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Review

Applications of monolithic materials for sample preparation

Tarang Nema[a,b], Eric C.Y. Chan[a], Paul C. Ho[a,*]

[a] Department of Pharmacy, National University of Singapore, 18 Science Drive 4, Singapore 117543, Singapore
[b] Waters Pacific Pte. Ltd., 1 Science Park Road, Singapore Science Park II, Singapore 117528, Singapore

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ABSTRACT

Recent advances in monolithic columns have made them an alternative to traditional packed columns used in liquid chromatography as well as capillary electrochromatography (CEC). The monolithic columns have been extensively studied and shown to possess several advantages that make them a promising and potential substitute for the particle packed columns. A large number of papers relating to monolithic columns have been published every year, focusing on different preparation techniques, characteristic evaluations as well as applications. This review highlighted the latest development of monoliths for other modes of analytical chemistry. In particular, this review will highlight the application of monoliths for sample preparation which is an important step of the entire analysis.

Contents

1. Introduction

High pressure liquid chromatography (HPLC) is one of the most versatile analytical and separation techniques in many fields of applications. Various inventions and discoveries have taken place to improve the performance and efficiency of the HPLC technique, column technology is one of them. The stationary phase in the form of a column is crucial and the most important element that governs the type of chromatography. Advancement in column technology has gained significant attention in the field of separation. Plenty of research has been carried out in developing highly retentive and selective column with the prospects of resolving components in a short duration of time in a cost-effective manner. Miniaturization of columns, reducing the internal diameter (i.d.) from millimeters to a fraction of millimeter in microbore or capillary columns is one of the techniques which have attained significant attention in recent years. Furthermore, miniaturization of column in analysis irrespective of its applications in proteomics, metabonomics, environmental analysis or any field of science for separation and quantitation, has become the research focus in separation science, because of the following advantages: (1) less solvent consumption which is related to the cost of analysis (cost of procurement and disposal of solvents); (2) more environmental friendly; and (3) greater sensitivity of the analysis [1,2]. However, limited loading capacity and difficult in preparation of miniaturized columns limit their popularity.

* Corresponding author. Tel.: +65 6516 2651; fax: +65 6779 1554.
E-mail address: phahocl@nus.edu.sg (P.C. Ho).

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Small molecules such as drugs are separated by columns with packed particles having pores in the range of 80–100 Å, whereas larger molecules like proteins are separated by columns with particle pores larger than 200 Å [3]. Generally, chromatographic column is packed with porous silica microparticles in the range of 2–10 μm. Chromatographic resolution is based on the size and distribution of the particles along with the quality of packing. Higher column efficiency and shorter analysis time are the key factors that every chromatographer desires [4–6]. Performance of such particulate columns also depends on the frit that is placed at the end to retain the particles within the column [7]. Ideally, the frits should be porous enough to allow uniform flow of mobile phase through the column. Failing to achieve it could lead to inefficient performance of particle packed column. Bubble formation and analyte reaction with the frit material are some other problems associated with the end frits that cause deterioration of the column performance. Even though many of the above mentioned problems with the frits have been resolved today, still a lot of skills and technique are required to prepare a highly permeable and robust end frits reproducibly.

In the continual search for suitable process for fabricating highly efficient columns, scientists have taken various approaches, leading to numerous developments in column technology. Particle packed columns with size less than 2 μm can provide excellent chromatographic performance. Further reduction in particle size could provide better sensitivity and resolution due to less eddy diffusion and shorter path length. However, increase in flow rates in these columns is limited due to the increased back pressure (as pressure is inversely proportional to square of particle diameter, according to Darcy's law) [7]. To achieve high separation efficiency with these columns, ultra high pressure liquid chromatography (UPLC) [8,9] and CEC [10,11] have been employed. Although these instruments solve the problem to great extent, the affordability to routine laboratory was difficult as well as their maintenance cost is high. For example UPLC requires high tensile strength expensive alloys for efficient performance of the pumps to deliver solvent continuously without any pulses. Design development, initial investment and maintenance cost are some of the major issues that limit their routine accessibility. This led to the search of highly permeable fritless surface active column which can be easily prepared and is economical. These properties were found to be the characteristic features of monolithic columns. The concept of monolithic columns was first conceived in the late 1970s when the scientists tried to use some organic monomers to prepare monolithic columns primarily to separate proteins [12]. In recent years, monolithic stationary phase has gained high acclamation and myriad of research has been carried out. The work has been reviewed extensively by Cabrera [13]. It is because of their ease in preparation, efficient properties and excellent performance compared to conventional packed columns which make them an efficient tool in HPLC [14]. According to Zou et al., monolithic stationary phase is a continuous unitary porous structure prepared by in situ polymerization of monomers (organic/inorganic) inside the column tubing [15,16].

Uniformity of bed with no end frits, higher permeability and the ability to design to desired length are the main advantages of monolithic stationary phase. There are various ways of preparing a monolith. Some of the most common approaches for their preparation include (1) polymerization of organic/inorganic monomers which differ in the chemistry, (2) fusion of microparticles with the monolith inside the capillary by sintering and (3) utilization of hybrid material [4]. Monolith based on organic monomers was the first and the most worked-on approach in the chromatographic field. However, the swelling of polymers in some solvents and mechanical instability limit the use of organic monomers as monolithic stationary phase [17]. These problems associated with organic monolith led to the introduction of inorganic based monoliths using monomers such as tetramethoxysilane, tetraethoxysilane and other functional monomers or combination monomers. These inorganic monoliths have the advantages of high mechanical stability and resistance to swell in solvents when compared to organic monoliths [5,17,18]. Another technique of using microparticles by sintering is one of the uncommon approaches for preparing monolithic columns. The difficulty in preparation and inconsistent column performance are the two major hindrances associated with this technique [19,20].

Although there are plenty of advantages associated with monolithic column which make them an efficient and promising tool in the separation technology, the monoliths still has limited popularity as a stationary phase. This can be attributed to cracking and shrinkage of the formed rod inside column tubing and difficulty in housing the detached rod in suitable cartridge [7]. In addition to these, post column modification to the desired chromatography is not only time consuming but also require careful control to the process, which could potentially affect reproducibility. The challenge leads to a wide spectrum of research area for the researchers to explore. Since the time of their invention, they are mainly utilized as columns. Therefore, little is known or explored about their other applications. Among them their applicability as a sample preparation tool had gained the attention recently. This review focuses on their applicability as solid phase extraction cartridges in different formats and chemistries. This review also focuses on some of the more recent approaches in the preparation of monolithic column to overcome the drawbacks associated with them. The review starts with the brief concept about the monolith followed by the discussion on the different types of monoliths, and the formats available with their application and finally their applicability as tool for extraction in various matrices.

2. Monoliths

In general, monolith means "column consisting of a single large block of stone". The word was derived from the Greek word monolithos with monos meaning "single" and lithos, the "stone." In chromatographic terms, it represents a continuous single rod of porous material [21]. It is characterized by high permeability due to uniform distribution of macropores and mesopores throughout the network enabling separation of many analytes. The macropores provides the permeability for solvents to flow through, whereas mesopores provides the high surface area for separation. As the formed network fills the column volume completely, interparticular voids are absent, resulting in 100% flow of mobile phase through the column. The need for packing as in particle packed column is also unnecessary, as the monolith can be prepared in situ by polymerization. However, this process of polymerization is restricted to capillaries (usually less than 200 μm in i.d.) due to the problem of shrinkage of the monolith in capillaries of larger i.d.

High porosity and low back pressure are the distinguishing features of monolith which has attracted many researchers and have been proven to be an efficient tool in column technology [22]. A comparison of the physical and surface properties between a particle packed column and a monolithic column is shown in Table 1. Pioneer efforts by Hjerten et al. [23], Svec and Frechet [24] on developing polymer monolith and later by Tanaka and Nakanishion developing silicon monolith [18,25] have revolutionized the field of column technology. Monolith which was initially confined to an academic practice has now been accepted as a legitimate member of stationary phases.

Other than permeability, monolithic columns have comparable phase ratio and enantioselectivity to conventional columns [13]. At the same time, monolithic columns are easier to prepare and modify to the desired porosity and pore diameter to suit different needs [26]. Specific selectors such as chiral selectors can be incorporated