Journal of Chromatography A, 1234 (2012) 50–55

Contents lists available at SciVerse ScienceDirect

Journal of Chromatography A

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

Comparative high-performance liquid chromatography enantioseparations on polysaccharide based chiral stationary phases prepared by coating totally porous and core–shell silica particles

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ARTICLE INFO

Article history:
Available online 3 February 2012

Keywords:
Enantioseparations
Chiral HPLC
Core–shell type chiral stationary phases
Polysaccharide-based chiral stationary phases

ABSTRACT

This article reports comparative high-performance liquid chromatographic separations of enantiomers with chiral stationary phases (CSPs) prepared by coating cellulose tris(4-chloro-3-methylphenylcarbamate) on totally porous and on core–shell type silica of comparable particle diameter. Several interesting observations were made: (1) the selectivity of separation was higher on core–shell type CSP compared to totally porous CSP at comparable content of chiral selector (polysaccharide derivative); (2) much flatter dependence of plate height on the mobile phase flow rate was observed for columns packet with CSP prepared with core–shell silica compared to the ones packed with CSPs prepared with totally porous particles; (3) at low mobile phase flow rates core–shell CSP provided lower resolving ability compared to a commercially available CSP having four times higher content of chiral selector along with higher retention of chiral analytes. However, at high flow rates core–shell type CSP performed similarly or better than the commercial column in regards of plate count (N) and peak resolution (R_s) per column length and within a given total analysis time. The advantage of CSP prepared with core–shell silica is obvious from the viewpoint of plate numbers and resolution calculated per unit time (i.e. speed of analysis).

1. Introduction

Chromatographic separation media based on core–shell silica particles are becoming very popular for high-performance liquid phase (HPLC) separations [1]. Shorter diffusion path-length and consequently higher column efficiency belong to the major advantages of these materials [1–5], as well as to a lesser extent more uniform particle-size distribution of core–shell particles [1,6] compared to their totally porous analogues. In addition to the above mentioned advantages, core–shell materials provide flatter dependence of column performance on the mobile phase flow-rate, primarily due to decreased resistance to mass-transfer compared to fully porous silica (a smaller C-term in the van Deemter equation), hence are better suited for high-speed separations [1]. While lower surface area belongs to certain limitations of these particles, lower retention and lower loadability of analytes result as a consequence. Intuitively, such material can be coated only with a limited amount of chiral selector compared to totally porous silica materials, which typically have higher surface area. To the best of our knowledge no journal article has discussed to date the use of core–shell silica materials for the preparation of chiral stationary phases (CSPs) for HPLC enantioseparations or the use of such CSPs in comparative studies with traditional chiral columns.

The goal of this preliminary study was to evaluate the feasibility of preparing polysaccharide-based CSP by coating core–shell silica and to perform initial comparative studies on this CSP and a CSP prepared by coating totally porous silica of same particle size, as well as on a commercial chiral column available with the same chiral selector.

2. Experimental

2.1. Materials

The chiral test compounds *trans*-stilbene oxide, benzoin and 2,2′-dihydroxy-6,6′-dimethylbiphenyl (Fig. 1A–C) were commercially available from Sigma–Aldrich (St. Louis, MI, USA). HPLC-grade n-hexane and 2-propanol were supplied by Karl Roth (Karlsruhe, Germany). The commercially available chiral column Lux Cellulose-4 used in this study (of 4.6 mm × 250 mm dimensions and packed with 3 μm particles) was supplied by Phenomenex Inc. (Torrance, CA, USA). The structure of the chiral selector in Lux Cellulose-4 is shown in Fig. 1D. Core–shell silica with 2.6 μm nominal particle diameter and 9 nm nominal pore size

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0021-9673/$ – see front matter © 2012 Published by Elsevier B.V.
doi:10.1016/j.chroma.2012.01.084

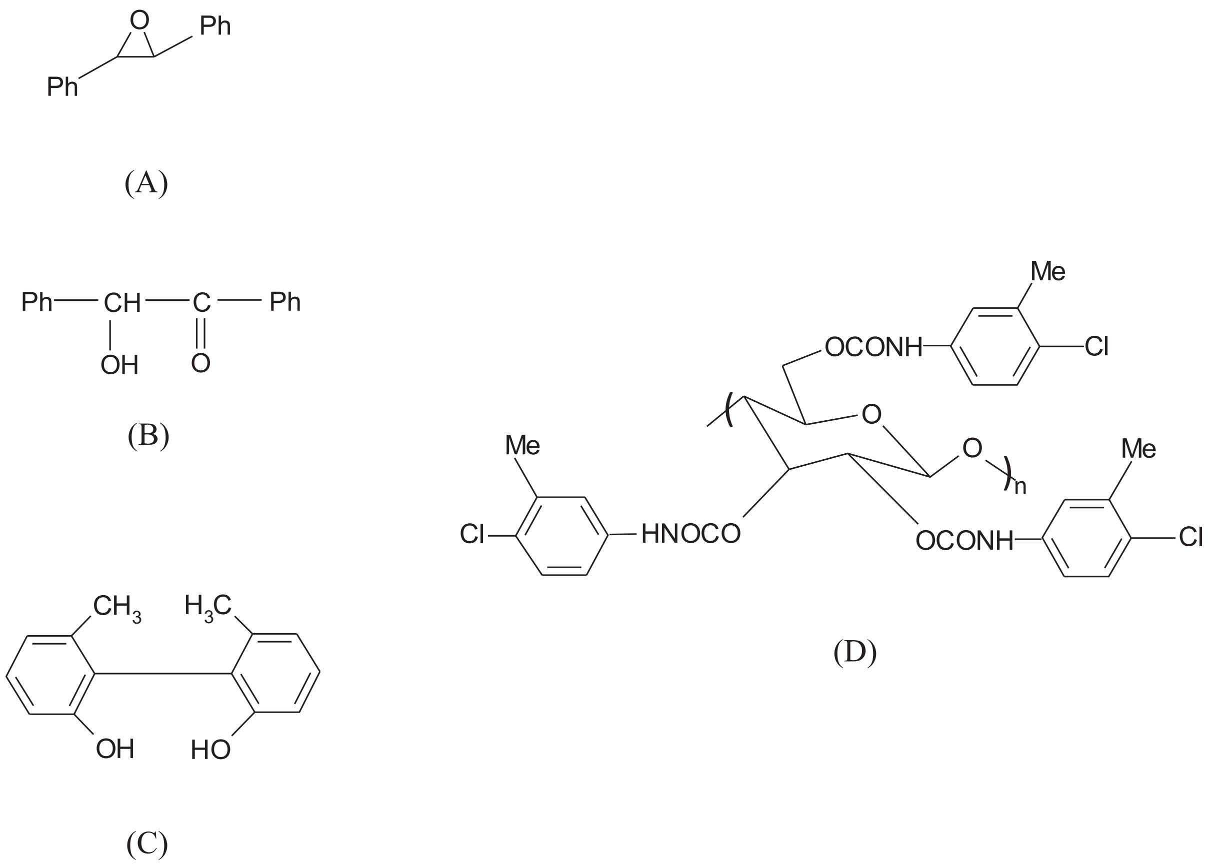

Fig. 1. Structures of *trans*-stilbene oxide (A), benzoin (B), 2,2′-dihydroxy-6,6′-dimethylbiphenyl (C) and cellulose tris(4-chloro-3-methylphenylcarbamate) (D).

was provided by Phenomenex. Totally porous silica with 3.0 μm nominal particle diameter and 10 nm nominal pore size was purchased from Daiso (Osaka, Japan). Cellulose tris(4-chloro-3-methylphenylcarbamate) used for coating core–shell and totally porous silica was synthesized according to a previously described method [7]. Coating of silica with the chiral selector was performed by dissolving of appropriate amount of cellulose tris(4-chloro-3-methylphenylcarbamate) in tetrahydrofurane, adding the weighed amount of silica to this solution, sonication applied for few minutes and evaporation of the solvent at reduced pressure, at room temperature, to dryness. The obtained powder was further dried under reduced pressure at 60 °C for 1 h and used for packing experimental HPLC columns. A slurry of the packing material was prepared in n-hexane/2-propanol mixture 9/1 (v/v), decanted two times and then packed at 500 bar in stainless-steel HPLC columns of 4.6 mm × 250 mm dimensions supplied by Phenomenex Inc.

2.2. Instruments

HPLC separations of test compounds dissolved in the mobile phase in the concentration 0.2 mg/ml were performed using a Knauer K1001 isocratic HPLC pump, a Knauer injection valve with 20 μl sample loop and Knauer K2001 fixed wavelength (220 nm) UV detector (Knauer, Berlin, Germany). The instrument control and data management were performed with Eurochrom software. Knauer K-1900 pneumatic pump was used for packing HPLC columns. Rotary evaporator used in this study was Rotavapor R-210/R-215 from BUCHI Labortechnik GmbH (Essen, Germany) with temperature control and an ultrasonic bath Sonorex RK-100 was from Bandelin (Berlin, Germany). Elemental analysis (N,C) was performed using an Elemental Combustion System CHNS-O, Model ECS4010 by Costech Analytical Technologies Inc. (Valencia, CA, USA). Pore size analysis was performed with the Tristar II Surface Area and Porosity System 3020 instrument made by Micromeritics (Norcross, GA, USA).

3. Results and discussions

3.1. Coating of core–shell silica with cellulose tris(4-chloro-3-methylphenylcarbamate)

Since the specific surface area of core–shell materials is lower and their density is higher compared to totally porous silica, coating of a polysaccharide phenylcarbamate derivative onto its surface is a challenging task due to formation of numerous particle aggregates. Due to this problem, the maximal amount of cellulose tris(4-chloro-3-methylphenylcarbamate) that could be coated with high yield (>80% of final packing material) onto the surface of core–shell silica was in the range of 5% (w/w) (Column 2). This material was used for further experiments together with the CSP prepared with totally porous silica also coated at nominally 5% cellulose tris(4-chloro-3-methylphenylcarbamate) (Column 3). Elemental analysis of CSPs packed in columns 2 and 3 showed that the actual amount of coated cellulose tris(4-chloro-3-methylphenylcarbamate) was 6.8% (w/w) onto the core–shell silica and 5.6% onto the totally porous silica.

3.2. Comparative chromatographic evaluation of experimental core–shell and totally porous CSPs of similar particle size and their comparison to the commercially available chiral column Lux Cellulose-4

The chromatographic performance of the commercial chiral column Lux Cellulose-4 (Column 1; made with 3 μm packing material) and two experimental columns prepared by coating of the same