



Size exclusion chromatography of synthetic polymers and biopolymers on common reversed phase and hydrophilic interaction chromatography columns



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ABSTRACT

This work describes the applicability of common reversed phase and HILIC columns for size exclusion chromatography of synthetic and natural polymers. Depending on the nature of the solute and column stationary phase, a “non-retention” condition must be created with the aid of the mobile phase to achieve a unique size-based separation in isocratic mode. The various bonded phases show remarkable differences in size separations that are controlled by mobile phase conditions. Polymer–mobile phase and column–mobile phase solvation interactions determine polymer hydrodynamic volume (or solute bulkiness) and polymer–column steric interaction. Solvation interactions in turn depend on polymer, mobile phase and stationary phase polarities. Column–mobile phase solvation interactions determine the structural order of the bonded ligands that can vary from ordered (extended, aligned away from the silica substrate) to disordered (folded, pointing toward the silica substrate). Chain order increases with increased solvent penetration into the bonded phase. Increased chain order reduces pore volume, and therefore decreases the size-separation efficiency of a column. Conversely, decreased chain order increases pore volume and therefore increases the size-separation efficiency. The thermodynamic quality of the mobile phase also plays a significant role in the separation of polymers. “Poor” solvents can significantly reduce the hydrodynamic diameter of a solute and thus change their retention behavior. Medium polarity stationary phases, such as fluoro-phenyl and cyano, exhibit a unique retention behavior. With an appropriate polarity mobile phase, polar and non-polar synthetic polymers of the same molecular masses can be eluted at the same retention volumes.

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1. Introduction

The characterization of polymers, both natural and synthetic, by liquid chromatography (LC) has always been an area of intense research activity. Modern LC methods for the separation of polymers can be divided into three major categories: (i) separations governed by entropy changes, *i.e.* exclusion; (ii) separations controlled by both enthalpy and entropy changes, but dominated by changes in enthalpy, *i.e.*, interaction; and (iii) separations governed by no changes in free energy where enthalpic and entropic contributions cancel each other, *i.e.*, critical conditions [1]. The separation mode is determined by the properties of the polymer, the stationary phase, and the mobile phase.

The simplest, best known and studied technique for the analysis of synthetic and natural polymers is size exclusion chromatography (SEC) [2–4]. The separation of polymers by this technique is entropy-driven, and occurs according to their hydrodynamic volumes with the larger molecules eluting first followed by the smaller ones [5]. The elution volume can be correlated with the molecular mass for a given type of polymer. However, SEC separations do not depend on molecular mass, but on hydrodynamic volume. If the polymer molecules have a different chemical composition, chain architecture, or functionality, but the same hydrodynamic volume, separation by a size-exclusion mechanism is impossible. To address this problem, researchers turned to reversed phase high performance liquid chromatography (RP–HPLC) for the separation of synthetic polymers, which has been an area of active research since the 1980s.

Successful separations of homopolymers and copolymers by interaction liquid chromatography (iLC) using reversed-phase (RP)

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and normal-phase (NP) stationary phases, where the separation is driven by enthalpy and entropy changes, have been reported. iLC can be performed in isocratic mode [6], as well as in gradient-elution LC (GELC) mode using binary solvent gradients [7–11] and ternary [12] solvent gradients. The retention mechanism is based on the adsorption of polymer molecules onto the stationary phase, however the main separation principle is hard to determine. Various processes such as adsorption, desorption, partitioning, exclusion, precipitation, and re-dissolution are taking place simultaneously [1]. The same principle is used in temperature gradient interaction chromatography (TGIC) [13,14]. Larger polymers are more strongly adsorbed, and therefore are retained longer, while smaller polymers are only slightly adsorbed and elute faster. While iLC exhibits better resolution than SEC, it is less universal than SEC. For a particular polymer, the magnitude of the enthalpic interaction has to be precisely controlled in order to achieve a reproducible separation. The combined selection of an appropriate stationary phase, mobile phase, and gradient (either solvent or temperature) is not always straightforward [14] and often requires extensive experimental work. Events such as precipitation of the polymers in the injector, breakthrough peaks, and irreversible adsorption of large polymers can result in incomplete recovery and therefore in inaccurate quantitation [1,15,16]. Nevertheless, iLC techniques offer orthogonal selectivity to that of SEC, and therefore can provide additional information on polymer molecular distributions (e.g. chemical composition distributions) that cannot be determined by any other technique.

Synthetic polymers can be effectively separated on the basis of their chemical composition, end-groups, or block length using conditions where enthalpic and entropic contributions compensate each other, so-called critical conditions (CC). LC under critical conditions (LCCC) uses RP columns and involves an adsorptive interaction of monomers with the stationary phase which is sensitive to small differences in chemical structure. The critical condition is created on the border of the exclusion and adsorption modes [17]. Under this condition, elution is independent of the molar mass of a polymer, and therefore all polymers with the same chemical composition elute at the total permeation limit of the column [1,18]. In spite of several advantages of LCCC, achieving and maintaining the critical conditions is a significant challenge, and in some cases even molar mass independence is uncertain [19]. Separations at the critical conditions are very sensitive to small variations in mobile phase composition and sample solvent, as well as to variations in temperature and pressure.

An attempt to separate polymers by chemical composition is not the only driving force to use reversed phase (RP) columns for polymer characterization. One of the major drawbacks of high-performance SEC is the need of multiple columns of different pore sizes, and oftentimes different chemistries that are typically more expensive than reversed-phase or normal-phase HPLC columns. In principle, any column containing packing of defined pore size can be used for SEC analysis irrespective of its chemical composition or surface chemistry in the case of silica packings. There are, however, two major requirements that must be met: (i) the absence of non-size-exclusion effects and (ii) sufficiently large pore volumes. To eliminate non-size-exclusion effects, mobile phase composition and oftentimes column temperature must judiciously be chosen to prevent adsorption in the case of non-ionic polymer samples, or ionic interactions, i.e., ion exchange and ion exclusion, in the case of ionic polymers. Pore volume, which must be maximized, plays a critical role in establishing SEC resolution. For a given packing, pore volume depends on its chemical nature and overall porosity; for example, polymeric cross-linked packings typically have greater pore volume than silica-based packings. The total pore volume of a packed column, which is easier to control, is proportional to the volume of a column.

In this work we demonstrate that RP columns, as well as hydrophilic interaction liquid chromatography (HILIC) columns, can be used for polymer molecular size characterization in the size exclusion mode. RP columns have major advantages over conventional SEC columns. SEC column technology has followed closely, but still lags behind RP column technology. There is strong interest in reducing particle diameters [20,21] and internal diameters in SEC columns in order to achieve better separations and column efficiencies. While typical particle sizes of SEC columns are 3–20 μm with a broad particle size distribution, typical particle sizes of RP columns are 1.7–5 μm with a narrow particle size distribution (typical 90%/10% particle diameter ratio is 1.55). Standard internal diameters of analytical SEC columns are 6–8 mm, compared to 2.1–4.6 mm of RP columns. The soft SEC polymeric resins compress under pressure and flow, which limits possible reductions in their particle size. SEC organic packing materials are subject to slow degradation over time. There is also a risk of unwanted components adsorbing on the surface of the packing. Packings can adsorb unwanted impurities during their preparation—polymerization, packing and washing. Unwanted impurities can also be adsorbed from the mobile phase and sample matrix [22]. Soft polymeric columns can swell or shrink during solvent changes. Any solvent changeover is a slow, time consuming process that sometimes requires an intermediate solvent. Moreover, polymer-based SEC columns are 3–4 times more expensive than silica-based RP (and HILIC) columns. Silica-based SEC columns overcome the swelling and reactive nature of polymeric columns. They're versatile and mechanically strong, which allows further improvement in performance via reduced particle size. However, they still have such drawbacks as a somewhat narrow pH stability range, and the presence of active surface silanol groups that promote interaction between solutes and the stationary phase. Recent advances have been observed with diol-modified silica that provides a significant reduction in silanol activity although some residual silanols still remain [4].

Particle size is a key factor that influences column efficiency in SEC. The smaller the particle size, the larger is the column efficiency. The evolution of RP columns in the last decade has resulted in the reduction of particle sizes typically employed from 5 μm to sub-2 μm , and produced stationary phases that are able to suppress unwanted silanophilic sites. Silica-based RP and HILIC column stationary phases are compatible with all chromatographically-relevant organic solvents and water, and can withstand pressures of 15,000 psi, which is important for wide pore columns. The ability to use RP and HILIC columns for size exclusion (SE) separations is extremely advantageous for polymer and protein separations since these applications centered upon HPLC can be directly transferred to UHPLC. While the development of small particle size SEC columns is still in its embryonic stage, the abundance of small particle, narrow bore RP and HILIC columns provides an immediate opportunity to utilize UHPLC for SEC. Because RP and HILIC columns have many favorable characteristics that make them very attractive for potential use in SEC, in this work, we probed the unexplored area of entropy-driven, size-exclusion separation of polymers using RP and HILIC columns. The unique SEC separation capability of RP and HILIC columns was demonstrated using synthetic and natural polymers of different sizes. Specifically, the influence of mobile phase composition and stationary phase polarity on the elution and size-exclusion separation of polystyrene and poly(methyl methacrylate) were examined.

2. Theory

RP-HPLC separates molecules on the basis of hydrophobicity. Solute molecules leave the polar mobile phase, that is commonly

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