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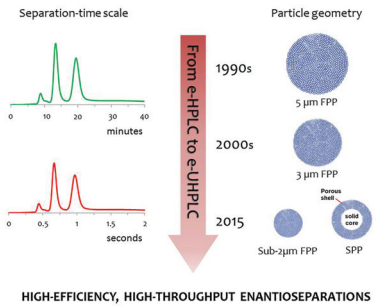

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Analyst

CRITICAL REVIEW

Recent advancements and future directions of superficially porous chiral stationary phases for ultrafast high-performance enantioseparations

Martina Catani,[a] Omar H. Ismail,[b] Francesco Gasparrini,[b] Michela Antonelli,[b] Luisa Pasti,[a] Nicola Marchetti,[a] Simona Felletti[a] and Alberto Cavazzini*[a]

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This review focuses on the use of superficially porous particles (SPPs) as chiral stationary phases for ultra-high performance liquid enantioseparations. In contrast to what happened in achiral separations where core–shell particles invaded the market, the introduction of SPPs in chiral liquid chromatography (LC) has been relatively recent. This is due in part to the technical difficulties in the preparation of these phases, and in part to scarce understanding of mass transfer phenomena in chiral chromatography. As a matter of fact, nowadays, the development of superficially porous CSPs is still in its infancy. This paper covers the most recent advancements in the field of core–shell technology applied to chiral separations. We review the kinds of chiral selectors that have been used for the preparation of these phases, by discussing the advantages of chiral SPPs over their fully-porous counterparts for high efficient high throughput enantioseparations. Notwithstanding the apparently obvious advantages in terms of the mass transfer of chiral SPPs, some critical aspects that could impact their development are presented.

1. Introduction

In 2006 Kirkland introduced the so-called second generation superficially porous particles (SPPs),[1,2] also referred to as core–shell, solid–core, Fused-Core™ or pellicular particles. Prepared by a proprietary nanoparticle technology, these 2.7 μm C_{18}

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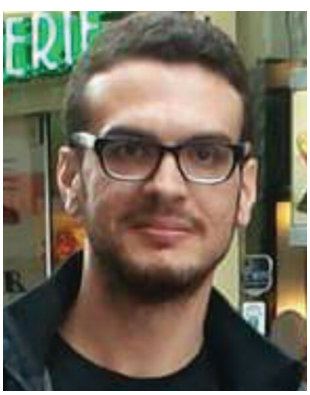
Martina Catani

Martina Catani was born in 1989. She received a master's degree in Chemistry in 2013 from the University of Ferrara. Since 2014 she has been a PhD student at the same university under the supervision of Prof. Alberto Cavazzini. Her research activity focuses around the investigation of the thermodynamics and the kinetics of separations in liquid chromatography with different kinds of stationary phases, including C_{18}, perfluorinated and chiral ones. During her PhD program, she spent periods of study in the groups of Prof. Gert Desmet (Brussels) and Prof. Attila Felinger (Pécs).



Omar H. Ismail

Omar H. Ismail was born in 1989. He has a master's degree in Medicinal Chemistry from the Sapienza University of Rome, and, currently, is a PhD student in Pharmaceutical Sciences at the same university. His scientific studies are focused on the theoretical evaluation of the new generation of sub-2 μm C_{18} columns in RP-ultra-high performance chromatography (RP-UHPLC). In addition, his research includes the development of sub-2 μm chiral stationary phases, using Pirkle/brush-type selectors, starting from the optimization of the synthetic strategies to the kinetic/thermodynamic evaluation of the columns packed with those CSPs. He is the co-author of 10 publications in peer-reviewed international scientific journals.

spherical particles were made of a 1.7 μm solid core surrounded by a 0.5 μm porous shell. The advantage of a porous zone occupying roughly three-quarters of the total volume of the particle is that it allows for a higher loading than the first generation core–shell particles developed in the late 1960s, which were made of a 50 μm solid core surrounded by a porous layer of only 1–2 μm.[3,4] Second generation core–shell particles have represented a breakthrough innovation into the market of chromatographic columns, providing efficiencies very similar to those of columns packed with 1.7 μm spherical fully porous particles but at a significantly lower back-pressure.[5–8] Since their introduction, a very large number of core–shell particles have been commercialized by different manufacturers with specific processes of preparation, surface chemistries and functionalization.[9–13]

The employment of SPPs in chiral liquid chromatography (LC) is more recent.[14–16] To the best of our knowledge, the first report about the use of chiral SPPs in LC is by Lindner's group in 2011.[17] They reported about the enantioseparation of amide type amino acid derivatives on a cinchona alkaloid-based anion exchanger CSP prepared on 2.7 μm fused-core particles. In this study, however, not much emphasis was given to the novelty of the CSP, nor to the advantages of the core–shell technology for efficient chiral separations.

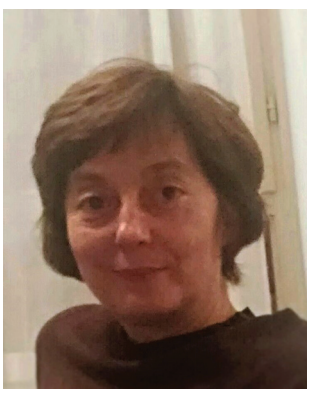
Chankvetadze and his group[18] were the first to investigate the characteristics of a pellicular CSP from a fundamental viewpoint. They used a polysaccharide-based CSP obtained by coating 2.6 μm pellicular particles. Following these authors, the principal advantages in using chiral SPPs compared to (chiral) fully porous particles (FPPs) lie in a higher enantioselectivity at a comparable content of the chiral selector, a limited dependence of the plate height on the mobile phase flow rate and a larger enantioresolution per analysis time.[18,19]

The most comprehensive work aimed at evaluating the performance of chiral SPPs for high-efficiency and high-throughput enantioseparations has been done by Armstrong and coworkers.[20–23] They have studied a wide variety of bonded brush-type CSPs prepared on 2.7 μm SPPs, including cyclofructan-6 based, β-cyclodextrin and macrocyclic antibiotics (among which are, in particular, teicoplanin, teicoplanin aglycone and vancomycin).[20] The emerging concept from these studies is that chiral SPPs outperform, in terms of kinetic performance, their FPP counterparts practically in all modes of chromatography, *i.e.*, reversed-phase (RP), normal phase (NP), polar organic and HILIC.[20–24] Thanks to the employment of very short columns (5 mm) packed with chiral SPPs operated at high flow rates, Armstrong and colleagues have very recently obtained striking results in the field of ultrafast chiral chromatography. By carefully reducing the extra-column volume of the equipment used in their measurements, they have indeed performed the sub-second separation of several enantiomers on various stationary phases (quinine- and teicoplanin-based) and under a variety of chromatographic modes.[25,26]

Looking at these extraordinary results, it would seem difficult to think about different approaches to achieve ultrafast chiral separations *via* LC. However, at the same time as Armstrong's group, Ismail *et al.*[27] published the first example of a sub-second enantioseparation performed on chiral FPPs. In particular, they report about the separation of *trans*-stilbene oxide enantiomers on a 10 × 3.0 mm column packed with 1.8 μm Pirkle-type Whelk-O1 FPPs in 0.9 seconds (retention factor of the more retained enantiomer is about 1.7). Furthermore, in this study, some of the above-mentioned advantages theoretically provided by SPPs over FPPs towards ultrafast chiral chromatography have been challenged. Essentially, on the one hand, these authors point out about

Francesco Gasparrini

Francesco Gasparrini, born in 1945, earned a Laurea in Chemistry in 1969 (University of Camerino). He has been a Professor of Organic Chemistry since 1986 at the Sapienza University of Rome; he was the Co-Chairman of the 2nd ISCD (1991) and of the 16th ISCD (2005). Awards: from the Italian Chemical Society (2001, "Structure Determination and Molecular Interactions" and 2011, award in the memory of Prof. Piero Pino); from the Analytical Chemistry Division of the Italian Chemical Society (2013, Arnaldo Liberti medal). He is the author of ca. *210 peer-reviewed papers and 15 patents (3 new CSPs are commercially available: DACH-DNB by Regis, USA; Chirobiotig-TAG and P-CAP by Sigma Aldrich, USA).*



Luisa Pasti

Luisa Pasti was born in 1964, and she has a master's degree in Chemistry and a PhD in Chemical Sciences from the University of Ferrara. She was a research fellow at the Vrije Universiteit Brussel, in the group of Prof. Désiré L. Massart from 1996 to 1998. She was employed in the Enichem Research Center. In 2007 she became an Assistant Professor at the University of Ferrara, until 2014 when she became an Associate Professor of Analytical Chemistry. Her research is concerned with both the theoretical and experimental development of chromatography and chromatography-like techniques. Practical applications of this research include the characterization of microporous and mesoporous materials.

the difficulty of achieving highly efficient packed beds by a slurry packing of polar SPPs (such as the chiral ones). On the other hand, they mention the importance of a deeper investigation of if and how the kinetics of adsorption–desorption depends on the surface density of chiral ligands. Not only could this be very important to understand how the resolution, selectivity and loading of chiral ligands are connected, but it is also fundamental to compare the performance of chiral SPPs and FPPs of similar particle sizes. The chemical functionalization of these particles (even if performed under identical experimental conditions) has been indeed shown to lead to different results in terms of the surface density of the chiral selector.[21,24,27] Following these authors, the assumption that chiral SPPs are the only support suitable to prepare chiral columns for ultrafast enantioseparations is therefore possibly premature.

The scope of this review is to provide an overview of the most important achievements in the field of fast and ultrafast chiral separations permitted by the use of core–shell technology. In doing so, the different CSPs that have been prepared in a pellicular format have been described; the fundamentals of mass transfer in chiral chromatography have been discussed; a critical comparison of the pros and cons of chiral SPPs and FPPs for ultrafast enantioseparations has been proposed by focussing on some critical aspects that, in our opinion, need to be further investigated for the successful implementation of core–shell technology in chiral separations.

2. Mass transfer in chiral chromatography

In this section, the fundamentals of mass transfer in chiral chromatography are shortly summarized. The equation from which this discussion starts from is the well-known van Deemter equation,[28] which correlates the height equivalent to a theoretical plate, H (or its adimensional form, $h = H/d_p$, where d_p is the particle diameter) to the mobile phase velocity. Since there is no flow inside the mesoporous silica employed in LC, the right velocity to refer to is the interstitial velocity, u_e, *i.e.* the velocity of the mobile phase moving between particles:[29]

$$u_e = \frac{F_v}{\pi {r_c}^2 \varepsilon_e} \quad (1)$$

or, in reduced coordinates:

$$\nu = \frac{u_e d_p}{D_m}. \quad (2)$$

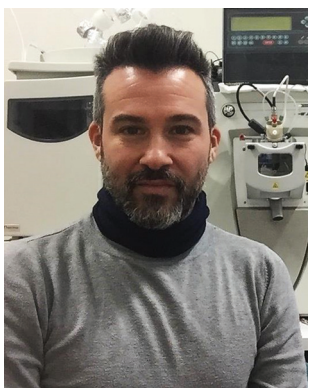
In eqn. (1) and (2), F_v is the flow rate, r_c the inner column radius, D_m the bulk molecular diffusion coefficient and ε_e the external column porosity, defined as:

$$\varepsilon_e = \frac{V_e}{V_{col}} \quad (3)$$

with V_{col} and V_e, respectively, the geometric and the external volume of the column. V_e can be determined, *e.g.*, through inverse size exclusion chromatography (ISEC) or pore blocking.[28,30–32] Under the hypothesis that the different mass transfer phenomena are independent of each other, the van Deemter equation, in reduced coordinates, is written as:

$$h = a(\nu) + \frac{b}{\nu} + c_s\nu + c_{ads}\nu + h_{heat} \quad (4)$$

where $a(\nu)$ is the eddy dispersion, b represents the longitudinal diffusion term, c_s is the mass transfer resistance across the stationary phase and c_{ads} is a term accounting for slow adsorption–desorption kinetics. This term is usually omitted in achiral RP LC, owing to the very fast adsorption–desorption process under these conditions (unless the separation of very



Nicola Marchetti

Nicola Marchetti was born in 1976. He has a master's degree in Chemistry and received his PhD in Chemical Sciences from the University of Ferrara in 2005. He was a post-doctoral research fellow at the University of Tennessee (Knoxville, TN, USA) from 2006 to 2008, in the group of Prof. Georges Guiochon. Then he returned to Italy and was appointed as a fixed-term Assistant Professor at the University of Ferrara from 2010. Now, he is a senior temporary Assistant Professor and his research activities focus on LC-MS and sample extraction, particularly the qualitative–quantitative analytical characterization of complex matrices of environmental, biological and food origin.

Alberto Cavazzini

Alberto Cavazzini (1970) has a master's degree in Chemistry and received his PhD in Chemical Sciences from the University of Ferrara in 2000. He was a research fellow at the University of Tennessee (Knoxville, TN, USA) and Oak Ridge National Laboratories (Oak Ridge, TN, USA) from 2000 to 2002, in the group of Prof. Georges Guiochon. In 2002 he returned to Italy after accepting an Assistant Professor position at the University of Ferrara, which he held until 2014 when he became a Professor of Analytical Chemistry. His research activities focus on separation science, particularly on liquid chromatography and chromatographic-like techniques.

large molecules, such as proteins, is considered). In chiral LC, on the other hand, the adsorption–desorption kinetics can be significantly slow also for low molecular-weight compounds and, particularly, for the second eluted enantiomer.[33] The term h_{heat} in eqn (4) accounts for the frictional heating due to the stream of the mobile phase against the bed under significant pressure. This contribution must be considered with columns packed with very fine particles, irrespective of whether they are chiral or achiral.[20,23,31,34]

The study of mass transfer in porous media has tremendously advanced in the last few years. Nowadays an accurate and independent evaluation of the individual factors contributing to peak broadening in LC is possible.[28,35–39] Conversely, the approach based on the nonlinear fitting of the experimental h data collected at different flow rates – traditionally employed for the estimation of van Deemter's equation coefficients – is to be avoided leading to parameters that are not physically meaningful.[40]

The longitudinal (or axial) diffusion term describes the band broadening due to the relaxation of the axial concentration gradient through the porous particles and the interstitial volume, in the absence of a flow. Since this is the only contribution to the band broadening when the flow is switched off, it is the best estimated through peak parking experiments. These consist of: (1) taking at a constant, arbitrary linear velocity as a sample zone somewhere in the middle of the chromatographic column; (2) suddenly stopping the flow; (3) leaving the band free to diffuse during a certain parking time, t_p; (4) resuming the flow rate to move the band out of the column. The variance (in length units) of the eluted peak, ${\sigma_x}^2$, is measured (${\sigma_x}^2 = L^2/N$, where L is the column length and N the number of theoretical plates) and the procedure is repeated (keeping the flow rate constant) for different parking times. The slope of the ${\sigma_x}^2$ *vs.* t_p plot gives an estimate of the D_{eff}, being:[34,41]

$$D_{eff} = \frac{1}{2}\frac{\Delta {\sigma_x}^2}{\Delta t_p}. \quad (5)$$

Through D_{eff}, the longitudinal diffusion term can be calculated. In reduced coordinates, it is:

$$b = 2(1 + k_1)\frac{D_{eff}}{D_m} = 2(1 + k_1)\gamma_{eff} \quad (6)$$

where γ_{eff} (= D_{eff}/D_m) is the dimensionless effective diffusion coefficient and k_1 is the zone retention factor, defined as:

$$k_1 = \frac{t_R - t_e}{t_e} \quad (7)$$

t_R being the retention time and t_e is the time spent by a species molecule in the interstitial volume. By invoking the ergodic hypothesis,[42–44] it is straightforward to show that:

$$k_1 \equiv \frac{n_{part}}{n_e} = \frac{1 - \varepsilon_e}{\varepsilon_e}\left[\varepsilon_p + (1 - \varepsilon_p)K_a\right](1 - \rho^3) \quad (8)$$

where n_{part} and n_e represent the number of molecules in the particle volume and in the interstitial volume, respectively,

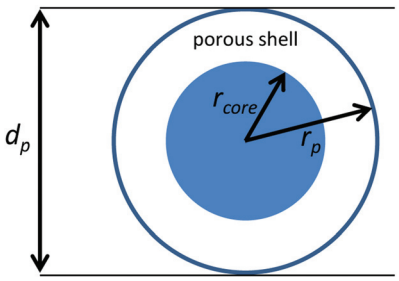


Fig. 1 Structure of a core–shell particle. d_p: particle diameter; r_p: particle radius; r_{core}: inaccessible core radius.

K_a is the distribution coefficient (equilibrium constant) of the sample between the porous zone and the eluent (see Fig. 1), $\rho = r_{core}/r_p$ is the ratio between the radius of the core and that of the whole particle (ρ is thus 0 for fully porous particles and 1 for non-porous ones) and ε_p is the particle porosity, *i.e.* the fraction of the particle volume that is occupied by pores:

$$\varepsilon_p = \frac{V_{pores}}{V_{part}} \quad (9)$$

V_{pores} and V_{part} being the pore and the particle volume, respectively. For core–shell particles, ε_p can be calculated as:[45]

$$\varepsilon_p = \frac{\varepsilon_t - \varepsilon_e}{(1 - \varepsilon_e)(1 - \rho^3)} \quad (10)$$

where ε_{tot} (= V_0/V_{col}, being V_0 the void volume) is the total column porosity.[46]

Finally, k_1 is connected to the more often employed phase retention factor, k(= $\frac{t_R - t_0}{t_0}$, t_0 being the void time), *via*:

$$k_1 = \frac{(1 + k)\varepsilon_{tot}}{\varepsilon_e} - 1. \quad (11)$$

Eqn (11) directly originates from the fact that the migration velocity of a retained component, u_R, can be referred to as either the migration velocity of an unretained compound, u_0, or as the interstitial velocity, *i.e.*:[47]

$$u_R = \frac{u_0}{1 + k} = \frac{u_e}{1 + k_1}. \quad (12)$$

The c_s term appearing in eqn (4) describes the solid–liquid mass transfer resistance due to the diffusion across the particle. Since there is an absence of flow inside the particles, this term is velocity-independent, which makes it easier to establish a theoretically-sound expression for this contribution. Following Kaczmarski,[48] for superficially porous spherical particles, this term can be written as:

$$c_s = \frac{1}{30}\frac{\varepsilon_e}{1 - \varepsilon_e}\left[\frac{k_1}{1 + k_1}\right]^2 \frac{1 + 2\rho + 3\rho^2 - \rho^3 - 5\rho^4}{(1 + \rho + \rho^2)^2} \frac{D_m}{D_{pz}\left(\varepsilon_p + (1 - \varepsilon_p)K_a\right)} \quad (13)$$

where D_{pz} is the diffusion coefficient in the porous zone, which can be estimated from D_{eff}, once a model of diffusion

through the porous medium has been defined.[28,35,36] For instance, in the simplest case of the so-called parallel or time-averaged model proposed by Knox (where all mass fluxes inside and outside the particle are considered additives),[49] D_{pz} is simply given by:

$$D_{pz} = \frac{(1 + k_1)D_{eff} - \gamma_e D_m}{k_1} \quad (14)$$

γ_e being the so-called obstructive geometrical factor. For a randomly packed column of impermeable spheres with a porosity of about 0.4, γ_e is approximatively 0.65 (ref. 50) (otherwise γ_e can be experimentally estimated through pore blocking[30]).

The expression of the term associated with a slow adsorption–desorption kinetics obtained by the Laplace transformation of the general rate model of chromatography,[45,51] is written in the case of superficially porous particles:[48,52,53]

$$c_{ads} = 2\frac{\varepsilon_e}{1-\varepsilon_e}\frac{1}{1-\varepsilon_p}\frac{1}{1-\rho^3}\left(\frac{k_1}{1+k_1}\right)^2\left(\frac{k_p}{1+k_p}\right)^2\frac{D_m}{k_{ads}{d_p}^2} \quad (15)$$

where k_p is:

$$k_p = \frac{1-\varepsilon_p}{\varepsilon_p}K_a \quad (16)$$

and k_{ads} is the kinetic adsorption constant. eqn (15) reveals that the calculation of c_{ads} requires the independent estimation of k_{ads} that – as will be discussed in the following – makes the estimation of this term *via* LC nontrivial.

The eddy dispersion term, $a(\nu)$ in eqn (4), is caused by the erratic flow profile in the through-pores of the packed bed. It includes *trans*-channel eddy dispersion, short-range inter-channel eddy dispersion, and *trans*-column eddy dispersion. Despite the fundamental work of Giddings that culminated in the well-known coupling theory,[42] there is still considerable debate in the literature regarding the values of the geometrical parameters needed to describe the complex structures of packed beds. Much work in this direction has been done by Tallarek and coworkers, who proposed a sophisticated approach based on the morphological reconstruction of the stationary phase structure and the calculation of the transport properties in the reconstructed materials.[38,39,54] In achiral systems, where the contribution of c_{ads} is negligible, the experimental estimation of $a(\nu)$ can be achieved by subtracting, from accurately measured h values (eqn (4)), both the longitudinal diffusion and the mass transfer terms (estimated, respectively, by eqn (6) and (13)).[28] In chiral systems, in contrast, this approach cannot be pursued since c_{ads} cannot be neglected. By ignoring frictional heating, indeed, the subtraction of b and c_s terms from h values, leads to

$$a(\nu) + c_{ads}\nu = h - \frac{b}{\nu} - c_s\nu \quad (17)$$

showing that an independent evaluation of the $a(\nu)$ and c_{ads} terms is not possible with this approach. Either $a(\nu)$ or c_{ads} must be estimated by different routes. As it was mentioned before, $a(\nu)$ can be quantified by the theoretical estimation of *trans*-channel, short-range, inter-channel and *trans*-column eddy dispersions.[33] Otherwise, $a(\nu)$ could be measured by employing achiral compounds eluted on the chiral column under investigation. Both approaches have some limitations. In the former case, the theoretical estimation of single terms of eddy dispersion, and thus $a(\nu)$, is difficult to assess. In the second case, one assumes that the eddy dispersion for achiral compounds is the same as for chiral ones, which could not be even in the case when they have similar retention factors.[55]

On the other hand, the determination of c_{ads} could be made by the microscopic model of chromatography, such as the so-called stochastic theory of chromatography.[42–44] This model focuses on the behavior of a single molecule during its chromatographic migration through the column. This erratic process is described as the sum of a random number of (random) events corresponding to visits in the stationary phase and movements in the mobile phase between two successive adsorptions. Accordingly, the time spent by a molecule inside the column is the sum of the times spent by the molecule in the stationary phase and those elapsed in the mobile phase between two successive adsorptions.[44,56–60] From the analysis of the peak shape, the stochastic model allows for the estimation of both the average adsorption time, τ_s, and the flying time, as well as of the number of adsorption–desorption steps. Thus, from the average adsorption time, the estimation of k_{ads} is possible, as follows:[42]

$$k_{ads} = \frac{1}{\tau_s}. \quad (18)$$

The difficulty of accurately measuring the adsorption–desorption kinetics has definitely slowed down the developments of high-efficiency, high-throughput enantioseparations, independent of whether core–shell or fully porous particles are employed.

3. Advantages and drawbacks of core–shell and fully-porous chiral particles for ultrafast high-efficiency enantioseparations

It is well known that core–shell particles offer some important advantages to speed up mass transfer compared to FPPs. The contributions to band broadening coming from both longitudinal diffusion (b-term of the van Deemter equation) and solid–liquid mass transfer resistance (c_s-term of the van Deemter equation) are indeed reduced by the presence of the inaccessible core. But, possibly, an even more important advantage of SPPs is that packed beds made of these particles are claimed to be more efficient than those packed with FPPs, even if admittedly this has been so far demonstrated only for hydrophobic C_{18} SPPs. It turned out that indeed columns packed with C_{18} SPPs are extremely efficient owing to their very low eddy dispersion.[32,34,61] Granted that the explanation of

this remains to a large extent unknown, the most accepted hypothesis is that the roughness of the C_{18} core–shell particles limits particle slipping after the release of the high pressure employed for the preparation of the packed bed by slurry-packing. This should basically reduce the bed heterogeneity in the radial direction and thus $a(\nu)$.[9,11]

The design and preparation of chiral SPPs has reflected the aim of exploiting the above mentioned advantages also in the field of chiral separations *via* LC. Recently, the proof-of-concept demonstration of ultrafast chiral separations on chiral SPPs was presented by Armstrong's group. In a series of publications, Armstrong and coworkers described several examples of subsecond enantioseparations performed on core–shell based CSPs.[20–23]

For all these reasons, core–shell CSPs have been considered the best candidate for the transition from traditional chiral-high performance LC (HPLC) to fast or ultrafast chiral ultra-high performance LC (UHPLC).

In spite of these very promising results, some of the authors of this review[27] have recently pointed out that to draw a definitive conclusion on whether SPPs are the only (or, possibly, the best) option towards the realization of high-efficiency, high-throughput CSPs, a deeper investigation of some aspects is necessary. In their study, Ismail *et al.*[27] compared the kinetic behavior of Whelk-O1 CSPs prepared on 2.6 μm core–shell particles and on both 2.5 and 1.8 μm FPPs. Two critical issues were identified. The first is about the experimental difficulty in the preparation, through high-pressure slurry packing, of efficient packed beds made of polar SPPs (in their case chiral Whelk-O1 SPPs). The second is the lack of information regarding the kinetics of adsorption–desorption on CSPs and, in particular, if and how the surface density of a chiral selector may affect it.

The difficulty to efficiently pack a chromatographic bed impinges on the kinetic performance of the column, basically through the *a*-term of the van Deemter equation. Following Ismail *et al.*, the slurry packing of polar SPPs is more difficult than that of C_{18} ones.[28,31,32] However, it is complicated to understand which are the critical factors determining the quality of the packing of chiral core–shell particles. Not only is the preparation of stable slurry suspensions of polar core–shell particles something that can create problems, but also the fine control of the experimental conditions of packing appears difficult to optimize, not to say, to standardize. Quite unpredictable results were obtained by changing some experimental conditions that are commonly varied to improve the quality of packing. For instance, it was observed that the kinetic performance (estimated through the minimum of the van Deemter curves) of chiral core–shell columns, otherwise packed under identical experimental conditions, changed dramatically by changing the time of compression of the bed.[27] However, this did not follow a clearly decipherable pattern. For instance, it was not possible to find any correlation between the compression time and column efficiency. On the other hand, these issues were not observed during the preparation of columns packed with fully porous chiral particles. These findings suggest that much work has still to be done to improve the packing of polar SPPs to get the maximum benefit in terms of column efficiency.

The second consideration by Ismail *et al.*[27] concerns the adsorption–desorption kinetics and, mainly, if and how it depends on the surface density of the chiral selector. While in achiral chromatography the kinetics of adsorption–desorption is practically never an issue (unless the separation of very large molecules is considered), in chiral chromatography even low molecular-weight molecules can exhibit slow adsorption–desorption. This can be particularly evident for the more retained enantiomer, which is often characterized by a strongly tailed peak.[18] However, there are no systematic studies in the literature aimed at investigating these features, while more attention has been paid to the dependence of thermodynamics (*e.g.*, the enantioselectivity) on the amount of the chiral selector bound to the surface.[18,19]

This is, however, particularly important as several research groups have independently reported about the experimental difficulty to obtain the same surface coverage (μmol m^{-2}) of the chiral selector on superficially and fully porous particles, even if the functionalization of both kinds of particles was carried out under identical experimental conditions.[21,24,27] Incidentally, these conditions are such that the amount of the chiral selector is always in a large excess with respect to the estimated number of reactive surface silanols. For instance, Ismail *et al.*[27] found that the functionalization of base SPPs leads to a significantly larger surface coverage of the chiral selector (roughly +20%) than that of native fully porous silica particles. They suggested that this could be due to different reasons, including a larger accessibility of the external layers of particles (with respect to the inner ones) or a different surface chemistry of base silica FPPs and SPPs. Both Spudeit *et al.*[21] and Patel *et al.*[20] reported very similar findings. On the other hand, Dolzan *et al.*[24] found the opposite behavior, the surface coverage of chiral selectors being larger on fully- than on superficially-porous particles. Obviously, since the specific surface area (m^2 g^{-1}) of FPPs is larger than that of SPPs, the total amount of the chiral selector bound per gram of base silica is always greater on FPPs than on SPPs. In light of these aspects, it is fundamental to know how the adsorption–desorption kinetics is affected by the surface density of the chiral selector. If the adsorption–desorption kinetics depended on the surface density of chiral selectors, this last one would possibly become one of the most important parameters to be considered during the preparation of high efficiency CSPs for ultrafast separations.

One last aspect that is worth discussing in this paragraph is regarding the effect of frictional heating. This is generated by the stream of the mobile phase against the packed bed of the column through which it percolates under a significant pressure gradient.[62–64] It can happen in RP as well as in NP, even though in the latter mode it is less evident due to a smaller back pressure under these conditions.[20] The heat produced locally is dissipated in both the radial and longitudinal directions of the column. This generates longitudinal and

radial temperature gradients, whose amplitude depends on the degree of thermal insulation of the column (either adiabatic or isothermal).[65–67] It is evident that, in this respect, chiral core–shell particles offer, at least theoretically, a significant advantage over fully porous ones exactly as it happens in achiral chromatography.

4. Chiral selectors prepared on SPPs

In this section, the classes of chiral selectors that have been prepared on superficially porous particles are briefly reviewed. Their structures are schematically represented in Fig. 2. Simultaneously, some of the applications for which they have been employed are described.

4.1 Polysaccharide-based CSPs

The first report about polysaccharide-based CSPs made on SPPs is that by Lomsadze *et al.* in 2012.[18] 2.6 μm SPPs were coated with cellulose tris(4-chloro-3-methylphenylcarbamate). These particles were used to prepare a packed column (250 × 4.6 mm, *L* × ID), whose chromatographic behavior was compared to that of the other two columns, namely, (i) a home-made 250 × 4.6 mm column packed with 3 μm FPPs functionalized in house with the same chiral selector and (ii) a commercial 250 × 4.6 mm Lux Cellulose-4 (from Phenomenex), also packed with 3 μm FPPs coated with cellulose tris(4-chloro-3-methylphenylcarbamate). The difference between the last two columns (apart from the packing) is the loading of the chiral selector that was almost four times larger on the commercial phase than on the home-made one. The authors concluded that the SPP column outperformed the FPP ones in terms of the plate number, resolution per unit time and optimal flow rate range. On the other hand, they observed that the commercial FPP column showed the highest selectivity, by virtue of a larger amount (in the paper by Lomsadze *et al.*,[18] this is the total amount per gram of base silica and not the surface density) of the chiral selector. Thus, this observation contrasts with that by Ismail *et al.*[27] who found a larger selectivity on the core–shell CSP with respect to the fully porous counterpart, in spite of a significantly smaller total amount of the chiral selector on the SPPs. The authors also mentioned about the difficulty of preparing polysaccharide-based CSPs on small silica particles due to the formation of numerous particle aggregates. The same CSPs were used by Fanali and co-workers to pack capillary columns for capillary chromatography and electrochromatography.[68,69] The authors encountered several difficulties to adequately operate these capillaries most likely owing to their inefficient packing.

In a recent paper,[19] Chankvetadze's group prepared two other polysaccharide-based CSPs on SPPs, by respectively coating cellulose tris(3,5-dimethylphenylcarbamate) on 2.8 μm particles and amylose tris(3,5-dimethylphenylcarbamate) on 3.6 μm particles. This study was aimed at demonstrating the potential of polysaccharide-based SPP CSPs to perform fast chiral separations. Indeed some interesting examples of enantioseparations performed in less than half a minute were reported by Chankvetadze's group (see Fig. 3), even though admittedly there is not enough information to evaluate the real kinetic performance of these CSPs.

4.2 Pirkle-type CSPs

By using a layer-by-layer self-assembly approach, Wu *et al.* synthetized SPPs with *trans*-(1*R*,2*R*)-diaminocyclohexane (DACH). These particles were tested as CSPs for LC towards the separation of several couples of enantiomers including binaphthol, bromo-substituted binaphthol and biphenantrol.[70] In a very basic approach (apparently based on the comparison of only two chromatograms, in addition to being recorded under different experimental conditions), these authors compared the performance of this column with that of a column packed with DACH-functionalized periodic mesoporous silica, by concluding that the SPP version of the CSP allows for a better performance and shorter analysis times than the FPP one.

The Whelk-O1 chiral selector was used by Ismail *et al.*[27] to functionalize 2.6 μm SPPs. The performance of a column packed with these particles was compared, under NP conditions, to that of the other two columns packed with 2.5 μm and 1.8 μm FPPs. Contrary to the initial expectations, the performance of the column packed with SPPs was worse than that of the column packed with 1.8 μm FPPs and quasi-comparable to that of the column packed with 2.5 μm FPPs. As it was widely discussed in previous paragraphs, this was presumably due to the combined effect of a slower adsorption–desorption kinetics and a greater contribution of eddy dispersion on the Whelk-O1 SPPs than on the FPPs. A series of chromatograms showing the ultrafast enantioseparation of *trans*-stilbene oxide enantiomers on two columns (10 × 4.6 mm and 10 × 3.0 mm, *L* × ID) packed with 2.6 μm SPP and 1.8 μm FPP Whelk-O1 particles are reported in Fig. 4. See the figure caption for details.

4.3 Macrocyclic antibiotic CSPs

Macrocyclic antibiotics including teicoplanin, teicoplanin aglycone (TAG) and vancomycin were employed by Armstrong and co-workers to prepare 2.7 μm core–shell CSPs.[20] Columns of different geometrical characteristics (either 10 or 5 mm long with a 4.6 mm I.D.) were slurry packed with these CSPs. The ultrafast separation (<30 s) of a wide range of amino acids was performed with teicoplanin and TAG CSPs.

The performance of a 10 × 4.6 mm vancomycin SPP column was compared to that of the commercial Chirobiotic V column of the same dimensions by Barhate and colleagues.[22] The former column exhibited better peak shapes, greater performance and a higher resolution for the separation of fluorinated and desfluorinated pharmaceuticals.

Finally, macrocyclic antibiotic SPP-based columns were employed for the sub-minute[20] and sub-second[25] screening of achiral and chiral compounds in various chromatographic modes. Some remarkable examples of sub-second enantioseparations are reported in Fig. 5. See the figure caption for more details.

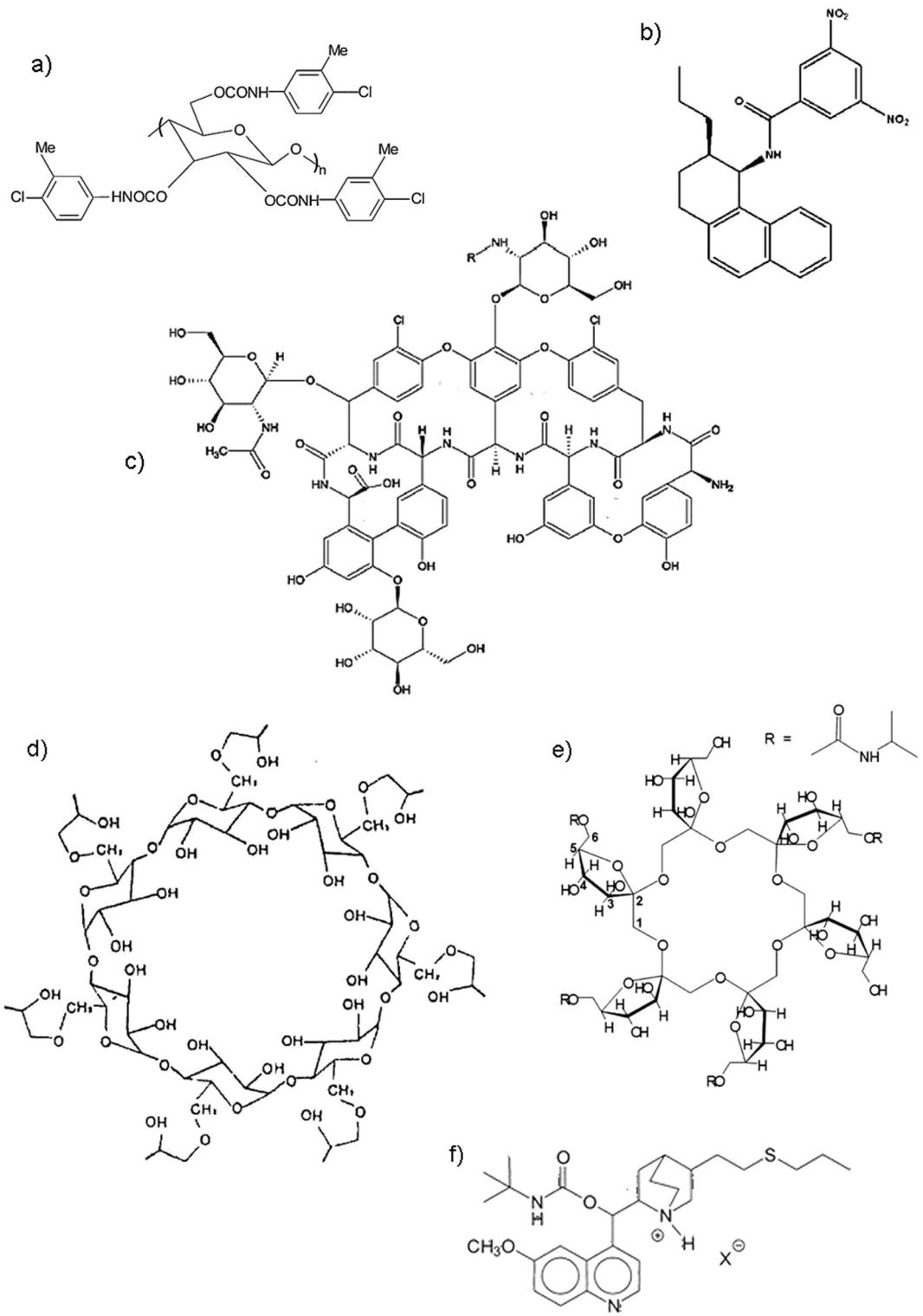


Fig. 2 Chemical structures of chiral selectors employed for the preparation of core–shell CSPs. (a) Cellulose tris(4-chloro-3-methylphenylcarbamate); (b) Whelk-O1; (c) teicoplanin; (d) cyclodextrin; (e) cyclofructan functionalized with the isopropyl carbamate group (CF6-P); (f) quinine carbamate derivative.

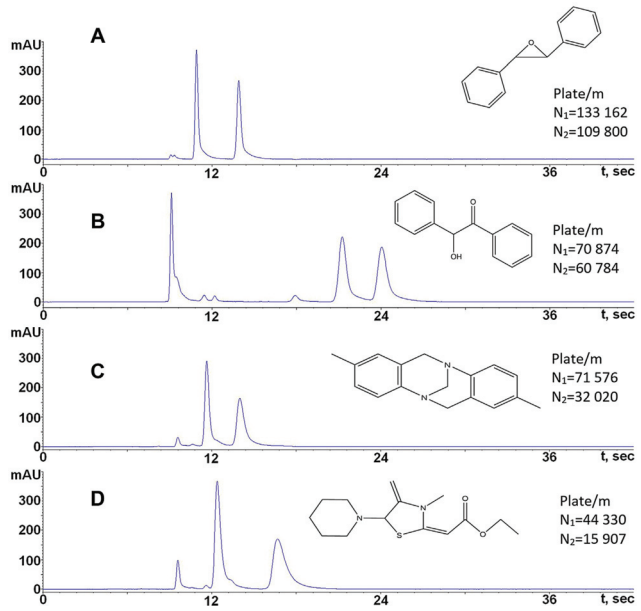


Fig. 3 Fast enantioseparations of the enantiomers of *trans*-stilbene oxide (A), benzoin (B), Tröger's base (C), and etozoline (D) performed on a 100 × 4.6 mm column packed with 3.6 μm SPPs functionalized with amylose tris(3,5-dimethylphenylcarbamate). Mobile phase: hexane/2-propanol 90 : 10 (case A and B) and methanol (case C and D). Flow rate: 5 mL min^{-1}. Reprinted with permission from ref. 19.

4.4 Cyclodextrin CSPs

Hydroxypropyl-β-cyclodextrin was used by Armstrong and co-workers to functionalize 2.7 μm SPPs, whose performance was compared to that of the two columns packed with 5 and 3 μm FPPs functionalized with the same chiral selector.[26] Small polar molecules such as nucleic acid bases, nucleotides, water soluble vitamins, β-blockers and salicylic acids were separated in the HILIC mode. Compared to FPP-based columns, the chiral SPP one exhibited better selectivities. No remarkable loss of efficiency was observed when the core–shell column was operated at high flow rates. Ultrafast separations were performed in less than 1 min.

This SPP-based CSP was also employed by Barhate and co-workers[22] to perform the ultrafast separation (analysis times <1 min) of fluorinated and desfluorinated pharmaceuticals.

4.5 Derivatized cyclofructan CSPs

Spudeit *et al.*[21] chemically bonded isopropyl cyclofructan 6 (CF6-P) to 2.7 μm SPPs. The column packed with this CSP was compared with other two FPP columns (5 and 3 μm particle sizes) with the same chemistry. The columns were operated under polar organic and normal phase modes for the separation of four pairs of enantiomers, including those of amlodipine and fipronil. The three columns showed comparable enantiomeric selectivity under constant mobile phase conditions, even though the SPP column was characterized by a higher surface density of the chiral selector. In contrast, the resolution measured on the SPP column was noticeably larger than that on the FPP columns. Shorter analysis times and wider optimal flow rates were achievable on the SPP-based column. Moreover, following these authors, the efficiency was enhanced thanks to a good packing quality. However, in the paper there is apparently not enough experimental

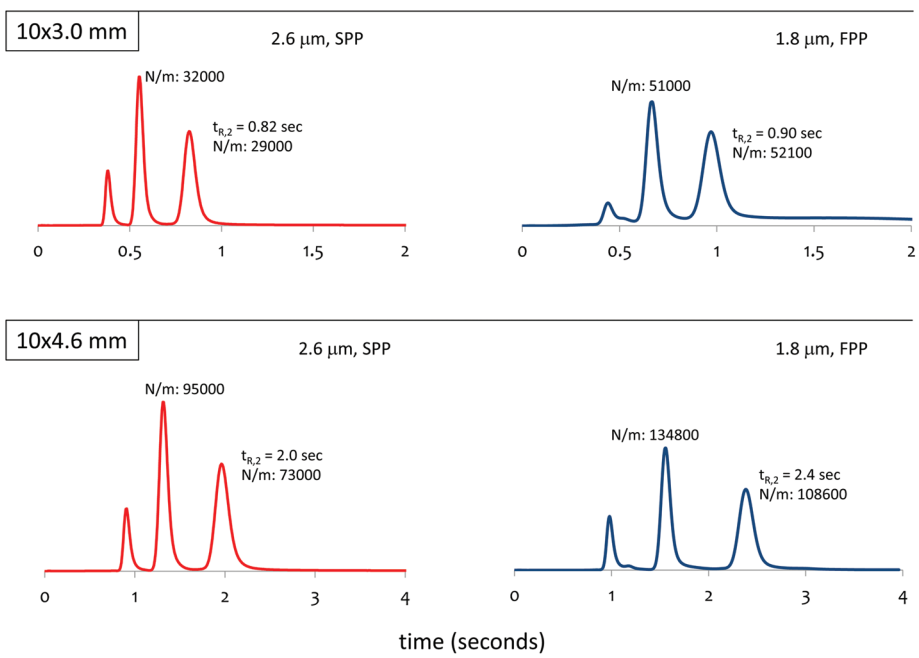


Fig. 4 Ultrafast enantioseparations on 10 × 3.0 mm (top) and 10 × 4.6 mm columns (bottom) packed with both 1.8 μm fully porous and 2.6 μm core–shell Whelk-O1 particles. Mobile phase 90 : 10, Hex/EtOH + 1% MeOH. Flow rate: 8 mL min^{-1}. The number of theoretical plates per meter and the retention time of the more retained enantiomer are indicated in each chromatogram. Unpublished data from ref. 27.

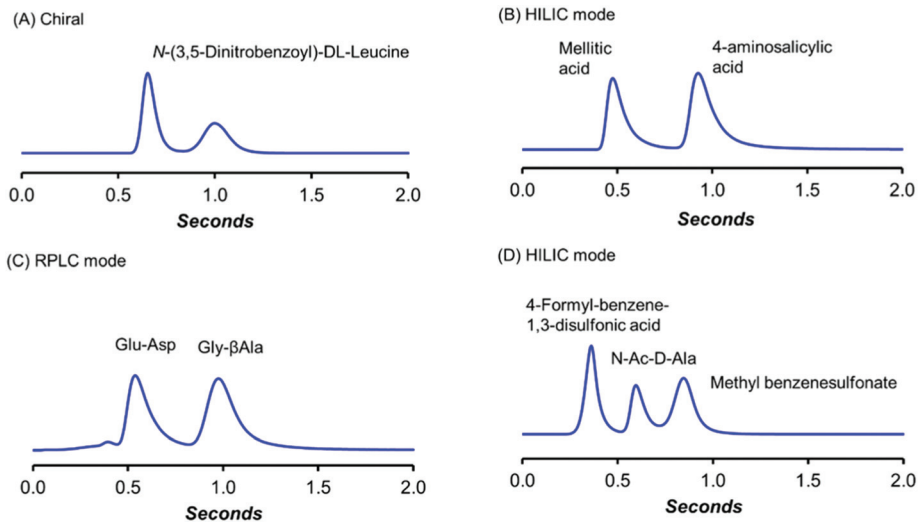


Fig. 5 Sub-second chromatography on various stationary phases using 50 × 4.6 mm I.D. columns: (A) SPP quinine (mobile phase 70 : 30, ACN/20 mM NH_4CO_2H, flow rate: 5 mL min^{-1}); (B) SPP silica (are indicated 94 : 6, ACN/15 mM $NH_4CH_3CO_2$, flow rate: 5 mL min^{-1}); (C) SPP teicoplanin (mobile phase 42 : 58, ACN/20 mM NH_4CO_2H, flow rate 5 mL min^{-1}); (D) SPP teicoplanin (mobile phase 70 : 30 ACN/water, flow rate 5 mL min^{-1}). Reprinted with permission from ref. 25.

information to support this hypothesis. The SPP-based CF6-P CSP was efficiently employed to perform the ultrafast separation of fluorinated and desfluorinated pharmaceuticals.[22]

Native cyclofructan 6 was employed as the chiral selector bonded to 2.7 μm SPP by Dolzan *et al.*[24] The performance of the column packed with these particles was evaluated under HILIC conditions and compared with that of the two FPP columns packed with 5 and 3 μm particles functionalized with the same chiral selector. This is the work where the functionalization of SPPs was found to lead to a lower surface density of the chiral selector than that of FPPs. The chiral core–shell column performed well in terms of analysis times and exhibited both higher optimal flow rates and efficiency than fully porous columns. However, the van Deemter curve measured on the 2.7 μm SPP column showed a comparable slope at high flow rates as that of the 3 μm FPP one, indicating that mass transfer was not advantageous on the former column. Following the authors, this was most likely due to a slow adsorption/desorption kinetics in the adsorbed water multilayer (typical of the HILIC mode).

Cyclofructan SPP-based CSPs were successfully employed to perform ultrafast separations (some of these in the sub-second domain) of achiral and chiral small molecules in different chromatographic modes.[20,25]

4.6 Ion and ligand exchange CSPs

The first ever report on SPP-based CSPs was that of Lindner and coworkers in 2011.[17] They reported about the preparation of a cinchona alkaloid based anion exchanger CSP on 2.7 μm SPPs. The column was employed for the separation of amide type amino acid derivatives.

A quinine-based CSP on 2.7 μm SPPs was employed by Armstrong and coworkers to perform sub-second separations of amino acid derivatives.[25]

5. Future directions

The reason for the great success of SPPs in achiral LC is that they have provided a reasonable compromise between two opposite tendencies. It is indeed well known that the tendency to improve analytical throughputs by using columns packed with smaller and smaller particles is limited by technical constraints, such as the very high pressures needed to operate these columns and the system extra-column volume. The future development of SPPs, even the chiral ones, towards particles of smaller diameters will necessarily require the availability of equipment with minimal extra-column volumes, that is also able to provide a very high back-pressure in the normal mode.

Another field where the development of a highly efficient chiral stationary phase for ultrafast separations is expected to have a tremendous impact is in supercritical fluid chromatography (SFC). Unlike what happened in LC, the technological advancement of SFC equipment has been much slower. The technical specifications of most of the instruments available nowadays on the market for SFC (*e.g.*, extra-column volume, maximum back-pressure/maximum flow-rate achievable, *etc.*) are indeed significantly worse than those for the instrumentation routinely employed in LC. Admittedly, with the packing particles already available, minor improvements in the characteristics of SFC equipment (for instance, a reduction in the volume of the detector cell that in many commercial instruments is excessively large) would permit the immediate achievement of extraordinary results in the direction of highest throughputs and ultrafast chiral separations.[71]

6. Conclusions

Chiral SPPs represent one of the most interesting advancements in the field of high-throughput ultrafast enantioseparations.

They were introduced as CSPs for LC more than five years ago. The scope was to exploit, in chiral liquid separations also, the advantages offered by core–shell particles and widely demonstrated in the literature (in particular, for the *b*- and *c*-terms of the van Deemter equation). Since then, different research groups all over the world have contributed to the development of these phases, to the resolution of many issues in their preparation and to the understanding of their properties in chiral separations.

It follows that the idea of using chiral core–shell particles the preparation of highly efficient chiral columns is not a new one. Several research teams have been devoted to making pioneering publications in the field of chiral chromatography, as it has been mentioned in the present publication. It should also be mentioned that most recently a patent in this area has been filed, including selectors already described in some of the previous publications.[72]

The consistent employment of chiral SPPs for the fast separation of several classes of compounds is more recent. It culminated in the latest demonstration by Armstrong's group of sub-second chiral separations achieved on chiral SPPs of a different nature.

However, to conclude from all this that SPPs are the ideal (or, possibly, the only) support to prepare highly efficient CSPs for ultrafast enantioseparations is in our opinion not obvious. To fully exploit the intrinsic advantages of SPPs even in the field of chiral separations some not trivial practical and theoretical aspects need further investigation. In particular, the achievement of efficient packed beds of polar SPPs, by high-pressure slurry packing, is significantly more difficult than that of hydrophobic C_{18} core–shell particles. Thus, one of the greatest advantages (possibly the greatest one) of C_{18} core–shell particles – namely, their ability to give extraordinarily well packed beds – is not said to be a characteristic of polar SPPs too.

In addition, in our opinion it is necessary to deeply understand if and how the surface density of a chiral selector impinges on the kinetics of adsorption–desorption, especially by considering that the chemical functionalization of chiral SPPs is apparently inherently different from that of FPPs.

The impact of both an inefficient packing and a slow adsorption–desorption kinetics on the column efficiency can be extremely negative in terms of the column performance especially at high flow rates.

As a conclusive remark, we point out that without this information the comparison of the kinetic performance of the chiral core–shell and fully porous particles lacks any scientifically sound basis. This becomes particularly important when the comparison is used to generalize concepts beyond the performance analysis of the application explicitly executed.

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