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Core-shell microspheres with porous nanostructured shells for liquid chromatography

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Abbreviations: CTAB, cetyltrimethylammonium bromide; CTAC, cetyltrimethylammonium chloride; DRIFTS, diffuse reflectance infrared Fourier transform spectroscopy; HILIC, hydrophilic interaction chromatography; HKUST-1, Hong Kong University of Science and Technology; LbL, layer-by-layer; MIL-100, Materials of Institute Lavoisier; MPTMS, 3-mercaptopropyltrimethoxysilane; MOFs, metal-organic frameworks; MW, molecular weight; OTMS, octadecyl trimethoxysilane; PDDA, poly(diallyldimethylammonium chloride); PMT, pseudomorphic transformation; PSD, particle size distribution; SOS, spheres-on-sphere; SPPs, superficially porous particles; TEOS, tetraethyl orthosilicate; UHPLC, ultra-high pressure liquid chromatography; UiO-66, Universitetet i Oslo; ZIF-8, zeolitic imidazolate framework.

Keywords: core-shell particles / superficially porous particles / spheres-on-sphere silica / hybrid particles / fast liquid chromatography

Abstract: Development of new stationary phase has been the key aspect for fast and efficient HPLC separation with relatively low backpressure. Core-shell particles, with solid core and porous shell, have been extensively investigated and commercially manufactured in the last decade. The excellent performance of core-shell particles columns has been recorded for a wide range of analytes, covering small and large molecules, neutral & ionic (acidic and basic), biomolecules, and metabolites. In this review, we firstly introduce the advance and advantages of core-shell particles (or more widely known as superficially porous particles) against non-porous particles and fully porous particles. This is followed by the detailed description of various methods used to fabricate core-shell particles. We then discuss the applications of common silica core-shell particles (mostly commercially manufactured), spheres-on-sphere particles, and core-shell particles with non-silica shell. This review concludes with a summary and perspective on the development of stationary phase materials for HPLC applications.

1. Introduction

LC has been widely used for separation, analysis, and detection. In addition to routine analysis of small molecules in chemistry and chemical-related industry labs, the use of LC covers separation of bio-related molecules (e.g., amino acid, peptide, proteins, lipids, glycans & saccharides, oligonucleotides), metabolic phenotyping, food science & nutrition, forensic toxicology, environmental analysis, and pharmaceutical analysis [1]. The development of modern LC has targeted fast and efficient separations, with improvements being made in innovative stationary phase and instrumentation [2]. The stationary phase materials in LC can be generally classified into two categories: microspheres and monoliths [3,4]. Monolith columns can offer fast separation and may be particularly useful for separation of large macromolecules. There are several products of monolithic columns on the market. However, the spherical packing materials (indeed silica spheres) are still dominating the market and widely investigated in research and development [3]. Because separation in LC is based on the adsorption/retention and desorption between the target molecules in the mobile phase and stationary phase, an increase in surface area of the stationary phase can increase the number of adsorption/desorption sites and enhance the separation efficiency. Both porous and non-porous spheres are used. While porous spheres exhibit high surface areas, non-porous spheres are mechanically more stable, which can be very important when operational pressure is high. The development and use of uniform and smaller silica microspheres has been the driving force for highly efficient HPLC. However, the backpressure generated by the use of small particles is a big obstacle for the HPLC systems.

To address the dilemma in high performance and high operational pressure for smaller particles, instrumentation design of the HPLC system with short narrow-bore columns has been shown to achieve fast separation with excellent sensitivity [2]. For the narrow-bore columns, the factors that can significantly compromise the analysis performance or reproducibility include extra-column volume (including injector system, connector tubing, detector cell), dwell volume, data acquisition rate, and injection cycle time [2]. The instrumentation design may also include coupling of on-line sample pre-treatment techniques with HPLC columns. This is to separate and enrich target

compounds in the complex samples with very low concentration. Typically, the sample pre-treatment techniques are solid phase extraction or liquid phase extraction [5]. To simply enhance separation efficiency and selectivity, column modulation via serial coupling of different columns under compatible conditions can be employed [6]. This, however, may cause a huge increase of operational pressure.

High operational pressure can give rise to detrimental effects of frictional heating generated by percolation of the mobile phase flowing through the packed particles. The heat generated is proportional to the pressure drop and flow rate [3]. This creates the temperature gradient along the axial direction (the flowing direction of mobile phase) and the radial direction (due to the high temperature in the central part and the heat loss through the wall), leading to serious band broadening and poor reproducibility. This effect may be reduced by using narrow-bore columns and reducing backpressure inside the column [2]. The high pressure can also lead to significant change in partial molar volume, which is the change of molar volumes of solute molecules bound to the stationary phase and solute molecules in the mobile phase. The changes are greater for large molecules such as proteins, peptides and other biomacromolecules. This can in turn influence the retention and separation of such molecules, as explained by the solvation parameter model [7].

Reducing operational pressure whilst maintaining high separation efficiency can generate multiple benefits. The use of core-shell silica particles, with solid core and porous shell, as column packing materials, has been regarded as a big step forward in HPLC column technology in the last decade or so [8]. These core-shell silica particles are also known by some of other commonly used names, such as fused-core, solid core or superficially porous particles (SPP) [4]. The main commercially available core-shell columns include Poroshell (Agilent), Cortecs (Waters), Accucore (Thermo Fisher Scientific), Ascentis Express (Sigma-Aldrich), Halo (Advanced Materials Technologies), Kinetex (Phenomenex), Ultracore (Advanced Chromatography Technologies) [4,9]. Core-shell columns with particles of 2.6-2.7 μm have been found to show better or the same efficiency compared with fully porous 1.7 μm column (BEH C_{18}), but with much lower back pressure [9]. The particle size distribution could also have a big impact on the operational pressure [9]. The core-shell particles columns have considerably enhanced the applications of HPLC in various areas [1,10]. There are, of

course, other exciting developments in the development of novel stationary phase materials, for example, for hydrophilic interaction chromatography (HILIC) [11], mixed-mode HPLC [12], size exclusion chromatography (SEC) [13], and metal-organic frameworks (MOFs) as new type of stationary phase for HPLC [14]. These new development may be based on core-shell silica particles or fully porous/non-porous silica particles.

In this review, we focus on the fabrication and use of core-shell particles for enhanced HPLC applications. The fabrication methods are particularly covered in details. The fundamentals for HPLC and core-shell particles and examples of the applications are also described.

2. Core-shell particles as packing materials and the mechanism for high performance

2.1 Columns packed with non-porous particles, totally porous, and core-shell particles

Separation of a complex mixture into individual species by LC requires the stationary phase to selectively retain analytes based on their chemical composition and structure [15]. Usually a column packed with porous micron-sized particles is employed in order to achieve the separation. In LC, the analytes are transported by a convective flow of the mobile phase through the packed column. Diffusion of solutes in a liquid phase is usually three orders of magnitude slower than in the gas phase for small to medium sized molecules. In addition, diffusion into and out of tortuous pores (pore diffusion) is reduced significantly as compared to diffusion in the liquid bulk phase. A central problem in HPLC is therefore to overcome the limited mass transfer of solutes due to pore diffusion by providing sufficient access to the interactive surface sites. One way is to reduce the average particle size of the packing to minimize the diffusion path length by using smaller microspheres [15].

2.1.1 Non-porous silica particles

Works on the use of non-porous sub-2μm silica particles in ultra-high pressure liquid chromatography (UHPLC) with columns id < 200 μm were carried out since late 1990s. The particles used were 1.0 and 1.5 μm. These particles were monodisperse and yielded very efficient chromatographic columns [16-18]. Spherical organosilica particles of 670 nm (stable in 1 < pH < 11) containing C18 moieties

were prepared by Cintron and Colon using a simple one-step synthesis process [19]. When applied in UHPLC for the separation of a mixture of ascorbic acid, hydroquinone, resorcinol, catechol and 4-methylcatechol, a fast analysis time (< 4 min) and high theoretical plates (500,000 plates/m) were achieved. Elevated-temperature UHPLC was performed using polybutadiene-coated 1 µm non-porous zirconia particles in a study by Lee and co-workers [20]. Five herbicides were separated in 1 min at 26,000 psi and 90 °C with a column efficiency of 420,000 plates/m. It was shown that non-porous particles with uniform size (below 1.5 µm) could form robust packed column and yield low plate height at elevated column pressure [17,20]. However, non-porous materials have comparatively lower surface area which results in low loading capacity.

For small non-porous particles, the challenge is the resulting high column pressure which renders the instrument dangerous and impractical to operate [19]. Xiang et al. studied the safety concerns of operating UHPLC with fused-silica capillary columns [21]. Liquid jets and high speed projectiles of silica particles due to rupture of the capillary or failure of the ferrule in the capillary connection might lead to injuries. To avoid the high pressure and potential danger, some studies using sub-2 µm silica were carried out on capillary electro-chromatography (CEC). CEC utilizes electro-osmotic driving force instead of pressure drop to achieve high separation efficiency [22-24].

2.1.2 Totally porous silica particles

In 2005, robust porous sub-2 µm silica materials were brought into the limelight for fast and efficient chromatographic separation [25]. Mesoporous sub-2 µm silica materials, being smaller in size compared to conventional 5 and 3 µm packing materials, could generate enhanced separation efficiency. The large surface area also significantly improves loading capacity. These factors allow for the use of column with shorter length and smaller id to achieve the same or even higher resolution in a very short analysis time compared to conventional HPLC [25].

Some researchers have focused on the development of chromatographic application using sub-2 µm materials and relatively high-pressure instrumentation. Wu and Clausen discussed the fundamental and practical aspects of UHPLC such as particle size, frictional heating, pressure drop, column diameter, pump and injection systems, detection as well as packing materials [26]. Since then,

153 the development of sub-2 µm porous silica material continues to contribute significantly to the field of
154 liquid chromatography. These silica materials have demonstrated good performance in achiral
155 chromatographic separations and have shown great promise in fast enantioselective separation of
156 racemic compounds.

157 The advantages of smaller fully porous particles compared with the conventional larger
158 particles are clearly demonstrated by the kinetic plots as shown in Figure 1a [27]. The pressure limit
159 of the conventional columns (used on conventional equipment) is assumed to be 400 bar, whereas that
160 of the smaller particles with UHPLC equipment is assumed to be 1200 bar. For separations requiring
161 up to about 100,000 plates, the curve for the 1.8 µm column lies well below that of the 3.5 µm
162 column, indicating that a faster analysis can be achieved.

163

164 2.1.3 Core-shell silica particles

165 A big forward in column technology came in 2007 with the introduction of SPPs by Advanced
166 Material Technologies [28], following the concept of pellicular particles which were initially tested in
167 the 1970s, with the particle size of 50 µm and the shells made of aggregates of nanoparticles only a
168 few µm thick [29]. Progress was then made on the preparation and evaluation of particles with
169 controlled surface porosity [30] and the SPPs with 5 µm core and 1 µm shell [31]. While the particle
170 size of the majority of SPP columns was of the order of 2.6 µm, columns packed with sub 2 µm SPPs
171 are now widely available and can give further improvements in performance, if appropriate
172 instrumentation is available [10, 32-34]. This improvement can be seen in the kinetic plots in Figure
173 1b where separations requiring around 20,000 plates can be achieved in around 1 minute on a 1.6 µm
174 SPP column, but require significantly longer on the totally porous column of about the same particle
175 size (1.8µm) [27].

176 The advantages of smaller core-shell particles (1.0-1.3 µm) have been investigated, including
177 commercially available 1.3 µm particles with 0.9 µm non-porous core and porous shell < 0.2 µm thick
178 [3, 35-37]. Minimum plate heights of 2.2 µm were observed, corresponding to a plate count of
179 450,000 plates/m. However, only short columns of 3-7.5 cm length operated at modest flow rates
180 could be used. Clearly, practical column dimensions and operation are severely limited by the

maximum pressure of current instruments, and their band spreading effects [3]. Only the instruments with the lowest extra-column bandspreading could be used to obtain these results [2]. Nevertheless, it was observed that the extra-column bandspreading could have a major impact on the apparent kinetic performance; significant plate count loss was noticed for retention factors <5, even with the best system used for the experiments, which had σ^2 (extra-column) = 2 μL^2 at the flow rates used [3]. It was also demonstrated that the loss in performance caused by frictional heating effects remained negligible, but the short column lengths and flow rates used should be taken into account when considering this observation. The performance of 1.3 µm particles was further studied in the gradient elution mode with both small molecules and peptides (which have different diffusion characteristics) [38,39]. The material appeared to be particularly well suited for fast separations, but the advantages were much more obvious for peptides than small molecules. This was due to the possibility of working closer to the optimum flow velocity for peptides, as they have smaller mobile phase diffusion coefficients.

Kirkland and co-authors debated whether sub-2 µm SPPs were really necessary in many practical applications [40]. While the introduction of SPPs in the sub-2 µm range as opposed to the original 2.5 - 2.7 µm particles allowed very fast separation, the efficiency advantage of these very small particles is not often realised nor sufficient to overcome some of the practical limitations and associated disadvantages. A 2.0 µm SPP was suggested to retain many of the advantages, while minimising some of the disadvantages [40].

2.1.4 High performance of core-shell particle columns

The advantages of SPP columns can be extended to their substitution for conventional larger 5µm totally porous particles [41]. The core-shell particle columns show a clear improvement in separation power over their fully porous counterparts by allowing faster separations (at the same efficiency) or a higher separation resolution (at the same analysis time). Van Deemter and kinetic plots showed that 5 µm SPPs provide a superior kinetic performance compared with the fully porous particles over the entire range of separation conditions, when both types of particles were evaluated at the same operating pressure. The same observations were recorded both for isocratic and gradient analysis [42].

It was demonstrated that the SPPs do not compromise sensitivity due to loadability issues, and that the columns could be used on conventional equipment without modification to obtain significant improvement in analysis time, especially if the columns of 4.6 mm id packed with larger particles (~2.6 µm) are utilised, which reduce the need for instrumentation giving a low extra column bandspreading contribution [43-45]. Gritti and Guichon found values of the minimum reduced plate height of 1.3-1.5 for a 4.6 x 150 mm column packed with 4.6 µm SPPs [46]. The separation speed and resolution of these columns was claimed to be equivalent to that of 2.5 µm totally porous particles for hold up times larger than only 10s, and virtually equivalent to that of 2nd generation silica monoliths. Care must be taken when comparing the performance of the packed columns with different physical dimensions, because the internal diameter of the column can influence the performance [43,46].

2.2 Theoretic aspects of high performance core-shell particles

Daneyko et al. undertook a comprehensive computational investigation of longitudinal diffusion, eddy dispersion and trans-particle mass-transfer in random packing of core-shell particles with varied shell thickness and shell diffusion coefficient [47]. An excellent summary of current knowledge of the various band spreading processes within a column was given. The van Deemter equation [48] written in modern terminology (equation 1) gives a simple description of these processes:

$$H = A + \frac{B}{u} + Cu \quad (1)$$

$$h = av^{0.33} + \frac{b}{v} + cv \quad (2)$$

$$\Delta P = \emptyset \frac{\eta L u}{d_p^2} \quad (3)$$

Equation (2) is the Knox equation and uses *h* and *v,* the reduced forms of *H* (Height of equivalent theoretical plate, HETP) and *u (*velocity) in equation (1). The Knox equation is often employed for physicochemical comparison purposes [49]. For equation (3) (the Darcy's law), ΔP is the backpressure generated, φ is the flow resistance, u is the average velocity, η is the viscosity of the mobile phase, L is the column length, and d_p is the diameter of the particles.

In the van Deemter equation, the A term includes contributions to flow biases taking place over different characteristic lengths in the column that can be divided into:
(i) transchannel (associated with the dimensions of the interparticle channels between neighbouring particles);
(ii) short-range interchannel (associated with the scale of a few particle diameters);
(iii) long range interchannel (associated with the distances between local defects in a packing);
(iv) transcolumn effects (associated with heterogeneities at the scale of the column dimensions).
The B term is related to an apparent, complex diffusion coefficient accounting for the sample diffusivity in the interparticle bulk eluent and in the pore network of the stationary phase. The C term (mass transfer term) accounts for all mechanisms resulting in a finite response time for transfer between solid and the bulk liquid mobile phase. It should be pointed out that the coefficients in the equation are semi-empirical and as a consequence cannot be directly related to a physical description of the individual mechanisms.

The *A* and *C* terms directly depend on the particle diameter, so the reduction in particle size enhances the performance of the separation. Nevertheless, this leads to an immediate drawback: columns packed with smaller particles have lower permeability and this causes higher backpressures, according to Darcy's law [Equation (3)]. This means that optimal flow rates for fully-porous particles will generate backpressures several times higher in columns packed with sub-3 μm or sub-2-μm particles than in the case of the 5-μm particles.

Gritti and Guiochon had previously outlined these processes, and proposed that the good performance of SPP columns resulted from a smaller B term (due to the non-porosity of the core giving a reduced packed bed volume accessible for diffusion) and a much reduced A term [50,51]. Other work indicated that the smaller A term in SPP columns was mainly due to a higher transcolumn homogeneity rather than an improved bed morphology on smaller length scales [52,53]. It was shown that when analysis of small molecules was performed at (for isocratic) or somewhat above (for gradients) the optimum flow, the eddy diffusion term contributed to the majority of the band broadening. The greatest contribution to eddy dispersion is from wall and/or border layer trans-

column effects. As the bed aspect ratio (the ratio of the column to particle diameter) increases, the column performance tends towards the infinite diameter column [54].

Due to their complex preparation procedures, the reproducibility of the manufacturing SPP columns might be questioned especially with regard to the reproducibility of the eddy diffusion and its effect on the total efficiency. However, studies have indicated that for several different commercially available products, reproducibility was good. Differences in column efficiency between columns were attributed merely to the random nature of the packing process and the resulting lack of homogeneity of the column bed [55]. For 2.6 µm SPPs in 2.1 x 100 mm column formats, the relative standard deviation (RSD) of the eddy diffusion contribution was less than 10% [56]. Similar results were found for SPPs with larger pore size designed for the separation of peptides [57]. The question of the monodispersivity of the particles and its influence on column performance has long been a subject of debate amongst researchers in this area. SPPs have a very narrow particle size distribution (PSD) and a considerably lower proportion of "fine" particulates left behind from the manufacturing process compared to preparation methods for totally porous particles. It seems possible that this could indeed influence the A term in some way. Horvath et al developed a theoretical framework for calculation of the effect of PSD on efficiency, demonstrating that a wider PSD was detrimental to performance [58]. A constant packing density was assumed in that work. The PSD mostly affects intraparticle diffusion; therefore, its effect is negligible in the case of small molecules. However, its influence increases as the size of solute molecules increases, because intraparticle diffusion becomes increasingly significant. Thus SPPs with a narrow PSD should be advantageous. It was shown that bimodal phases (which consist of deliberate mixtures of particles of different size) had no advantage over monodispersed particles - manufacturers are known to sometimes add small quantities of larger particles to a UHPLC packing in order to reduce the operating pressure [3].

Recently, fully porous particles with narrow PSD have become available, and their properties have been briefly reviewed [59]. By comparison of their performance with SPP columns, some elucidation of the factors leading to high efficiency might be possible. Guiochon and co-workers studied 2.1 and 3.0 mm id columns packed with 1.9 µm porous particles [60,61]. These materials had particle size distribution of 10 % RSD, intermediate between that of classical porous particles (~20 %)

and SPPs (~5 %). The Titan material exhibited low reduced plate height (h) values of 1.7-1.9, which is low for totally porous particle columns although does not reach the even lower values exhibited by some SPP columns. The authors pointed out that it was tempting to therefore assume a correlation between narrow PSD and high efficiency. However, they attributed the performance instead to the unusually small diffusivity of analytes across the porous particles (about a factor of 3 lower than for typical porous C18 particles), leading to the lowest h values being obtained at low reduced velocities (around 5 instead of 10). Therefore the performance is attributable to a reduction in the B term. An undesirable consequence however, is a larger C term, which leads to poorer performance at high mobile phase velocity. Increasing the pore size of the material from 80-120 Å produced improvements in the efficiency of the columns when applied to the analysis of peptides and small proteins [62,63].

2.3 Suitability of core-shell silica particles for separation of large molecules

The effect of shell thickness has been studied particularly with regard to the separation of large molecules. In general, studies have suggested a compromise between a short diffusion path (which leads to high efficiency) and adequate retention / mass load tolerance. SPPs with thinner porous shells show marginal improvement in column efficiency for small molecules, but improved performance for larger molecules which have much smaller diffusion coefficients and thus give higher mass transfer contributions to band broadening [64]. This particular study indicated the best compromise for large molecules was a 0.2 µm shell thickness. This value represents a rather thinner shell than used in the original materials, which reflect the slower diffusion of large molecules and its consequent negative influence on mass transfer [64].

For the separation of larger molecules such as peptides and proteins [65], the pore size must be large enough to accommodate the solutes. SPPs with larger pores are now commercially available [66]. Wagner et al. estimated that solutes with MW >5000 could show restricted diffusion and poor performance in 2.7 µm shell packings with a conventional pore size (e.g. 90 Å) while the SPPs with pore size 160 Å were limited to solutes of MW <15000 [67]. SPPs with pore size of 400 Å were suitable for separation of proteins with MW 400 kDa or higher [67]. Further work explored in more detail the added effect of shell thickness on performance using particles of diameter 3.4 µm with

different pore sizes in the range of 90-400 Å and shell thickness 0.15 - 0.5 µm for the separation of proteins [68]. It has confirmed that large molecules (even up to 500 kDa) have unrestricted access to the bonded phase of the material.

2.4 Important characteristics of core-shell silica particles

Langsi and co-workers studied the preparation of SPPs of overall diameter 1.5, 1.7 and 1.9 µm coated with a thin 50 nm shell [69]. These particles were manufactured via micelle templating using short chain alkylammonium halide surfactants followed by hydrolysis/condensation of a pure silica precursor. The materials were comprehensively characterised using scanning and transmission electron microscopy (SEM, TEM), dynamic light scattering, gas adsorption, elemental and thermogravimetric analysis, DRIFTS, and inverse size exclusion chromatography. The minimum reduced plate heights were around 4; this value was larger than that for a 1.7 µm packing with a thicker 150 nm shell [69].

While much work has centred on the question of the pore size of SPPs, it seems that the pore size distribution of these materials is considerably wider than that of totally porous particles, as demonstrated using inverse size exclusion chromatography [70]. This observation is perhaps unsurprising in view of the completely different methods used to produce these different kinds of particles, and must arise from the method of shell synthesis.

The loading capacity of SPPs is sometimes questioned in that only a proportion of the particle is porous and therefore accessible to solutes. The capacity of SPPs was found to be not greatly reduced compared with totally porous particles yielding similar efficiency [71,72]. The original small particle SPP columns had a value of ρ of 0.63 [73], indicating that about 75 % of the particle is porous compared with a totally porous particle of the same particle diameter. Other popular commercial varieties of SPP column have a solid core of diameter 1.9 µm and an overall diameter 2.6 µm, implying a value of $\rho = 0.73$ and around 61 % of porous volume. The ρ value of close to 1 for the original pellicular particles indicates a very much smaller porous volume. In view of these values, there is no particular reason to suspect that for these small "thick" SPPs the loading capacity should be drastically compromised, although clearly their capacity might be expected to be somewhat

reduced. However, this favourable assessment may not be true for more recently developed SPPs with different shell thickness. In addition, other factors may be involved in loading capacity; it is possible that solutes do not penetrate completely to the centre of totally porous particles, indicating that some of their potential capacity could be redundant. Furthermore, the specific surface area of the porous shell (in m^2/g of shell material) may be different from that in totally porous particles [3].

3. Fabrication of core-shell particles for separations

Core-shell particles are usually fabricated by a two-step or multiple step method; the core is usually formed first and the shell is generated on the core subsequently. The shell may be formed from aggregated nanoparticles, nanofibers, or nanorods. The interstitial spaces between the nanostructures generate the porosity, which is the platform for HPLC separation. Different methods have been used to prepare such core-shell particles [74]. Among them, some methods are frequently used to produce core-shell microspheres for HPLC.

3.1 Silica core-shell particles by layer-by-layer deposition

The layer-by-layer (LbL) method is mostly used for the preparation of core-shell packing materials, particularly for those commercially available ones. The fabrication by the LbL technique starts with a non-porous solid sphere (as the core), followed by alternate deposition of the charged polymer and oppositely charged nanospheres (Figure 2). This method utilizes the electrostatic interaction and other forces such as hydrogen bonding, covalent bonding, van der Waals interaction between the positively charged and negatively charged molecules/particles [4]. For commercial core-shell particles used in chromatography, the whole particle is usually made of the same material silica.

This concept was first introduced 50 years ago using glass beads or polymer particles as the cores coated with the shell of a few micrometers thick [29]. These particles were intended for the analysis of macromolecules and were expected to provide high loading capacity and a low solid-liquid mass transfer [29, 75]. The development of core-shell particles was slow over the years and it took time before reliable procedures had been developed. This led to the introduction of second generation of core-shell particles appearing in 1992 [31]. Poroshell columns were introduced later. The core was

made from totally porous particles which were densified at 1050 ºC. The core-shell particles were made by spray-drying a suspension of silica core and silica sol. A subsequent elutriation was required to produce the desired size and distribution [31]. The particles met with limited success in spite of improved separation of proteins compared fully porous particles [76, 77]. The limitations were addressed with the introduction of modern 2.7 µm Halo core-shell particles (third generation) developed by Kirland et al. [28, 73]. The technology gained considerable attention with the achievement of a minimum reduced plate height of 1.5 for small molecules separations [28,78]. With this achievement, smaller particles such as Phenomenex 2.6µm and then Kinetex 1.7 µm were later introduced, exhibiting exceptional performance [79,80].

These core-shell particles show very narrow PSD of $d90/d10 \leq 1.25$. This is achieved by the use of monodisperse non-porous solid microsphere cores, which are prepared via a Stöber method [81]. The Stöber method can be modified to obtain sub-2 µm and sub-3 µm non-porous silica particle to be used as core for core-shell particles [82]. The second step is building a stable and homogenously thick layer of porous silica shell around the non-porous core [83,84]. A large percentage of core-shell silica particles used for chromatography are prepared via the LbL approach. Several successive steps are needed to build a homogenously thick layer of sol shell [85]. The thickness of the shell and the size of the silica sol can be tuned to suit different types of chromatographic applications as it strongly affects the mass transfer for different analytes [32, 33]. But it is also important to achieve a sufficient shell thickness to improve retention factor and sample capacity. The formation of the shell is driven by electrostatic interaction of silica sol onto the core, which is firstly coated with cationic polymers [33].

Manufacturing core-shell silica particles with the LbL method is time-consuming, resulting in low productivity as it requires extensive dilutions of reagents below 0.5 w/w% to reduce agglomeration and repetitive washes of excess reagents in order to main the narrow particle distribution [33,86]. Several studies were carried out to optimise the method by examining various factors such as the effect of polymer chain, pH and temperature on improving the deposition of sol onto the surface [33, 86]. Thicker shell can be obtained depending on the molecular weight of the polymer as it can absorb several layers of sol particles at one time as the charge density of the

electrolyte is increased allowing more extended conformation. Thus, rather than forming monolayer deposition per coating cycle, altering the polymer chain results in change in the polymer behaviour in solution, which allows longer segments to protrude into the suspension and hence maximize the amount of nanoparticles adsorbed. This approach has considerably improved the productivity of the synthesis and higher level of porosity of the silica shell. The behaviour of the polymer in solution is influenced by suspension ionic strength and pH.

Colloidal silica sol is regarded as an important part in controlling shell roughness and physical properties (Figure 3). A number of sol layers are needed to produce a sufficient surface area, which is commonly around 50-100 m^2/g. This is generated from the inter-particle voids of the sol particles during shell growth, with the pore size usually in the range of 8-10 nm (the size of silica sol control the finial pore size) [33, 86]. Thus, to obtain the desired surface area for separation of small molecules it is recommended that porous layer thickness should be between 24-41% of the total particle diameter [32, 64, 87]. For the separation of large molecules, the shell thickness less than 13% was recommended by Horvath and co-workers for increased efficiency and mass transfer [87]. It should be noted that these values refer to the whole mass of the particles that includes non-porous cores. Taking into account the volume fraction of the porous shell only, specific surface area would be equivalent to fully porous particles. Synthesis parameters such as drying and sintering temperatures can lead to shell deformity and loss in surface area and pore volume [88]. Drying a wet-shell by lyophilisation can better preserve the structure of the porous shell and contribute to a higher surface area. Sintering temperature was also investigated ranging from 550 – 1050 ^oC to improve the stability of shell without significant shrinkage or melting of the shell [89]. The stability of the shell increased with increasing sintering temperature, accompanied by a decrease in shell thickness. With temperatures above 1000 ^oC, shell melting and deformity occurred with a significant loss in surface area < 40 m^2/g. The data obtained indicated an approximately 5% drop in surface area with 100 ^oC increase in sintering temperature [89]. Nonetheless, it was determined that 950 ^oC gave the optimum mechanical strength without substantial loss in specific surface area, pore volume and pore size.

3.2 One-pot synthesis of spheres-on-sphere silica particles

The current core-shell particles preparation method does have its limitation, e.g., poor quality control in multiple-step synthesis, time consuming, and a large amount of waste, particularly when constructing the particles with thick shell. Recently, a unique type of core–shell particle with large macropores was reported by Ahmed et al. [90]. The particles were prepared by one-pot synthesis at room temperature from a single precursor 3-mercaptopropyltrimethoxysilane (MPTMS). The obtained particles showed a unique spheres-on-sphere (SOS) structure (Figure 4). Monodisperse SOS particles can be prepared from the one-pot synthesis, without the need to elutriate and classify the particles as normally employed by the commercial manufacturing process [91]. Both the core and the shell are formed within a single vessel in the synthesis. A time study was carried out to track the formation of particles throughout the reaction using SEM. The data suggested two stage nucleation process occurred at different intervals which was induced by use of appropriate silica source and reagents. The first stage was the formation of the core microsphere. Within a short time after the core formation, the second stage of nucleation led to shell formation which was made up of nanoparticles ≤100nm in diameter on the surface of these microspheres [90]. The shell thickness and porosity can be controlled by fine-tuning preparation conditions such as pH and solvent ratios. The interstices from the assembly of the nanospheres on solid microspheres generate the interconnected macroporosity which serves as the foundation for HPLC separation. These SOS particles are highly efficient as stationary phase for separation of small molecules and particularly peptides and large biomolecules [91-93]. This method is an alternative to the mainstream approach of producing solid core-shell silica particles which use the time-consuming LbL approach and could potentially offer easier quality control and lower manufacturing cost.

For the SOS particles, both the nanospheres and microspheres are nearly free of mesopores, although a type I nitrogen isotherm was generated implying the presence of microporosity less than 1 nm in size after low temperature sintering [90]. Surfactants have minimal contribution in microposity formation and the microporosity is believed to be mainly from the decomposition of MPTMS during sintering. Further studies were carried out to co-condense other silanes, such as the commonly used tetraethyl orthosilicate (TEOS) and octadecyltrimethoxysilane (OTMS), into the SOS framework, promoting the formation of surfactant-templated mesopores [94]. The resulting particles exhibited

higher surface area reaching up to 680 m^2/g and bimodal micropore (<1nm) and mesopore (2-3nm) distribution (Figure 5). However, these mesopores are too small for efficient HPLC separation which usually requires minimum pore size of 7 nm. The mesopores in these SOS particles could be further expanded to about 8 nm by a solvothermal swelling approach with pore expanding reagents N,N-dimethyldecylamine and 1,3,5-trimethylbenzene [94]. The type of pores within the particles is critically important for certain applications and entrapment of other materials. There is great interest in utilisation of SOS particle macroporosity rather than mesopores as it can offer higher mass transfer for biomolecules [91-93]. The surface structure of the assembled nanoparticles of SOS particles also allows the growth of interesting materials such as metal-organic framework (MOF) nanoparticles [95,96].

The one-pot synthesis strategy has also been adopted by other researchers. For example, the rods-on-sphere silica particles were prepared from solutions containing cetyltrimethylammonium chloride (CTAC), Na_2SiO_3, and formamide in water with magnetic stirring at 35 ^oC. The prepared particles were calcined in air at 550 ^oC to remove the surfactant templates [97]. Like the SOS particles, the column packed with the rods-on-sphere particles showed good HPLC separation with low backpressure [97].

3.3 Core-shell particles with silica-core and MOF-shell.

MOFs are crystalline microporous materials which are formed by metal ions or clusters linked with organic ligands. MOFs are usually synthesized via one-pot reactions between ligands and metal ions in solutions between room temperature and 250 ^oC. This results in crystalline pore structures with uniform pore size and pore shape with better interconnectivity. Its porosity can be systematically adjusted due to the enormous variety of metal ions and organic ligands available [98-100]. MOFs have been widely investigated for applications such as gas storage, drug delivery, catalysis, medical sensors and separation [98 and the references in the issue]. For chromatographic separation, having well-defined interconnected pores with minimal dead volume can offer better efficiency [101]. MOF materials have been used as stationary phase for HPLC [14]. This includes direct packing of MOF particles [101-104] and incorporation of MOF into porous silica [105]. The issues with MOFs as

stationary phase are: (i) The micropores are not suitable for separation of smaller molecules in liquid phase; (ii) MOF particles are not spherical and are difficult to pack into column, leading to low efficiency; (iii) Poor mechanical stability of MOF particles leads to crush of the particles under operational pressure; (iv) Most of the MOFs are not stable in the presence of water, particularly under acidic and basic conditions.

The core-shell particles with silica core and MOF shell have emerged as promising stationary phase, combining the advantages of MOFs and easy packing/mechanical stability of silica spheres. However, only water-stable MOFs are usually employed to produce silica-MOF core-shell particles or other MOFs if the mobile phase contains no water. The MOFs mostly investigated in this regard include ZIF-8 [96, 106-109], UiO-66 [110-112], and HKUST-1 [95]. This is likely because these MOFs can be readily formed on the silica surface with reasonable coverage and not too harsh conditions. MIL-100 (Fe) and some other MIL-typed MOFs have good stability and are used as stationary phase directly [104, 113-115]. However, the reports on core-shell particles with MIL-100 (Fe) are not as much as the other MOFs, which may be attributed to the difficulty in forming MIL-100 (Fe) coating on silica.

A number of strategies have been attempted to prepare the microspheres with MOF coating. This involves either a single or multiple depositions using metal source and organic ligand to achieve a uniform growth of MOF crystals. This can be achieved by simple synthesis of MOFs in the presence of microspheres or pre-modifying the surface of the microspheres with the functional group that can form linkage with the organic ligand. Figure 6 shows the scheme of the latter method in synthesizing silica spheres with UiO-66 coating [111]. This is expected to promote the formation of stable coatings.

Han et al. reported the synthesis of MIL-68(Al) onto silica at 403 K by introducing both $AlCl_3.6H_2O$ and terephthalic acid [116]. This resulted in MIL-68(Al) coated onto silica but particle fusion and random growth of MOF crystals was observed. A similar attempt was carried out by hydrothermal synthesis of UiO-66 (Zr) onto silica particles at 120 ^oC [110]. The particles were collected by centrifugation with successive washes, but silica@UiO-66 (Zr) microsphere could not be isolated from the UiO-66 (Zr) nanocrystals. The resulting composite material was applied in LC

separation of small aromatic molecule demonstrating the unique selectivity of MOF. A control growth of these crystals on the surface seems to be an issue with further reported studies by Tanaka et al. who prepared chiral (R)-CuMOF-1 on silica [117]. Nonetheless, this does not seem to diminish the column performance but does affect mass transfer kinetic resulting in broader peaks.

In recent years, methods have been developed to simplify the separation of core-shell MOF particles from liquid suspension by combining magnetic particles with MOFs. For example, Fe_3O_4 nanoparticles was used as a scaffold for the growth of HKUST-1 [118] and MIL-100(Fe) [119] shell. Step-by-step assembly growth strategy using ethanolic solutions of metal source and ligand was used and desired shell thickness was achieved by repetitive deposition process. Qin et al. used the composite magnetic silica particles as scaffold for the synthesis of UiO-67 (Zr) coating [120]. The step-by-step coating was again utilised to achieve the desired coating. The composite core-shell particles were packed into HPLC column for the separation of polar aromatic molecules. The selectivity of the MOF molecules was illustrated, but peak shape was poor [120].

The surface functionalities of silica may provide strong interaction with metal ions, hence to drive more controlled growth of MOF crystals and better isolation from liquid suspension. Ahmed et al. showed the surface functionality could be a critical factor in improving interaction, adherence and coverage on the microspheres [95, 96]. The SOS particles were functionalized with –COOH groups and $–NH_2$ groups which promoted the formation of HKUST-1 [95] and ZIF -8 [96] nanoparticles. This methodology has also been demonstrated with different supports such as polymer [100], alumina [121] and silica [107, 108]. Sorribas et al. [108] and Naik et al. [122] manipulated the growth kinetics of ZIF-8 using a seeded growth technique followed by secondary and repetitive crystal growth cycles, achieving a sufficient thickness. Figure 7 shows the morphology of mesoporous silica spheres, seeded microspheres, and the change in thickness of ZIF-8 coatings under 1 and 2 growing cycles [108]. The seeded growth and carboxylic acid functionality are believed to be the key factors in obtaining uniform and homogenous coverage of ZIF-8 shell.

The surface morphology of the microspheres may play a significant part in crystal nucleation and growth. This has been evidenced by the use of SOS particles for the controlled growth of HKUST-1 [95] and ZIF-8 [96] MOF crystals. Surface roughness, functionality and type of solvent

were found to significantly influence the crystal morphology, attributing to interaction with metal ions and crystal growth kinetics [96].

3.4 Core-shell particles with silica-core and other inorganic shell

Although core-shell particles are mainly prepared from silica for LC, other types of shell materials have also been used in chromatographic applications. The coating shell using different materials on silica core could increase functionality, selectivity and stability. The synthesis of such materials is achieved through a one-pot method or a multiple-step process on a pre-synthesised core via different approaches such as LbL coating, polymerisation of the shell on solid core [74, 123].

Continuous flow synthesis was used to coat colloidal silica with Ag nanoparticles [124]. A solution of silver nitrate, colloidal silica, formaldehyde were mixed with ammonia solution at the T-junction of the flow system. The result showed a consistent and reproducible formation of patchy silica@Ag particle at high yields. The distribution of Ag nanoparticles was affected by the flow rate of mixing. Faster mixing led to accelerated nucleation and random growth in between the colloidal silica. Hanisch et al. reported the synthesis of Ag nanoparticles necklaces coating on silica particles without addition of reducing or templating agents [125]. It was found that ammoniacal silver complex was essential for necklace formation, at a high pH typically around pH 11. The silica surface dissolution at this pH interacted with the ammoniacal silver complex and promoted the formation of Ag nanosized necklaces [125].

Dun et. al. applied the LbL method for the deposition of ZrO_2 nanoparticles on silica core [126]. Stable and homogenous layers of ZrO2 were formed in the present of SDS by alternate adsorption steps, reaching up to 326 nm shell thickness on the silica core particles. Zirconyl chloride octahydrate was used to synthesize the ZrO_2 nanoparticles. The particle size increased from 3.5 µm to 3.8 µm, and the removal of SDS resulted in surface area ranging from 131 to 326 m^2/g with 84 Å pores depending on the number of layers [126]. SiO_2@ZrO_2 particles were used in chromatographic separation of inorganic and organic ions. Both pH and the strength of Lewis base of the mobile phase played an important role in retention behaviours. The particles exhibited excellent pH stability at pH 12 after 5000 column volumes [127]. A follow-up study was reported without the use of surfactant

571 during the synthesis [128]. Electrostatic attachment of positivity charged zirconia nanoparticles onto
572 negatively charged silica particle was achieved by adjusting the pH around 6. This also led to further
573 condensation reaction between the Si-OH and Zr-OH groups on the surface resulting in the formation
574 of Zr-O-Si linkages. Sub-2 µm silica particles were used for the formation of SiO_2@ZrO_2 particles. A
575 dense coverage of nanoparticles on the surface was formed. The reported surface area (42 m^2/g) after
576 calcination was much lower than the LbL method, with smaller pores around 32Å. However, this did
577 not reduce the chromatographic performance of the composite particles [128].

578 Ge et al. prepared silica core-shell particles with porous titania shell by alternate adsorption of
579 SDS and titania nanoparticles on 6 µm silica core particles [129]. The titania sol was prepared using
580 tetrabutyl titanate acetic acid in ethanol, which resulted in nanoparticles around 12 nm in diameter.
581 The shell thickness reached about 300 nm after 6 layers coating with surface area almost doubled
582 from 110 to 200 m^2/g and an increase in particle density [129]. The surface areas decreased
583 significantly when the treatment temperature increased to above 500 ^oC. The silica@titania particles
584 were found to offer good stability under extreme chromatographic conditions for basic compounds
585 separation [130]. In another report, liquid phase deposition was utilised for the coating of titania
586 nanoparticles onto silica surface [131]. Ammonium hexafluorotitanate ($(NH_4)_2TiF_6$) was incubated at
587 35 ^oC in boric acid/silica solution for 16 hours. The boric acid acted to stabilize the fluoro complex
588 and facilitate oxide precipitation onto the silica surface. The deposition was repeated to obtain
589 different levels of titania coating and more surface roughness with an estimated 3 nm shell thickness.
590 The material was successfully applied for the separation of Adenosine phosphate compounds due to
591 the Lewis-acid-base interaction with the phosphate groups [131].

592 Cationic polymer poly(diallyldimethylammonium chloride) (PDDA, Mw = 100 – 200 kDa)
593 was used in the LbL assembly of iron oxide nanoparticles on silica core [132]. The PDDA-coated
594 silica was added into the Fe_2O_3 nanoparticle suspension and the process was carried out under
595 sonication to reduce aggregation. No pH control was performed, similar to the silica LbL method. The
596 homogeneity of the shell coating and thickness was somewhat random. These core-shell particles
597 showed superior performance in removing methylene blue and methyl orange, attributing to the
598 electrostatic interactions between the dyes and iron oxide coating [132].

3.5 Surfactant-templated shell in core-shell particles

Sol-gel synthesis can be combined with surfactant templating to fabricate mesoporous silica materials [133]. By using amphiphilic triblock copolymers as the structure-directing agents, silica materials with well-ordered uniform pore sizes up to 300 Å, thicker wall, and greater hydrothermal stability can be formed [134, 135]. However, most of these materials are irregularly shaped porous powders, limiting their use in areas such as HPLC. Yang and others developed the methods to fabricate mesoporous spheres [136-138]. They focused on optimizing the one-pot reactions through adjustment of pH, stirring rate, adding co-solvent, using spray drying, etc., to control the particle size, particle size distribution, pore size, and pore size distribution simultaneously. However, the synthesized materials usually have either broad particle size distributions or small pore sizes.

The surfactant-templating approach has been used to synthesize SPPs. One method is similar to the LbL method, where a thin porous silica shell was grown on non-porous particles for multiple times in the presence of a surfactant. At least seven layers were required to form final particles with a surface area of 100 m^2/g [32]. Recently, Min et al. reported the preparation of dandelion like core−shell silica microspheres with hierarchical pores [139]. Although it has the potential to be used as the stationary phase in HPLC separation, an etching process was involved to create the porosity in the shell, which significantly reduced the mechanical stability of the structure. Furthermore, the pore sizes were still too small (<7 nm).

Different from bottom–up methods, such as coacervation and LbL, a top–down method called "pseudomorphic transformation" (PMT) was used to form mesoporous microspheres as stationary phase for HPLC. The process was firstly used to transform amorphous porous spheres to micelle-templated totally porous particles with the same particle morphology [140-143]. By starting the transformation process with non-porous spheres (either silica or metal oxide spheres) and controlling the reaction time, it is possible to fabricate solid-core porous-shell particles [65, 144-145]. In this process, non-porous silica particles are dispersed in an alkaline solution with the presence of surfactant. When the temperature increases, the silica on the particle surface first dissolves and the resulting silicic acid subsequently precipitates around ordered, positively charged micelles on the

627 silica particles without changing the overall particle morphology. The advantages of this process are
628 that the properties of the particles and the properties of the pore structure can be independently
629 optimized. The particle size and size distribution are determined by the starting materials and the
630 intra-particle pore structure is controlled by the PMT variables such as surfactant, swelling agent, pH,
631 and reaction time.

632 Wei et al. explored the process of PMT and developed an improved process for the SPPs with
633 a unique shell pore structure [146]. The thin porous shell contained elongated pore channels normal
634 to the particle surface (Figure 8). The impact of the novel pore structure on the performance of these
635 particles was evaluated by measuring van Deemter curves and constructing kinetic plots. Reduced
636 plate heights as low as 1.0 was achieved on conventional LC instruments, suggesting greater
637 efficiency of such particles compared to conventional totally porous and superficially porous particles.
638 It is interesting to note that these preparations provide radially ordered pore systems within a single
639 shell whilst the pore structure in the common SPPs is rather random (Figure 8).

640 Qu et al. produced monodisperse silica spheres with solid core and fibrous shell synthesized
641 using a bi-phase reaction using cyclohexane to slow down the rate of silane hydrolysis and
642 condensation [147]. Both the thickness and the pore size of the fibrous shell could be finely tuned by
643 changing the stirring rate during synthesis. When the stirring rate was adjusted from 0 to 800 rpm, the
644 thickness of the shell could be tuned from 13 to 67 nm and the pore size from 5 to 16 nm. By
645 continuously adjusting the stirring rate, fibrous shells with hierarchical pore structure ranged from 10
646 to 28 nm and thickness up to 200 nm could be obtained in one pot (Figure 9). The particles were
647 functionalised with a C18 bonding and packed in to a column showing separation performance as high
648 as 2.25 × 105 plates m^{-1} for naphthalene and back pressure as low as 5.8 MPa. The columns were not
649 evaluated for reduced parameters or kinetic performance.

650

651 3.6. Core-shell particles with non-silica core

652

653 Materials other than silica have also been used as the core to construct core-shell particles. Deng et al.
654 developed a method to coat polystyrene particles with an organo-silica shell, although these materials

were intended for applications outside HPLC such as in medicine or catalysis, where core-shell materials have also attracted considerable attention [148]. Carbon particles, derived from oxidised and carbonised polystyrene-divinylbenzene particles, were used as the core to fabricate core-shell particles [149]. The LbL deposition of amine containing polymer and nanodiamonds onto these carbon cores was performed. The particles showed good mechanical, thermal and pH stability, and good separations of essential oils [149]. A commercially available variant of this material with pore size 180 Å was evaluated for the separation of proteins [150]. As the stationary phase contained some protonated amine groups within the structure, the retention mechanism was expected to be a mixture of hydrophobic interaction and anion exchange, although it appeared the former was the dominant mechanism [150]. The robustness of the material allowed the effect of temperature variation over the range of 30-80 ºC to be studied even with the use of larger amounts of trifluoroacetic acid (TFA) in the mobile phase than usual (0.2-0.5 % compared with the usual 0.05-0.1 %), which was necessary to improve efficiency. Changing the temperature over this range hardly affected the peak capacity of the gradient separations, but did give interesting selectivity changes.

Wide pore materials (120, 180 and 250 Å) with carbon core and nanodiamond-polymer shell were evaluated for the separation of proteins [151]. The largest pore size gave the best performance for large molecules. The particles were shown to be smooth (Figure 10) and therefore do not benefit from the proposed packing advantages of rough silica SPPs, a property which may improve the radial homogeneity of the packing and reduce eddy dispersion. However, it was considered that eddy diffusion may not be a significant contributor to band broadening for large molecules. The smooth surface morphology of these particles in this case could be an advantage for separation of large molecules [151].

4. Applications of silica Core-Shell particles

The need for stationary phases with improved kinetic performance has increased in demand. Among them, increasing sample analysis throughput has become a target in many applications. Core-shell silica columns offer faster analysis without compromising separation efficiency. The short path diffusion in the porous shell improves mass transfer and minimises peak broadening, which in turn

683 increases resolution and efficiency for complex mixtures. This was demonstrated by comparing
684 Kinetex and Zorbax columns for the separation of three alkylphenones [41]. There was a clear
685 improvement in peak width and 30% in plate height. With increasing flow rate this difference in plate
686 height was more than 75%. Fekete et al. evaluated Ascentis Express C18 (2.7 µm) for the separation
687 of a mixture of steroids against sub-2 µm particles stationary phase [152]. The separation was
688 completed in less than 2 min with lower backpressure due to its higher column permeability. Column
689 permeability is significantly influenced by core-shell particle size. Bobály et al. showed that Kinetex
690 1.3 µm particles suffered from low permeability compared to Kinetex 1.7 µm particles under optimal
691 linear velocity [39]. The best comparison to Kinetex 1.7 µm particles was Cortecs 1.6 µm particles
692 with similar permeability, but the Cortecs 1.6 µm particles possessed excellent kinetic performance
693 with reduced plate height (H_{min}) of 2.66 µm (Kinetex 1.7 µm H_{min} of 3.17 µm).
694
695 ## 4.1 Applications in food and drinks
696 The core-shell technology is still evolving and the number of commercially available core-shell
697 particles is rapidly growing to cover a wider range of applications. Core-shell particles have been used
698 in determining several classes of antioxidant and natural contaminants in food or drinks.
699 Comprehensive characterisation of a variety of complex flavones and phenolic acids matrices in wine
700 were successful explored and resolved within 30 min at elevated temperature 60 °C [153]. A study
701 was carried out to compare fully porous sub-2 µm BEH particles to Halo 2.7 µm C18 particles for the
702 separation of 10 phenolic compounds in canned artichoke [154]. The obtained results for core-shell
703 column in term of efficiency, speed, resolution and pressure, demonstrated the potential of SPPs over
704 totally porous particles (Figure 11).
705 Manns and Mansfield explored complex antioxidant compounds from red and white juices
706 and wines produced from Vitis vinifera and hybrid cultivars [155]. The resolving power of Kinetex
707 2.6 µm C18 column successfully resolved the first two classes of antioxidant nonanthocyanin and
708 phologucinolysis. Anthocyanins antioxidant was better separated using Kinetex 2.6 µm PFP particles,
709 which offered different selectivity due to π-π and H-F interaction. The unique selectivity of PFP phase
710 (Kinetex 2.6 µm) was also demonstrated in the separation of positional isomers chlorogenic acids and

711 sesquiterpene lactones in chicory root without additional purification step [156,157]. Simonovska et
712 al. developed a rapid separation method to determine lutein in spinach pigments extract (xanthophylls,
713 all-trans-violaxanthin, all-trans-zeaxanthin and all-tran-lutein, chlorophylls and all-trans-β-carotene)
714 on a Kinetex 2.6 µm C18 column in less than 13 min [158]. The column did show some limitation,
715 with poor resolution observed for targeted compounds lutein and structural isomer zeaxanthin.
716 Fontana et al. developed an approach for rapid determination of multiclass polyphenols in grape
717 pomaces using Kinetex 2.6 µm C18 column under 12 min [159]. Considering the complexity of the
718 grape pomaces extract, which includes high quantity of target analytes of different chemical nature,
719 classes and concentrations, high sensitivity and rapid analysis were demonstrated for the core-shell
720 particles column. The method was suitable for quantification and determination of 20 polyphenols
721 from different grape varieties.

722 Natural contaminants mycotoxins are low molecular compounds and can be very toxic at low
723 concentrations closer to 1 µg/kg. In routine control of red wines, quantification of Ochratoxin A levels
724 below 2µg/L levels is mandatory [160]. The low operation pressure of core-shell column, e.g.,
725 Kinetex 2.6 µm C18 particles, facilitated the use of conventional HPLC for these analysis, detecting
726 Ochratoxin A at the concentrations as low as 0.0028 µg/L with high efficiency.

727 Core-shell columns were also applied into agricultural products such as determination of five
728 Alternaria toxins and citrinin in tomato juice [161], microstins in drinking water [162], and
729 deoxynivalenol in cereal products [163]. The analysis of bisphenol compounds contamination caused
730 by the coating inside the soft-drink (such as cola, orange soda, energy drinks, etc) containers was
731 reported by Gallart-Ayala et al. [164]. Supelco Ascentis Express 2.7 µm C18 column was used to
732 carry out a gradient elution of bisphenol compounds coupled to MS. Fast elution was achieved in less
733 than 3 min with higher resolution of five bisphenolic compounds in beverages [164].

734

735 ## 4.2 Applications in separation of large biomolecules

736 Macromolecules are highly complex and chromatographic analysis is still challenging. The
737 importance of analysing biomacromolecules is increasing due to its substantial development in life
738 science and biomedical applications. Wide pores in the porous shell are required to allow free

739 diffusion of such molecules. It has been claimed that the pore should be at least four to ten times the
740 hydrodynamic diameter of the biomolecule, which would allow less restriction on mass transfer and
741 better separation efficiency [165]. Close et al. investigated the influence of pore size 80, 150 and
742 400Å on nucleic acids chromatographic resolution [166]. Triethyammonium acetate and
743 tetrabutylammonium bromide were used as the ion pair reagent in conjunction with Accucore 2.6 µm
744 C18 column. The results for the separation of oligonucleotides, a 2'-deoxythymine ladder (dT 19 –
745 24) and large dsDNA/RNA fragments showed that the increase in pore size resulted in increased
746 resolution, rapid and high throughput analysis. The effect of having wider pore size for the diffusion
747 of large proteins up to at least 400 kDa such as ribonuclease A and insulin was investigated by
748 Wagner et al. [67]. In this study, Halo 2.7 µm C18 columns with pore size of 90, 160 and 400 Å were
749 used and the results showed significant improvement in peak shape, resolution and selectivity (Figure
750 12).

751 Due to significant advances in biopharma sector, there are still needs for larger pores to
752 sustain biomolecules greater than 350 kDa. The preparation of core-shell prototype particles with pore
753 size of 1000 Å was recently reported and the packed column was evaluated against the columns
754 packed with core-shell particles of smaller pores around 400 Å [167]. The results suggested that both
755 400 and 1000 Å pores were well suited for biomolecules up to 350 kDa. The advantage of large pores
756 showed better efficiency for the separation of base pair DNA (~660 kDa) with minimal peak
757 broadening.

758

759 ## 4.3 Core-shell particle columns for HILIC

760 Some hydrophilic molecules are poorly retained by conventional reversed stationary phase. As an
761 alternative, HILIC has been used to offer higher efficiency for the separation of small polar
762 compounds using the solvents with lower viscosity [168]. Heaton et al. demonstrated that the kinetic
763 performance of bare silica core-shell (Cortecs) was superior compared with hybrid and conventional
764 bare silica phases and exhibited higher diffusion of hydrophilic base cytosine [169]. In another study,
765 the Halo silica 2.7 µm columns were used in HILIC mode to separate a mixture of acidic and basic
766 compounds, with acetonitrile (85%): ammonium formate as the mobile phase. When three columns

were coupled together with a length of 45 cm, over 100,000 theoretic plates were achieved, with the total backpressures generated only 280 bar [170]. Kirkland et al. used the modified Halo silica (2.7 µm, pore size 90 Å) with ligands for HILIC to provide efficient separation of compounds that are difficult to separate by reversed-phase HPLC [171]. This modified column with a highly polar poly-hydroxylic phase, Halo Penta-HILIC (100 mm x 2.1 mm), could efficiently separate a mixture of drugs of abuse and selected metabolites, using a mobile phase of acetonitrile (95%)/5 mM ammonium formate aqueous solution (5%). This column can be used for separation of highly polar solutes such as sugars, peptides, and nucleic acids, as demonstrated by the separation of 15 compounds containing nucleosides and bases within 8 minutes with a gradient mobile phase [171].

4.4 Core-shell particle columns for SEC

Separation based on the size difference of molecules has been developed as an effective technique known as SEC. Given the complexity of protein and peptides-based parenteral therapies, a set of complementary separation techniques, including SEC, are required to monitor the critical quality [172, 173]. SEC are predominantly favoured for routine and validated analysis to monitor protein aggregation. Totally porous silica particles have been employed in SEC separation, provided that a significant reduction in silanol activity is achieved. In recent years, SPPs have been used in SEC separation. Pirok et al. investigated the feasibility of using core-shell particles in SEC [13]. The results showed good resolution and performance, depending on pore size of the core-shell particles. The core-shell particles with large pores of 1000 Å were used for the separation of polystyrene standard (17.5 kDa to 1.8 mDa) [167].

The total pore volume is reduced for the core-shell particles, and hence a lower loading capacity, when compared to the standard SEC porous particles. But this is usually compensated for by an increase in efficiency. Schure and Moran compared the SEC performance between fully porous SEC particles and core-shell particles with different pore sizes of 160 and 1000 Å (Figure 13) [174]. Having large pore volume is thermodynamically advantageous for better separation, but the porous shell faster diffusional kinetic compensate for the loss of pore volume and thermodynamic limitations.

Thus, diffusion length is much shorter, offering narrower peaks which tend to preserve the peak capacity of the separation and higher efficiency.

5. Applications of SOS particles

The large interstitial space between the nanospheres of the SOS shell provide high porosity and good performance in HPLC separation. The studies published have covered a range of HPLC applications from small to large molecules [91-94] and polycyclic aromatic hydrocarbons [92, 95-96]. The focus has been on separation of large molecules, because of the large pores and high surface area. Nevertheless, modifications of SOS particles offer the option of tuning the particles properties to optimise the separation of small molecules by creating mesopores across the shell [94].

The reduced intra-particle mass resistance allows the use of high flow rates, which particularly helps the fast separation of large molecules [93]. The good performance of C4 bonded SOS particles was shown in gradient mode analysing large molecules such as insulin, myoglogin, BSA, mbA and reduced ADC (antibody drug conjugate) in comparison to commercial fully porous and core-shell materials usually used for proteins separation such as Aeris C18, Halo Protein C4 and BEH300 C18 (Figure 14) [93]. The findings highlighted the SOS capabilities in fast separation showing lower retention at high flow rate (0.4 mL/min) compared to the other materials, with good peak shapes and high peak capacities [93]. In the same study, the efficiency of SOS columns was examined by obtaining h-v plots with butylparaben, decapeptide and glucagon in isocratic mode, resulting in minimum reduced plate height h_{min} = 2.6, 3.3 and 3.3 respectively [93]. The analysis resulted in the average column permeability at different batches as $K_v = 1.09 \times 10^{-10}$ cm^2, and the lowest separation impedance as $E_{min} \sim 48000$, categorising the SOS particle at the performance level of the best state-of-the art commercial columns [93].

The large pores of SOS particles and the lack of mesoporosity limit their application for small molecules separation. The separation of small molecules was explored through modifying SOS particles using CTAB templates [94]. The modified synthesis process resulted in 6.9 nm mesopores on the particles shell that allowed improved column efficiency (up to 68000 plates/m), but it also resulted in higher retention time due to the increased surface area [94]. The separation of toluene,

nitrobenzene and nitroaniline isomers in normal phase HPLC resulted in 39 bar backpressure and 6 min retention time, while a different normal phase mixture using toluene, 2,4-di-tert-butylphenol, o-nitroaniline and cinnamyl alcohol showed separation within 5 min [94]. Furthermore, the modified SOS particles did not have significant loss of performance in the separation of large molecules because the separation occurred in the shell interstitial space. This was demonstrated by the separation of ribonuclease A, cytochrome c, lysozyme, trypsin and BSA under reverse phase mode [94]. The separation completed in 2.5 minutes showing comparable column efficiency to the un-modified SOS particles based on similar peak capacities (17 and 19 respectively). The addition of carbonic anhydrase to the mixture expanded the analysis to 3 minutes showing a backpressure of 355 bar [94].

The performance of SOS particles in separating compounds with a wide range of molecule sizes has been further demonstrated. The normal phase HPLC analysis of toluene and nitroaniline isomers using 6.5 µm calcined SOS particles showed fast separation in 2 min, a very low back pressure of 18 bar, and a higher permeability (K_v =2.79×10^{-13} cm^2), compared to the commercial Hypersil silica columns (K_v =1.24×10^{-13} cm^2) [92]. A modification of the SOS with diol groups improved the efficiency further to 65,380 plates/metre and reduced the backpressure to 14 bar. The HILIC separation for sugars mixtures for the same column showed column efficiency of 43,900 plates/m and a backpressure of 17 bar. The separation of a large proteins mixture containing ribonuclease A, cytochrome C, lysozyme, tryspin, and BSA was achieved using the C8-modified SOS particles [92]. The results of this reverse phase testing under gradient mode showed fast separation within 2.5 min, a backpressure of 222 bar, and efficiency of 91,705 plates/m [92].

The applications on separation of small molecules was also reported by Hayes et al. [91] using C4 bonded 2.9 µm SOS particle columns in order to examine a selection of peptides comprised of 5 small molecules and a selection of proteins. The separation data for the small molecules revealed fast separation within 3 min and 259 bar backpressure. The good performance of the column was also highlighted by reverse phase HPLC analysis for the proteins mixture showing retention time within 6 min and a maximum backpressure of 400 bar. However, the use of CTAC instead of CTAB for the preparation of the SOS particles produced smaller pores (< 100 Å) that limited the efficient separation

of the larger proteins such as BSA, thyroglobulin and myoglobin resulting in peak tailing and poor defined peaks [91].

In order to further explore the separation of small molecules, other modifications of SOS particles were performed, for example, by coating with MOF nanocrystals [95]. Two types of MOFs, HKUST-1 [95] and ZIF-8 [96], were formed on SOS particles. The columns packed with SOS@HKUST-1 particles showed the separation of xylene isomers although with relatively poor resolution and low efficiency. The original SOS column itself could not separate the xylene isomers [95]. For the SOS@ZIF-8 particles, the packed column exhibited fast separation of aromatic compounds with column efficiency up to 19,000 plates/m [96]. The good chromatographic analysis obtained by these two materials highlights the potential and possibilities for further development of the SOS particles to tune their composition for more efficient separations.

6. Applications of core-shell particles with non-silica shell

Although not many, there are applications of core-shell particles with non-silica shell for HPLC. The limited chemical stability of silica in the pH range of 2-8 is the drive for the use of different metal oxides forming the shell around the silica solid cores. Such metal oxides include titania [129, 175] and zirconium [126-128] nanoparticles coated on silica microspheres. The titania-coated silica particles showed a strong ability to separate adenosine phosphate compounds and good column reproducibility [175]. The column efficiency was reported to be up to 30000 plates/m when separating basic compounds using 35% acetonitrile as the mobile phase [175]. This study also reported low permeability of $K_v = 1.56\times10^{-14}$ m^2 and good column stability under extreme pH condition (pH 7-12) [175]. In another study, the octadecyl-bonded TiO_2/SiO_2 showed good stability at high pH (pH 9-10) using phosphate buffer [129]. In addition, aromatic isomeric compounds were well separated under normal phase conditions using n-hexane as the mobile phase (0.7 mL/min) and also the separation of nitroanilline using hexane-ethanol (70/30 at 1 mL/min) [129]. However, the column efficiency (~4000 plates/m) was relatively low compared to those of the SOS particles [129]. Nevertheless, the separation of 15 basic compounds under the reverse phase showed high symmetrical peaks and good separation of pyridine and aniline derivatives [129].

Zirconia-coated silica particles produce a similar performance in HPLC applications, demonstrating the high stability over a wide pH range. One study using such particles showed the separation of hydroxybenzenes, basic compounds, organic and inorganic anions [127]. The inorganic anions were examined by modulating the pH from 3 to 5 using 20 mM of potassium chloride, showing increased retention with pH > 4 due to the weaker anion exchange [127]. The organic anions tested at pH 4.6 and 5.5 showed longer retention time and weakness to elute all the components. On the other hand, good separation of basic compounds using different contents of ethanol as mobile phase confirmed the advantage of these hybrid particles over silica particles. Efficient separation and symmetrical peaks were also observed at the other end of pH range when three acidic compounds were analysed (hydroxybenzenes) using ethanol/hexane (25:75) as mobile phase, highlighting the excellent stability across a wide range of pH [127]. Another study reported a column efficiency of 5000 plates/m, short retention time, and symmetrical peaks during the separations of neutral compounds, isomers and aromatic compounds using hexane-isopropanol (85:15) as the mobile phase [128]. The column stability was demonstrated at pH10 using a sodium phosphate solution and a 70:30 methanol-water mobile phase. The results showed that the stationary phase was stable at pH 10 without noticeable changes on the retention factor or peak width of the solutes [128]. The stability of C18 bonded ZrO_2/SiO_2 phase was also examined at pH 12 [126]. The tests were performed via the separation of a mixture of aromatic hydrocarbons using methanol-water (80:20) as the mobile phase at ambient temperature. After the columns were conditioned for 30 min with the mobile phase, the data showed good stability at pH 12 without noticeable changes on the capacity factor or peak width. The explanation was the creation of a hydrophobic shield consisting of a cross–linked alkylpolysiloxane layer that protected the Zr-O-Si bonds from hydrolysis [126].

It can be concluded that the hybrid core-shell particles present good stability over a wide range of pH for HPLC applications. This advantage is highly appealing for all types of particles used for HPLC separations, and quite possibly indicating the need of creating hybrid core-shell particles for even wider range of applications.

7. Summary and perspectives

It has been demonstrated by various reports that core-shell particles (e.g., 2.6 μm particles) show at least the same or better column efficiency in HPLC separation, compared to sub-2 μm fully porous silica particles. But the backpressure is much lower, which makes the traditional HPLC systems usable. The LbL technique is most widely used for the production of core-shell silica particles, particularly the commercial ones. The one-pot synthesis of SOS particles has provided an effective route to novel columns offering fast separation with low backpressure, particular for large biomolecules. Other developments, e.g., hybrid particles with silica core and metal oxide shell/MOF shell, are also covered in this review. These non-silica shell particles offer high stability under basic and acidic conditions or new functionality that separates mixtures (e.g., isomers) that would otherwise not be separated using silica as stationary phase. Although not intended to be exhaustive, we provide examples to show how different types of core-shell particles have been used for HPLC separation over a range of test mixtures, usually with high efficiency/sensitivity, fast separation, and low back pressure.

For future development, new methods/procedures should be continuously developed and optimized to produce uniform core-shell particles with controlled thickness and tuneable pores (particularly wide pores) in the shell. Although hybrid particles with metal oxide shells have been fabricated and evaluated and their high stability has been demonstrated, the column efficiency is still rather low. This may be improved by controlling the thickness and pore size of the shell. MOF@silica particles have been intensively investigated but the controls on the shell thickness and the porosity between the assembled MOF nanoparticles are very limited. Another development, which has been rarely reported, is the shell formed of porous graphitic carbon or nanodiamonds. Their chemical stability will be highly attractive for chromatographic separations under extreme conditions. Similarly, the challenge will be the control of the shell thickness and the formation of wide pores. In addition, finding parameters/conditions to fabricate monodispersed particles will be highly beneficial because that will exhibit sharp peaks, high selectivity, and high separation efficiency.

Another important aspect will be applying newly developed core-shell particles for separation of complex mixtures. When selecting suitable core-shell particles, the important characteristics include shell thickness, pore size, particle size, chemical stability, and surface functional groups. This

933 will inform the best ways to utilize the columns, e.g., in reverse phase mode, normal phase mode,
934 HILIC, ion exchange chromatography, or SEC. Different analytes, i.e., small molecules, large
935 molecules, peptides, proteins, oligonucleotides, ionic compounds, or drug metabolites, will require the
936 columns with corresponding characteristics. Instrumentation of the HPLC system will be required in
937 order to achieve the best selectivity, sensitivity, and efficiency for complex mixtures.
938
939 **References**

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Figure Captions

Figure 1. Kinetic plots showing the progress (vertical and horizontal arrows) made recently for (A) sub-2 μm fully porous (FP) particles (red data) vs FP particles before the introduction of sub-2 μm particles. (B) Inclusion of core-shell (CS) particles. Plots are for small MW compounds, measured on C18 columns in reverse phase mode. Reprinted with permission from ref [27].

Figure 2. The schematic representation for the preparation of core-shell microspheres by the layer-by-layer deposition method. Reprinted with permission from ref [4].

Figure 3. Comparison of colloidal silica size on the preparation of core-shell silica particles from 0.9 μm non-porous silica spheres, 0.5 wt % poly(diallydimethylammonium chloride) (Mw = 100 – 200 kDa, cationic polymer), and 10 % colloidal silica dispersion (pH = 3.5) by one coating process. (A) Nyacol Nexsil125 (125 nm); (B) Nyacol NexSil85 (85 nm); (C) Nalco 1060 (60 nm); (D) Nalco 1030 (13 nm); (E) Ludox, AS-30 (12 nm); and (F) Nyacol NexSil8 (8 nm). Reprinted with permission from ref [33].

Figure 4. Monodisperse SOS particles prepared under modified reaction conditions with polyvinylpyrrolidone and cetyltrimethylammonium chlroide. The inset image shows a close-up look of a SOS particle. Reprinted with permission from ref [91].

Figure 5. Effect of organic templates on surface morphology and pore size distribution of modified SOS particles. (A, B) cetytrimethylammonium bromide (CTAB) + TEOS; (C, D) OTMS + TEOS, both added at t = 30 min. Reprinted with permission from ref [94].

Figure 6. The scheme shows the preparation of silica-UiO-66 core-shell particles by modifying the silica sphere first. Reprinted with permission from ref [111].

Figure 7. SEM images showing the morphology (A) mesoporous silica microspheres; (B) the seeded microsphere; (C) one cycle growth of ZIF-8; (D) two cycle growth of ZIF-8. And the TEM images showing the thickness of ZIF-8 coating: (E) the core-shell sphere with one ZIF-8 coating; (F) the core-shell sphere with two ZIF-8 coatings, where the particles were embedded in epoxy resin and cut with ultramicrotomy. Reprinted with permission from ref [108].

Figure 8. High-resolution SEM comparison between (A) PMT-SPPs and (B) SPPs made from the multilayer method. Cross-section view of pore channels on (C) PMT-SPPs and (D) SPPs made from the multilayer method. The sol-aggregated pore structure made by the multilayer process shows a more tortuous diffusion pathway than the ordered elongated channel made by pseudomorphic transformation. The red line/curve illustrate the differences in pore structures and diffusion pathways. Reprinted with permission from ref [146].

Figure 9. The structure of core-shell silica particles with solid core and nanofibrous shell. Reprinted with permission from ref [147].

Figure 10. SEM images of the carbon core (A) and the finished material (B) of carbon-based nano-diamond wide-pore particle. Reprinted with permission from ref [151].

Figure 11. Chromatograms of artichoke extract obtained with the BEH-C18 column (A) and Halo-C18 column (B) at different linear velocities. Mobile phase: MeOH/water (88:12); injection volume: 2 μL; UV detection: 320 nm. Reprinted with permission from ref [154].

Figure 12. Effect of pore size in core-shell particles on the separation of the mixture containing 7 compounds. Columns: 4.6 mm×100 mm; particles: 2.7 μm; mobile phase – A: 10% acetonitrile/aqueous 0.1% trifluoroacetic acid; B: 70% acetonitrile/aqueous 0.1% trifluoroacetic acid; gradient: 0–50% B in 15 min.; flow rate: 1.5 mL/min; temperature; 30 °C; injection: 5 μL; instrument:

Agilent 1100; detection: 220 nm; peak identities: (1) Gly-Tyr – 238 g/mol, (2) Val-Tyr-Val – 380 g/mol, (3) methionine enkephalin – 574 g/mol, (4) angiotensin II – 1046 g/mol, (5) leucine enkephalin – 556 g/mol, (6) ribonuclease A – 13,700 g/mol, (7) insulin – 5800 g/mol; peak widths in minutes measured at 50% height for ribonuclease A and insulin. Reprinted with permission from ref [67].

Figure 13. Chromatographic elution data of polystyrene solutes and toluene superimposed on the time axis. (A) SPPs with pore size of 160 Å; (B) SPPs with pore size of 1000 Å; (C) Fully porous particles with pore size of 200 Å; (D) Fully porous particles with pore size of 1000 Å. All data shown here are from the flow rate of 0.25 mL/min. Reprinted with permission from ref [174].

Figure 14. Representative chromatogram of reduced ADC (brentuximab-vedotin). Columns: Prototype SOS (sphere-on-sphere) C4 (100 mm × 2.1 mm, ~2.5 m), Halo Protein C4 (150×2.1mm, 3.4 m), BEH300C18 (150×2.1mm, 1.7 m) and Aeris Widepore C18 (150 × 2.1 mm, 3.6 m). Mobile phase A: 0.1% TFA, mobile phase B: 0.1% TFA in acetonitrile. Flow-rate of 0.4 mL/min, gradient: 27–42%B in 12 min on the SOS column and 30–45% B on the other columns, temperature: 80 ^{o}C, UV detection was carried out at 280 nm. Reprinted with permission from ref [93].