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Review

Advances in the development of organic polymer monolithic columns and their applications in food analysis—A review

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ABSTRACT

Monolithic continuous separation media are gradually finding their way to sample pre-treatment, isolation, enrichment and final analytical separations of a plethora of compounds, occurring as food components, additives or contaminants, including pharmaceuticals, pesticides and toxins, which have traditionally been the domain of particulate chromatographic materials. In the present review, recent advances in the technology of monolithic columns and the applications in food analysis are addressed. Silica-based monoliths are excellent substitutes to conventional particle-packed columns, improving the speed of analysis for low-molecular weight compounds, due to their excellent efficiency and high permeability. These properties have been recently appreciated in two-dimensional HPLC, where the performance in the second dimension is of crucial importance. Organic-polymer monoliths in various formats provide excellent separations of biopolymers. Thin monolithic disks or rod columns are widely employed in isolation, purification and pre-treatment of sample containing proteins, peptides or nucleic acid fragments. Monolithic capillaries were originally intended for use in electrochromatography, but are becoming more frequently used for capillary and micro-HPLC. Monoliths are ideal highly porous support media for immobilization or imprinting template molecules, to provide sorbents for shape-selective isolation of target molecules from various matrices occurring in food analysis. The separation efficiency of organic polymer monoliths for small molecules can be significantly improved by optimization of polymerization approach, or by post-polymerization modification. This will enable full utilization of a large variety of available monomers to prepare monoliths with chemistry matching the needs of selectivity of separations of various food samples containing even very polar or ionized compounds.

Contents

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1. Introduction

As early as in 1967, Kubín et al. [1] reported on in situ preparation of a porous organic polymer block in a glass column, which however showed poor permeability to be of practical use as a separation medium. Later, Hjertén introduced compressed gels in the form of continuous beds [2]. Tennikova et al. [3] and Švec and Fréchet [4] developed "continuous separation media" consisting of one piece of porous organic polymer material filling whole volume of a cylindrical column, which were later named "monolithic stationary phases" [5]. Monolithic columns based on inorganic matrices were introduced by the beginning of 1990s [6]; the silica monolith fabrication by a sol–gel approach was later reported by Tanaka group [7].

Since then, two types of monolithic materials have been available for chromatographic separations – monoliths based on inorganic precursors and those based on organic polymers. The structure of monolithic media can be represented as a network of small mesopores, which are responsible for the retention and separation selectivity, interconnected by large flow-through pores. This morphology provides good bed permeability and enables fast separations at high flow of the mobile phase and moderate backpressures, in comparison to particle-packed columns with similar efficiency [8]. Hence monolithic columns show higher permeability and lower flow resistance than the conventional LC columns packed with small particles, providing thus significantly shorter separation times at moderate operation pressures. Due to these favorable properties, monolithic columns have been widely applied almost in all LC application areas, including environmental, pharmaceutical, clinical, forensic, industrial, and food analysis.

Silica-based monolithic columns are available commercially in the conventional column format (4 mm i.d. rods, encased in a cladding tube to produce the final column), or larger diameter columns for preparative applications; monoliths prepared in situ in fused silica capillaries < 0.2 mm i.d., and recently in the most difficult narrow bore format (2 mm i.d.). Because of their high efficiency, bare silica or chemically modified commercial monolithic columns have been used routinely as the time-saving substitutions of conventional HPLC columns packed with porous particles for the separations of small molecules and biomolecules in many application areas, including food analysis, with excellent resolution and short times of separation. Recently, preparation of titanium-based monolithic columns was reported, even though the chromatographic applications have been so far relatively rare [9].

Some earlier applications of silica-based monolithic columns in food analysis were covered in the review by Cabrera [10]. More recent specific reviews focused on the applications of monoliths in the analysis of milk and diary products [11] and on the applications in the analysis of proteins in food [12]. Some applications of monolithic columns are included in a recent review on new trends in fast liquid chromatography for food analysis [13].

The present review focuses on the remarkable progress recently achieved in the development of monolithic stationary phases, with attention to the reversed-phase, normal-phase, HILIC and ion-exchange applications of monolithic LC columns in food analysis. The applications of molecularly imprinted monolithic polymer media (MIPs) in sample treatment and monolithic columns in two-dimensional separations (2D LC) are also addressed.

2. Silica and organic polymer monoliths – types, morphology, characterization and recent improvements

In spite of the internal structure with dominant flow-through pores, the silica-based and the organic polymer monoliths show significant differences in pore morphology. The differences are apparent from the micrographs in Fig. 1. Silica-based monoliths have a bimodal pore structure with significant representation of 7–12 nm mesopores (~13%) and relatively high specific surface area of several hundred m^2/g, similar to the specific surface area 150–400 m^2/g, typical for particles packed in conventional columns [7,14]. Hence the silica monoliths are ideal for separations of small molecules, as the mesopore size allows easy penetration to the adsorption sites and fast diffusion, resulting in high efficiency of separation.

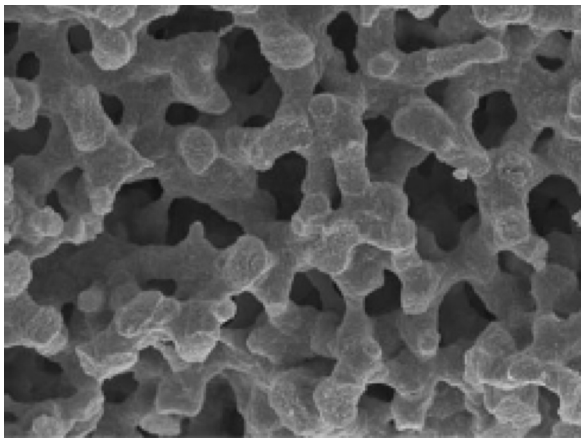

Fig. 1. The bimodal pore structure of silica-based monoliths.

Like silica gel particles, the monolithic silica rods can be chemically modified by binding a variety of non-polar, weakly or strongly polar functionalities for various LC applications. Chemically bonded silica-based monoliths allow fast separations of low-molecular samples with column efficiencies up to 100,000 theoretical plates/m [15]. Commercial silica-based monolithic columns with chemically bonded alkyls such as Chromolith C_{18} or Chromolith C_8 from Merck (Darmstadt, Germany) have been widely used for separation of various low-molecular compounds, including applications in food analysis [10]. These columns provide lower column pressure and large decrease in separation time in comparison to columns packed with fully porous and even fused-core particles, even though in some cases at the cost of lower chromatographic efficiency. Also the method transfer between the particulate and monolithic columns often requires adjustment of separation conditions [16,17].

Unfortunately, silica monoliths generally show less good performance for separations of macromolecular compounds such as proteins and other biopolymers. The separation of polymers requires different conditions than the separations of small molecules. The penetration of macromolecules requires wide pores (at least 15–100 nm) and relatively low specific surface area (10–150 m^2/g), to allow easy access to the interactive adsorbent surface within the pores. Further, limited stability at temperatures higher than 60 °C and pH > 8.5, similar to silica particles, might limit their performance for the analysis of polar – especially basic – compounds.

The polymer-based monoliths have a heterogeneous structure, which resembles rather a net of interconnected non-porous cauliflower-like microglobules with significantly lower surface area (Fig. 2). This morphology is more suitable for separation of polymers requiring large pores (15–100 nm) with relatively low specific surface area (10–150 m^2/g) [18]. The small pores in the microglobules of organic monoliths are generally inaccessible for large molecules of biopolymers, which are therefore not subject to slow diffusion decreasing the separation efficiency, unlike the silica-based monoliths with partially accessible larger mesopores [19]. In the past, organic monoliths have shown low efficiency for