Available online at www.sciencedirect.com
ScienceDirect
Analytica Chimica Acta 576 (2006) 84–90
ANALYTICA CHIMICA ACTA
www.elsevier.com/locate/aca

Use of β-cyclodextrin bonded phase with *s*-triazine moiety in the spacer for separation of aromatic carboxylic acid isomers by high-performance liquid chromatography

Chen-Hsing Lin [a], Chih-Yu Chen [b], Shu-Wen Chang [a], Jong-Chang Wu [a], Ching-Erh Lin [a,c,*]

[a] *Department of Applied Chemistry, Fooyin University, Kaohsiung County, Taiwan*
[b] *Department of Environmental Engineering and Science, Fooyin University, Kaohsiung County, Taiwan*
[c] *Department of Chemistry, National Taiwan University, Taipei, Taiwan*

Received 10 April 2006; received in revised form 30 May 2006; accepted 3 June 2006
Available online 16 June 2006

Abstract

The separation and retention behavior of five aromatic carboxylic acid isomers was investigated by means of high-performance liquid chromatography (HPLC) using a β-cyclodextrin bonded phase with *s*-triazine ring in the spacer. The influence of mobile phase pH on the retention was examined. The presence of *s*-triazine moiety in the spacer enhances greatly the selectivity of the isomers of aromatic carboxylic acids. Baseline separations of the five aromatic carboxylic acid isomers were achieved. In particular, the isomers of toluic, aminobenzoic, nitrobenzoic and hydroxybenzoic acid were successfully and effectively separated. The chromatographic results indicate that, in addition to inclusion complexation, π–π interaction and hydrogen bonding interaction between the bonded phase and analytes play significant roles in the retention of these acid isomers. Different elution orders were observed for these acidic solutes with different substituents. Possible retention mechanisms are discussed.

Keywords: High-performance liquid chromatography; Cyclodextrin bonded phase; Retention mechanism; Aromatic carboxylic acids; *s*-Triazine

1. Introduction

Cyclodextrins (CDs) are toroidal shaped molecules with an overall appearance of a truncated cone. They possess relatively non-polar cavities which allow for inclusion of non-polar moieties of guest molecules through hydrophobic interactions. Thus, they are capable of forming inclusion complexes with various organic molecules. The stability of such inclusion complexes is mainly determined by the relative size and geometry of the guest molecule in relation to the cavity size of the host molecule. However, the stability of the inclusion complex may also be affected to a considerable degree by additional interactions through hydrogen bonding or through steric hindrance [1–4].

The selective inclusion property of CDs has been advantageously utilized in many separation techniques [4–8]. In high-performance liquid chromatography (HPLC), two approaches have been designed for separation of various compounds by using either CDs as mobile phase additives in a mobile phase [9–13] or CD bonded stationary phases [4,7,14–22]. Separation is favored for molecules possessing aromatic and hydrogen bonding moieties so that the inclusion mechanism can be enhanced and additional separation mechanisms are involved. As a matter of fact, cyclodextrins have been widely used in liquid chromatographic separations for a variety of aromatic compounds, structural isomers, positional isomers, and enantiomers [4–7,14–22].

Aromatic carboxylic acids and their derivatives are metabolites of numerous toxic substances, drug and catecholamines and are widely used as anti-phlogistic, anti-rheumatic, antipyretic and anaesthetic drugs to treat many diseases [23,24]. Therefore, the separations of aromatic carboxylic acids are of importance. The separations of the isomers of aromatic carboxylic acids using β-CD bonded phases by HPLC have been reported [15–18,25–29]. However, the positional isomers of some aromatic carboxylic acids such as toluic, aminobenzoic, nitrobenzoic, and hydroxybenzoic acid were still not success-

* Corresponding author. Tel.: +886 7 7811151x667; fax: +886 7 7826732.
E-mail addresses: sc030@mail.fy.edu.tw, celin@ntu.edu.tw (C.-E. Lin).

0003-2670/$ – see front matter © 2006 Elsevier B.V. All rights reserved.
doi:10.1016/j.aca.2006.06.025

fully separated [15]. Efforts to improve the chromatographic selectivity and resolution are still warranted. In previous publications [19,22], it has been shown that the presence of the *s*-triazine ring in a β-CD bonded phase plays an important role in the enhancement of the selectivity of disubstituted benzene derivatives. In view of these, better separation and selectivity for some aromatic carboxylic acid isomers on the *s*-triazine–β-CD bonded phase are expected. In this study, five aromatic carboxylic acids, including toluic, aminobenzoic, nitrobenzoic, hydroxybenzoic and naphthoic acid are selected as analytes. The separation and retention behavior of the positional isomers of these acidic solutes on the *s*-triazine–β-CD bonded phase are examined. Possible retention mechanisms are discussed.

2. Experimental

2.1. Chemicals and reagents

β-Cyclodextrin was purchased from Sigma (USA). Cyanuric chloride was purchased from Merck (Germany). The silica gel used was Nucleosil (pore size 10 nm; particle size 10 μm; surface area 350 $m^2\ g^{-1}$) obtained from Macherey-Nagel (Germany). 3-Aminopropyltriethoxysilane (APS) was supplied from Janssen (Belgium). Synthesis reagents for preparing the stationary phase and solutes of aromatic carboxylic acid isomers were purchased from various suppliers. The methanol of LC grade was purchased from Mallinckrodt (USA). Water was purified by ion exchange followed by treatment in a Milli-Q water purification system (Millipore, USA).

2.2. Preparation of s-triazine–β-CD bonded phase

2.2.1. Derivatization of β-CD with s-triazine

The derivatization of β-CD with *s*-triazine was previously described [19]. A solution of cyanuric chloride (1.84 g) in 20 mL of acetone was added with agitation to a solution of Na_2CO_3 (1.06 g) and β-CD (3.24 g) in 100 mL of water. The mixed solution was reacted at room temperature for 4 h. The product was precipitated by adding excess acetone, filtered, washed well with acetone several times, and then dried over P_2O_5 at reduced pressure.

2.2.2. Silane-modified silica gel

The preparation of silane-modified silica gel was described previously [19]. The silane used was 3-aminopropyltriethoxysilane.

2.2.3. s-Triazine–β-CD bonded phase

The *s*-triazine–β-CD bonded phase was prepared as follows. Silane-modified silica gel (3 g) was suspended in 150 mL aqueous solution to which the derivative of β-CD (2.5 g) was added. A solution of $NaHCO_3$ was slowly added with agitation to this suspension while the pH was kept at about 8. The reaction temperature was kept at about 50 °C. After 48 h of reaction, the product was filtered off, washed thoroughly with water, methanol, and acetone successively, and then dried at 60 °C at reduced pressure for 2 h. The average number of cyanuric chloride molecules attached to each molecule of β-CD was estimated to be 1.87 and the loading capacity of the *s*-triazine–β-CD bonded phase was found to be 155 $\mu mol\ g^{-1}$ by elemental analyses. The reaction schemes and the possible schematic structures of this stationary phase are shown in Fig. 1.

2.3. Apparatus and chromatography

All chromatographic studies were carried out with a liquid chromatographic system that consisted of a Waters 501 solvent delivery system, a U6K injector, and a Waters 486 variable-wavelength UV detector. The recorder was a SIC Chromatocorder 21. The column (30 cm in length; 4 mm i.d.) was packed by a balanced density slurry method described previously [19]. The mobile phase was composed of 4 mM potassium dihydrogen phosphate buffer and methanol (50:50) and the pH was adjusted with phosphoric acid (85%). It was filtered through a 0.45 μm membrane filter and degassed by ultrasonic vibration. The flow-rate of mobile phase was 1.0 mL min^{-1}. The wavelength of UV detector was set at 254 nm. Experiments were carried out at room temperature. Sample solutes were dissolved in methanol and an adequate amount of a sample solution was injected.

3. Results and discussion

3.1. Effect of mobile phase pH on retention

The pH of the mobile phase is an important parameter to optimize the separation of ionizable analytes. Extensive research has been conducted on the optimization of separation through manipulation of mobile phase pH [30–36]. As β-CD forms inclusion complexes more easily in aqueous methanol than in other aqueous–organic solvents, such as water–acetonitrile or water–tetrahydrofuran, in reversed-phase liquid chromatography [17,18], methanol–phosphate buffer was chosen as the mobile phase.

Fig. 2 shows the effect of mobile phase pH on the retention of the five aromatic carboxylic acids studied in the pH range 2.7–3.6. The isomeric peaks of substituted aromatic acids were identified by spiking with each acid isomer. As can be seen, the elution order of the three isomers for each acidic solute does not alter when the pH of the mobile phase varies in the pH range studied. The elution of toluic acid isomers follows the order *ortho* < *meta* < *para*. This elution order agrees with the order expected from the relative stability of their CD inclusion complexes [18] and is consistent with the results obtained on the amine–β-CD bonded phases reported in the literature [15]. This chromatographic result clearly indicates that the formation of inclusion complexes is primarily responsible for the retention of toluic acid isomers on the *s*-triazine–β-CD bonded phase.

It is noted that the retention factors (k) of the *para* isomer obtained on the *s*-triazine–β-CD bonded phase are nearly three times larger than those obtained on the amine-β-CD bonded phase reported by Fujimura et al. [15]. Thus, the selectivity of the *para* isomer to *meta* isomer is significantly enhanced. This retention behavior is attributed to the chemical nature of the spacer of the bonded phase, i.e., the *s*-triazine moiety in the spacer.