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Review

Retention and selectivity of stationary phases for hydrophilic interaction chromatography

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ABSTRACT

More and more polar stationary phases have become available for the separation of small polar compounds in the past decade as hydrophilic interaction chromatography (HILIC) continues to find applications in new fields (e.g., metabolomics and proteomics). Bare silica phases remain popular, especially in the bio-analytical area. A wide range of functional groups (e.g., amino, amide, diol, sulfobetaine, and triazole) have been employed as polar stationary phases for HILIC separation. This review provides a survey of the popular stationary phases commercially available and discusses the retention and selectivity characteristics of the polar stationary phases in HILIC. The purpose of the review is not to provide a comprehensive overview of literature reports, but rather focuses on findings that demonstrate retention and selectivity of the polar stationary phases in HILIC.

Contents

1. Introduction

Twenty years after Alpert coined the term hydrophilic interaction chromatography (HILIC) [1], HILIC has been widely recognized as a distinct chromatographic mode and has enjoyed nearly a decade of rapid growth since its potential in separating very polar compounds was rediscovered by the scientific communities in the early 2000s [2–5]. HILIC has been applied to both small and large molecules and is becoming an increasingly important tool in proteomic and metabolomic research [6–8]. From a practical perspective, HILIC offers an attractive alternative to normal phase chromatography (NPC) to separate very polar compounds. The solvent used for the mobile phase in HILIC is similar to that in reversed-phase liquid chromatography (RPLC), thus eliminating the need to maintain dedicated instruments for normal phase methods. Secondly, organic solvents (e.g., acetonitrile) in HILIC mobile phases are more compatible with mass spectrometry, and the high organic content necessary to maintain retention in HILIC

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significantly increases ESI-MS sensitivity due to improved ionization efficiency [9,10]. Thirdly, some biological samples can be directly injected onto HILIC columns without the need for evaporation and reconstitution after protein precipitation with acetonitrile, thus simplifying sample preparation in bio-analysis. In addition, counter ions (e.g., Na^+, K^+, Cl^-, Br^-, amines and acids) that are commonly used to form pharmaceutical salts can also be retained and separated on HILIC columns [11,12]. Thus, counter-ion analysis of pharmaceutical salts can be performed using HILIC columns on regular HPLC instruments instead of using ion-exchange columns on specialized ion-chromatography (IC) instruments.

Along with increasing popularity of HILIC, stationary phases for HILIC have received a lot of attention from both academic researchers and column manufacturers, and many new stationary phases and columns have become commercially available in the past decade [2,13]. In HILIC, stationary phase chemistry is more diversified with a wide variety of functional groups employed (Table 1). Newer stationary phases have the potential to offer different retention and selectivity for polar compounds, and also provide method development chemists with opportunities to find an appropriate stationary phase for the desired separation. At the same time, it is challenging to select the optimal phase in a systematic manner in a short time.

HILIC has been the subject of a few excellent reviews in recent years [2,14–16]. Irgum's review in 2006 was comprehensive with special attention to the HILIC mechanism [2]. Tanaka published a review in 2008 focusing on the efficiency, and to a lesser extent, the retention of various columns used in HILIC [14]. Another review by Hao et al. systematically discussed the impact of column temperature and mobile phase components on the selectivity in HILIC [15]. However, there has not been a topical review focusing on the retention and selectivity of various stationary phases commonly used in HILIC. In light of recent development in HILIC stationary phases, it is fitting to review the subject. This review covers the commercially available stationary phases commonly used in HILIC, but is not intended to be comprehensive. Uncommonly used stationary phases are not included due to the lack of data to make comparison with the more commonly used ones. In this review, the HILIC stationary phases are classified based on the charge characteristics of their functional groups. This classification facilitates comparison of their retention and selectivity. Various retention models for HILIC are also discussed to further understanding of the difference in retention and selectivity of various stationary phases. In addition to the stationary phase, chromatographic parameters (e.g., organic solvent content, mobile phase pH, salt type and concentration, and column temperature) have significant effects on the retention and selectivity in HILIC. Although the effect of the chromatographic parameters on the retention and selectivity should be considered together and viewed as a whole, this review focuses on the effect of the mobile phase pH and salt concentration since organic solvents and column temperature have been extensively discussed in other reviews [2,15].

2. Stationary phases in HILIC

Similar to normal phase chromatography, polar stationary phases are typically used to retain polar solutes in HILIC. In fact, most HILIC separations were performed on normal phase columns (e.g., amino, cyano and silica phase) in the 1990s and early 2000s since only a few HILIC columns (e.g. amide and aspartamide phases) were commercially available [1,17–20]. Conventional silica and amino columns packed and stored in reversed-phase solvents are now commercially available. Many specialty phases with diverse functionalities have been developed exclusively for HILIC in recent years.

Underivatized silica remains a popular phase for HILIC, particularly in the bio-analytical field [16,21]. Irreversible adsorption of solutes and irreproducibility of retention have plagued silica columns in normal phase chromatography, but are not as problematic in HILIC due to the presence of significant levels of water in the mobile phase [22]. Many silica columns for normal phase chromatography have been tested for HILIC separations with varying degrees of success. Olsen reported a significant difference in retention among the silica columns (Type A and B silica) from different manufacturers [23]. This might be attributed to different purity of the silica material or different column preparation procedures. Many column manufacturers have developed silica columns specifically designed for HILIC, such as Atlantis HILIC, Zorbax HILIC plus and YMC pack silica columns. Silica columns promoted for HILIC applications are typically packed and stored in aqueous/organic solvents (e.g., water and acetonitrile) instead of normal phase solvents. In addition, the silica columns packed with sub-2 μm particles (e.g., Acquity 1.7 μm and Epic 1.8 μm silica columns) are also available for UHPLC applications [24]. The superficially porous silica column based on fused-core™ technology has been applied to HILIC separations and has been shown to have greater resistance to overloading by ionized basic compounds than silica-based reversed-phase columns [25].

In addition to underivatized silica columns, a wide variety of functional groups have been incorporated into the stationary phases for HILIC. The majority of the bonded phases are silica-based and prepared either as a monomeric phase or with a polymer coating covalently bonded to the silica. Table 1 shows the bonded stationary phases currently used for HILIC applications. These are referred to in this review by the conventional names of the functional groups, and representative columns are provided as an example of the corresponding phases. The stationary phases in Table 1 are classified into three categories based on the charge characteristics of the functional groups, namely, neutral, charged, and zwitterionic phases.

2.1. Neutral stationary phases

The functional groups in this category (e.g., amide, cyano, diol, and cyclodextrin) cannot be charged in the pH range typical for the mobile phase in HILIC. This category includes most bonded phases in HILIC and represents a wide variety of functional groups, which are all polar in nature. In this category the amide phase is one of the most popular and has found many applications in HILIC [26–29]. In addition, new amide phases have also been developed for HILIC recently, but few applications have been reported [30]. The amide moiety is attached to the silica surface either through a propyl linker or a proprietary linkage. The aspartamide phase is worth special mention since it was the first stationary phase especially developed for HILIC separations [1]. Its preparation has been discussed in detail in Ref. [1] and also in Irgum and Tanaka's reviews [2,14]. The aspartamide phase is a polymeric phase prepared by bonding a layer of polysuccinimide to aminopropylated silica, then treating the polymer with ethanolamine to generate the final stationary phase. The aspartamide phase has been applied to the separation of small polar compounds, peptides and proteins [31–33]. However, it is not as widely used as the amide phase, possibly due to lower efficiency and limited long-term stability.

Although the cyano and diol phases in this category are commonly used in normal phase separations, their applications in HILIC are still very limited. Both cyano and diol phases are monomeric phases directly attached to the silica surface through a propyl linker. Cyano groups lack hydrogen bond donor capability and are also less hydrophilic. This leads to insufficient retention for most polar compounds, and only a few special applications have been reported on the cyano phase [34]. In comparison, the diol