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Comparison of superficially porous and fully porous silica supports used for a cyclofructan 6 hydrophilic interaction liquid chromatographic stationary phase

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ABSTRACT

A new HILIC stationary phase comprised of native cyclofructan-6 (CF6) bonded to superficially porous silica particles (2.7 μm) was developed. Its performance was evaluated and compared to fully porous silica particles with 5 μm (commercially available as FRULIC-N) and 3 μm diameters. Faster and more efficient chromatography was achieved with the superficially porous particles (SPPs). The columns were also evaluated in the normal phase mode. The peak efficiency, analysis time, resolution, and overall separation capabilities in both HILIC and normal phase modes were compared. The analysis times using the superficially porous based column in HILIC mode were shorter and the theoretical plates/min were higher over the entire range of flow rates studied. The column containing the superficially porous particles demonstrated higher optimum flow rates than the fully porous particle packed columns. At higher flow rates, the advantages of the superficially porous particles was more pronounced in normal phase separations than in HILIC, clearly demonstrating the influence that the mode of chromatography has on band broadening. However, the minimum reduced plate heights (h_{min}) were typically lower in HILIC than in the normal phase mode. Overall, the superficially porous particle based CF6 column showed clear advantages over the fully porous particle columns, in terms of high throughput and efficient separations of polar compounds in the HILIC mode.

1. Introduction

Superficially porous particles (SPPs), also called core–shell, porous shell or fused core particles [1,2], are state-of-the-art support materials used in the production of HPLC columns. Historically, the concept of shell particles (pellicular particles) was firstly proposed by Horvath et al. during the 1960s and they were developed as ion exchange materials for the analysis of large biological molecules [2–4]. SPP technology was advanced by Kirkland, who prepared 50 μm particles in the 1970s and 5 μm particles in the 1990s [5–7]. Concurrent improvements in the manufacturing of high-quality fully porous particles (FPPs) inhibited the application of SPPs [8]. FPPs with diameters of 3 μm (1990s) and sub 2 μm (2004) came in vogue along with liquid chromatographs that could operate at higher pressures (i.e., ≥1000 bar) [3]. However, recent improvements to SPP technology have moved them to the forefront of HPLC packing materials. These, more successful core–shell particles have thicker porous shells compared to the early pellicular particles. For example, columns are now available with SPP sizes of 1.7, 2.6 or 2.7 μm and porous shell thicknesses of 0.23, 0.35 and 0.5 μm, respectively [9]. This generation of SPPs markedly improved its chromatographic performance, due to its morphology, which consists of a solid inner core surrounded by a porous layer, where analytes and mobile phase can diffuse [2]. The presence of the solid core results in a shorter path for diffusion and decreases band broadening caused by poor mass transfer for systems with slow mass transfer kinetics, such as large molecule separations and some chiral separations. These factors permit analyses at high flow rates without a significant loss in efficiency. Further, SPP columns can be very well packed (particularly from the wall to the center

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Table 1
Physical parameters and bonded selector loading of the stationary phases.

	Particle diameter (μm)	Porosity %	Pore size (Å)	Surface area (m^2/g)	CF6 content (μmol/m^2)[a]	CF6 content (mass%)[a]
FPP[b] 5 μm	4.3	100%	93	465	0.72	32.2
FPP 3 μm	3.0	100%	100	300	0.91	27.9
SPP 2.7 μm	2.7	75%	120	120	0.86	12.8

Legend: FFP and SPP mean fully and superficially porous particles, respectively.
[a] Values obtained from the percentage of carbon.
[b] FRULIC-N

of the column) and therefore exhibit decreased band broadening due to eddy diffusion [3]. Columns packed with superficially porous particles have been used for high throughput separations by improving efficiency while keeping methods robust [8–10].

In recent years, the number of publications involving HPLC columns based on SPP has increased [1,3,8,11–16]. Many SPP HILIC columns can be purchased from different companies, but the majority of the marketed HILIC packing material is simply unmodified silica [9]. Silica gel does not always offer acceptable HILIC separations [17,18]. Thus, it is both timely and important to produce and evaluate newer, more promising, HILIC separating agents bound to SPPs.

Native cyclofructan-6 (CF6) has been reported to be a powerful selector in separation of polar compounds in the HILIC mode [17,18]. The column based on CF6 chemically bonded to FPPs is commercially available (FRULIC-N) and it has demonstrated advantages over other popular commercial columns in separating several compounds such as nucleic acid bases, nucleosides, nucleotides, xanthines, β-blockers, carbohydrates, etc. [17,18]. Further, the native CF6 phase is hydrolytically stable, whereas evidence of dissolution of silica and polar/polar embedded phases in the HILIC mode has been reported [19,20].

In this work, a HILIC stationary phase based on native CF6 was evaluated when bonded to SPPs as the support material (2.7 μm), and it was compared with 3 μm and 5 μm FPP based columns. The columns were also tested in the normal phase (NP) mode, to evaluate the influence of aqueous and non-aqueous containing mobile phases in performance. Results in terms of efficiency, analysis time and resolution were evaluated, demonstrating clear advantages of the new CF6 HILIC column based on SPPs.

2. Experimental

2.1. Materials

Anhydrous N,N-dimethylformamide (DMF), anhydrous toluene, anhydrous pyridine, 3-(triethoxysilyl)propylisocyanate, ammonium acetate (NH_4OAc), trifluoracetic acid (TFA) and all analytes tested in this work (5-phenylvaleric acid, ferulic acid, pyridoxine, L-ascorbic acid, uracil, adenosine, cytosine, thymidine 3′:5′cyclic monophosphate (cTMP), adenosine 2′:3′cyclic monophosphate (cAMP), guanosine 2′:3′cyclic monophosphate (cGMP), cytidine 2′:3′cyclic monophosphate (cCMP), 1,3-dinitrobenzene (1,3-DNB), α-tocopherol, (R)-(+)-2′-amino-1,1′-binaphthalen-2-ol (NOBIN) and 1,3,5-tri-*t*-butylbenzene) were purchased from Sigma–Aldrich (Milwaukee, WI). The CF6 was provided by AZYP, LLC (Arlington, TX). Acetonitrile (ACN), heptane (Hep), isopropyl alcohol (IPA) and ethanol (EtOH), used for the chromatographic separations, were obtained from EMD (Gibbstown, NJ). Water was purified by a Milli-Q Water Purification System (Millipore, Billerica, MA).

The FPPs with 5 μm of diameter (d_p) were purchased from DAISO (Osaka, Japan), the FPPs with 3 μm of d_p were obtained from Glantreo (Cork, Ireland), and the SPPs with 2.7 μm of d_p (0.5 μm of thickness of the porous layer and a solid core having a 1.7 μm diameter) were obtained from Agilent Technologies (Santa Clara, CA, USA).

2.2. Synthesis of native cyclofructan 6 (CF6) based stationary phases

Native CF6 was chemically bonded to silica gel according to literature [17]. The same procedure was used to develop all the stationary phases applied in this work, just changing the silica particles used as supporting material (previously described on Section 2.1). The products were characterized by elemental analysis (CHN), and the loading of CF6 in the stationary phases could be calculated. Some physical parameters of the silica particles and the developed stationary phases are listed in Table 1.

2.3. Instruments

A total of three columns were prepared in this work (150 mm × 4.6 mm i.d.). All the chromatographic separations were conducted on an Agilent HPLC series 1200 system (Agilent Technologies, Santa Clara, CA), equipped with a quaternary pump, an autosampler and a multiwavelength UV–vis detector. For data acquisition and analysis, the Chemstation software version Rev. B.03.02 [341] was used. The injection volume was 0.5 μL for all analyses. The temperature was maintained at 30 °C. The mobile phases (MP) used in the HILIC mode were composed of 75–95% of ACN and 5–25% of 25 mM NH_4OAc, except for the cyclic nucleotides, for which the MP was composed of ACN/100 mM NH_4OAc (70/30, v/v). For the separations carried out in the normal phase, Hep/IPA and Hep/EtOH in different ratios were used and 0.1% of TFA was added to the EtOH phase for the analysis of ferulic acid. Efficiencies were measured using the peak width at half height.

3. Results and discussions

3.1. Preparation of the stationary phases

The results obtained for the elemental analysis of the packing materials are important in understanding the performance of the columns and also to evaluate synthetic implications of the varying particle morphologies. The CF6 content on the stationary phase was calculated from the percentage of carbon obtained in the elemental analysis. The μmol/m^2 loading (CF6/surface area of the silica particle) is important in understanding the effective/relative coverage of each phase (Table 1).

As can be seen, the effective coverage (μmol/m^2) ranged from 0.72 for the FPP 5 μm media to 0.91 for the FPP 3 μm media. The SPP material had an effective coverage of 0.86 which was greater than the commercial 5 μm FPP column (0.72) indicating that the selector density was slightly higher and that sufficient coverage was achieved. It is important to consider coverage/surface area for a bonded/modified SPP based HILIC phase in order to know how much silica character has been masked by the bonded HILIC selector. As can be seen (Table 1), the stationary phase particles of the SPP have less than a half of the absolute amount of CF6 present