra, Marina, & Crego, 2013; Ward & Ward, 2012).

In our recent study, we first prepared an open tubular capillary column for use in the ligand-exchange enantioseparation of amino acids (Aydoğan & Denizli, 2014). In this study, an application of the open tubular column to the chiral ligand-exchange separation of D- and L-malic acid enantiomers without derivatization by OT-CEC was demonstrated. Parameters affecting the enantioseparation and resolution of malic acid were investigated. The determination of enantiomers of malic acid was successfully achieved in apple juice solutions with different malic acid content.

2. Material and methods

2.1. Materials

ACN, NaOH as well as the derivatization reagent, 3-trimethoxysilylpropyl methacrylate (TMSPM) (98%) were purchased from Sigma–Aldrich chemical (Milwaukee, WI, USA). Fused-silica capillary (i.d. 50 μm and, o.d. 360 μm) was supplied by Polymicro Technologies (Phoenix, AZ, USA). $CuSO_4{\cdot}5H_2O$, $(NH_4)_2SO_4$ were supplied from Merck A.G. (Darmstad, Germany). The single enantiomers of malic acid were supplied from Sigma (St. Louis, MO, USA).

2.2. Instruments

All OT-CEC experiments were performed on a Prince CEC-760 (Prince Technologies B.V. Cornelis Houtmanstraat 267825 VG Emmen, The Netherlands) equipped with a photodiode array detector, a high voltage power supply (−30 kV and +30 kV). A centrifuge, Zentrifugen, Universal 32 R (Germany) was used for sample preparation.

2.3. Preparation of open tubular column

The silanization and preparation procedures of the open tubular column were described in our previous study (Aydoğan, Çetin, & Denizli, 2014). The preparation of the open tubular capillary column included the following steps: a fused silica capillary was rinsed with 0.5 M NaOH for 2 h and water for 15 min, respectively. Then, the capillary was functionalized by filling with TMSPM (50%, v/v) in N,N-dimethylformamide (DMF). The silylated capillary was rinsed with methanol to remove the unreacted monomers. 15.5% (v/v) HPMA-Cl and 0.75 mg/mL AIBN as initiator dissolved in toluene were applied into the capillary column. After the two ends of the capillary were sealed with GC septa, the capillary column was heated to 80 °C in an oven and kept at the temperature for 6 h. HPMA-Cl grafted capillary was rinsed with 2.0 M L-His solution adjusted pH 5.2 for 30 min submerged into a water bath at 60 °C for 10 h. Finally, the column was washed with methanol for 30 min and water 30 min, respectively, before use. After conditioning with $CuSO_4$ solution, the Cu(II) ions grafted on the surface of the open tubular column.

2.4. Chiral LE-OT-CEC principles

All experiments were carried out in long capillary mode of CEC system (anode at inlet and cathode at outlet). A capillary with a total length of 37 cm and an effective length 30 cm was used. Sample solutions were injected by electrokinetic method for 0.05 min at −10 kV. An electrolyte solution of $CuSO_4{\cdot}5H_2O$ and 20.0 mM $(NH_4)_2SO_4$ (60/40%) for run buffer was prepared. The concentration of $CuSO_4$ solution between in the range of 1.0–20.0 mM was used. Stock solutions of D- and L-malic acid enantiomers were individually prepared by dissolving in water and stored at +4 °C. At the beginning of each day throughout analysis, the column was flushed with water for 5 min, and then $CuSO_4$ solution for 20 min, respectively. The apple juice was purchased from a local market and it was centrifuged at 2000 rpm for 30 min. 0.50 mL sample of the resulting supernatant was mixed with 3.0 mL water for 2 h. Each standard solution of malic acid was freshly prepared by diluting before use. D- and L-Malic acid enantiomers were detected at 214 nm. Thiourea, unretained compound, was used as an EOF marker. Capacity factors for each enantiomers and separation factors (α) were calculated according to our previous work (Aydoğan, Yilmaz, Çimen, Uzun, & Denizli, 2013).

3. Results and discussion

3.1. EOF of the open tubular column

The EOF in the unmodified and L-His modified open tubular columns was examined with the use of thiourea as the EOF marker. Fig. 1 shows the effect of running buffer pH on EOF, in which negative values of EOF represent anodic EOF, whereas the positive values represent cathodic EOF. As it is shown here, the prepared open tubular column could generate both anodic and cathodic EOF. Compared with the bare capillary column, L-His modified open tubular column could change the EOF and provide reverse polarity. These results should be explained by the fact that the EOF in the open tubular column may be ascribed to the net surface charge density of all charged groups such as amine groups and carboxylic

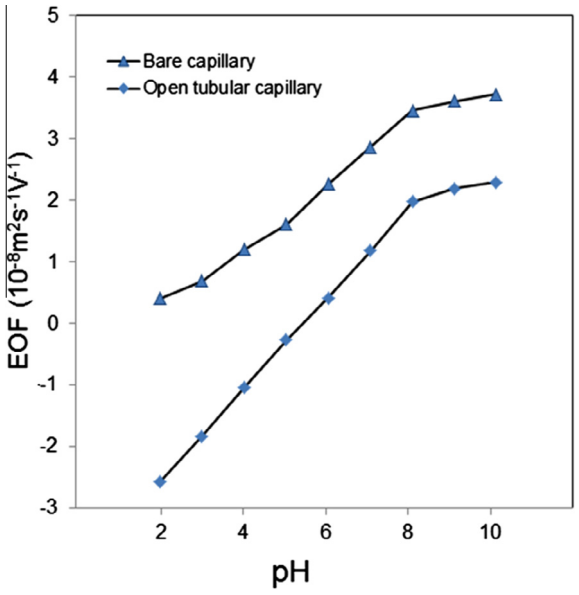

Fig. 1. The effect of buffer pH on EOF in both the bare and the open tubular capillaries. Conditions; 60:40% ACN/Phosphate buffer (5.0 mM): injection: −10 kV, 0.05 min: applied voltage −20 kV: DAD at 200 nm: thiourea was used as EOF marker.