Journal of Chromatography A, 1354 (2014) 56–64

Contents lists available at ScienceDirect

Journal of Chromatography A

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

Impact of biomolecule solute size on the transport and performance characteristics of analytical porous polymer monoliths

Ivo Nischang*

Institute of Polymer Chemistry, Johannes Kepler University Linz, Welser Strasse 42, A-4060 Leonding, Austria

ARTICLE INFO

Article history:
Received 3 March 2014
Received in revised form 25 April 2014
Accepted 20 May 2014
Available online 27 May 2014

Keywords:
Adsorption
Gel porosity
Mass transfer
Pore-fluid gel interface
Retention
Size exclusion

ABSTRACT

Porous monolithic poly(styrene-*co*-divinylbenzene) stationary phases in 4.6 mm ID analytical format have been investigated with respect to their transport properties probed by solutes of biological origin varying vastly in size. Elucidation of several properties of these benchmark and robust materials gave complementary insight. These are: (i) the porous polymers' apparent dry-state microscopic appearance, (ii) the columns porosity probed by the biomolecules and modulated by mobile phase solvent composition, (iii) the impact of probe solute size on apparent retention at varying mobile phase solvent compositions, and (iv) the elution performance under both nonretained and retained elution conditions. By varying the volume percentage of acetonitrile in the mobile phase, it is demonstrated that the monolithic scaffold shows a variable porosity experienced in particular by the larger sized solutes, while the smaller solutes are gradually less affected. The nanoscale swelling and solvation of porous monolithic adsorbents resulting in gel porosity varied with mobile phase solvent composition was, therefore, indicated. The plate height curves for the solutes under nonretained conditions show a moderate increase at increased flow velocity while approaching plateau values. These plateau values were in conjunction with a trend of a decreased performance at an increased molecular weight of the solute. The systematic shape of the plate height curves at increased flow velocity indicates pre-asymptotic dispersion. This is because the column bed aspect ratio of length-to-diameter is equal or smaller than 10. Imposing retention on the solutes at a constant flow velocity deteriorates isocratic elution performance, more pronouncedly for the larger sized solutes at even weak retention. This is explained with slow pore fluid–gel interface diffusion. Additionally, the apparent retention factor for elution of the probe solutes becomes a function of flow rate, consequently a function of imposed pressure experienced by the scaffold.

1. Introduction

Porous polymeric monoliths based on a cross-linked material intertwined by micrometer-sized flow-through pores have found significant attention in recent years. Typically, the scaffolds are prepared *via* free radical cross-linking (co)polymerization of small organic precursors in porogenic diluents. Such entities enable excellent separation of large molecules in gradient elution mode [1–8]. This application for large molecules has been extended to that of smaller proteins, peptides, and amino acids with varying success in the monoliths' application [9–12]. Another recent focus of the monolithic column technology can be found in the exploitation of a variety of chromatographic modes such as hydrophobic interaction chromatography [13–15], ion-exchange chromatography [16,17], affinity chromatography [18–20], size-exclusion chromatography [21], and hydrodynamic chromatography [22]. Recent efforts were also directed towards scaling capabilities, microfluidic applications, and novel types of functionalization to explore a diversity of implementations [4,6,23,24].

More detailed studies on the chromatographic properties of porous polymeric monoliths, however, indicated that the properties measured in the dry-state of the materials allow only limited interpretation regarding their nanoscale dynamics and associated transport performance [25–28]. These materials, no matter how heavily cross-linked, develop gel porosity on a nanometer-scale, a porosity that is absent in the dry-state of the cross-linked polymeric materials and which stems from nanoscale solvation and swelling of the polymer scaffold features [27]. Such phenomena have not yet been investigated for large molecule transport, since gradient elution conditions leave limited room for interpretational efforts of mass transfer processes [28,29]. In general, the poor performance of polymeric monoliths in isocratic (equilibrium) elution

* Tel.: +43 732 671547 66; fax: +43 732 671547 62.
E-mail address: ivo.nischang@jku.at

http://dx.doi.org/10.1016/j.chroma.2014.05.053
0021-9673/

mode is frequently reported. The poor performance under isocratic elution conditions could partly be associated with the flow-through pore heterogeneity [28–33] resulting in convective flow dispersion, an aspect that has received significant attention in recent years. Experimental elucidations in this direction via reconstruction [31–33], and modelling attempts [31,32], have already been reported. The efficiency analysis in the elution of small (retained) solutes also reveals the strong impact of nanoscale gel porosity [25–29]. This aspect is detached, but interplays, with the morphological aspects of polymer monoliths [28]. In fact, polymer monoliths show heterogeneities on the nano-, micro-, and potentially confine-associated length scales. These heterogeneities have their origin in the very early beginnings of the uncontrolled, though adjustable, free-radical polymerization processes and the inherently associated phenomena of scaffold formation [25,27].

The separation of small molecules, peptides, and (small) proteins is well-served by silica-based monoliths [34,35], packed beds of porous adsorbent particles, and the recent revival of core-shell particles [36,37] with a noticeable extension to the analysis of large molecules [38]. Theoretical description of transport and performance characteristics through such types of porous media has advanced considerably in recent years and this has allowed correlation to actual elution experiments with small solutes [39,40].

Using such small solutes with typical polymer monoliths enters a regime of chromatography that is ruled by convective flow dispersion, as well as diffusion and partitioning into the solvated polymer gel matrix. This is irrespective of what happens to the solutes once they are adsorbed, or whether mesopores are present in the dry-state or not [28]. Such small solutes have sizes that correspond to the molecular framework openings of the heterogeneously cross-linked and solvated polymeric material found in porous monoliths [25–29]. From a fundamental point of view, this marks the major difference to hard matter silica-based (meso)porous materials [29]. In turn, polymer monoliths are analogous to their earlier generation bead-based polymeric counterparts [29,41–46]. A practical and easy indication for the discussed issues on column performance can be found by estimating the resistance to mass transfer contribution (classically C-Term contribution) to the plate height of small molecules. Once systematically probed, isocratic performance is impacted by the solute properties such as size, functional group content, as well as retention [47]. The C-Term contribution to band broadening can dominate overall achievable performance of these materials even at very low flow velocities [27].

We have introduced and explored the concept of gel porosity as an important tool for indirectly studying the soft matter structure of macroscopically rigid polymer monoliths [25]. It has helped in explaining the performance of porous polymeric monoliths for small molecules including the impact of mobile phase composition. The mobile phase composition modulates both polymer nanoscale gel structure and associated retention-dependent performance in the elution of small retained solutes for both methacrylate and styrene/divinylbenzene based chemistries [25,26,47]. In both of these cases, a specific mobile phase solvent composition translates to a variably solvated polymer scaffold. Direct demonstration of this aspect has been possible by confocal Raman spectroscopy [48]. While water was seen almost exclusively in the large, micrometer-sized flow-through pores of a hydrophobic porous polymeric poly(styrene-*co*-divinyl benzene) material, acetonitrile was present even within individual globular features. The resultant distribution equilibrium of mobile phase components (associated with a dynamic gel porosity) likely then also determines variable permeation of the cross-linked polymer gel by small-sized solutes based on partition and adsorption. Varying polymer monoliths feature size, and cross-link density then are aspects that have to be considered for a straightforward performance analysis. Small molecules permeate the cross-linked polymer to a varying degree under operation in liquid chromatography [28,47].

Porous polymer monoliths show their optimum performance for gradient elution of large molecules. The impact of gel porosity on the metric description of transport performance for larger solutes such as proteins should yet require significant attention [28,29]. Related studies in this area mostly utilize the nonretained elution regime in the absence of adsorption at the pore-fluid gel interfaces and indicate a rapid loss of efficiency at even low retention [49,50]. The recently introduced modelling approaches for flow [31] and dispersion [32] in commercial monolithic disc materials further found disagreement of both shape of plate height curves, and magnitude of large molecule transport performance under nonretained conditions. Theoretically predicted values of the (reduced) minimum plate height for a limited model domain of a commercial CIM^{TM} disc material were smaller than 10 μm and were increasing with flow velocities. This shape of the derived plate height curve was in contrast to experimental results [32]. A knowledge of the theoretically achievable performance should spur efforts and further interest in improving the polymer monoliths' chromatographic performance.

It is purpose of the current experimental study to have a closer look at the characteristics of porous polymeric monoliths for elution of biomolecular solutes under isocratic conditions. The experiments involve a set of small peptides and proteins ranging in molecular weight from 181.19 to 44,287 Da. This study is complemented by the use of a suitable low molecular weight mobile phase velocity tracer as a reference.

2. Experimental

2.1. Chemicals and materials

Solutes uracil (112.09 g/mol), tyrosine (181.19 g/mol), val-tyr-val (379.45 g/mol), [D-Ala^2]-leucine enkephalin (569.65 g/mol), [Asn^{1}Va^5]-angiotensin II (1031.17 g/mol), insulin (from bovine pancreas) (5733 g/mol), cytochrome *c* (from equine heart) (12,384 g/mol), myoglobin (from equine skeletal muscle) (17,699 g/mol), albumin (from chicken egg white) (44,287 g/mol), and the mobile phase additive formic acid were acquired from Sigma–Aldrich (Vienna, Austria). LC–MS grade acetonitrile was received from VWR (Vienna, Austria). Water was purified on a Milli-Q Reference water purification system from Millipore (Vienna, Austria). Sample solutions were prepared in running mobile phases containing various volume percentages of acetonitrile/water (v/v) and 0.1% (v/v) formic acid.

2.2. Equipment, column, and chromatographic measurements

A 1290 Infinity UPLC system (Agilent Technologies, Vienna, Austria) was used for all chromatographic experiments. The overall measured dead volume from injection to detection with the column replaced by a zero dead-volume connection was 25.7 μL. The data acquisition rate was set to 160 Hz to allow sufficient data sampling for the very short residence times and small peak widths. This is particularly important with rapid elution times that can have serious effects on estimated performance [51].

The commercially available Thermo Scientific (TM) ProSwift (TM) RP-1S and RP-3U monoliths with a poly (styrene-*co*-divinylbenzene) cross-linked macroporous monolithic polymer were a kind gift from Thermo Fisher Scientific (Sunnyvale, California) as research samples. The total extra-column volume was calculated to be ≤4% of the overall column volume according to the provided bed dimension of 4.6 × 46 mm (RP-1S) or 4.6 × 40 mm (RP-3U), respectively. The (macroscopic) bed aspect