Journal of Chromatography A, 1490 (2017) 166–176

Contents lists available at ScienceDirect

Journal of Chromatography A

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

# Ordered mesoporous silica functionalized with β-cyclodextrin derivative for stereoisomer separation of flavanones and flavanone glycosides by nano-liquid chromatography and capillary electrochromatography

Mariana Silva[a,b], Damián Pérez-Quintanilla[a], Sonia Morante-Zarcero[a], Isabel Sierra[a], María Luisa Marina[c], Zeineb Aturki[b], Salvatore Fanali[b,*]

[a] Departamento de Tecnología Química y Energética, Tecnología Química y Ambiental, Tecnología Mecánica y Química Analítica, E.S.C.E.T, Universidad Rey Juan Carlos, C/Tulipán s/n, 28933 Móstoles, Madrid, Spain
[b] Institute of Chemical Methodologies, Italian National Council of Research, Area della Ricerca di Roma, Via Salaria Km 29, 300-00015 Monterotondo Scalo, Rome, Italy
[c] Departamento de Química Analítica, Química Física e Ingeniería Química, Facultad de Biología, Ciencias Ambientales y Química, Universidad de Alcalá, Ctra. Madrid-Barcelona Km 33.600, 28871 Alcalá de Henares, Madrid, Spain

## ARTICLE INFO

*Article history:*
Received 28 November 2016
Received in revised form 6 February 2017
Accepted 7 February 2017
Available online 9 February 2017

*Keywords:*
Nano-liquid chromatography
Capillary electrochromatography
Ordered mesoporous silica
Chiral separations
β-Cyclodextrin stationary phase
Flavanones

## ABSTRACT

In this paper a chiral stationary phase (CSP) was prepared by the immobilization of a β-CD derivative (3,5-dimethylphenylcarbamoylated β-CD) onto the surface of amino-functionalized spherical ordered mesoporous silica (denoted as SM) via a urea linkage using the Staudinger reaction. The CSP was packed into fused silica capillaries 100 μm I.D. and evaluated by means of nano-liquid chromatography (nano-LC) and capillary electrochromatography (CEC) using model compounds for the enantio- and the diastereomeric separation. The compounds flavanone, 2′-hydroxyflavanone, 4′-hydroxyflavanone, 6-hydroxyflavanone, 4′-methoxyflavanone, 7-methoxyflavanone, hesperetin, hesperidin, naringenin, and naringin were studied using reversed and polar organic elution modes. Baseline stereoisomer resolution and good results in terms of peak efficiency and short analysis time of all studied flavonoids and flavanones glycosides were achieved in reversed phase mode, using as mobile phase a mixture of $MeOH/H_2O$, 10 mM ammonium acetate pH 4.5 at different ratios. For the polar organic mode using 100% of MeOH as mobile phase, the CSP showed better performances and the baseline chiral separation of several studied compounds occurred in an analysis time of less than 10 min. Good results were also achieved by CEC employing two different mobile phases. The use of $MeOH/H_2O$, 5 mM ammonium acetate buffer pH 6.0 (90/10, v/v) was very effective for the chiral resolution of flavanone and its methoxy and hydroxy derivatives.

## 1. Introduction

The separation and analysis of chiral compounds are an important research topic in different fields also including analytical chemistry, and the separation of chiral compounds is increasingly in demand in various application fields such as, pharmaceutical, agrochemical, biomedical, environmental and nutraceutical areas [1]. Therefore, to meet the requirements related to the resolution and quantification of enantiomers, several stereoselective separation methods have been developed [2–7].

Different analytical techniques have been used for separation of enantiomers [2,5–11]. In high-performance liquid chromatography (HPLC), the direct resolution method employing chiral stationary phases (CSPs) is very popular, and numerous chiral selectors (CSs) have been used for this purpose [2,5–8,12–14]. In that respect, in the last years, thanks to recent achievements in the field of materials science, chromatographic methods for separation of enantiomers utilizing other CSPs have been developed [15]. In addition, the recent progress of miniaturized analytical techniques such as nano-/capillary liquid chromatography (nano-LC/CLC) or capillary electrochromatography (CEC) has opened new horizons in the field of separation science. These techniques can be considered complementary or alternative to HPLC or CE and offer good separation efficiency and resolution, shorter analysis time and rapid

---

* Corresponding author.
*E-mail address:* salvatore.fanali@imc.cnr.it (S. Fanali).

http://dx.doi.org/10.1016/j.chroma.2017.02.012
0021-9673/

optimization of experimental conditions [3,4,16,17]. Developments in the preparation of nanoparticles and monoliths as stationary phases for miniaturised liquid phase separation techniques have been reviewed recently [18].

The discovery of ordered mesoporous silicas (OMSs) in the early nineties marked the beginning of research in the development of high surface area materials with controlled porosity [19]. The dramatically higher surface area of OMSs in comparison to commercially available chromatographic grade silica enhances resolution of molecules by increasing retention factors to allow effective separations of analytes. Up to date, a variety of OMSs have been proposed as stationary phases or supports to prepare stationary phases for solid phase extraction [20,21] and chromatography [22].

Among the CSs used to develop CSPs, β-cyclodextrin (β-CD) and its derivatives have been extensively used for chiral separations employing different chromatographic modes [11,23–31]. Although many CSPs based on CDs are commercially available nowadays, there is a need to develop new packing materials offering high enantioselectivity in short analysis time. In some studies, the use of OMSs as supports for CSPs preparation has improved enantioselectivity and resolution with respect to conventional silica. Some of these articles have been discussed recently in a review paper [15] but, to the best of our knowledge, papers dealing with the use of CSPs based on OMSs for capillary/nano-LC have not been reported yet. In CEC only one paper has demonstrated the application of submicron OMS modified with phenylcarbamoylated-β-CD as CSP [32].

The first high coverage stable bonded phase CD-based CSP was developed by Armstrong and DeMond in 1984 [33]. β-Cyclodextrin was bonded to silica gel via an ether linkage and the resulting CSP could separate many compounds in reverse phase mode [33]. Thereafter, the application of CSPs based on chemically anchored CDs and the understandings of their properties have been broaden tremendously. Thus, numerous publications about synthethic routes and/or immobilization strategies have emerged during the last years, in order to develop well-defined CD-based CSPs. These strategies have been reviewed recently by Xiao et al. [34] and after this review other interesting strategies have also been published [35,36].

In our paper, a spherical mesoporous silica (denoted SM) with 3-D wormhole-like porous framework was used for the first time as support to prepare a CD-CSP. For this purpose, mono(6-azido-6-deoxy)perfunctionalized β-CD was first synthesized and purified, and then this derivative was immobilized onto the surface of amino-functionalized SM (SM-$NH_2$). The Staudinger reaction, which was applied for the first time by Zhang et al. [37] for inmobilization of CD onto aminised silica gel, was used. Under optimized conditions, the chemical anchoring of the β-CD derivative onto the SM support was effective via the hydrolytically stable urethane linkage (Fig. 1) and the current procedure afforded a structurally well-defined β-CD based CSP. In this regard, the objective of this work was to investigate the enantioselectivity of the prepared CD-CSP, by using nano-LC and CEC, for the enantiomeric and diastereoisomeric separation of some selected flavanones as model analytes.

## 2. Experimental

### 2.1. Chemicals and samples

All chemicals were of analytical reagent grade and used as received. Tetraethylorthosilicate 98% (M = 208.33 g/mol, d = 0.934 kg/$m^3$), poly(ethylene glycol)-block-poly(propylene glycol)-blockpolyethylene glycol, Pluronic 123 ($M_{av}$ = 5800 g/mol, d = 1.019 kg/$m^3$), cetyltrimethylammonium bromide (CTAB) 98%, (M = 364.46 g/mol), β-CD, ethyl acetate, pyridine, 3,5-dimethylphenyl isocyanate 99%, sodium azide and 3-aminopropyltriethoxysilane were purchased from Sigma-Aldrich (St. Louis, MO, USA). Hydrochloric acid 37%, acetone, ethyl ether, ethanol and anhydrous sodium sulphate were purchased from Scharlau (Barcelona, Spain). Acetonitrile (AcN), methanol (MeOH), ethanol (EtOH), isopropanol (i-PrOH), acetone and n-hexane (n-Hex) were purchased from Carlo Erba (Milan, Italy). A Milli-Q system (Millipore Waters, Milford, MA, USA) was employed to purify water. Ammonium acetate (500 mM), utilized for the chromatographic runs, was obtained by titrating the appropriate volume of acetic acid with concentrated ammonia solution to pH 4.5. Mobile phases employed for the nano-LC experiments were daily prepared by mixing suitable volumes of buffer solution, water and organic solvents (AcN or MeOH). LiChrospher 100 RP-$C_{18}$, 5 μm particle diameter, was from Merck (Darmstadt, Germany). Fused silica capillaries, 100 μm I.D. × 360 μm O.D. were purchased from Polymicro Technologies (Phoenix, AZ, USA). The selected flavonoids (flavanone, 2′-hydroxyflavanone, 4′-hydroxyflavanone, 6-hydroxyflavanone, 4′-methoxyflavanone, 7-methoxyflavanone, hesperetin, hesperidin, naringenin, and naringin) were from Sigma–Aldrich. Stock standard solutions of each flavonoid (1 mg/mL) were prepared in MeOH, and stored at 4 °C. Further dilutions were daily done with water and AcN (60/40, v/v) to obtain the final concentration of 100 μg/mL. Fig. 1S. shows the chemical structure of the studied compounds (supplementary material).

### 2.2. Instrumentation

A BASIC 20 pH meter (Crison, Barcelona, Spain) was employed for accurate pH measurements in aqueous buffer solution. A Series 10 LC HPLC pump (Perkin Elmer, Palo Alto, CA, USA) a Decon model FS 100b (Hove, UK) ultrasonic bath and a Stereozoom 4 optical microscope (Cambridge Instruments, Vienna, Austria) were employed for the capillary packing process.

The nano-LC experiments were carried out with a laboratory-assembled instrumentation utilizing a LC10 HPLC pump (Perkin Elmer, Palo Alto, CA, USA), a modified injection valve (Enantiosep GmbH, Münster, Germany), and an UV–vis on-column detector (Spectra Focus PC1000, Thermo Separation Products, San Jose, CA, USA), set at 200 nm. The HPLC pump, delivering continuously MeOH isocratically, and the injector were connected so as to obtain a passive split-flow system needed to reduce the flow rate at nL/min levels. The capillary column was directly inserted into the modified injector equipped with 50 μL loop. Both pump and injection valve were joined to a stainless steel T piece (Vici, Valco, Houston, TX, USA) by means of 500 μm I.D. stainless steel tubes with lengths of 70 and 5 cm, respectively. The third entrance of the T was connected to the MeOH reservoir of the pump, through a fused silica capillary (50 μm I.D. × 50 cm) achieving a continuous recycling of the organic solvent.

Sample and mobile phase were introduced into the nano- LC system through the injection valve. Injections were done by filling the loop with the sample solutions, switching the valve for appropriate time and then flushing the loop with the mobile phase. The flow rate was estimated by connecting a 10 μL syringe (Hamilton, Reno, NV, USA) to the capillary column outlet through a Teflon tube (TF-350; LC-Packing, CA, USA) and by measuring the volume of mobile phase accumulated over 5 min. Data were collected using Clarity$^{TM}$ Advanced Chromatography Software (DataApex Ltd., Prague, Czech Republic).

CEC experiments were performed on a HP$^{3D}$ CE apparatus (Agilent Technologies, Waldbronn, Germany) with on-column UV-