Solid Core - Understanding the Technology of Today to Help Design the Particle of Tomorrow

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The introduction of liquid chromatography saw the development of two types of stationary phase, fully porous and pellicular. The latter was a technology introduced initially by Horvath [1], and developed at DuPont by Jack Kirkland and others. The development of the pellicular material saw an interesting journey to full commercialisation, although since the successful introduction in 2006 by AMT in a more convenient form for chromatographers, the use of this material has grown substantially. Gaining a full understanding of the mechanism by which these particles work, and also understanding how to improve the synthetic process will be the major challenges over the coming years as this technology becomes more mainstream.

Review of Previous Work

In a previous edition of Chromatography Today [2] there was a wide range of contributions reviewing solid core chromatography particles. These included insights into the benefits of the technology, introducing the concept 'bar for bar better separations', as well as an article that suggested that the reports of the death of fully porous media were greatly exaggerated. This provided data which suggested that the efficiencies for a fully porous and a solid core material of the same size were in fact comparable.

Modelling Work

The underlying reasons for the advantages in performance benefits associated with solid core materials has been the subject of much debate and also a high degree of marketing from various manufacturers.

In order to better understand how the morphology of solid core particles improves the chromatographic performance, it is necessary to investigate the individual terms of the van Deemter equation **[3]**, to determine the effect of the dispersion of the solute molecules within a packed bed environment. The models to describe this were initially devised by Desmet **[4,5]** and later mirrored by work from Guiochon **[3]** and are centred on three key benefits:

Accucore Vanquish C18 1.5 µm 100 x 2.1 mm, flow 380 µL / min	
Time / mins	%B
0	20
4	40
7	80

Solid Core C18 2.6 µm 100 x 2.1 mm, flow 650 µL / min	
Time / mins	%B
0	20
6.9	40
12.1	80

Figure 1
Separation of 18 pesticides in elution order (desethylatrazine, metoxuron, simazine, cyanazine, methabenzthiazuron, chlorotoluron, atrazine, monolinuron, diuron, isoproturon, metobromuron, metazachlor, sebuthylazin, propazine, terbuthylazine, linuron, metolachlor)
Mobile phase a – Water, B – Acetonitrile

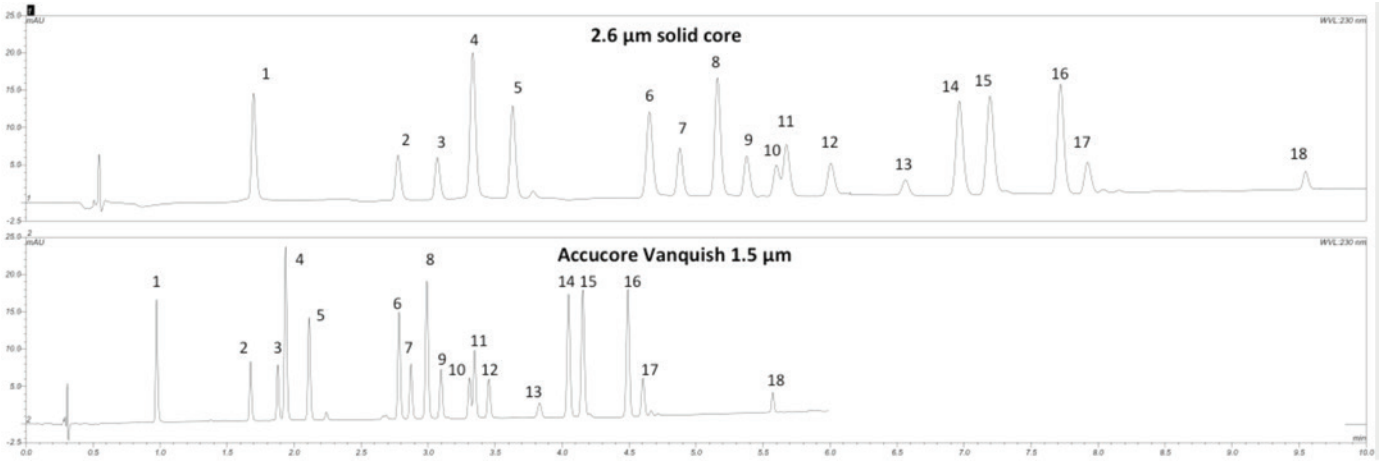
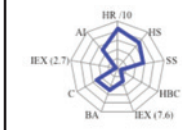
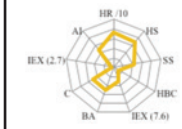
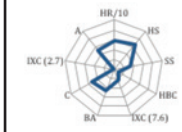
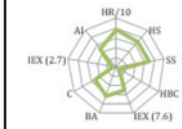
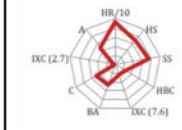
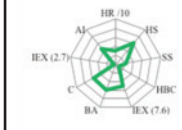
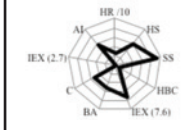
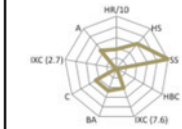
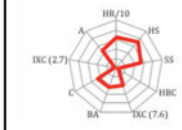


Table 1 - List of available phases from Thermo Fisher Scientific (HR – Hydrophobic Retention, HS – Hydrophobic Selectivity, SS – Steric Selectivity, HBC – Hydrogen Bonding capacity, IEX (7.6) – ion exchange at pH 7.6, BA – base activity, C – Chelation, IEX (2.7) – ion exchange at pH 2.7, AI – Acid Interaction)

Column Name	Column Characterisation	Particle size (µm)	Pore sizes available (Å)
Accucore Vanquish C18 Accucore C18 Accucore XL C18 Accucore 150-C18		1.5, 2.6, 4.0	80 150
Accucore RP-MS		2.6	80
Accucore C8		2.6, 4.0	80
Accucore aQ		2.6	80
Accucore Polar Premium		2.6	150
Accucore Phenyl-Hexyl		2.6	80
Accucore PFP		2.6	80
Accucore Phenyl-X		2.6	80
Accucore C30		2.6	150
Accucore HILIC	NA	2.6	80
Accucore Urea-HILIC	NA	2.6	80
Accucore 150-C4		2.6	150
Accucore 150-Amide-HILIC	NA	2.6	150

1. the high homogeneity and roughness of the particles improves the packing process of the column;
2. the solid nuclei reduce longitudinal diffusion;
3. the existence of a reduced porous region in the particles reduces the band broadening.

In turn these benefits can be aligned with the three terms described in the van Deemter equation **[3]**, with 1 being related to the A term, 2 to the B term and 3 to the C term.

Effect of Temperature Dispersal

The initial offerings associated with the use of the solid core technology were aligned to obtaining comparable efficiencies to those obtained using sub 2 µm particles but with the pressures associated with a larger particle, 2.6 µm. However, as the product became more popular, so sub 2 µm solid core particles were developed with the associated increase in the pressure to allow optimal performance. It has previously been shown by several authors that increasing the pressure can affect the retention of individual compounds **[7,8]**, and that with higher pressure drop, significant longitudinal and radial temperature gradients can exist within the column. It has also been shown that radial thermal gradients can have a detrimental effect on the peak shape **[9,10]**, since the temperature will affect the fundamental dispersion processes.

Manufacturers are becoming increasingly aware of the importance of temperature in liquid chromatography, particularly with the never ending drive to reduce the particle size with the resultant increase in pressure associated with this. The importance of adiabatic column ovens **[11]** will grow as the particle size is reduced, with forced air ovens providing an option for the use of thermal gradients **[12]**, which is sadly under utilised in this form of chromatography, primarily due to misconceptions relating to column stability and compound stability at the higher temperatures typically used. Column pre-heating will also become more standard as this will have a greater effect on peak shape with increases in the longitudinal temperature gradient **[11]**.

Reducing the Particle Size

Recently Thermo Fisher Scientific launched the Accucore™ Vanquish™ 1.5 µm solid core material. Combining the Thermo Scientific™ Vanquish UHPLC system (Thermo Fisher

Scientific, Germering, Germany) with the efficiency of the Accucore Vanquish columns (Thermo Fisher Scientific, Runcorn, UK) results in separations such as is demonstrated in Figure 1. In this test example a series of 18 pesticides have been separated with an improved resolution and a reduction in the analysis time of 40% when compared to the 2.6 µm equivalent column. It should be noted that the flow rates has been increased when employing the smaller particle sized column, using the column volume as a scaling factor. Although the flow rate has been increased the resolution has improved, which is a result of the improvement in the chromatographic efficiency obtained with the smaller particles.

Available Phases

The use of high efficiency columns provides chromatographers with sharper peaks but does not necessarily provide them with the tools to drive a separation. For this to occur, optimisation of the resolution equation has to happen. There are three terms associated with the Purnell [6], or resolution equation, as given in equation 1:

$$R = \frac{\sqrt{N_2}}{4} \cdot \frac{k_2'}{k_2' + 1} \cdot \frac{\alpha - 1}{\alpha}$$

Where:

R – resolution

k_2' – retention factor for the second peak

α – separation factor for peaks 1 and 2

N_2 – efficiency of the second peak

It has been demonstrated that the most important parameter in driving the separation is the separation factor, particularly at high retention factors [13]. This term is affected by several experimental parameters, including stationary phase, mobile phase composition, temperature, and latterly it has also been found that the pressure, and hence flow rate, can also affect the selectivity [14,15,16]. The initial lack of selectivities with solid core media gave fully porous media greater versatility. However, there has been a substantial increase in the number of stationary phases available and also the number of manufacturers producing solid core over the past few years. The number of offerings from Thermo Fisher Scientific has now been extended to thirteen, existing in a range of pore sizes and particle sizes. A full list of the available offerings is given in Table 1. The axes relate to different interactions that the column exhibits. In general it is not possible to state that having a high or low value is good, unless the physiochemical properties of the molecule are considered. The individual labels have been described in detail elsewhere [17]. The shapes of the plots are an ideal way to determine columns that are similar and those that are very different. This approach has been successfully applied to the characterisation of reversed phased columns, although there is not a universal approach to the classification of HILIC columns and hence the HILIC columns have no data supplied.

Development of New Morphologies

The early generations of pellicular particles are covered in the review by Guiochon et al. [18] and Kirkland et al. reported the preparation of superficially porous particles ('Poroshell'), which were composed of an ultra-pure solid silica core with a thin porous shell. A co-spraying method was initially used, but with the disadvantage of forming some totally porous microspheres that could not be effectively separated from the rest of the Poroshell particles [19].

A large percentage of core-shell silica particles for chromatography are now prepared by a layer-by-layer (LbL) approach, particularly those that are commercially available. This approach utilises the electrostatic interaction (and also hydrogen bonding, covalent bonding, van der Waals interactions, etc.) between the positively charged (cationic) and negatively charged (anionic) species to assemble multiple layers together. Colloidal particles with suitable surface charges are used as the core and alternative layers (of oppositely charged species, for example, negatively surface charge silica nanospheres and cationic polymer poly(diallyldimethylammonium chloride) are built up onto the core colloidal particles. The core silica particles are firstly bound with a polyelectrolyte (e.g., negatively charged silica particles with a cationic polymer). Excess polyelectrolyte is removed by rinsing. The coated core particles are then immersed in a colloidal dispersion of nanoparticles with charges opposite from those of the organic polyelectrolyte. This process is repeated by alternating immersions between the polyelectrolyte solution and the nanoparticle suspension until the desired shell thickness is obtained [20]. The resulting particles can then be treated thermally to remove the organic polyelectrolyte and produce solid-core porous-shell particles.

The productivity of manufacturing core-shell silica particles is low and tedious. This is due to the numerous centrifugation steps that are needed to remove extra and loosely bound species in each coating cycle to avoid particle aggregation. A multilayer (ML, film of more than one layer)-by-multilayer approach was developed to speed the process [21,22]. The silica shells created by the ML-by-ML method have a higher level of porosity than those obtained by the traditional LbL process. The multilayer adsorption phenomenon was attributed to the formation of nanoparticle aggregates, reduced repulsive force between nanoparticles and increased non-electrostatic attraction between nanoparticles and polyelectrolytes [22].

Shell Synthesis on Pre-formed Cores

The Stöber method is commonly used to prepare uniform nonporous silica microspheres and nanospheres, where a base catalyst such as ammonia is often used in a system also including water, alcohol and tetraalkoxysilane [23]. The introduction of surfactants/porogens such as cetyltrimethylammonium bromide, CTAB, or non-ionic surfactants Pluronic P123 and F127 result in the production of mesoporous silica microspheres, either in the core shell or on the particles on the outer layer [24,25].

One-pot Synthesis and Spheres-on-sphere (SOS) Silica Particles

Spheres-on-sphere then offers an interesting alternative to the mainstream approach of producing solid core-shell silica particles which uses time-consuming LbL approach. A one-pot synthesis of core-shell particles would be highly advantageous, offering potential benefits on reaction time, easier quality control, materials costs, and process simplicity for facile scale-up. There have been limited reports on the one-pot synthesis of core-shell silica microspheres which are suitable for HPLC [26,27 but these approaches have not yet been employed for commercial use.

Ahmed et al. reported the one-pot synthesis of core-shell silica microspheres with the spheres-on-sphere (SOS) morphology from one single precursor 3-mercaptopropyltrimethoxysilane (MPTMS) [28]. The same approach, with slightly modified reaction conditions, has also been utilised in the generation of more complex structures that are more akin to the structure of a fractal [29], where the porosity of the spheres is removed, resulting in the surface area being attributable to the morphology of the particle rather than the pore diameter.

The concept of fractals was first introduced

in the 17th century by the mathematician Leibniz, who was investigating the possibility of recursive self-similarity . Recursive self-similarity is when a shape looks the same no matter how close the viewer is. So zooming into the text on this page will look different, however zooming into a line will always look the same. This science of fractals was not followed for another century until it was developed by Weierstrass [30], Cantor [31], Klein [32], Poincaré [33] and Koch [34]. These academics developed the first mathematical understanding in terms of self similarity, a non-differential curve and the concept of a fractional dimension and also presented the first pictorial representations of a fractal shape, in particular with the Koch curve, figure 2. The morphology of the porous structure within silica is inherently stochastic, but it has been determined that it has a fractional dimension [35]. However the random nature of the pore morphology means that there is no scale dilation associated with traditional silica's. This has a dramatic effect when considering the mass transfer effects of large molecules into and out of the pores, where the diffusion affects the rate of equilibration of the analyte concentration between the pressure driven regime and the diffusional regime. With fully porous media the dispersive contribution at elevated flow rates becomes significant, and will effectively limit the analysis time, due to the reduction in the performance of the chromatography.

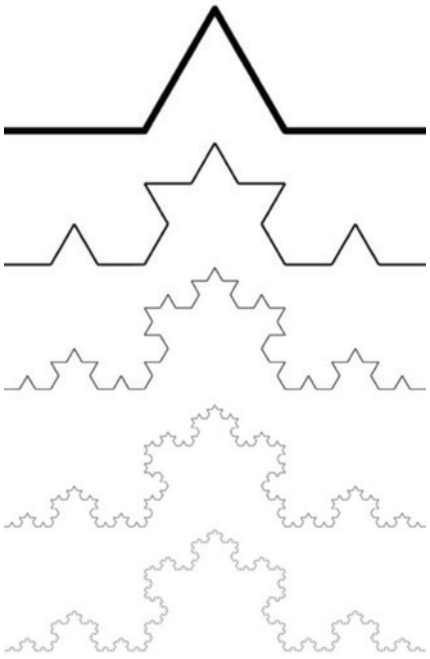


Figure 2
An example of a scalable fractal, the Koch curve. The figures demonstrate how to make the curve by self replicating the original structure, resulting in a structure that looks the same no matter what the scale and also one that does not have an integer dimension.

The development of sphere on sphere technology leads to the possibility of generating a scalable fractal structure [36]. A truly fractal structure with a fractal dimension and self similarity would offer increased surface area capacity without the detriment of increased mass transfer equilibration associated with more traditional materials. For smaller molecules where the diffusion rates are much higher this effect is not so noticeable, and so there is not a requirement to produce these types of particles in this arena. Figure 3 shows some of the first structures that demonstrate a degree of scalability with Figure 4 demonstrating an example separation.

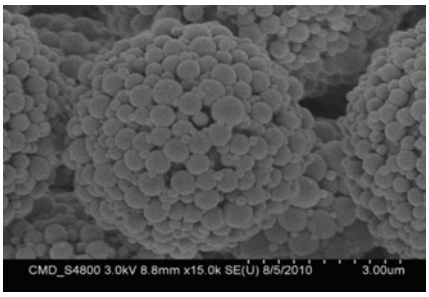


Figure 3
Potential fractal structure starting to appear with spheres on spheres on spheres of solid silica

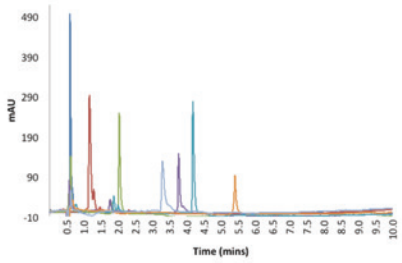


Figure 4
Separation (in elution order) of Ribonuclease A, Insulin, Lysozyme, BSA, Myoglobin, Carbonic anhydrase, Ovalbumin

Conditions	*Mobile phase A. Water + 0.2% TFA*
	B. Acetonitrile + 0.2% TFA
Gradient	*30-75% B in 10 minutes*
	400 µL/min, 50 °C

Up to now this technology has not been compared directly to fully porous or indeed solid core technology, but looking at the initial chromatography obtained, there is clearly substantial degree of promise shown by the new technology.

There is still some way to go with the concept of fractal chromatography. Currently only a few examples have been investigated and substantial more work has to be done in applying the technology for the analysis of large molecules, however the initial work does look promising. If the further testing is successful then the product will be invariably commercialised, allowing protein chemists to develop separations which are not feasible on traditional fully porous materials.

Conclusion

The advantages of solid core technology and an understanding of how the technology works compared to fully porous media has been provided. The Accucore Vanquish™ media has also been discussed and data presented which demonstrates the incredible performance that can be obtained when combining UHPLC and solid core. The future design of particles has been discussed and the concept of fractal chromatography introduced, with some of the first scalable fractal structures that have been synthesised shown. It is the possibilities that the new synthetic pathways allows that offers the most exciting opportunities in terms of separation science, offering high performance separations of large protein molecules without excessive pressures.

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