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Review

New developments and possibilities of wide-pore superficially porous particle technology applied for the liquid chromatographic analysis of therapeutic proteins

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ABSTRACT

This review paper discusses the success of columns packed with superficially porous particles (SPP) in liquid chromatography for the analysis of peptides and proteins. First, it summarizes the history of SPP, including the development of different SPP generations from particles of 50 μm to sub-2 μm. It also critically discusses the improved kinetic performance of SPP particles in comparison to fully porous particles. The current trends and applications of columns packed with SPPs for the analysis of peptides and proteins (including mAbs and ADC at the intact and sub-unit levels) are shown, as well. Finally, some of the potential perspectives for this technology are also described, including the radially oriented mesopores or the applicability of the technology for chiral separations.

Contents

1. History of columns packed with SPP particles

In the recent development of particle technology targeted for liquid chromatography (LC), the use of superficially porous (SPP or often called as shell, core-shell, fused-core or partially-porous) particles has received considerable attention [1,2]. SPPs manifest the advantages of porous and nonporous particles. Knox was the first to recommend the use of thin films of the stationary liquid phase in liquid–liquid chromatography [3]. The concept of superficial stationary phases in LC, was first introduced by Horváth and co-workers in the late 1960s [4,5]. Horváth applied 50 μm glass bead particles covered with styrene-divinylbenzene based ion exchange resin, known as pellicular packing material for the separation of nucleotides. Later, Kirkland showed, that 30–40 μm diameter SPPs provide much faster separations, compared to the large porous particles used earlier in LC [6]. The motivation behind the development of such materials was that columns packed with

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partially porous particles would have a higher efficiency than those packed with fully porous particles, because diffusion through the thin porous layer surrounding the particles would be faster than diffusion through the whole particles [2]. This acceleration of diffusion would reduce the time required for solute equilibration between the porous layer and the mobile phase or, more exactly, would effectively reduce the resistance to mass transfer through the stationary phase [2]. This feature should be especially beneficial for the separation of large molecules possessing low diffusivity. This idea made sense at a time when the average particle sizes were ca. 80 μm. Therefore 30–50 μm particles with very thin porous shell have been commercialized in the 1970s under different brand names such as Zipax, Corasil and Pellicosil [6–8].

In the 1990s, non-porous particles have also been considered as a valuable option for protein separations. Issaeva et al. showed an extremely high speed separation of proteins and peptides using 1.5 μm non-porous particles (Micra) [9]. Barder et al. also demonstrated that the efficiency of columns packed with non-porous silica particles was considerably higher than that of columns packed with porous particles, especially at high flow-rates [10]. Non-porous particles can indeed provide lower mass transfer resistance and higher efficiency than porous particles, but they are suffering from a smaller specific surface and sample loading capacity. Seifar et al. estimated a 50-fold higher sample capacity for porous particles compared to non-porous particles of the same size [11]. In another work, the loading capacity for the 1.7 μm fully porous Acquity C18 particles was found to be 16 times larger than for non-porous 1.5 μm particles [12]. Another issue related to the use of non-porous particles is its very low retention capacity compared to fully porous ones. It was shown that the average carbon load for 1.5 μm non-porous particles was about 56 times lower than for 1.7 μm Acquity C18 porous particles [12]. The lower carbon load provides a lower phase ratio for non-porous particles, which leads to significantly lower retention. Due to the above mentioned limitations, non-porous materials never had too much success.

SPP materials had a regain interest in the year 2000 and the second generation of SPPs then appeared [13]. At this time, the commercial 5 μm particles, having an average pore size of 300 Å and 0.25 μm shell thickness (it was called Poroshell), showed excellent efficiency for macromolecule separations. Few years later a new generation of SPPs has been developed and particles having standard-pores (90 and 100 Å) were successfully applied for small molecules separations. These were the so-called sub-3 μm SPPs and their structure was very close to the optimum morphology, offering a good compromise between column efficiency and loadability. They were commercialized under the brand names of Halo, Ascentis Express and Kinetex [14–16]. A sub-3 μm particle with the pore size of 160 Å packing was introduced in 2010 by Advanced Material Technology (AMT) and Supelco under the brand names of Halo Peptide ES-C18 and Ascentis Express Peptide ES-C18, respectively [17,18]. An average pore size of 160 Å allowed the unrestricted access of molecules up to approximately 15 kDa, depending on the molecular conformation [19]. Kirkland et al. compared the efficiency of the 160 Å and 90 Å SPPs for mixtures of peptides and small proteins [18]. Small proteins (i.e., ribonuclease, insulin, cytochrome C and lysozyme) exhibited broadened peaks with the 90 Å SPP, indicating restricted diffusion, but they eluted in narrow peaks from the 160 Å SPP column.

In 2012, a larger (3.6 μm) SPP wide-pore material (0.2 μm shell thickness) was launched under the name Aeris Widepore, and seemed to be particularly promising for large protein separations including monoclonal antibody (mAb) fragments [20,21]. Its relatively large particle diameter afforded low column pressures, which could help to minimize potential on-column degradation of pressure sensitive proteins, by avoiding high shear forces, and to minimize pressure induced increases in hydrophobic retention that can contribute to peak broadening [22,23]. To analyse intact large proteins and their sub-units, the particle size and shell thickness were further optimized [24]. Both theory and previous experimental studies indicated that a thin shell should be used to compensate for the low diffusion coefficients of large molecules. To find the optimum particle morphology, three different batches of 3.4 μm particles with 400 Å pores and thick shells of 0.15, 0.20 or 0.25 μm were compared in an experimental study [24]. It was found that a 0.20 μm shell thickness (400 Å) provided the highest chromatographic performance for proteins. This material is now commercially available under the brand name HALO Protein. It was found that the larger pore size actually had more impact on the kinetic performance achieved with mAbs, than the particle size and shell thickness. The SPPs with larger particle size (3.5 μm) and pore size (450 Å) showed the highest resolution for mAbs [25]. This results led to the optimal particle design with a particle size of 3.5 μm, a thin shell of 0.25 μm and pore size of 450 Å. This material is now commercialized as AdvanceBio RP-mAb. Later, SPPs with 1000 Å pores designed specifically for separating large biomolecules and industrial polymers have been described and showed benefits compared to 300–400 Å SPPs [26]. Very recently, another wide-pore silica-based SPP with a high coverage phenyl bonding has been released and successfully applied for the analysis of mAbs and antibody-drug conjugates (ADCs) [27]. This new material (BioResolve RP mAb polyphenyl) is based on 2.7 μm particles having a shell thickness of 0.40 μm and average pore size of approximately 450 Å.

Today, mostly two processes are employed to prepare SPPs. One is called “multilayer technology” or “layer by layer” in which solid silica cores are repeatedly coated with layers of colloidal nanoparticles by alternating layers of oppositely charged nanoparticles and polymers containing amino-functional groups, until the particles reach the desired sizes [28]. The other procedure involves a one-step coacervation, where solid silica spheres are suspended in a coacervation reaction mixture including urea, formaldehyde, and colloidal silica sol under acidic conditions. A coacervate of urea-formaldehyde polymer and ultrapure silica sol is thus formed and coats the solid cores [28]. The urea-formaldehyde polymer is then removed, and the particles are then strengthened by sintering at high temperatures.

As shown, there is still a continuous development in SPP technology and more and more efficient stationary phases are regularly released. Various particle morphologies (i.e. particle size, shell thickness, pore size) are now available for protein separations, and Fig. 1 illustrates the history of SPP development. The aim of this paper is to review the latest developments and applications of wide-pore SPPs applied for large molecule separations and provide some guidelines for method development. Some future perspectives are also presented.

2. Advantages of SPP technology

The peak dispersion in chromatography is generally characterized by the theoretical plate height (H) and the number of theoretical plates (N). The treatment of mass transfer processes and the distribution equilibrium between the mobile and stationary phase in a column lead to equations which link the theoretical plate height to the properties of the chromatographic systems, such as the linear velocity. First, van Deemter proposed an equation, which described the column performance as a function of the linear velocity [29]. Since then, several plate height and rate models were derived for LC, by numerous researchers. Knox suggested a useful empirical three term equation to describe the dependency of the theoretical plate height of a column as a function of linear velocity [30]. In this well-known equation, the three parameters