Advantages and Applications of Revolutionary Superficially Porous Particle Columns in Liquid Chromatography

Introduction

The current trend in liquid chromatography is towards the achievement of higher kinetic efficiency and shorter analysis time. Different types of column packings are now available for attaining very fast and high resolution separations without changing instruments, worrying about high backpressure or compromising column longevity. In recent developments of particle technology, the use of superficially porous particles has received considerable attention.

This white paper gives an overview about the theory behind the success of superficially porous particles technology and presents a summary of its latest applications.
1. What are superficially porous particles?

Superficially porous particles (also called: shell, fused-core™, core-shell™, partially porous, pellicular) are made of a solid, non-porous core surrounded by a shell of a porous material that has properties similar to those of the fully porous materials conventionally used in HPLC. The terminology of “fused-core” was introduced by Jack Kirkland. As the name implies, fused-core particles are manufactured by “fusing” a porous silica layer onto a solid silica particle (Figure 1).

The initial intent of applying superficially porous particles was for the analysis of large biomolecules. The rationale behind this concept was to improve the column efficiency by shortening the diffusion path that analyte molecules must travel and, doing so, to improve their mass transfer kinetics [1,2]. Figure 2 shows a schematic representation on the impact of the trans-particle mass transfer in superficially porous and in fully porous particles. The concept of shell-type (pellicular) particles was imagined by Horváth and co-workers in the late 60’s for the analysis of macromolecules such as peptides and proteins [3]. Shortly after, Kirkland demonstrated, that 30–40 μm diameter superficially porous packing provide much faster separations, compared with the large fully porous particles used earlier in liquid chromatography [4]. Later on, the core diameter was further reduced and the thickness of active layer was cut down to only 0.5 μm and was used for fast separation of peptides and proteins.

In recent times, the pressing needs to improve analytical throughput forced the manufacturers to find a better compromise between the demand for higher column efficiency and the need for columns that can be operated with conventional HPLC instruments (back-pressure up to 400 bar) [1,2]. In 2007, a revolution started by the commercialization of the first sub-3 μm superficially porous particle [5]. This material was made of 2.7 μm particles that consist in a 1.7 μm solid core covered with a 0.5 μm thick shell of porous silica. Up to now, this particle structure is one of the references in superficially porous particle technology since this particle morphology seems to be a good compromise between fully porous and non-porous materials. It manifests the advantages of porous and some benefits of nonporous particles. This design solved the problem of the low loading capacity of columns packed with the large, early pellicular particles because 75% of the volume of these particles is porous. Since then, several vendors commercialized different types of superficially porous particles. Now shell packing materials are commercially available in various diameters (5 μm, 4 μm, 3.6 μm, 2.7 μm, 2.6 μm, 1.7 μm and 1.3 μm), with different shell thicknesses (0.6 μm, 0.5 μm, 0.35 μm, 0.25 μm, 0.23 μm, 0.20 μm and 0.15 μm).

With this current state-of-the-art column technology, very high efficiencies (comparable to sub-2 μm fully porous particles) can be attained at moderate back pressure. Therefore, these columns packed with superficially porous particles can provide faster separations for both small and large molecules compared to columns of the same size packed with fully porous particles. The new generation of superficially porous particles became very popular in pharmaceutical, biomedical, food and environmental analysis in the last few years and allows faster and more efficient separations than was possible before.

2. Why do superficially porous materials provide better kinetic performance?

The peak dispersion in chromatography is generally characterized by the theoretical plate height (H) and the number of theoretical plates (N). The treatment of the mass transfer processes and the distribution equilibrium between the mobile and stationary phase in a column lead to equations which link the theoretical plate height to the properties of the chromatographic systems such as the linear velocity. First, Van Deemter proposed an equation, which described the column performance as a function of the linear velocity. Knox suggested a useful empirical three term equation to describe the dependency of the theoretical plate height of a column as a function of linear velocity:

$$H = Au^{1/3} + \frac{B}{u} + Cu$$

where A, B, and C are constants, determined by the magnitude of band broadening due to eddy dispersion, longitudinal diffusion, and mass transfer resistance, respectively and u is the mobile phase linear velocity. The constant A depends on the

![Figure 1. Schematic structure of superficially porous particle.](image1)

![Figure 2. Schematic representation of the expected trans-particle mass transfer of high molecular weight analyte in columns packed with (A) superficially porous particles and (B) with fully porous particles.](image2)
quality of the column packing such as (1) the homogeneity of the packed bed structure, (2) the arrangement of the particles in the wall and the central regions of the column and probably (3) on the particle size distribution. The B- and C-terms of the plate height equation depend on analyte retention. The B-term increases with analyte retention as more time is available for diffusion to take place in the stationary phase (surface diffusion). The C-term expresses the resistance to mass transfer and can be divided to trans-particle (or intra-particle) and external- (or film-) mass transfer resistance contributions. It is expected by the theory that the eddy dispersion contributions to the efficiency of columns packed with superficially porous particles would correspond to the external diameter of the particle, but the internal mass-transfer resistances and longitudinal diffusion would correspond to the thickness of the porous layer. The initial idea of preparing SPP particles was to improve the column efficiency by reducing the mass transfer resistance across the particles. However, it seems that trans-particle mass transfer resistance is far from being the dominant contribution to band broadening in HPLC [1,2]. Indeed, the columns packed with the new generation of shell particles are successful, but for other reasons [1].

According to the theory, the intra-particle diffusivity depends on the ratio of the diameter of the solid core to that of the particle in a superficially porous particle. As this ratio increases, the mass transfer kinetics becomes faster through the particles. Similarly, the external mass transfer also depends on the structure of the particles. According to some recent experimental measurements, the mass transfer kinetic is mostly accounted for by the external film mass transfer resistance across the thin layer of the mobile phase surrounding the external surface area of the particles [1]. This suggests that the initial idea of preparing shell or superficially porous particles with the purpose of increasing the column efficiency by reducing the mass transfer resistance across the particles might provide only modest practical gains for the separation of low or medium molecular weight compounds [1]. To conclude on the mass transfer resistance, approximately 2 times lower C-term is expected with current superficially porous particles than with the same size fully porous particles. The gain in the C-term is more important for large biomolecules.

On the other hand, the presence of a solid core inside the particles has a direct consequence on the longitudinal diffusion term (B-term in eq. 1) observed for a column, since it decreases this contribution to the plate height by about 20% when the ratio of the core to the particle diameter is \( \rho = 0.63 \) (Halo®, Ascentis® Express, Brownlee SPP) [1,2]. However, the reduced internal porosity of the superficially porous particles brings a limited improvement in their efficiency. Experimentally, it was implied that the solid core reduced the B-term by no more than 30% in comparison with fully porous particles [6]. As a conclusion, it can be stated that recent superficially porous particles manifest a gain of approximately 20-30% in the longitudinal diffusion. This causes only a gain of a ~10% increase in the total column efficiency compared to that of columns packed with fully porous particles.

Finally, according to several experimental results, the eddy diffusion term (A term in eq. 1) of the columns packed with superficially porous particles is significantly smaller (~30-40%) than that of the column packed with fully porous particles [1,2]. It is still unclear whether this significant improvement in efficiency is due to the narrow particle size distribution (PSD) of superficially porous particles. Some recent studies have indeed suggested that particles displaying a very narrow PSD can lead to unprecedented low minimal plate heights [1,2]. Figure 3 shows a schematic representation on the expected impact of the particle structure and particle size distribution on the eddy dispersion.

![Figure 3. Schematic representation of the eddy dispersion in columns packed with (A) superficially porous particles (narrow PSD) and with fully porous particles (wide PSD).](image)

It is however uncertain whether this finding can be purely related because there are other factors that might influence the packing quality. Superficially porous particles have indeed a higher density and some of them are rougher than fully porous particles [1,2]. This might also have had an influence on the achieved packing quality, apart from the PSD.

To conclude on the efficiency of superficially porous particles, the success of these materials in the separation of small molecules is not primarily a result of the decrease in the C-term, as it is often claimed in commercial brochures [1]. Most importantly, the exceptional performance of columns packed with superficially porous particles is probably caused by the important reduction of the eddy dispersion term.

The application of reduced parameters is common in chemical engineering to compare the performance of columns in unit operations. It is useful to convert \( H \) and \( u \) to dimensionless parameters according to the following simple formulas:

\[
h = \frac{H}{d_p} \quad 2
\]

\[
v = \frac{ud_p}{D_M} \quad 3
\]
where \( h \) is the reduced plate height, \( d_p \) the particle size of the column packing material, \( \nu \) the reduced linear velocity and \( D_M \) the analyte diffusion coefficient in the mobile phase. The particular advantage of this approach is the ability to compare the performance of columns packed with particles of different sizes or structures (morphology). Based on the above theoretical discussion, some model calculations were made to show the effect of particle structure on the expected achievable reduced plate heights. Figure 4 shows theoretical Knox type plots of fully porous particles and superficially porous particles of the same size. As expected, SPP particles (\( h_{\text{min}} \) of 1.5) significantly outperforms fully porous particles (\( h_{\text{min}} \) of 2), in terms of kinetic performance.

3. Do loadability, retention and resolution remain acceptable with superficially porous particles?

The most important differences between the characteristics of columns packed with core-shell and with porous particles are in their hold-up times (total porosity), retention factors (and therefore possibly in resolution), and their loading capacities.

The sample loading capacity and the expected retention of a given solute in liquid chromatography are proportional to the stationary phase volume. Therefore, obviously both the sample loading and retention are expected to be lower on superficially porous materials than on fully porous ones.

The volume of the porous shell surrounding a particle is \( 4\pi(R_e^3 - R_i^3)/3 \), where \( R_e \) and \( R_i \) are the radius of the particle and of its solid core, respectively. The volume fraction of the porous material of the shell in the column is \( 1 - (R_e/R_i)^3 \). For example, the shell thickness of the Brownlee SPP particles (\( R_e = 1.35 \mu m, R_i = 0.85 \mu m \)) is 0.5 \( \mu m \). The volume fraction of the porous shell in these particles is 75%. Figure 5 demonstrates the relationship between the fractional volume and the relative shell thickness [1].

The separation power of superficially porous particles increases with decreasing shell thickness if the strength of the mobile phase is decreased to compensate for the retention change caused by the decreased surface area of the stationary phase [1]. On the other hand, according to the theory, if the mobile phase remains the same, the retention factor will decrease and therefore the resolution of low molecular size compounds may also decrease. The smaller the diffusivity of the solutes, the larger the increase in the separation power is compared to that of fully porous particles. In the one hand, the theory suggests that the thickness of the porous layer should be decreased drastically in order to increase the separation efficiency of the columns for large molecular size compounds. On the other hand, there is a strict limitation in the decrease of the thickness of porous layer, as it markedly decreases the loadability of the column, making the column easily overloaded, which decreases the separation efficiency [1]. Therefore, the optimum shell thickness is likely to be a compromise between efficiency, sample loading capacity and analyte retention, and is strongly sample dependent. Overload problems are likely to be more severe for both sub-2 \( \mu m \) porous particles as well as shell particles due to the very high efficiencies produced by both types of columns.

In practice, quite similar retention factors were observed on superficially porous particles and on fully porous sub-2 \( \mu m \) particles or even on...
silica based monolith. The loadability of commercially available superficially porous materials also seems to be similar to that of sub-2 µm particles. Due to the very high kinetic efficiency of superficially porous materials, the slight possible decrease of retention factor does not adversely affect the resolution. Theoretically, with the structure of commercially available superficially porous materials, resolution should not decrease (even an increase is expected).

Probably sample loading and retention become critical when the volume fraction of the porous material is very low (e.g. < 40%), which is not the case with the current 2.7 µm SPP particles.

4. Which chromatographic system is adapted to use superficially porous particles?

As expected from the theory, columns packed with 2.7 µm superficially porous particles provide an efficiency comparable to that obtained with columns packed with totally porous sub-2 µm particles, but at reasonable operating pressure (e.g. P < 400 bar). Because superficially porous sub-3 µm packings can operate at the half or at third pressure compared to fully porous sub-2 µm particles, it is theoretically possible to operate such columns on conventional HPLC instruments. Regarding the required system pressure, it is true that only the half or the third pressure is required to operate with a column packed with 2.6 - 2.7 µm particles, compared to a column packed with 1.5 - 1.7 µm particles, in agreement with the Darcy’s law and Karman-Kozeny equation.

In generic conditions, when using acetonitrile-water mobile phase, there is no need for more than 400 bar pressure with columns packed with 2.7 µm particles. However, higher pressure capability may be useful when using more viscous organic modifiers in the mobile phase such as methanol and isopropanol in order to tune the selectivity and/or for sustainable purposes. The maximum viscosity of acetonitrile-water mixture is around 1 cP at 25 °C, while the viscosity of methanol-water and isopropanol-water mixtures can reach 1.6 and 2.9 cP at 25 °C, respectively. The nominal mechanical stability of 2.7 µm superficially porous columns is 600 bar, while the pressure capability of conventional HPLC systems is 400 bar. To sum up, the pressure capability of conventional HPLC systems seems to be appropriate with columns of 2.7 µm superficially porous particles but all the benefits of these phases cannot be attained. Indeed, operating these columns at a pressure higher than 400 bar can provide faster separations but also allows for the use of alternative solvents.

In terms of system volume and variance, the conventional HPLC systems are not able to maintain the high efficiency of small columns packed with 2.7 µm superficially porous particles. The commercially available LC systems can be classified into three groups, (1) optimized systems for fast separation with very low dispersion (σ²ec < 10 µL²), (2) hybrid LC systems recommended by the vendors for both fast and conventional separations (σ²ec =10-50 µL²), and (3) conventional LC systems with an extra column variance over 50 µL² [2]. The extra-column peak variances of several commercially available instruments were reported in the literature. As expected, the difference between system variances of current instruments could be quite important. Some model calculations were performed to illustrate its importance, which are based on experimental column efficiency data observed with columns packed with superficially porous 2.7 µm packing. Figure 6 shows the effect of extra-column variance on the remaining column efficiency for 50 x 2.1 mm, 50 x 3 mm and 100 x 4.6 mm columns (moderate retention of k = 5 is assumed).

Clearly, when using the standard bore column (4.6 mm), no significant impact on the column efficiency is observed. This column dimension can be used with almost any conventional HPLC systems without important loss in efficiency. When working with 3 mm I.D. columns, significant amounts of the intrinsic column plate number can be lost when this column is operated with conventional systems (e.g., ~50% loss at 100 µL² system variance). It becomes even more critical with narrow bore columns of 2.1 mm I.D. These small, very efficient columns can only be used on dedicated UHPLC systems. Only 75% of the intrinsic column efficiency can be achieved when working with a system having a 10 µL² variance.

To benefit from the advantages of these very efficient columns of 2.1 and 3 mm I.D., optimized systems are mandatory. The HPLC system optimization means the reduction of all the possible non-desired peak dispersions. It is recommended replacing (1) the injector loop with a lower volume loop, (2) the standard detector flow cell with a low volume one, and (3) the standard tubing with ≤ 0.005” I.D. tubing. As additional suggestions, the column switching valves, if there are any,
should be bypassed and the detector sampling rate and time constant should be optimized.

In a systematic study, it was shown that the combination of several changes in the system or the method allowed for the achievement of the full potential of superficially porous phases using a conventional LC system [7]. The first modification was the reduction of the extra-column volume of the instrument, without increasing its back pressure contribution too much, by changing the needle seat volume, the inner diameter and the length of the capillary connectors, as well as the detector cell volume of a standard conventional HPLC instrument. The second adjustment consisted of injecting a volume of weak eluent (less than half the elution strength of the mobile phase) right after the sample. Experimental results showed that these changes could provide most of the efficiency expected from the true column performance. After applying these changes, the resolution of the 50 x 2.1 mm, 50 x 4.6 mm, and 100 x 4.6 mm columns for compounds having retention factors close to 1 (worst case) were increased by about 180, 35, and 30%, respectively.

If gradient mode has to be employed, the dwell volume should also be considered. The system dwell volume could become critical when performing fast analysis with short narrow bore columns. Conventional HPLC systems possess 0.5 – 5 mL dwell volumes which is clearly unacceptable for fast gradient separations. Similar to extra-column band dispersion, the dwell volume of the system has to be also minimized. However, adjusting the dwell volume of a low-pressure mixing system is not as simple as changing a detector cell or a tubing. When small dwell volume is required, high-pressure mixing systems are preferred. Several vendors offer different volume mixers for their instruments to select the most appropriate mixing volume for the required flow rate (column dimension). Conventional HPLC systems with low pressure mixing systems are only acceptable for standard bore columns.

5. What type of applications should superficially porous particle technology be used for?

As previously discussed, superficially porous particle technology is simply an extension of regular HPLC, allowing improved kinetic performance (faster separation or higher resolution) through the modification of particle morphology. Because the mobile phase nature and stationary phase chemistry remain identical with superficially porous particles, the fields of application are similar to that of regular HPLC.

As shown in Figure 7, superficially porous materials have been successfully applied in many different fields of application during the last few years, including the following:

- Qualitative and/or quantitative determination of drugs and metabolites in biological fluids (e.g. oral fluid, human plasma, blood, urine).
- Pharmaceutical applications (e.g. impurity profiling, stability-indicating assay, cleaning validation, quality control).
- Characterization of large biomolecules under their intact or digested form (e.g. peptide mapping).
- Analysis of plant extracts (e.g. quality control of plant samples, profiling and fingerprinting for comparison of plant species, dereplication, metabolomics. See Figure 8b for example of separation of Ginsenosides on a PerkinElmer Brownlee SPP column).
- Determination of various types of contaminants (e.g. drugs, pesticides, herbicides) in environmental matrices (surface water, ground water, waste water).

- Quantitative determination of residues (e.g. mycotoxins, perfluorinated chemicals, pesticides, herbicides, veterinary drugs) in food samples (fruits, vegetables, eggs, fish, meat, honey, palm oil).

All these applications prove that superficially porous particles can now be considered as a mature strategy, compatible with simple and complex matrices. In addition, depending on the goal and the complexity of the sample, either faster separation or higher resolution can be attained thanks to the advantageous particle morphology. When coupling superficially porous columns with MS detection, it is recommended to use state-of-the-art devices since the acquisition rate may be insufficient for the narrow peaks produced by the column. This behaviour is illustrated in Figure 8a showing the determination of 151 pesticides in food sample [8]. In this example, time-scheduled MRM was required to reach a sufficient number of data points across the peaks.

6. What are the new and next developments of superficially porous particles?

Due to the success of C18 columns packed with superficially porous particles of 2.7 µm introduced in 2007, numerous additional solutions have been proposed by providers to extend the possibilities offered by superficially porous materials. There are three directions followed by providers to further develop superficially porous material, namely i) reduce or increase the particle size of original phases, ii) preparation of widepore phases for biomolecules analysis, and iii) extend the chemistries for RPLC, but also for HILIC conditions.

6.1. Reducing or increasing the particle size

Similar to what is observed with fully porous particles, the reduction of particle size leads to a decrease of band broadening (improvement of kinetic efficiency). In addition, as the mobile phase flow rate is increased faster separations are achieved. For this reason, some providers now offer columns packed with superficially porous 1.7 and even 1.3 µm particles (the latter has only been available since 2013) for ultra-fast separations. However, since back pressure is inversely proportional to the square of particle size, these stationary phases packed with sub-2 µm particles are not appropriate for use with conventional HPLC instrumentation. In addition, it has been demonstrated that such columns were less adapted to achieve very high resolution (N > 30000 plates), compared to the regular columns packed with 2.7 µm superficially porous particles. Last but not least, packing columns with sub-2 µm particles is much more difficult and challenging than with 2.7 µm particles so the efficiency achieved with these
innovative phases is often lower than theoretical expectations. From our point of view, the main interest of superficially porous technology lies in the possibility to have kinetic performance close to that of columns packed with fully porous sub-2 µm particles without the high backpressure constraints. Using superficially porous sub-2 µm particles invalidates this statement since such particles require powerful UHPLC instrumentation.

Alternatively, some columns packed with superficially porous particles in the range of 4 - 5 µm have been commercially available from multiple suppliers since 2012. Depending on the size of the particle, performance is close to or better than that of columns packed with porous 3 µm particles. It is important to notice that larger superficially porous particles can be packed with even greater bed homogeneity than the already excellent homogeneity of beds packed with 2.7 µm superficially porous particles [9]. A separation of 11 phenolic compounds is shown in Figure 9. It compares the performance attained from a regular column packed with fully porous 5 µm particles and a column packed with 5 µm superficially porous particles. As expected, the peaks have a smaller volume (sharper peaks) on the superficially porous column corresponding to higher resolution and peak capacity.

An additional advantage of using large particle size is related to the reasonable backpressure generated. These new 4 – 5 µm superficially porous particles can easily be employed on any conventional HPLC systems, as the pressure always remains systematically far below the upper limit of 400 bar.

6.2. Increasing the pore size of superficially porous materials

The first superficially porous particles were originally developed by Horvath and Kirkland to improve the performance of RPLC with large biomolecules (i.e. peptides, proteins, monoclonal antibodies) by reducing the diffusion pathlength (improving the C-term in eq. 1). During the last few years, these innovative phases were mostly employed for the analysis of small molecules. However, some providers recently developed and commercialized superficially porous particles possessing pore sizes in the range 150 – 400 Å. It is important to consider that analyte diffusion in the pores significantly slows down as the pore size becomes smaller than approximately 10-fold the size (hydrodynamic diameter) of the analyzed compound. Therefore, small peptides can be analyzed with particles possessing conventional pore size (90 - 120 Å), but larger porous packing materials (up to 400 Å) are beneficial for the analysis of proteins or monoclonal antibodies.

Figure 10 demonstrates the possibility to analyze peptides on various types of regular superficially porous particles and also confirm that the peak capacities are very close to that of a UHPLC column packed with 1.7 µm fully porous particles [10]. As expected, the backpressure was lower with superficially porous sub-3 µm particles vs. fully porous sub-2 µm particles.

6.3. Extending stationary phase chemistries for RPLC and HILIC modes

Since the commercialization of the first C18 columns packed with superficially porous particles of 2.7 µm, there have been some significant progresses made by suppliers to extend the range of available chemistries. Indeed, during the initial screening procedure of RPLC method development, it is recommended to test at least 4 - 5 different stationary phase chemistries and various mobile phase conditions. Thanks to this fast procedure, the highest selectivity can be attained. In addition, depending on the physico-chemical properties of the analyzed substances, some particular compounds may be not properly retained on a C18 phase. For all these reasons, some alternative phases are required. Today, the superficially porous RPLC technology is mature and there are many companies that commercialize superficially porous type particles bonded with C30, C18, C8, C4, C3, phenyl-hexyl, pentafluorophenyl (PFP), phenyl, C18 with embedded polar group, and C18 with polar endcapping. All these types of phase can be characterized and appear to be very different in terms of hydrophobic selectivity, cationic exchange properties, hydrogen bonding capacity or shape selectivity, which may be beneficial for method development.

Hydrophilic interaction liquid chromatography (HILIC) is a chromatographic mode that has gained in importance during the last decade, due to its possibility to increase the retention of polar and hydrophilic compounds, achieve orthogonal selectivity and increase MS sensitivity, compared to reversed phase liquid chromatography (RPLC). The number of superficially porous polar chromatographic surfaces that can be employed for HILIC experiments is growing in importance and today, bare silica and amide are commercially available for HILIC operation. It is also worth mentioning that these polar phases may also be considered for normal phase liquid chromatography (NPLC) and supercritical fluid chromatography (SFC).

6.4. Some perspectives with superficially porous technology

Because of the interest in superficially porous particle technology from the separation science community, some attempts have been made to develop chiral stationary phase (not yet commercially available) as well as preparative columns (offered since 2013) made with superficially porous particles of 2.7 and 5 µm. Despite some promising results related to the high efficiency produced by these particles, the choices in terms of column chemistries and dimensions are still too limited to be employed at a larger scale.

As previously discussed, columns packed with superficially porous 2.7 µm particles possess an upper pressure limit of 600 bar. It has been recently demonstrated that better separation could be achieved if those columns would be compatible with pressures up to 1200 bar. This is particularly true in the case of high resolution separations requiring several superficially porous columns coupled in series.
7. Conclusion about the superficially porous particle technology

The most remarkable progress in column technology from the last years came with the introduction of the 2.7 µm superficially porous particles and more recently its 4 - 5 µm version. In conventional HPLC, the main motivation to transfer method from regular 5 µm fully porous particles to the 4 - 5 µm superficially porous materials is related to the improvement of kinetic performance that can be achieved (faster separation and higher resolution) and its compatibility with conventional HPLC systems. In the case of ultra high performance liquid chromatography (UHPLC), it could be of interest to consider columns packed with 2.7 µm superficially porous particles instead of sub-2 µm fully porous particles, since the kinetic performance (analysis time and efficiency) remains quite close between these two approaches, but the backpressure is reduced by a factor 2 to 3, for a similar column length and flow rate. Besides the kinetic performance, it is also important to notice that the selectivity, retention, loading capacity and peak shape achieved with the superficially porous technology were comparable to that obtained with fully porous particles. In addition, there is today a wide range of chemistries both for RPLC and HILIC modes, which makes the technique more attractive.

One of the main constraints observed with sub-3 µm superficially porous particles is instrumental. Indeed, conventional HPLC systems may be hardly compatible with such highly efficient stationary phases, due to a large extra-column volume, important dwell volume and limited upper pressure limit. To avoid these issues, it is possible either to improve the performance of the available HPLC instrument (by reducing tubing length and diameter, changing mixing chamber), or to use columns of 3 or 4.6 mm I.D., at a temperature of 40-50 °C and exclusively with acetonitrile as organic modifier. Alternatively, the full performance of these innovative phases of 2.1 mm I.D. columns can easily be attained on a modern UHPLC system.

Finally, if the readers wish to obtain additional information about the superficially porous particle technology, there are two interesting and up-to-date review papers [1,2].

References