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Recent advances in development and characterization of stationary phases for hydrophilic interaction chromatography

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ABSTRACT

Hydrophilic interaction chromatography (HILIC) has been one of the most fascinating research branches in chromatographic field with great interest in separating polar and hydrophilic compounds. Many new stationary phases have been developed by introducing a variety of functional groups like zwitterionic groups, hydrophilic macromolecules and ionic liquids, which can provide a wide range of selectivity and applications. Accordingly, it poses a challenge for choosing an appropriate one from the diversity of HILIC columns. Some evaluation models and methods are constructed and used to characterize the retention mechanism of HILIC columns, such as the solvation parameter model and hydrophilic-subtraction model *etc*. The present review mainly summarized the development of HILIC stationary phases and the establishment of characterization approaches in the last five years.

Contents

1. Introduction

Hydrophilic interaction chromatography (HILIC) was proposed by Alpert in 1990 [1], and has attracted increasing attention and gained great progress in recent years. In fact, this chromatographic mode had been employed in separating some hydrophilic compounds before that, such as the separation of carbohydrates in 1975 [2,3]. As a complementary approach to reversed-phase liquid chromatography (RPLC), HILIC can retain polar and hydrophilic solutes, and the aqueous-organic mobile phase in HILIC is beneficial to dissolve polar and hydrophilic samples relative to the nonpolar solvent in normal phase liquid chromatography (NPLC). Moreover, HILIC is very convenient to be coupled to mass spectrometry (MS) without ion pair reagents and the dominated organic solvent in

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mobile phase can improve MS sensitivity. Therefore, HILIC has displayed outstanding performances in separating polar and hydrophilic small molecules, carbohydrates, glycans and peptides *etc*. It has been applied in various fields, including food safety, bioanalysis, environmental and pharmaceutical analysis, proteomics and metabolomics. The previous two reviews have provided a general overview for the progress of HILIC before 2012 [4,5]. Afterwards, the recent two reviews described respectively the stationary phases for HILIC separation of nucleosides and nucleotides as well as the advances of HILIC in UHPLC [6,7].

In the present review, we focus mainly on the important development in HILIC stationary phases since 2011 and the recent advances in characterizing HILIC systems.

2. Stationary phases for HILIC

Among various experimentally chromatographic factors, the nature of stationary phases displays the greatest effect on the separation performance of a HILIC system [8]. The development of stationary phases plays a crucial role in the progress and application of HILIC technique. Several previous reviews [9–12] gave a detailed overview for most HILIC stationary phases before 2011, including the initially used stationary phases originated from NPLC such as amino, diol, cynno-modified silica and bare silica, as well as some exclusive HILIC stationary phases including amide, carbohydrates/saccharides (mono-, linear and macrocyclic oligosaccharides) and zwitterionic molecules modified materials. The following sections summaried the newly developed HILIC stationary phases since 2011, and the main progresses are listed in Table 1.

2.1. Zwitterionic stationary phases

Due to their good hydrophilicity, zwitterionic stationary phases have gained great concern for the HILIC applications recently. Different types of zwitterionic stationary phases were developed with the various charge orientations and spatial arrangements as shown in Fig. 1. Conventional zwitterionic stationary phases (ZIC-HILIC, ZIC-cHILIC and ZIC-*p*HILIC) were obtained by bonding sulfobetaine or phosphorylcholine moieties on the surface of silica or polymeric materials. The permanently charged groups are distributed inversely and the spatial orientation tends to be perpendicular to the supporting substrates as shown in Fig. 1A. Besides, some other types of zwitterionic stationary phases were developed by researchers. New zwitterionic stationary phases were prepared by bonding 3-P,P-diphenylphosphonium-propylsulfonate on the surface of silica and exhibited stronger retention, higher column efficiency and better peak symmetry than ZIC-HILIC column towards a variety of polar solutes, including β–blockers, nucleosides and bases, and water soluble vitamins [13]. In our previous work, surface-bonded imidazolium-based zwitterionic silica materials were obtained as stationary phases for HILIC, which possessed a positively charged imidazole ring and a negatively charged sulfonate group. The resulting zwitterionic stationary phases presented good separation selectivity towards typical polar compounds with very high column efficiency near 100,000/m for cytosine [14]. Another imidazoline type stationary phase was developed and possessed zwitterionic properties from a quaternary ammonium and a carboxylic acid group in a certain pH range [15].

In another case, two oppositely charged groups are in a straight chain linked to silica and can form a perpendicular arrangement to the surface of supporting materials, as shown in Fig. 1B. Shen et al. [16,17] prepared a novel zwitterionic stationary phase (Click TE-Cys) by bonding cysteine to vinyl silica through "thiol-ene" click chemistry. The positively and negatively charged moieties were in a branched chain linked with the straight chain and could be parallel to the surface of silica gel, making charges more uniformly distributed. The unique configuration facilitates the resulting Click TE-Cys stationary phase exhibiting good hydrophilicity and selectivity with high column efficiency. The zwitterionic nature was based on nonpermanent charges of amino and carboxyl groups, and ζ-potential measurement indicated a slight switch of surface net charge from positive to negative within pH 3–7. Similarly, some other types of zwitterionic stationary phases were prepared by grafting lysine (Click-Lysine) [18], arginine (Click-Arginine) [19], N-benzyl iminodiacetic acid (Click-IDA) [20] or glutathione (Click TE-GSH) [21] onto silica particles through click chemistry, and they showed good separation performances under HILIC mode.

Besides the above mentioned zwitterionic stationary phases, some other zwitterionic materials were obtained with both negatively and positively charged moieties separately and covalently immobilized on supporting substrates, as shown in Fig. 1C. Qiu et al. [22] presented copolymerization of anionic and cationic monomer pairs of an ionic liquid on the surface of silica to prepare new surface-confined ionic liquid stationary phases, and the strategy provided a new point of view to develop zwitterionic functionalized materials. Cheng et al. [23] prepared a new amino-phosphate zwitterionic HILIC stationary phase (APS) by separately immobilizing phosphate and amino groups onto silica. Their recent work presented another zwitterionic stationary phase by separately bonding positively charged tertiary amine moiety and negatively charged carboxyl moiety on the surface of silica materials with a controllable ratio [24], which could provide a possible way to adjust separation selectivity.

2.2. Hydrophilic macromolecules bonded phases

Some macromolecules, such as cyclodextrins (CDs), cyclofructan (CFs) and cucurbit[n]urils (CBs), usually contain multiple polar groups on the interior, which can provide especially good hydrophilicity and present great prospects as HILIC ligands. The structures of three macromolecules are shown in Fig. 2A. Cyclodextrins (CDs) are cyclic oligosaccharides consisting of six, seven or eight D-glucose units linked through 1,4-glycosidic bonds, and have been used as chiral selectors for enantiomeric separation under RPLC or NPLC mode owing to their molecular recognition properties from the unique topological configuration and hydrophobic cavities. As multiple hydroxyl groups on the exterior rim of CD molecules provide sufficient hydrophilicity, CDs or derivatized CDs bonded phases have also shown typical HILIC retention behavior in separating polar solutes [25,26].

As another new kind of macrocyclic oligosaccharides, cyclofructans (CFs) are composed of six or more β–(2→1) linked D-fructofuranose units, and the unique structure endows them good hydrophilicity with great potential as HILIC selector. Among cyclofructans, cyclofructan 6 (CF6) received greatest attention due to its highly defined geometry and availability in pure form. Native CF6 bonded silica materials were prepared as HILIC stationary phases, which exhibited advantageous separation performances over some popular commercial columns (such as ZIC-HILIC and Astec Diol column) [27]. At present, CF6 bonded silica columns named FRULIC-N can be obtained commercially and have presented a promising prospect in separation of nucleotides [28] and therapeutic peptides [29]. Moreover, some derivatized cyclofructan based stationary phases were further developed for HILIC, including sulfonatedcyclofructan 6 (SCF6) [30] and isopropyl carbamate CF6 bonded silica column (Larihc-P) [29,31]. Recently, native CF6 and isopropyl carbamate CF6 bonded superficially porous silica columns were developed and presented obviously superior efficiency over the corresponding fully porous silica columns [32,33].

Cucurbit[n]urils (CBs, n = 5–8, 10) are an attractive family of pumpkin-shaped host macromolecules composed of glycoluril monomers linked by methylene bridges. They bear a symmetrically