Retention mechanism of hypercrosslinked polystyrene silica hybrid phase in normal phase chromatography☆

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ABSTRACT

Hypercrosslinked polystyrene phases have been described as quasi-normal phase because they lack discrete polar sites. Retention on the HC-Tol column is investigated using the Snyder–Soczewinski model. Solvent strength of different hexane-solvent binary mobile phase compositions can be predicted with solvent strength of pure dichloromethane (DCM, 0.159), tetrahydrofuran (THF, 0.22), and benzene (0.127). The HC-Tol column is shown to be a localizing adsorptive phase. Also, site-competition delocalization on HC-Tol demonstrates that whatever its adsorption groups are, they are able to participate in lateral interactions.

1. Introduction

Macroporous polystyrene–divinylbenzene (DVB) columns have been developed [1,2] to circumvent the pH stability and other limitations of traditional silica phases [3,4]. However, macroporous polystyrene–DVB stationary phase have poor adsorption capacity and efficiency compared to silica based columns [2]. Later, a new generation of homogeneous and rigid stationary phase called hypercrosslinked (HC) polystyrenes was synthesized [5–10]. HC polystyrene phases possess long polystyrene chains that are extensively crosslinked by connecting phenyl groups with methylene groups [11]. HC polystyrene phases have large inner surface area (up to 1000–1500 m^2/g), shows better efficiency and adsorption capacity than macroporous polystyrene–DVB [4,8]. HC polystyrene phases are pH stable and compatible with polar, non-polar and aqueous mobile phases. These properties have resulted in HC polystyrene phases becoming very promising new stationary phases that have been used for solid-phase extraction (SPE) [12,13], and both reverse and normal phase LC [10,14].

Normal phase HPLC separations have been performed with fully polymeric hypercrosslinked polystyrene [5,8] and with silica particles possessing a thin layer of highly crosslinked aromatic network [15–22]. The Carr group developed these polymer–silica hybrid phases as acid and thermal stable phases for RPLC [15–22]. The highly crosslinked aromatic network prevents loss of stationary phase under extreme conditions. HC polystyrene silica hybrid phases can provide orthogonal selectivity in ultra-fast, 2D-LC [16]. A wide array of HC polystyrene silica hybrid phases have been developed, including HC-COOH [19], HC-C_8 [19], and HC-Tol [22]. Recently, our group observed that the HC-Tol phase provided unique and useful group-type selectivity for nitrogen containing compounds under normal phase conditions [23]. In the HC-Tol phase, the silanized silica is primarily cross-linked, secondarily crosslinked and finally derivatized with toluene [24]. Despite the good reproducibility, high efficiency and better acid stability than most of the commercialized acid stable phases, these silica-base HC phases are not widely used [16]. This lack of usage may be due in part to the lack of understanding of the retention mechanism.

Davankov and co-workers [4,5,8] referred to normal phase LC on HC polystyrene phases as *quasi-normal phase* to reflect the lack of discrete polar groups in the structure of HC polystyrene. HC-Tol column also does not have polar sites on the surface. This work seeks an understanding of the quasi-normal phase retention on the HC-Tol column. Hopefully, to promote the use of silica-base HC phases.

2. Theory

Normal phase chromatography (NPLC) uses a polar stationary phase and a less polar mobile phase. NPLC stationary phases usually

possess discrete polar sites, such as silanols, amines or cyano groups [25]. NPLC mobile phases are mixture of a nonpolar solvent A such as hexane and a polar solvent B such as DCM. The latter one acts as the strong mobile phase component.

To understand the quasi-normal phase retention of the HC-Tol column, it is important to understand the dependence of the retention factor on the mobile phase composition and solvent strength. In this paper, we use the widely accepted Snyder–Soczewinski model [26–30]. In the 1960s, Snyder developed a model for liquid solid chromatography [31–34]. Later, Soczewinski and co-workers [35] suggested an alternative model. The two models are equivalent [36] and so are generally referred to as the Snyder–Soczewinski displacement model. The Snyder–Soczewinski model fit and explained normal phase retention data better than the other models [26,37], and has provided understanding of retention on silica [32], alumina [33,34], bonded phases [30] and other phases [38] under normal phase conditions.

According to the Snyder–Soczewinski displacement model [26–30], a solvent monolayer is present on the surface of the stationary phase. Solutes must replace an adsorbed solvent molecule of comparable size to be retained. Solvent–solute interactions are lost in the mobile phase but the same interactions are gained on the stationary phase, and so the net effect balances out and is ignored. The relative retention of a solute under two mobile phase conditions is [27]

$$\log\left(\frac{k_2}{k_1}\right) = \alpha A_i(\varepsilon_1 - \varepsilon_2) \tag{1}$$

where k is the retention factor, ε is the solvent strength parameter, α is the stationary phase activity factor, and A_i is the relative area of interaction between the solute and the adsorbent surface.

A_i is the area occupied by an adsorbed molecule on the adsorbent. For flatwise adsorption, A_i can be calculated from the van der Waal's radii of the atoms corrected for the less efficient arrangement of molecules on a chromatographic surface than in a crystalline phase (i.e., an 0.5 Å increase in the van der Waal's radius of all atoms) [31]. A_i for benzene was defined as 6 in units of 8.5 Å^2 [31].

Expressions for retention in reverse phase LC usually substitute the composition of the strong mobile phase B% for the solvent strength [25]. Such a substitution is not possible in normal phase because the solvent strength is not linearly related to B% [25]. For a binary mobile phase of A and B in normal phase LC, the solvent strength ε_{AB} of the mixture may be determined from the solvent strengths of pure A (ε_A) and pure B (ε_B):

$$\varepsilon_{AB} = \varepsilon_A + \frac{\log(N_B 10^{an_b(\varepsilon_B - \varepsilon_A)} + 1 - N_B)}{\alpha n_b} \tag{2}$$

where N_B is the mole fraction of B in the mobile phase, n_b is the relative area of the stationary phase occupied by solvent B (A_i of solvent B). Combining Eqs. (1) and (2) provides an expression for the dependence of $\log k$ on the logarithm of the mole fraction of polar solvent B N_B.

An important aspect of the Snyder–Soczewinski model is the *localization* of the solute and solvent on the stationary phase surface [28,39–41]. Fig. 1a represents *non-localized* retention, where a less polar solute and a less polar mobile phase adsorb in a non-oriented fashion to the stationary phase. There is not a specific one-to-one interaction formed between the solute or eluent molecules with the stationary phase. Rather there are multiple transient interactions between the solvent/ or solute and the stationary phase. Retention of solute involves replacement of an appropriate number of mobile phase molecules necessary to occupy the same area. For instance, as depicted in Fig. 1a, adsorption of bromobenzene would displace two DCM molecules.

Fig. 1b schematically represents a *localized* interaction. There is a distinct interaction (indicated by a bold double-headed arrow) between a specific functional group of a polar molecule and a discrete polar site (e.g. silanol) on the stationary phase surface. Retention of the solute involves disruption of the one-to-one interaction between the eluent and stationary phase, and formation of a one-to-one interaction between the solute and stationary phase. Mobile phase and solute compete directly for a polar site on the surface of stationary phase. Retention of solute involves replacement with an appropriate number of mobile phase molecules to occupy the same number of polar sites.

3. Experimental

3.1. Apparatus

All experiments were performed on an Agilent 1260 Infinity LC (Agilent, Santa Clara, CA, USA) consisting of a quaternary pump, an on-line degasser, an auto-sampler performing a 1 μL partial loop injection, a column heater at 35 °C, and a variable wavelength detector set at 254 nm (280 nm when benzene was used as mobile phase) with a response time of 1 s. Data acquisition at 10 Hz was controlled using Agilent Chemstation software. All tubing and fittings were stainless steel (0.17 mm i.d.). The length of all connecting tubing was minimized.

The retention factors were calculated using the dead time t_0 based on the first peak caused by injection of pure hexane [42].

3.2. Chemicals

All solutes were >90% purity. Optima grade hexane, DCM, THF and benzene were from Thermo Fisher Scientific Inc. (Fairlawn, NJ, USA). Chrysene and picene were from K & K Laboratories (Carlsbad, CA, USA). All other solutes were from Sigma–Aldrich (St. Louis, MO, USA). Solute solutions were 0.05–5 mg/mL, and filtered through 0.20 μm Millex syringe filters (EMD Millipore Corporation, Billerica, MA, USA) prior to injection.

3.3. Column

The HC-Tol phase was synthesized according to reference [24,43]. Silanization and crosslinking were carried out on the surface of Zorbax RX-Sil Type B silica (5 μm, 180 m^2/g, 80 Å pore size, Agilent Technologies Inc., Wilmington, DE, USA). The substrate silica was first silanized with dimethyl-chloromethylphenylethylchlorosilane, primary cross-linked by triphenylmethane, then secondary cross-linked by 2,4,6-tris-(bromomethyl)-mesitylene, and finally derivatized with toluene.

After synthesis, the HC-Tol phase was slurry packed into a 50 mm × 4.6 mm i.d. stainless steel column with 2 μm frits (Grace Davison Discovery Science, Deerfield, IL, USA) using a Haskel nitrogen-driven fluid pump (Burbank, CA, USA). One gram of HC-Tol particles was sonicated in 10 mL isopropanol for 15 min to wet the pores. The slurry was transferred into a 10 mL stainless steel reservoir (Lab Alliance, State College, PA, USA) and packed downward into the column jacket. The packing pressure was increased from 0 to 6000 psi (414 bar) over 30 s and then maintained at 6000 psi until 200 mL of isopropanol was driven through the column.

4. Results and discussion

Unique and useful selectivity has been observed on hypercrosslinked (HC) polystyrene under normal phase conditions [4–12,14]. Retention on HC polystyrene under normal phase conditions has been termed *quasi-normal phase*, as the HC phase