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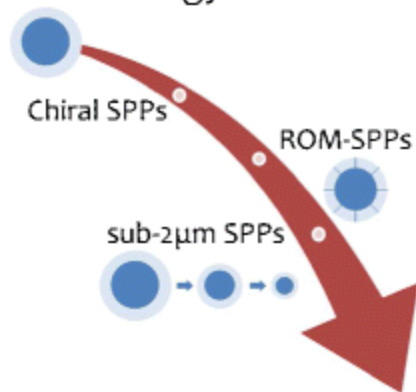
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Superficially porous particles technology



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2	Article Sub-Title		
3	Article Copyright - Year	Springer-Verlag GmbH Germany, part of Springer Nature 2018 (This will be the copyright line in the final PDF)	
4	Journal Name	Analytical and Bioanalytical Chemistry	
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76		e-mail	None
77		Received	30 October 2017
78	Schedule	Revised	5 December 2017
79		Accepted	15 December 2017
80	Abstract	<p>About ten years after their introduction to the market (happened in 2006), the so-called second generation superficially porous particles (SPPs) have undoubtedly become the benchmark as well as, very often, the preferred choice for many applications in liquid chromatography (LC), when high efficiency and fast separations are required. This trend has interested practically all kinds of separations, with the only exception of chiral chromatography (at least so far). The technology of production of base SPPs is advanced, relatively simple and widely available. The deep investigation of mass transfer mechanisms under reversed-phase (RP) and normal-phase (NP) conditions for achiral separations has shown the advantages in the use of these particles over their fully porous counterparts. In addition, it has been demonstrated that SPPs are extremely suitable for the preparation of efficient packed beds through slurry packing techniques. However, the research in this field is in continual evolution. In this article, some of the most advanced concepts and modern applications based on the use of SPPs, embracing in particular ultrafast chiral chromatography and the design of SPPs with engineered pore structures or very reduced particle diameter, are revised. We describe modern trends in these fields and focus on those aspect where further innovation and research will be required.</p>	
81	Keywords separated by ' - '	<p>Superficially porous particles (SPPs) - Sub-2 μm SPPs - 2.0 μm chiral SPPs - Highly ordered radially oriented mesopore SPPs - High efficient ultrafast (chiral) separations</p>	
82	Foot note information		

New frontiers and cutting edge applications in ultra high performance liquid chromatography through latest generation superficially porous particles with particular emphasis to the field of chiral separations

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Received: 30 October 2017 / Revised: 5 December 2017 / Accepted: 15 December 2017
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Abstract

About ten years after their introduction to the market (happened in 2006), the so-called second generation superficially porous particles (SPPs) have undoubtedly become the benchmark as well as, very often, the preferred choice for many applications in liquid chromatography (LC), when high efficiency and fast separations are required. This trend has interested practically all kinds of separations, with the only exception of chiral chromatography (at least so far). The technology of production of base SPPs is advanced, relatively simple and widely available. The deep investigation of mass transfer mechanisms under reversed-phase (RP) and normal-phase (NP) conditions for achiral separations has shown the advantages in the use of these particles over their fully porous counterparts. In addition, it has been demonstrated that SPPs are extremely suitable for the preparation of efficient packed beds through slurry packing techniques. However, the research in this field is in continual evolution. In this article, some of the most advanced concepts and modern applications based on the use of SPPs, embracing in particular ultrafast chiral chromatography and the design of SPPs with engineered pore structures or very reduced particle diameter, are revised. We describe modern trends in these fields and focus on those aspect where further innovation and research will be required.

Keywords Superficially porous particles (SPPs) · Sub-2 μm SPPs · 2.0 μm chiral SPPs · Highly ordered radially oriented mesopore SPPs · High efficient ultrafast (chiral) separations

Introduction

One of the main challenges facing chromatographers is developing high efficient and fast separation methods. A fundamental aspect of this process is the choice of the liquid-chromatography (LC) column, in particular regarding the physico-chemical and geometric characteristics of packing particles. Their size and morphology (either fully or

superficially porous) indeed dramatically affect the kinetic performance of columns not only by modifying the volume available for the diffusion of molecules but also through the “quality” of the resulting packed bed [1–3].

As a matter of fact, for a long time, the main approach followed by column manufacturers to improve the efficiency of separation has been to prepare columns made of particles with smaller and smaller diameter. Sub-2 μm spherical fully porous particles (FPPs) are nowadays widely commercialized and routinely employed. The downside of this approach is in the very high pressure required to use these columns at their full potential (up to 1200–1500 bars or more) [4], since pressure drop along the column increases by a square function of the inverse of particle size [5].

In 2006, the so-called second generation superficially porous particles (SPPs) – alternatively named core-shell, fused-coreTM or porous shell particles – were launched [6]. Since then, columns packed with SPPs invaded the market,

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25 representing an effective and concrete alternative to sub-
26 $2\ \mu\text{m}$ FPPs in terms of efficiency and speed of separation,
27 but originating much less back pressure [7]. As an example,
28 columns packed with C_{18} $2.7\ \mu\text{m}$ SPPs provide efficiencies
29 close to those of columns of the same geometry packed with
30 $1.7\ \mu\text{m}$ fully porous C_{18} particles but operating at a
31 backpressure that is 50-75% smaller than that of FP particles
32 [8, 9].

33 Second-generation SPPs are made of a nonporous solid
34 silica core surrounded by a porous silica shell, exactly as
35 the pellicular particles introduced in the sixties by Horváth
36 and Lipsky [10]. The main advantage of these particles
37 with respect to first generation ones is their higher loading
38 capacity achieved thanks to a specific design, where the
39 porous zone occupies roughly $3/4$ of the total particle
40 volume [11].

41 The rationale behind the introduction of a solid core
42 into the particle was not only to improve solid-liquid
43 mass transfer (c_s -term of the van Deemter equation) by
44 shortening the diffusion path length across the particle but
45 also to reduce the contribution of longitudinal diffusion (b -
46 term of the van Deemter equation) by decreasing the pore
47 volume accessible to analyte molecules [8, 12–14]. Later on,
48 it turned out that SPPs are characterized by very low eddy
49 diffusion (a -term of the van Deemter equation, accounting
50 for any kind of flow inhomogeneity and unevenness in
51 the packed bed), which largely contributes to the overall
52 efficiency of a column [8, 15].

53 A countless number of papers and reviews have been
54 published describing the fundamentals, developments and
55 applications of SPPs in areas as different as food chemistry,
56 biological applications, environmental chemistry, “omics”
57 sciences, bi-dimensional chromatography, etc. [8, 11, 16–
58 24]. Readers interested in these topics are addressed to
59 specific literature.

60 On the other hand, in this paper, we focus on some
61 of the most interesting solutions and ideas proposed to
62 push further the limits of performance and the field of
63 applications of SPPs. These innovations embrace different
64 fields and sectors of activities. First of all, they pertain to
65 high efficient and ultrafast chiral chromatography, where
66 results that were unimaginable even only a few years ago
67 have been recently achieved [16, 25–28]. For instance,
68 several examples of chiral separations performed in less
69 than one second with chiral SPPs as stationary phases have
70 been published. Even if some fundamental aspects need
71 further understanding [16, 25], these works represent the
72 turning point between an old concept of chiral separations
73 by LC and a new one based on columns exhibiting
74 performance (in terms of efficiency and speed of separation)
75 very similar to those of chips employed for high-speed
76 enantioseparations [29]. We may reasonably expect in the
77 next few years the appearance on the market of many

chiral stationary phases based on these concepts, since the
technology of production of chiral SPP particles is mature
enough to find its way into commercial products.

In other less fortunate cases, very innovative and
promising concepts of SPPs are still at the level of
prototypes. Among these, it is worth to mention the so-
called highly ordered radially oriented mesopore (ROM)
SPPs [30, 31]. Engineered to achieve superior kinetic
performance thanks to their highly ordered mesopore
network, these SPPs have however exhibited some issues
in terms of chemical and long term stability, limiting the
extensive evaluation of their potential for high efficient
separations. Another remarkable example of precursors is
represented by SPPs of very reduced diameter (down up to
 $1.1\ \mu\text{m}$) and porous layer thickness. In this case, the major
barrier to large scale production and commercialization
has been essentially practical, coming from actual limits
of even state-of-art instrumentation, whose extra column
void volume is incompatible with the efficiency of these
particles. Admittedly, also the slurry packing of smallest
SPPs ($1.1\ \mu\text{m}$) into very narrow tubes presents important
difficulties [32, 33].

Chiral SPPs: the future of high efficient and ultrafast enantioseparations?

The employment of high efficient particles – either sub-
 $2\ \mu\text{m}$ fully porous or second-generation superficially ones
– in chiral LC has been relatively recent. This delay,
with respect to achiral separations, depends on different
reasons. They include both practical issues and theoretical
problems. Among the former, the most relevant ones are the
difficulty to adapt in some cases pre-existing methods in
use for the functionalization of larger chiral FPPs to very
small particles; particle agglomeration during synthesis;
the non uniform coating of chiral particles. On the other
hand, from a theoretical viewpoint, the lack of complete
understanding of the complex mass transfer phenomena
in chiral chromatography is a relevant limitation to the
development of very efficient chiral particles [1, 16, 34].
Last but not least, conservative commercial strategies by the
most important producers of chiral columns may also be
advocated to explain the delay.

As a matter of fact, until 2011, SPPs were not used as
base material for the preparation of chiral stationary phases
(CSPs) [35, 36] (for the sake of information completeness,
the first report on the use of $1.9\ \mu\text{m}$ fully porous chiral
particles is dated 2010 [37, 38]). Since then, different
classes of CSPs have been produced as porous shell
materials and the debate about pros and cons of chiral
SPPs over FPPs has begun. Chankvetadze and his group
were most active in the preparation of polysaccharide-based

superficially porous CSPs [39, 40]. Their studies about the comparison of kinetic performance between these CSPs and their fully porous counterparts of comparable content of chiral selector and particle size led to the conclusion that SPP chiral columns can provide higher separation factors, higher efficiency and flatter van Deemter curves.

The most complete works on the evaluation of the performance of SPPs in chiral chromatography are those from Armstrong's group [28, 41–46]. Armstrong and coworkers have evaluated, from a kinetic viewpoint, a wide class of chiral selectors prepared on 2.7 μm SPPs including cyclofructan-6 and β -cyclodextrin, macrocyclic antibiotics (teicoplanin, teicoplanin aglycone and vancomycin) and quinine-based ones. In agreement with Chankvetadze's findings, they also have demonstrated that chiral SPPs perform systematically better than fully porous ones under RP, NP, hydrophilic interaction (HILIC) and polar organic mode LC. Remarkably, the employment of very short columns (5 mm long) packed with chiral SPPs and operated at a very high flow rate, permitted to achieve ultrafast enantioseparations (sub-second timescale) [27]. At the same time, also Gasparini and coworkers reported about the possibility of performing sub-second separations by using SPPs functionalized with Whelk-O1 chiral selector [1, 16]. As an example, Fig. 1 shows some remarkable cases where – thanks to the use of high flow rates (up to 8 ml/min) and very short columns (length 5–10 mm) packed with latest generation chiral particles – separations of enantiomers in less than one second were achieved (see figure caption for details).

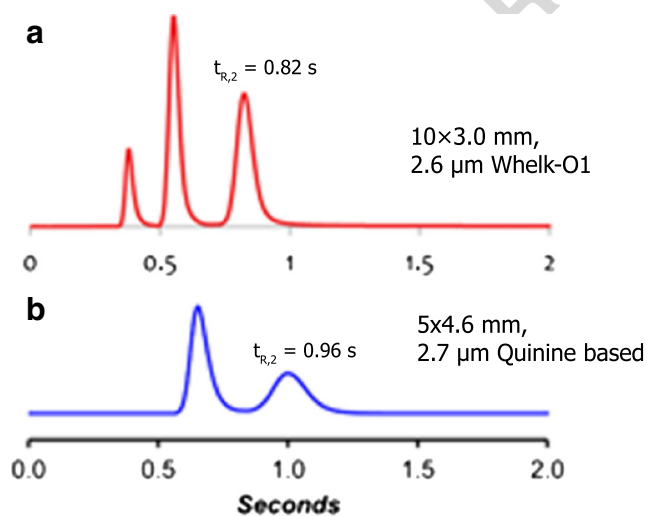


Fig. 1 Ultrafast enantioseparations of **a** *trans*-stilbene oxide enantiomers on a 10×3.0 mm column packed with 2.6 μm Whelk-O1 SPPs, MP: hexane/ethanol 90:10 % (v/v)+1% methanol, flow rate: 8 mL/min; **b** N-(3,5-dinitrobenzoyl)- DL-leucine enantiomers on a 5×4.6 mm column packed with 2.7 μm Quinine based SPPs, MP: acetonitrile/20 mM ammonium formate 70:30%(v/v), flow rate: 5 mL/min. (Modified with permissions from Refs. [16] and [27], respectively)

These proof-of-concept experiments demonstrate the state of the art of chiral LC and allow to predict a great future for this technology in the field of ultrafast enantioseparations. However, in spite of these very promising results, it is the opinion of the authors of this paper that there are still some fundamental aspects that require a deeper investigation to truly understand the potential and limits of these particles. They concern essentially two interconnected aspects. The first one is about the importance of the adsorption-desorption kinetics on the performance of modern, ultra-high efficient chiral LC columns [47, 48]. In particular, questions such as:

- if (and how) the adsorption-desorption kinetics varies by changing the surface density of chiral selector;
- if (and how) the surface density of chiral selector varies across the particle diameter (this is particularly important when considering the comparison between chiral SPPs and FPPs);
- if (and how) the chemical environment surrounding the chiral moiety anchored to the surface affects the adsorption-desorption kinetics (physico-chemical properties of bare silicas can be very different);

need serious consideration. To date these points have been only marginally addressed in the literature.

The other aspect that needs more fundamental work is about the very complex problem of evaluating the contribution of eddy dispersion to band broadening and the factors on which it depends [49]. It concerns, clearly, also the study of packing of particles into chromatographic columns and how it possibly changes depending on the surface characteristics of particles themselves [50]. According to the experience of the authors of this work, packing apolar or polar particles (such as chiral ones), be they FPPs or SPPs, [16, 25] can be intrinsically different. Even the most advanced approaches to study mass transfer in chiral chromatography, indeed, cannot provide independent estimations of contributions to band broadening coming from eddy dispersion and adsorption-desorption kinetics [51].

These considerations show that the apparently obvious statement according to which columns packed with chiral SPPs must outperform those made of chiral FPPs in terms of efficiency (in agreement with what happens in achiral RP LC) [43, 44], must be taken with great caution. Indeed, some experimental facts showing that the above mentioned generalization cannot be always applied have been reported. Ismail et al. [1], for instance, compared the efficiency of chiral columns for ultrafast high-efficient separations packed with both Whelk-O1 SPPs (2.6 μm) and FPPs (1.8 and 2.5 μm). Contrary to initial expectations they found that, especially for the more retained enantiomer, the efficiency of the column packed with SPPs was worse than

210 that of the 1.8 μm FPP column and quasi-comparable to
 211 that of the column made of 2.5 μm FPPs [16]. The authors
 212 reported about the possible combination of both a slower
 213 adsorption-desorption kinetics and a larger eddy dispersion
 214 in the column packed with chiral SPPs as the reasons to
 215 explain this behavior. On the one hand, they correlated
 216 the unusual low performance of SPPs to the larger surface
 217 density of chiral selector found on the SPPs (+20%) with
 218 respect to the fully porous ones (even if particles were
 219 prepared under identical experimental conditions) and, on
 220 the other hand, to the empirical difficulties encountered
 221 during the packing of chiral SPPs.

222 Quite recently, the same group pushed beyond the limit of
 223 high efficient chiral particles, by featuring the first example
 224 of a (teicoplanin-based) CSP prepared on 2.0 μm SPPs [26].
 225 The kinetic performance of the column packed with this
 226 new particles was compared to that of other two columns
 227 packed with 2.7 μm SPPs and 1.9 μm FPPs of narrow
 228 particle size distribution (TitanTM particles), functionalized
 229 with the same chiral selector. At the minimum of the van
 230 Deemter curve, the new 2.0 μm SPP CSP was found to
 231 overcome the other two for the separation of both achiral
 232 and chiral compounds in HILIC conditions, with efficiency
 233 close to 300,000 plates/meter. On the opposite, at higher
 234 flow rates, even with the new 2.0 μm teicoplanin-based SPP
 235 column a significant loss of performance (especially for the
 236 second eluted enantiomer) was observed. This finding is
 237 consistent with the observation made with Whelk-O1 CSPs
 238 (see before).

239 To conclude this paragraph, Fig. 2 reports another extraor-
 240 dinary example, in addition to those given in Fig. 1, of
 241 the outstanding results that can be achieved with the new
 242 2.0 μm teicoplanin-based SPPs. This figure shows the sepa-
 243 ration of a mixture of haloxyfop and ketorolac enantiomers
 244 in about 8 seconds with a resolution larger than 2.0 (see

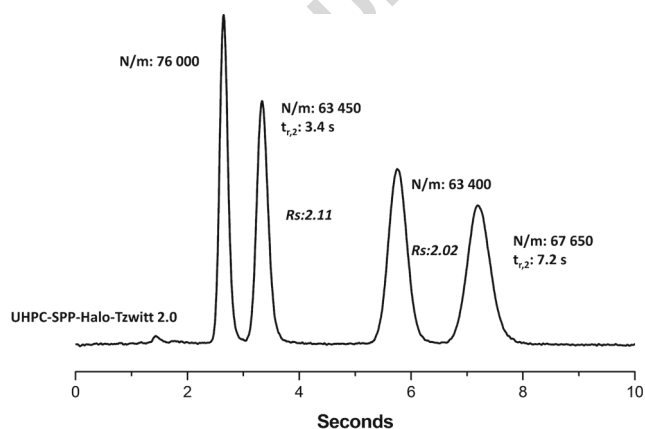
figure caption for details) [26]. Incidentally, we mention
 here that teicoplanin and teicoplanin-based derivatives have
 been for a long time considered “slow” selectors, unsuitable
 for high efficient and ultrafast separations.

249 Highly ordered radially oriented mesopore 250 SPPs: reaching unexplored efficiency limits 251 through engineered particles

252 In 2016, an innovative approach named pseudomor-
 253 phic transformation (PMT) micelle templating has been
 254 described to produce a new type of SPPs characterized by:
 255 (i) narrower particle size; (ii) thinner porous layer with high
 256 surface area; and, most importantly, (iii) a pore network
 257 made of highly ordered radially oriented mesopores [30].
 258 PMT process is based on the dispersion of non-porous sil-
 259 ica particles (which will form the core) in a silica-dissolving
 260 alkaline solution with self-organizing surfactant molecules.
 261 Fig. 3 reports SEM images of ROM-SPPs (squares *a* and *c*)
 262 and traditional SPPs (squares *b* and *d*). Cross-section views
 263 (Fig. 3c and d) show how the presence of ROM limits diffu-
 264 sion only to the radial direction. This is thus fundamentally
 265 different from the randomly distributed and tortuous dif-
 266 fusion pathways in conventional SPPs (Fig. 3d). Prototype
 267 columns packed ROM-SPPs with an overall diameter of
 268 5 μm have been demonstrate to produce minimum reduced
 269 plate height values about 0.5-1 units lower than those
 270 achievable with fully porous and traditional SPPs of the
 271 same particle size, respectively. This represents the lowest
 272 value reported for analytical columns [30].

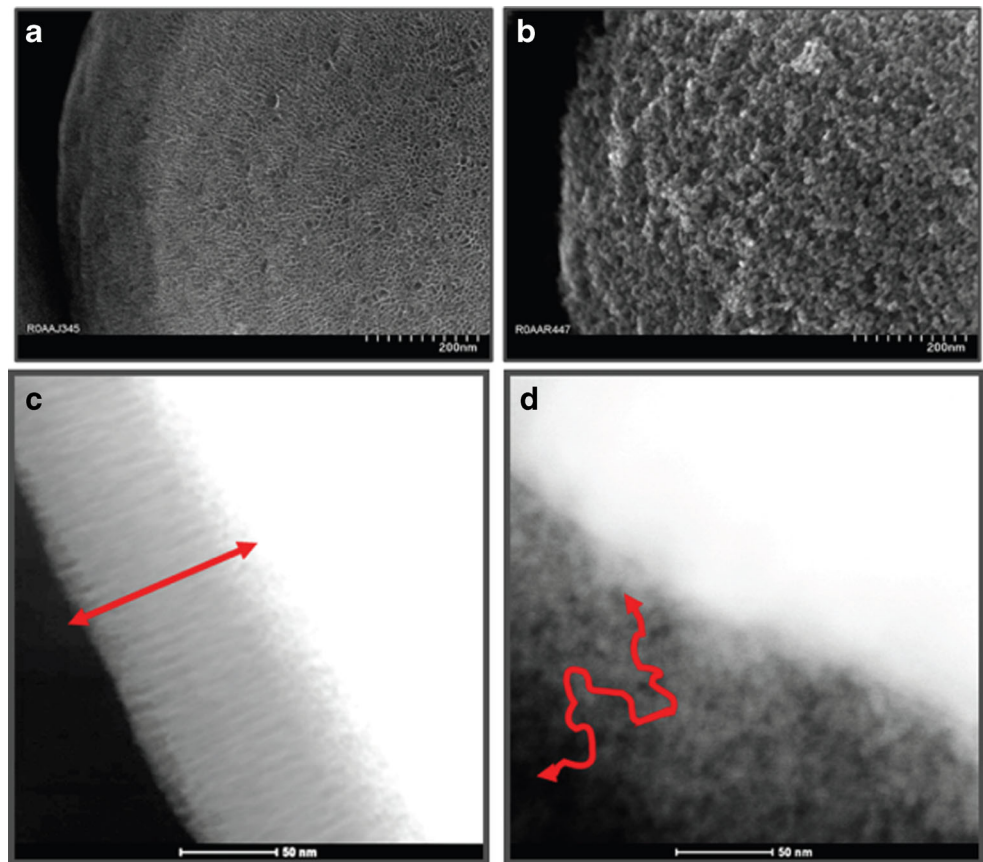
273 In a remarkable theoretical study by Deridder et al. [31],
 274 computational fluid dynamics (CFD) was used to compare
 275 mass transfer properties and band broadening in perfectly
 276 ordered beds made of: ROM-SPPs; traditional SPPs; and,
 277 finally, FPPs. To allow for a fair comparison, the same
 278 particle arrangement, the same values for the mobile zone
 279 and porous zone diffusion coefficients, as well as the same
 280 retention factor have been assumed for the three particle
 281 types. The results of this study can be summarized with the
 282 help of Fig. 4, where the theoretical van Deemter curves
 283 obtained for the three types of particles are reported. The
 284 advantage in terms of mass transfer given by ROM-SPPs is
 285 evident. The ordered pore structure allow these particles to
 286 outperform the others, thanks to a dramatic reduction of the
 287 *b*-term contribution.

288 Deridder et al. demonstrated the longitudinal diffusion
 289 to be independent of the retention factor. It remained at
 290 its minimal value (corresponding to that of unretained
 291 molecules) instead of increasing with retention, as it
 292 happens for particles with isotropic internal diffusion. This
 293 depends on the fact that when retained molecules reside
 294 in the porous layer of ROM-SPPs, their diffusion in the



295 **Fig. 2** Ultrafast enantioseparation of a racemic mixture containing
 296 haloxyfop (firstly eluted pair of peaks) and ketorolac (secondly eluted
 297 pairs of peaks) on a 20×4.6 mm (L×I.D.) column packed with 2.0 μm
 298 teicoplanin SPPs. Modified with permission from [26]

Fig. 3 High resolution SEM images of a ROM-SPP (a, c) and a SPP (b, d). Pictures (c) and (d) are cross-section views of the mesoporous network, showing the differences between the diffusion pathways in the two types of particles. Taken with permission from [30]



295 circumferential direction is completely blocked. Therefore,
 296 the only remaining route available for diffusion is the
 297 interstitial volume between particles. This advantage in the
 298 b -term is achieved without affecting the c_s -term, which does
 299 not increase, as it should be expected. Another important
 300 aspect that would affect the performance of ROM-SPPs
 301 is the geometrical shape of mesopores. From a theoretical
 302 point of view, Gritti has demonstrated that conical shaped

mesopores would produce roughly 80% lower c_s -term than
 cylindrical ones [52].

In spite of these important advantages, the development
 of ROM-SPPs apparently is not any longer supported, due
 to (no better specified) both chemical stability problems and
 low mechanical resistance.

Sub-2 μm SPPs: when instrumental constraints are the bottleneck to reaching highest efficiency

The reduction of the particle size to increase efficiency
 and favour faster separation has been pursued also with
 SPPs. Already a few years after the introduction of second
 generation SPPs in the format of 2.7 μm (HaloTM particles),
 sub-2 μm SPPs were produced and commercialized. Very
 high efficiency and reduced analysis times were found by
 several authors by using columns packed with 1.7 μm SPPs
 [7, 17, 53–55]. Later on, the particle diameter of SPPs
 has been further decreased to 1.3 μm , which represents
 the smallest dimension of SPPs available to date in the
 market. Fekete et al. characterized columns packed with
 these particles from a kinetic viewpoint [4, 56]. They
 found exceptionally low reduced plate heights and high

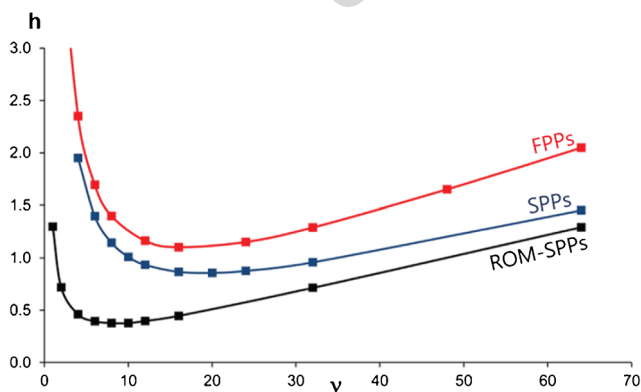


Fig. 4 Theoretical reduced van Deemter curves (h vs. v , being h the reduced plate height and v the reduced interstitial velocity) for packed beds made of FPPs (red data), traditional SPPs (blue data) and ROM-SPPs (black data). Modified with permission from [31]

325 peak capacities with cutting edge applications, especially in
 326 the field of fast separation of peptides. However, it appeared
 327 evident that instrumental constraints of even state of the art
 328 equipments prevent the full deployment of particle technology.

329 Figure 5 compares the van Deemter curves of Kinetex™
 330 SPPs of different sizes (including 1.3 μm ones). As it can
 331 be evinced from this plot, the minimum of the van Deemter
 332 curve for 1.3 μm particles is barely reached. This depends
 333 on the back-pressure limitations of commercial UHPLC
 334 equipments, which are not able to supply the pressure
 335 needed to push, through beds made of very small particles,
 336 the mobile phase at reasonably high linear velocities. As a
 337 matter of fact, for the current operating pressure limit, these
 338 particle format look advantageous only for the separation
 339 of large molecules (having a lower optimal velocity range
 340 than that of small molecules) both in isocratic and gradient
 341 elution mode [4, 56].

342 The reasearch was pushed forward by Blue and
 343 Jorgenson who featured the first example of 1.1 μm SPPs,
 344 the smallest SPP ever produced, through an innovative
 345 layer-by-layer synthetic approach [32, 33]. The information
 346 contained in Fig. 5 let us glimpse the highest potential of
 347 this material. Indeed, one might expect the van Deemter
 348 curve of 1.1 μm SPPs to be significantly lower than those of
 349 the other particle formats, potentially permitting to achieve
 350 incredibly high efficiency.

351 However, the expectation was not satisfied. Blue and
 352 Jorgenson report about the importance not only of an
 353 extremely precise control of experimental conditions for
 354 the synthesis of these particles but also of the slurry
 355 packing procedure, which can have a major impact on the
 356 efficiency of the column, in their case made of a 30 μm
 357 I.D. capillary. This last aspect, in particular, was claimed
 358 to be responsible for the performance observed with their
 359 capillaries, significantly lower than the theoretical values
 360 predictable for 1.1 μm particles.

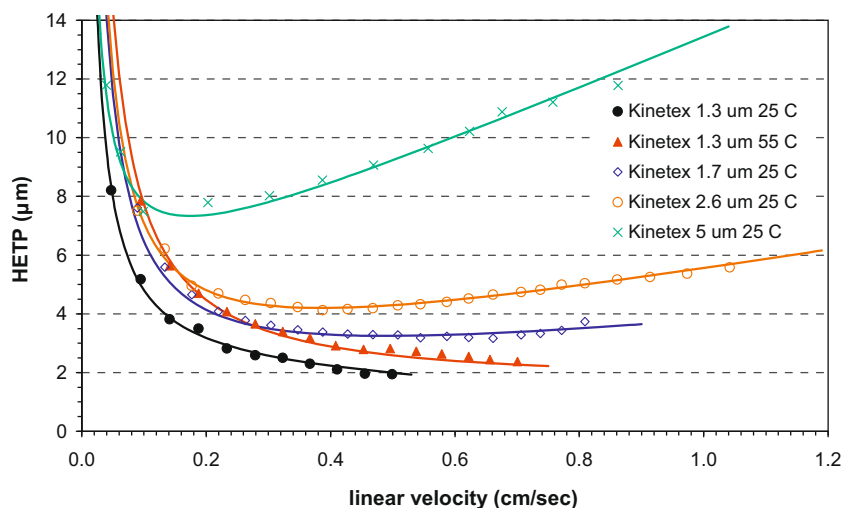
In addition, the other very important instrumental factor
 limiting the development of this technology comes from the
 contribution to efficiency given by band broadening in the
 extra-column void volume (including injector, connections,
 column frits, detector, etc.) of modern UHPLC equipments,
 which is larger than that produced by particles of these
 intrinsic characteristics [57]. Finally, it is worth to mention
 that a practical problem of columns packed with very small
 particles is that they can behave as traps for particulate
 matter dissolved in the eluent, with important consequences
 on the lifetime of these columns if mobile phases and
 samples are not carefully filtered prior analysis.

Outlook

The technology not only of production but also of
 functionalization of SPPs to prepare very small particles
 with extremely enhanced properties in terms of mass
 transfer has come a long way. With the remarkable
 exception of RP achiral separations for particle not smaller
 than 1.7 μm, however, the potential of latest generation
 SPPs remains still largely unexplored due to a series of
 limitations, mainly instrumental ones, which have impeded
 the development of techniques and methods based on them.

The further advancement of the field requires an
 important contribution by LC instrument manufacturers
 for the production of equipments suitable to provide very
 large back pressure and, simultaneously, characterized by
 extremely low extra-column volume through innovative
 designs for detectors, injectors, column fittings, etc. This
 is particularly important (see below) for supercritical
 fluid chromatography (SFC), where the development of
 enhanced instrumentation is particularly necessary. Column
 manufacturers, on the other hand, should develop the
 technology to prepare very short columns with optimized

Fig. 5 Experimental van Deemter curves of butylparaben in reversed-phase conditions measured on columns packed with Kinetex 1.3, 1.7, 2.6 and 5 μm SPPs. Taken with permission from [4]



394 hardware (including column frits) to reduce extra-column
395 band broadening. Advancement in 3D printing technology
396 and CFD studies are fundamental to drive this change.

397 From a more theoretical viewpoint, an extension of our
398 understanding of the packing process of slurry suspensions
399 into chromatographic columns is necessary, by focusing in
400 particular on the factors (including the rheology of particles,
401 slurry density, etc. [49]) that affect it and which could have
402 an impact on the performance of the resulting packed bed
403 (e.g., through the α -term of the van Deemter equation).

404 In parallel, the investigation of the fundamentals of mass
405 transfer is expected to provide information that will help the
406 design of SPPs with still more advanced kinetic properties.
407 For instance, the study of adsorption-desorption kinetics in
408 chiral chromatography might suggest important indications
409 on how to functionalize particles (e.g., in terms of density
410 of chiral selector) for optimum performance.

411 It is precisely in the field of enantioseparations by LC
412 that, in the nearest future, we can expect a real revolution
413 thanks to the use of chiral SPPs of latest generation.
414 Over the year, this field has fallen behind compared to
415 achiral RP separations as regards ultrafast and high efficient
416 separations. However, new developments in chiral particle
417 technology let us predict an inversion of this trend. The
418 market of chiral technology is already a very important
419 one but it is expected to remarkably grow thanks to the
420 new technology. In particular, extraordinary results and very
421 fast enantioseparations are expected by the employment of
422 latest generation chiral particles in SFC [58]. Moreover,
423 chiral stationary phases made on SPPs could be suitable,
424 thanks to their high efficiency, in the case of challenging
425 enantiomeric separations (e.g., chiral impurity profiling),
426 where an extremely low concentration of one enantiomer
427 has to be detected [59].

428 Another field where chiral SPPs will find application is
429 2D-chromatography. Very short columns packed with SPPs
430 can be efficiently used as second dimension for very fast
431 separations in comprehensive applications [22].

432 **Acknowledgements** The authors thank Dr. Ercolina Bianchini of the
433 University of Ferrara for technical support.

434 **Compliance with Ethical Standards**

435 **Conflict of interests** The authors declare that they have no conflict of
436 interest.

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