

An Extension to Core-Shell Particle Technology – New Applicability at High pH.

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This article discusses the use of core-shell particles in terms of the current market trends, where they are and what is next in the evolution of the technology. There is no doubt that core-shell particles provide very high efficiency separations at reduced backpressures, however quite how these particles work is still the subject of much on-going work and discussion. What also isn't in doubt is that manufacturers will continue to expand the offerings available. One important such development will be the extension of core-shell's ability to operate at extremes of pH. This can be achieved successfully through a surface grafting technology, Fortis SpeedCore pH Plus, to give a material, which possess an increased lifetime at high pH for basic drug analysis.

Introduction

Core-shell particles for use in HPLC have become the current trend in the last few years, commercially introduced in their current small particle form by Advanced Materials Technology in 2006, they provide the capability for high speed, highly sensitive, rapid separations. DuPont first described the technology for use in separations of peptides and proteins back in the 1970's, but this was utilising relatively large particles sizes and featured lower control over the outer shell thickness than current technology.

Now since various small particles, 1.7 μm , 2.6 μm , 5 μm are commercially available from several manufacturers [1], the technology has seen an increase in uptake, being one of the most discussed subjects at conferences, the introduction of new core-shell particles and phases outstripping traditional porous particles by 10:1 according to Majors [2].

Due to the mass transfer, reduced band broadening and particle morphology of these core-shells, analysts are utilising these particles effectively to operate at high speed, whilst achieving high resolution, high efficiency separations. However there is still a steep learning curve for practitioners seeking to know more about the attributes of these particles and the way that the mechanisms operate [3]. Initially thoughts were based around the particle size distribution being tighter and the reduced

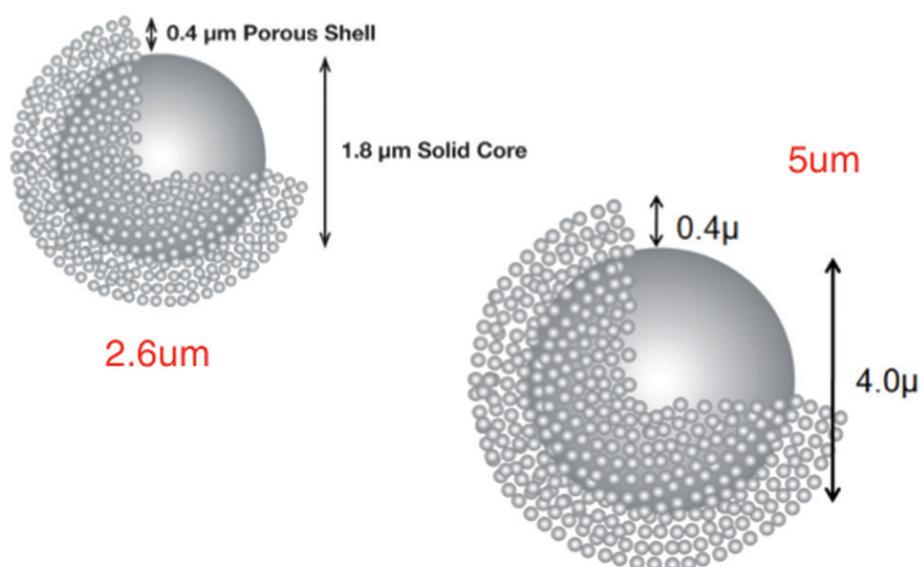


Figure 1. 2.6 μm and 5 μm core-shell particle sizes available for scaling separations

mass transfer. However now it would appear that the A and B terms in the Van-Deemter equation are playing a bigger role than first thought since these core-shell particles can be packed more effectively with smaller 'dwell-volume' [4].

Limitations

Core-shell particles have a very attractive attribute in efficiency gains, however can they totally replace traditional fully porous particles? Well there are several limitations

[5], scaling from small 2.6 μm to larger 5 μm and 10 μm particles to provide preparative capabilities, selectivity choices, lifetime, and pH range to name a few. If all of these can be overcome then potentially yes, it remains to be seen if it is achievable, but there is certainly a lot of hard work to be done to get to that point.

There are a few products now available (Figure 1) where you can scale from one particle size to another, although it will need to be shown that the separation is

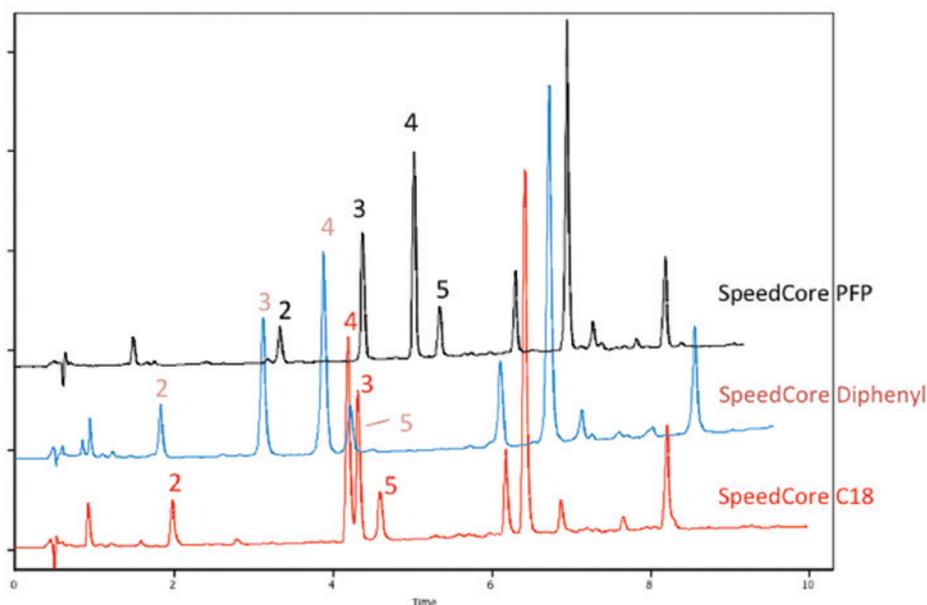


Figure 2. Selectivity options on core-shell provide orthogonal selectivity options to increase resolution.

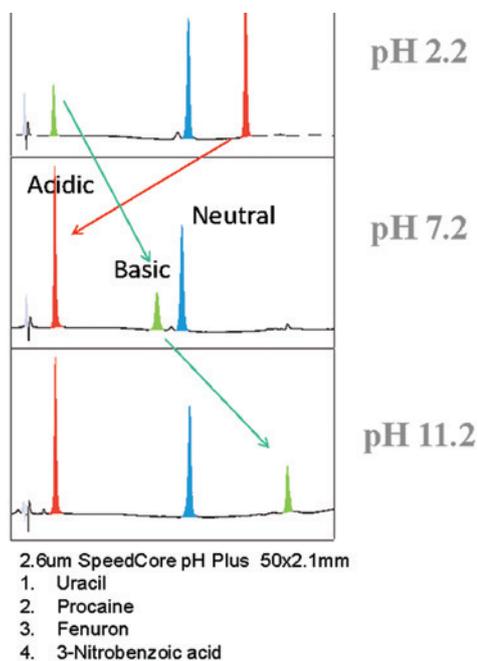


Figure 3. Use of pH to provide a method development screening option. SpeedCore pH+ 50x2.1 mm phosphate buffer at three pH values.

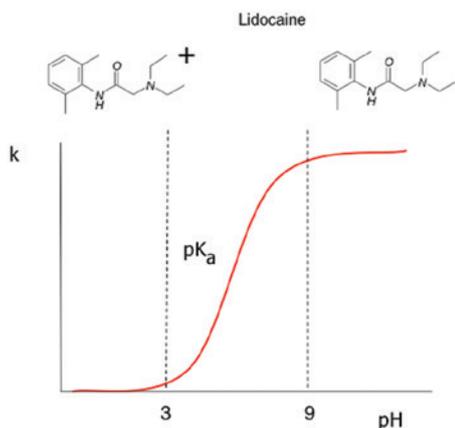


Figure 4. Basic analyte retention as a function of pH

above 9, which provided an increased performance and lifetime at high pH values. Several other manufacturers have since introduced high pH fully porous particles that operate by either a hybrid concept or a surface coverage/protection mechanism.

Moving forward the marriage of high pH and core-shell is clearly something that will provide the next generation of core-shell particles. It provides extra selectivity options (Figure 3), as the analyst gains the ability to screen methods quickly for unknown acid, bases and neutral analytes. At the start of the discovery process compounds tend to be screened quickly to find suitable starting conditions, many times using a multi column switching system. If the full pH range can also be screened then it gives the option to learn more about the pK_a of unknown compounds. Acids will retain with good peak shape at low and mid pH values whereas basic analytes with pK_a >7 will tend to be unretained. Then the reverse happens as you move up to pH 9-10, acids will exhibit lower retention whereas the basic analytes now in their neutral form will retain by the hydrophobic mechanism offered them by reversed phase C18. Meanwhile neutral species will be largely unaffected by the changes in pH. Improved peak shapes will also be a feature of the bases retaining at high pH above their pK_a values, since the interaction mechanism should now be a single one, hydrophobicity, as opposed to hydrophobicity and silanol interactions with a charged base.

Since most LC systems have quaternary solvent systems then it can be simple and effective to screen unknown compounds, by using one low pH, one mid pH and one high pH buffer with the fourth solvent being the organic modifier (ACN or MeOH generally). It provides the most data possible without moving to column screening of a multitude of stationary phase chemistries. Utilising pH correctly may also potentially produce very robust methods, if you are 1.5 units above the pK_a of the basic analyte in question then it should be in its fully neutral state rather than a mixed ionised state leading to less variability in retention (Figure 4).

It is also usually this ionisation of bases that causes the polarity to prevail in the retention process, retention of polar basic analytes that are not achievable in low pH

not altered by any subtle changes in physical characteristics, shell thickness, shell uniformity, pore structure or carbon loading.

Stationary phase selectivity is certainly a variable that many manufacturers are focused on; as most have vast experience having produced many phase chemistries on traditional fully porous particles. There is no reason why any of these chemistries (Figure 2) cannot be applied to core-shell particles. Therefore expect a continuous flow of product additions in this space. If you do not increase selectivity options many difficult applications, positional isomers, metabolites and homologous series, will continue to prove a challenge with the use of high efficiency alone.

Another limitation with core-shell particles is the useable pH range; a look through the current literature suggests that pH 2-8 is the most recommended range with some manufacturers claiming 2-9 or possibly 2-10. Since this is generally marketing literature with fine print such as 'the gradient pH range is different to the isocratic range', or 'only certain organic buffers may be used', then it is clearly debatable how robust these phases currently are.

Producing a high pH stable silica based stationary phase is not new. In 1999, Waters launched the first organic/inorganic hybrid columns, being the first to try and bridge this gap in silica's capabilities. These were the first porous silica particles to extend the upper pH range significantly

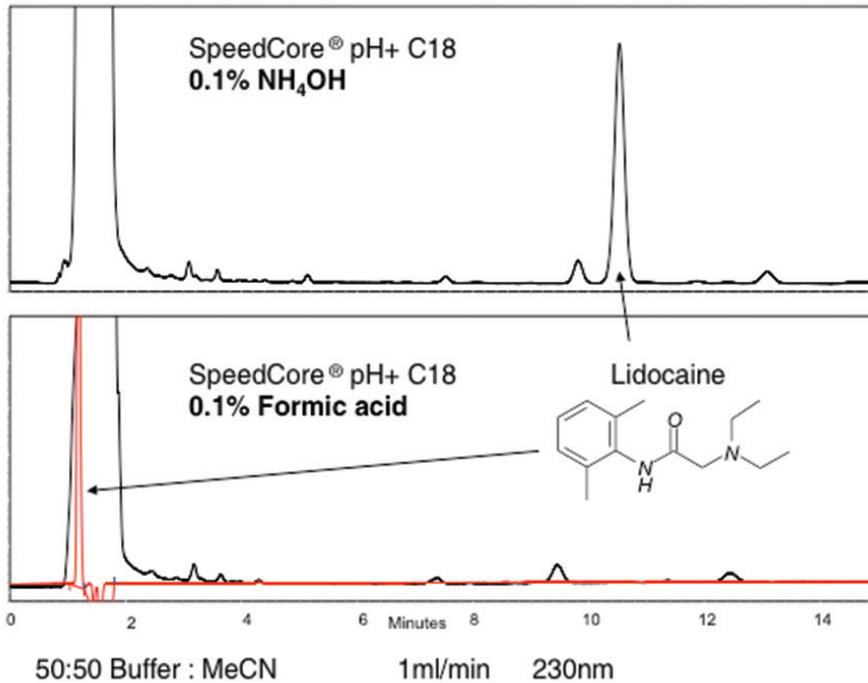


Figure 5. Retention of Lidocaine in low pH (formic acid) and high pH (Ammonia). Column geometry and ratio of buffer to organic remain the same throughout.

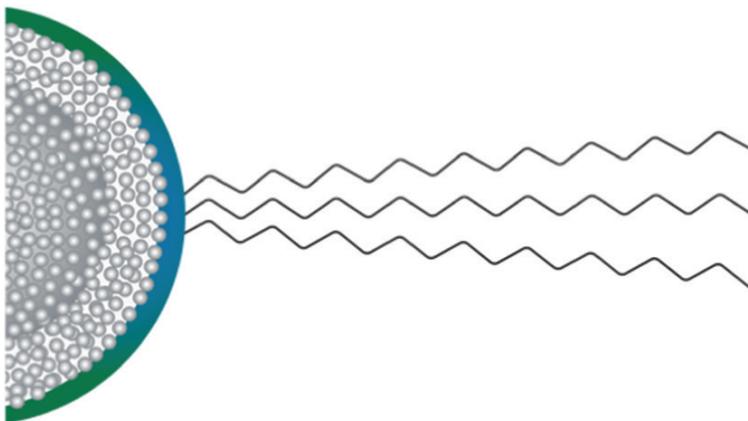
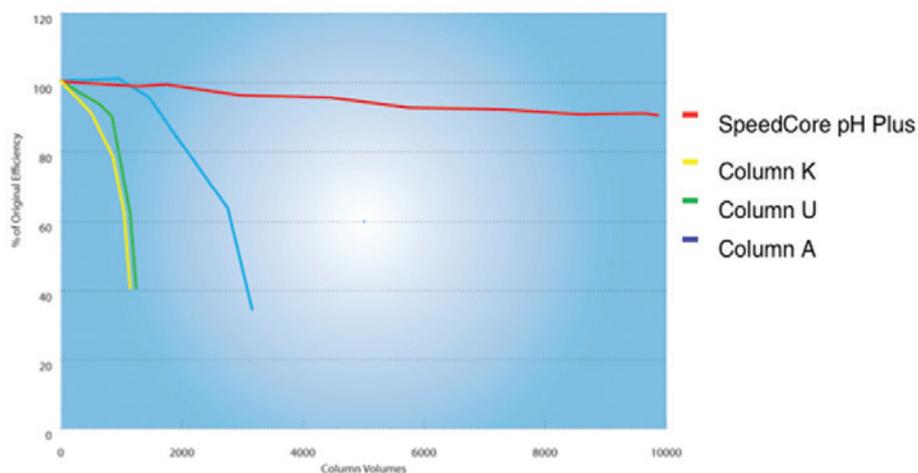


Figure 6. Surface grafting technology – cross-linked surface modification of a core-shell particle to eliminate surface interaction and attack at extremes of pH



Stability - 50mM Ammonium Bicarbonate pH 10 Temperature 50°C

Figure 7. pH stability of four core-shell columns at elevated pH and temperature. Ammonium Bicarbonate pH 10 and 40°C

mobile phases are suddenly well retained in high pH conditions (Figure 5). High pH may also lead to extra loadability [6] for basic analytes if scaling up an analysis to preparative scale using newly available larger 5µm core-shell particles.

Surface Grafting Technology (SGT)

In order to increase the usable pH range with a core-shell particle a unique surface grafting technology (SGT) has been developed (Figure 6), this has a cross-linked surface modification, which eliminates surface interaction and attack, therefore providing extreme pH range stability. If you can protect the silica surface in this way efficiency should not be compromised by changing the particle morphology in any way, since utilising hybrid technology can lead to lower carbon loading and low surface area values due to the very nature of substitution of silica with organic ethylene linkers.

Other variables that will be improved if a surface protection route as opposed to a hybrid route is used, is that you should also see improvements in equilibration, retention and peak shape. This will be due to the single mechanism of interaction remaining, hydrophobicity. With a surface protection the silica surface is covered and produces a more homogenous mechanism with the C18 ligands to retain analytes, equilibrate quickly with the mobile phase and produce the necessary adsorption/desorption mechanisms required for suitable peak shape. In the process any secondary interactions that would normally occur with residual silanols are minimised. With the organic/inorganic hybrid particles having multiple mechanism (hydrophobic, ion-exchange and hydrogen bonding) which can impact the desired separation.

Another advantage of SGT combined with newer silica supports is that they allow for the use of 'softer' MS friendly buffers. When we are screening unknown samples then our detector of choice will typically be MS so use of inorganic phosphate buffers due to incompatibility becomes an issue with the MS ionisation process. Using formic, acetic acid, ammonium acetate and ammonia allow diversity in pH range but at the same time compatibility with the detector. With older generations of silica the use of these 'soft' buffers would not have been sufficient to mask the negative silica surface interactions present.

To test this new SGT technology an enhanced stress test is performed in order

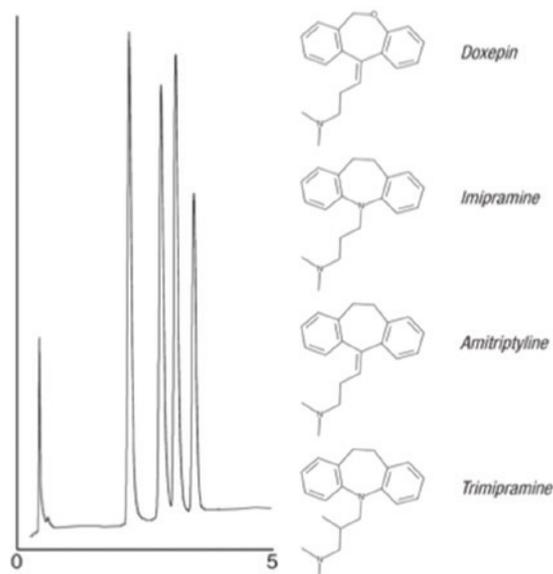


Figure 8. Separation of tricyclic antidepressants. 2.6 μ m Speed-Core pH+ 50x4.6mm. 60-80% B in 10 minutes (A: 10 mM Ammonium bicarbonate pH 10, B: MeOH) Flow 1 ml/min Temp 40°C

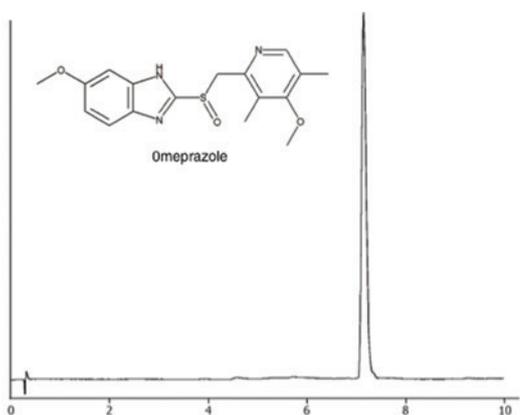


Figure 9. Separation of omeprazole 2.6 μ m SpeedCore pH+ 50x3.0 mm 10-80% B in 10minutes (A: 10 mM Ammonium bicarbonate pH 10, B: MeOH) Flow 0.6 ml/min Temp 40°C

to compare the technology with the current core-shell phases to see the potential lifetime differences. Elevated temperature and pH were combined across all columns to produce this amplified test.

(Figure 7) show the results of the study, in which three commercial core-shell particles and one surface grafted technology (Fortis SpeedCore pH Plus) were compared. It can quite clearly be seen that the SGT improves the lifetime of the core-shell well past that achievable by the three other commercial core-shell particles, two of the core-shell particles being irreversibly compromised in less than 1000 column volumes of mobile phase, whilst the third core-shell shows slightly better lifetime of \approx 3000 column volumes.

The reason that column volumes are highlighted is due to the increased flow rates utilised with these core-shell particles. Number of injections is not a direct

comparison to older fully porous particles which are run at half or less of the linear velocity, whereas volume of solvent through the column is a fair comparator.

Applications

If a core-shell column is able to operate at these extended high pH ranges then it opens up the possibility to retain and separate problematic basic analytes quickly and with excellent peak shapes. Operating above the pKa values of compounds such as tricyclic antidepressants (pKa 9-9.7) (Figure 8) and being able to meet the stipulations of the EP, USP for methods such as omeprazole (Prilosec Figure 9), which is particularly harsh on columns, containing a disodium hydrogen phosphate mobile phase means that method development options are expanded. Altering pH is also a powerful tool in the initial screening of unknown compounds when trying to determine analyte physicochemical properties, since acids and bases retain very differently to the alteration of mobile phase pH as previously discussed.

Conclusion

Core-shell particles for use in HPLC are highly popular due to the very high efficiency and high-speed separations that are achievable without the need for excessive pressures. If the selectivity of these particles can be extended further by adding in the use of a much higher pH range in order to retain and separate complex polar basic analytes then it will offer the next generation of core-shell particle. Obviously it needs to be proven that robust, reproducible methods are produced, but in theory these particles will be more forgiving and robust if they cover the complete pH spectrum since they will not be running at an 'extreme' at any point. Operating a particle at pH 7 on a phase that has a stated pH range of 2-8 will be less robust than a particle with a pH range up to 12.

As the technology moves forward there

is no doubt that analysts will learn more about the theory of core-shell particles, in particular the flow dynamics and construction mechanisms and how they are affected, there will be a flow of information between the theoretical practitioner and manufacturers of core-shell particles which will improve the knowledge, capabilities and potential of the particles. Understanding the mathematics will lead to smaller more efficient particles, with optimised shell to core ratios for both small and large molecules. It will lead to better packing of the particles to optimise potential efficiency gains.

Of most importance is what it will offer to the analyst! They wish to improve throughput, efficiency and resolution of their separation process; they want to screen 'unknowns' quickly and effectively (not be near any prospective pKa values). So at this moment in time, there is the ability for high efficiency separations, new options for selectivity of stationary phases and most recently the possibility to operate at elevated pH ranges, particularly high pH with a new surface grafting technology. This gives the analyst a new option towards their goals of reducing method development time, increasing productivity, increasing robustness and dealing with the cost implications of these.

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