Symbiosis of Core-Shell and Fully Porous Particles: Implementing Both for Better HPLC and UHPLC Results

(Via LCGC North America)

Learn to capitalize on the advantages of both core-shell and thermally modified fully porous stationary phase media to expand the options for HPLC and UHPLC method development.

High performance liquid chromatography (HPLC) and ultra-high performance liquid chromatography (UHPLC) method development options can be extended significantly by tapping into the distinct advantages of core-shell and fully porous particles. Integrating these media platforms into everyday work can result in enhanced performance and selectivity. This report examines the morphology and performance of two types of stationary phase media (Luna® Omega Thermally Modified Fully Porous Particles and Kinetex® Core-Shell Particles from Phenomenex) and their application in method development work.

Overview of Particle Technology

To fully appreciate and harness the utility of the core-shell and thermally modified fully porous particles, it is helpful to first understand the differences in the morphology of these materials (Figures 1 and 2). Understanding the strengths and weakness of both formats can help chromatographers take advantage of their benefits for specific needs.

Fully porous particles. Most chromatographers are familiar with conventional fully porous stationary phase material. These particles are silica microspheres that are highly porous
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and similar in structure to a sponge. As a sample travels through the column in the mobile phase, it diffuses in and out of the pores, interacts with the stationary phase, and becomes separated.

**Core-shell particles.** Core-shell particles are a relatively new development in the field of liquid chromatography. The internal volume of the core-shell particle is occupied by a solid, impermeable silica inner core. Surrounding the solid core is a porous layer of silica that forms the outer shell. The ratio of the internal solid core to the external porous shell varies depending on the manufacturer, but the basic structure of these materials is the same. The dense solid inner core is inaccessible to analytes in the mobile phase; analytes interact only with the outer porous layer of conventional silica.
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Differences in Performance

The different morphologies of fully porous and core-shell particles have dramatic effects on column performance.

Core-shell particles. Coreshell particles yield very high efficiency relative to fully porous particles of equivalent diameter. Peaks will elute as narrower bands, which facilitates...
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greater resolution of closely eluting components and often increases sensitivity. Because the core-shell particle interior has less surface area than fully porous particles, less retention of ten occurs, which means better performance in less time. Pressure values are essentially the same as for an equivalently sized fully porous particle.

As particle size decreases, the performance advantages slightly decrease. The 5 μm core-shell material is about 80% more efficient than a 5 μm fully porous material, but at sub-2 μm sizes, the performance advantage is only about 20%.

Kinetex Core-Shell particles are available in 1.3, 1.7, 2.6, 3.5, and 5 μm sizes suitable for UHPLC to preparative HPLC, and utilize a variety of bonded phases for standard reversedphase. Specialty phases are also available for work at high pH or for separating very polar materials under HILIC conditions.

**Fully porous particles.** A relatively broad range of pore size distribution occurs during the formation of the porous silica microspheres. Even though they may be labeled as 100Å, this number is just an average, as both large and micropores actually exist. Because of their incredibly small size, micropores are inaccessible to bonding, so residual silanol activity is common here and leads to peak tailing. Additionally, poor diffusion kinetics associated with these micropores also contributes to brand broadening. As a result, manufacturers are moving toward producing material with more homogenic particles sizes and pore size distribution for better performance.

**Thermally modified fully porous particles.** A thermally treated fully porous particle is now available, Luna® Omega (Phenomenex), which offers several distinct advantages in performance over the conventional fully porous material used for small-molecule work. The thermal treatment process greatly strengthens the silica and reduces the presence of
micropores, which contribute to peak tailing by way of free silanols (see Figure 2). This process results in a 20-30% increase in efficiency and, therefore, much better resolution with the Luna Omega material when compared with standard fully porous material. The thermal treatment also provides a more inert surface, which results in better peak shape overall for basic analytes that might display strong peak tailing on a conventional silica particle.

In comparison with the core-shell particles where much of the inner volume is impermeable, the Luna Omega particles have a relatively high surface area, which results in improved retention. And, by utilizing this greater retention to move compounds further from the beginning of the run, they will be less affected by strong organic solvents that may otherwise cause peak distortion.

The higher surface area of fully porous material also improves loadability. This loading capability is advantageous for analysts running stability-indicating assays or trying to characterize low-level impurities where large injection amounts are required to see impurities that are present at very low levels. Typically in these cases, poor loadability can lead to band broadening, which obscures closely eluting impurities.
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**Figure 2:** Thermally modified pore structure Luna® Omega.

<table>
<thead>
<tr>
<th></th>
<th>Total Surface Area (m²/g)</th>
<th>Microporous Surface Area (m²/g)</th>
<th>Surface Area Ratio of Microporous to Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre-Treatment</td>
<td>441</td>
<td>51</td>
<td>11.6%</td>
</tr>
<tr>
<td>Post-Treatment</td>
<td>256</td>
<td>16</td>
<td>6.3%</td>
</tr>
</tbody>
</table>

**LC System Considerations**

Your HPLC or UHPLC platform guides the selection of which type of core-shell or fully porous media and particle size is most appropriate for method development (Figure 3).
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Conventional HPLC-UV (400–600 Bar pressure limit).

For conventional HPLC systems, column efficiency varies relative to different particle sizes and morphologies. A standard fully porous 5 μm material will yield about 100,000 plates/m. The Luna Omega 5 μm material is about 20% more efficient than the standard material. Kinetex 5 μm particles show a large increase in efficiency at about 180,000 plates/m.

For 3 μm materials, the same trend occurs. The Luna Omega 3 μm particle is about 15–20% more efficient (~200,000 plates/m) than conventional fully porous 3 μm particles (~180,000 plate/m). The Kinetex 3.5 μm material is even more efficient, while the Kinetex 2.6 μm
material shows the largest boost in efficiency with more than twice the efficiency (280,000 plates/m) of conventional fully porous 5 μm material on HPLC-UV systems. This performance is essentially equivalent to a 1.7 μm fully porous particle used for UHPLC (see Figure 4).

![Figure 4: Conventional HPLC-UV: efficiency levels (plates/m).](image)

*Comparative separations may not be representative of all applications*

Overall, core-shell columns offer superior efficiency on conventional HPLC instrumentation, with chromatographic improvements that are further enhanced by overall productivity gains due to shorter run times.

Figure 5 compares core-shell and thermally modified fully porous columns on a standard HPLC-UV using a separation of several cannabinoids. Selectivity and elution order are identical, but the difference in the peak width is quite apparent. For the two peaks highlighted in the pink box, one can see a significant improvement in resolution with the core-shell column versus the fully porous column. Improved performance happens in a fraction of the time as well with the core-shell column.
For core-shell particles, performance advantages are apparent but do tend to decrease as particle size is reduced, which is relevant to the sub-2 μm formats used in UHPLC. Column efficiency for different materials used in UHPLC varies in similar ways as with conventional HPLC, but the differences may not be as dramatic. Kinetex 1.7 μm particles generate about 20% more efficiency than fully porous 1.7 μm particles. However, the thermally modified Luna Omega 1.6 μm fully porous material demonstrates almost identical performance to the Kinetex 1.7 μm. Kinetex 1.3 μm material offers somewhat higher efficiency, though performance is heavily instrument dependent. Overall, core-shell particles under UHPLC conditions are not yet able to demonstrate the huge performance benefits seen for HPLC systems (see Figure 6).
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**LC-MS.** Some notable differences are apparent in performance relative to particle type for LC-mass spectrometry (MS) versus LC-UV systems. In mass spectrometry, the ionization of analytes in the source is highly dependent on the amount of organic solvent present. Ionization is favored in organic solvents, and analytes are not ionized as well in aqueous mobile phases. LC-MS methods are generally run on short columns, so the amount of organic solvent changes rapidly over time. When a peak elutes later in a run, it is eluting in a higher percentage organic solvent, which results in a better ionization response from the MS detector. So, the MS detector response somewhat counteracts the core-shell efficiency gains. In this case, fully porous particles, which favor retention, can at times be a better option than core-shell particles.

For LC-MS, particle choice is a toss-up. In some cases, the core-shell provides the resolution needed to separate two isomers or to separate the target analyte from a matrix interference. But, in other cases, the improved retention achieved on fully porous material yields sensitivity gains, which may be important in meeting limit of quantitation (LOQ) or limit of detection (LOD) objectives.

**Method Development Challenges**

Some typical chromatographic challenges faced by chromatographers can be met with
improved results based on the choice of core-shell or thermally modified fully porous particles.

**Improving the retention of extremely polar bases.** For the analysis of highly polar bases under typical low-pH operating conditions, a C18 reversed-phase column is usually the first choice. If the analyte is not retained on a C18 column, a Biphenyl phase can be used as it has multiple interaction mechanisms as opposed to only hydrophobic interactions. These interactions may be aromatic, pi–pi, weak dipole, hydrophobic, or hydrogen bonding, but overall promote greater retention.

**Figure 7** shows the separation of three isobaric species. The separation in the top chromatogram on a Kinetex C18 uses a water–methanol–formic acid mobile phase. The first two isomers are barely retained on the column because its main retention mechanism is hydrophobicity. Under identical separation conditions, except for the use of a Kinetex Biphenyl column that contains multiple interaction mechanisms, the three polar isomers are well retained and well separated from one another.
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A polar-modified C18 column can also be used for polar bases. The Polar C18 stationary phase is available in both core-shell and fully porous formats. In these columns, a traditional C18 is found alongside a polar modified silica surface. This polar modification provides enhanced selectivity for polar analytes (particularly bases and some neutrals) and stability in 100% aqueous mobile phase conditions. By having both an overall dual polar and non-polar selectivity, the Polar C18 stationary phase yields even more significant increases in retention for a wide variety of compounds.

This makes the Polar C18 is a good option for screening multiclass panels such as pesticides, which contain analytes with a broad range of selectivities and chemistries.

**Improving retention of polar acids.** Biphenyl and Polar C18 do not deliver as much separation improvement for polar acidic compounds as for polar bases. As a result, the Luna Omega PS C18 (“positive surface”) column was developed for greater retention of polar
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acidic analytes. This phase contains C18 bonding with TMS endcapping, and a positively charged (proprietary) functional group is added to the silica’s surface. This process, in effect, creates a mixed-mode phase with a combination of hydrophobic interaction and ion-exchange capacity for small polar acid molecules. While this phase enhances selectivity for polar acids, it also has some benefits for the analysis of basic drugs.

Figure 8 illustrates increased polar selectivity for small acid molecules such as ethyl sulfate (ETS), which is highly polar and does not retain well on a standard C18 column. On the Polar C18 column (top of Figure 8), ETS is separated well from the urinary isobaric interference earlier in the run. Under the same conditions using the Luna Omega PS C18 column, retention is dramatically increased.

In summary, for polar bases, Biphenyl and Polar C18 are good choices. For polar acids, the
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PS C18 provides the selectivity needed to retain the polar acids and move them away from the early part of the run, as well as potentially providing better selectivity and separating closely related polar acids. In addition, the positive charge on the PS C18 particles’ surface has a secondary function of protecting the underlying silica from interacting by means of ion exchange with strong bases that might lead to peak tailing.

This level of performance for basic compounds can also be maintained over a wide loading range. If, for example, a large amount of an active pharmaceutical ingredient is injected onto a conventional core-shell or fully porous C18 column, band broadening can result. Using the Luna Omega PS C18 column, however, results in much less band broadening and a wide range of sample loading under identical conditions.

**High pH mobile phases.** Using special stationary phases can be effective for improving retention of highly polar molecules, but mobile phase pH adjustments can also be used. Retention of analytes in reversed-phase HPLC is a function of ionization. If analytes are ionized, they are less retained. However, moving polar bases closer to their pK value can significantly improve retention and peak shape.

Conventional silica LC material, however, is not stable at the high pH range needed to neutralize bases. The siloxane bridges that link adjacent silica atoms are stable only up to about neutral pH. Above that, the silica begins to dissolve, and a column will die within about 10 injections. This problem can be overcome by replacing some of the siloxane bridges with organo-silica bridges, such as an ethane linkage. The organo-silica bridge allows for long column lifetime even at high pH.

The Kinetex EVO C18 material, which can be operated from pH 1 to pH 12, is made up of
core-shell particles in which the outer fully porous layer of silica has been replaced by organo-silica high-pH stable material.

In an analysis of nicotine metabolites (see Figure 9) under typical LC–MS conditions (i.e., water, methanol, formic acid mobile phase) and using the Kinetex 2.6 μm EVO C18 column, almost no retention occurs under acidic conditions because the analytes are ionized. Using a midrange buffer, such as ammonium acetate (pH 5), allows some peaks to emerge, although peaks 1 and 2 still elute near the front of the chromatogram. At a higher pH mobile phase, such as ammonium bicarbonate (pH 10), the components are nicely retained and well separated.

Sensitivity concerns for LC–MS analyses can also be addressed by adjusting gradient mobile phase conditions. By reducing the ionization state of the molecules through a high pH mobile phase, they stick to the column longer and elute later in the gradient profile. The molecules enter the source in a higher percent acetonitrile or methanol mobile phase, which
facilitates greater ionization and results in a better signal response.

**Orthogonal selectivity.** C18 columns are used in about 80% of analyses and should be considered first in method development. A C18 run can easily be used as a baseline and then adjustments can be made to the mobile phase or other factors depending on the results. But C18 columns don’t work for every sample. Adjustments to the stationary phase can offer distinct orthogonal selectivity that complements a C18. The Kinetex Biphenyl phase discussed earlier is very structurally different from C18. It has hydrophobic mechanisms of interaction, as well as some highly useful secondary interactions, and offers a good orthogonal selectivity.

**Figure 9B** shows the separation of two sets of steroids, prednisone/prednisolone eluting first and then cortisone/ cortisol. When a Kinetex Biphenyl phase is used (bottom), the order of the two pairs of steroids reverses. In both cases, the hydroxyl form elutes later in the run. The hydrogen-bonding capacity of the Biphenyl phase favors the hydroxyl group.
Prednisone and prednisolone have an additional double bond, whereas the cortisone and cortisol do not. Because the Biphenyl ligand is capable of pi–pi interactions with the double bond, it is more retained on the Biphenyl phase. The elution order is reversed on the C18 column because it lacks the capacity to interact by pi–pi interaction. In short, if a C18 column is not providing the needed selectivity, a Biphenyl phase is the next logical choice.

**Conclusion**

Conventionally fully porous particles and core-shell particles are the most commonly used media platforms for HPLC and UHPLC, and the new thermally modified fully porous Luna Omega material offers an additional avenue for performance gains. A fundamental understanding of the differences in morphology and performance of both thermally modified fully porous and core-shell particles allows an analyst to integrate both platforms into everyday work in a complementary fashion to solve most method development challenges.

Find more information on Core-Shell and Thermally Modified Fully Porous products here: www.phenomenex.com/kinetex and www.phenomenex.com/lunaomega

To see the full webinar, click here.
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