Journal of Chromatography A, 1345 (2014) 57–67

Contents lists available at ScienceDirect

Journal of Chromatography A

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

A dextran-bonded stationary phase for saccharide separation

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ARTICLE INFO

Article history:
Received 27 February 2014
Received in revised form 25 March 2014
Accepted 27 March 2014
Available online 4 April 2014

Keywords:
Dextran
Oligosaccharide
Glycopeptides
HILIC
MS

ABSTRACT

A-saccharide-based stationary phase for hydrophilic interaction liquid chromatography (HILIC) is presented. The method uses carbonyl di-imidazole (CDI) as a cross-linker for dextran in aqueous solution. Different from the traditional immobilized saccharide stationary phase, this method was using a simple way to bond the high molecular of polysaccharide immobilized on the silica gel. The new method avoided the time-consuming process but had a very considerable loading result. With silica-based surface bonded, it will be possible to develop well-defined surface modifications that promote the hydrophilic with dextran and high mechanical strength with silica. For all tested compounds, including polar compounds and carbohydrates, this dextran-bonded stationary phase performed well in terms of separation efficiency and column stability, and the retention mechanism matched that of typical HILIC retention. Moreover, good selectivity was achieved in the separation of oligosaccharides and glycopeptides.

1. Introduction

Along with development of hydrophilic interaction liquid chromatography (HILIC), separation materials for this technique have attracted increasing attention in recent years [1]. The main characteristic of the HILIC stationary phase is that the polar group on its surface (amino, hydroxyl or amide) has affinity for water. NPLC stationary phases, including bare silica, amino, and diol, were used during the early stages of HILIC development [2–4]. In recent years, new separation materials have been generated that mainly contain strong polar molecules, such as amide [5,6], carbohydrate [7–9] or zwitterionic groups [10–12] bonded to silica. Saccharides having multiple hydroxyl groups were utilized as a functional group for the HILIC stationary phase [9]. Carbohydrate-modified silica materials were mainly employed for the effective separation of oligosaccharides.

Dextran, a type of saccharide, displays a high degree of polymerization. For several decades, dextran was marketed as 'Sephadex Gels', which practically revolutionized the science of separating biological molecules [13]. Cross-linked dextran hydrogelbeads (Sephadex) are routinely used as the first purification step to isolate natural products for column chromatography applications [14,15]. With the development of dextran applications, accumulating studies have reported non-toxic aqueous methods to covalently immobilize dextran on material surfaces [16]. Dextran was initially selected as the hydrophilic part due to its excellent biocompatibility, aqueous solubility and non-fouling properties, which have valuable application in chromatographic analysis [16–18]. However, owing to a number of unsuitable characteristics, such as poor mechanical strength, chemical and thermal stability, inappropriate and uncontrollable pore structure and surface area, dextran is not an ideal material for HPLC packing.

Click chemistry has been successfully used in surface bonding technology. In our earlier work, the Click CD stationary phase was developed as HILIC separation material for sugars, amino acids, peptides, etc. [8,9]. Other noncyclo-oligosaccharides, such as Click glucose [9], Click maltose [19], and Click lactose [20], etc. prepared using click chemistry, were effectively used in HILIC separations. Moreover, a click chitooligosaccharide stationary phase for HILIC also developed *via* click chemistry achieved significant separation of polar compounds [21]. However, the use of click chemistry for surface bonding requires the introduction of an azide or alkynyl

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http://dx.doi.org/10.1016/j.chroma.2014.03.076
0021-9673/

group on saccharide, which is difficult to achieve on polysaccharides due to their poor solubility in organic solvent. Recently, we developed a saccharide-based stationary phase prepared using a simple method based on ionic interactions [22]. However, this method was restricted to ionic polysaccharides, and the stationary phase showed strong ionic exchange properties, which may pose some potential problems during analysis of ionic compounds. Thus, a more efficient method of polysaccharide immobilization is needed for preparation of the hydrophilic stationary phase.

Here, we have generated a stationary phase by bonding dextran to the surface of silica. The saccharide-based stationary phase was prepared by reacting dextran with carbonyl di-imidazole (CDI)-activated silica [16,23,24]. Silica-based surface bonding should facilitate well-defined surface modifications that promote hydrophilic interaction with dextran and high mechanical strength from the silica. The dextran-bonded column demonstrated high performance in terms of separation and high column stability for the polar compounds examined, in particular, carbohydrates or oligosaccharides, and the retention mechanism matched that of typical HILIC. Thus, dextran-bonded materials not only display the performance in isolation and analysis in glycobiology, but also have potential application in glycopeptide analysis in glycoproteomics.

2. Experimental

2.1. Chemicals and materials

Spherical silica (particle size: 5 μm; pore size: 100 Å; surface area: 300 $m^2 g^{-1}$) was from Fuji Silysia Chemical Ltd. (Kasugai, Japan). Dextran (M_W 20,000) and 3-aminopropyltriethoxysilane was from Aladdin Reagent Co. Ltd. (Shanghai, China). Hydrochloric acid, toluene, methanol, acetone and 1,4-dioxane were from Shanghai Lingfeng Chemical Reagent Co. Ltd. (Shanghai, China). Carbonyl di-imidazole (CDI) and human serum immunoglobulin G (IgG) were from Sigma Aldrich (St. Louis, MO, USA). Ammonium bicarbonate (NH_4HCO_3), ammonium formate (NH_4FA), ammonium acetate (NH_4Ac) and formic acid (FA) were from Acros (Fair Lawn, NJ, USA). Sequencing grade modified trypsin was from Promega (Madison, WI, USA). Acetonitrile (ACN) was from Merck (Darmstadt, Germany). GELoader tips were from Eppendorf (Hamburg, Germany). Water used in experiments was purified using a Milli-Q system (Millipore, Bedford, MA, USA). SunFire C18 (5 μm, 100 Å, 150 mm × 4.6 mm i.d.) was supplied by Waters (Milford, MA, USA). XCharge C18 (5 μm, 100 Å, 150 mm × 4.6 mm i.d.), Unitary NH_2 (5 μm, 100 Å, 150 mm × 4.6 mm i.d.) and Click XIon [12] (5 μm, 100 Å, 150 mm × 4.6 mm i.d.) were from Acchrom (Beijing, China). Click maltose [25] (5 μm, 100 Å, 150 mm × 4.6 mm i.d.) and CC-100 [22] (5 μm, 100 Å, 150 mm × 4.6 mm i.d.) were all prepared in-house.

2.2. Apparatus

Experiments were performed on a Waters HPLC system, which consisted of autosampler, heated column thermostat, Waters 2695 HPLC pump, Waters 2998 photodiode array detector (PDA) and Waters 2424 evaporative light-scattering detector (ELSD) (Waters, Milford, MA, USA). Chromatograms were recorded on a computer with Empower workstation software. Mass spectrometry (MS) was carried out on an X'TremeSimple nano-LC system (Micro-Tech Scientific, Vista, CA) coupled to a quadrupole time-of-flight (Q-TOF) mass spectrometer (Waters, Manchester, UK).

2.3. Synthesis and column packing

Silica was firstly activated by 2.5 M HCl at 100 °C for 6 h [26]. This treatment hydrolyzed any siloxane bridges to yield isolated silanols that were available for reaction with the silane reagent. Synthesis of the dextran-bonded stationary phase began with 160 mL of anhydrous toluene added to a flask containing 10 g of activated silica. After exposure to vacuum to get rid of air inside the pores, the flask was filled with nitrogen. 16 mL of 3-aminopropyltriethoxysilane were gradually added to the solution, with magnetic stirring at 300 rpm. The reaction was kept at 110 °C with stirring for 24 h [27]. The silica gel intermediate was filtered out, washed successively with anhydrous toluene, acetone and methanol and dried at 80 °C overnight.

To the dried silica gel intermediate, 60 mL of anhydrous 1,4-dioxane containing 3.9 g of CDI were added and the reaction was stirred for 4 h at room temperature. The resulting material was filtered, washed with anhydrous 1,4-dioxane and dried at 80 °C overnight to obtain the CDI activated silica intermediate.

One gram of dextran was dissolved in 100 mL water at 50 °C. Five gram of CDI activated silica intermediate was added to the solution and the mixture was stirred for 30 min. The silica particles were then collected by centrifuging, washed with water, and dried at 80 °C overnight to obtain the dextran-bonded stationary phase. The elemental analysis is in Supplementary content (Table S1). Structural parameters of silica before and after bonding are also in Supplementary content (Table S2).

Packing material (2.5 g) was slurry-packed into a stainless steel column (150 mm × 4.6 mm i.d.) with methanol as both the slurry solvent and propulsion solvent under a pressure of 50 MPa.

2.4. Material characterization

Elemental analysis was measured using a vario EL III Cube (Elementar Analysen systeme GmbH, Hanau, Germany). Surface coverage was calculated from the increase in nitrogen content based on the equation of Kibbey and Meyerhoff [28]. Nitrogen sorption experiments were performed on a Micromeritics Tristar II 3020 apparatus (USA). The samples were dried at 200 °C under vacuum overnight prior to measurements. Surface areas and pore-size distribution were measured using the Brunauer–Emmett–Teller (BET) and Barrett–Joyner–Halenda (BJH) methods, respectively. The ζ-potential measurements were carried out using a Malvern Zetasizer Nano-ZS90 instrument (Malvern, UK). Stock sample solutions were prepared by suspending 50 mg of material in water (30 mL). The final samples for ζ-potential measurement were prepared by mixing stock sample (1 mL) with ammonium acetate or ammonium formate buffer (100 mM, 2 mL), and then diluting with water to 10 mL. After a suspension was made, it was thoroughly mixed and immediately thereafter transferred to the measurement cell.

2.5. Chromatographic conditions

For chromatographic evaluations, the flow rate was 1.0 mL min^{-1} and the column temperature was 30 °C unless otherwise specified. Mobile phases consisted of three components: ACN, water and buffer salt, respectively. The void time of the column was measured using toluene as the t_0 marker, resulting in a t_0 of 1.68 min and a system dwell time t_d of 1.12 min.

MS experiments were performed on a quadrupole time-of-flight (Q-TOF) mass spectrometer. 5 mL of fraction solution was directly infused into the nano-ESI source operating under positive ion mode with nano-spray voltage at 2.0 kV. MS data was acquired at m/z 500–2000 [29].

For glycopeptides enrichment, a glycoprotein standard was first digested with trypsin as previously described [30]. The dextran-bonded material was slurry packed into a GELoader tip and the microcolumn was conditioned and equilibrated. After loading the IgG, the column was rinsed to remove nonglycopeptides using eluent ACN/H_2O (75:25 v/v) (20 μL) 2 times and ACN/H_2O (70:30