Sub-2 μm Silica Particles in Chiral Separation

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Abstract

For decades, the race for the shortest chromatogram with the best resolution and separation efficiency has been the focus of researchers and manufacturers. Considerable advancement has been attained in the field of separation science with the widespread applications and outstanding performance of nanomaterials. According to the van Deemter equation, sub-2 micron particles employed in a conventional HPLC short column should subsequently result in analysis time reduction and efficiency improvements without the drawbacks of high pressure associated with sub-2 micron particles. This chapter provides comprehensive discussion about the applications of the new sub 2 microns silica particles in chiral separation of racemates.

Keywords: CHIRALPAK IG-U, HPLC, chiral separation, sub-2 micron, nanomaterials

1. Introduction

1.1. Nanomaterials as stationary phases in separation science

Nanomaterials are nanostructures with sizes in the range of approximately 1–100 nm [1]. These nanomaterials frequently have chemical and physical characteristics that are distinct from those of their macroscopic counterparts [1]. They can be exploited in many fields of science and technology including separation science [2]. In separation science, this term often refers to nano-materials-based stationary phases used to separate chemical compounds [2]. Widespread applications and outstanding performance of nanomaterials have not only accelerated the development of separation science, but also offer many opportunities in other related disciplines, and have a significant impact on many fields of science [2, 3]. Separation science is based on the application of broad chromatographic techniques to achieve separations of compounds. Such separation is achieved by regulating the magnitude of the distribution
coefficient between two distinct phases namely the stationary and the mobile phases [1–3]. The components separate as they migrate with different rates depending on their unique distribution coefficients, they separate [4]. Different chromatographic techniques are available depending on the type of the phases [4]. The chromatography is known to be liquid chromatography (LC) when it employs a liquid mobile phase [5]. The most sophisticated form of LC is High-performance liquid chromatography (HPLC) where the mobile phase passes through the stationary via a pump at high pressures [4, 5].

2. Applications of nanomaterials in HPLC stationary phases

In conventional HPLC, the stationary phase (SP) plays a pivotal role in the separation technique [5]. The packing particles constituting the SP are of several micrometres in diameter with nanometre-sized pores [6]. Therefore, the industry has pushed researchers to investigate new packing materials as an attempt to achieve high throughput with robust analysis [6]. Packings in HPLC can be divided into three types-polymeric, inorganic, and hybrid materials. At present, inorganic materials, which include silica, hydroxyapatite, graphite, and metal oxides, etc. are widely used in research and applications [7]. Among these materials, silica is almost ideal support given its favourable characteristics, for example, good mechanical strength, high chemical and thermal stability, controllable pore structure and surface area, etc. [7]. Therefore, silica has been developed as the most widely used HPLC packing material [6, 7]. Throughout the years, many silica stationary phases (both porous and non-porous) have been commercialised and widely applied for analysis of pharmaceutical and biological samples [8].

2.1. Porous and non-porous nanomaterials

Non-porous and porous particles are the two major types of spherical packing materials used in HPLC [8]. The significant difference between both particles is that porous particles

![Nonporous MS and Porous MS](image)

*Figure 1.* The difference in surface area between porous and non-porous microspheres under scanning electron microspray [2].
have resistance to mass transfer contribution from the stagnant mobile phase in the pores [8]. Decreasing the particle size and increasing the diffusion coefficient can improve the mass transfer of solutes in the mobile phase [3, 5]. Non-porous particles can provide lower mass transfer resistance and higher efficiency than porous particles [8]. However, porous particles have higher surface areas (Figure 1) and can provide much higher sample loading capacity [8].

On the other hand, in porous particles, solutes transfer from the mobile phase exterior to the particles into the mobile phase within the pores to interact with the chiral stationary phase (CSP) [9]. Following this interaction, the solute molecule must diffuse out of the particle and continue its journey down the column ahead of the solute [5, 9]. This slow rate of mass transfer into and out of the porous particle is a source of HPLC band broadening [9]. Figure 2 illustrates a reduction in particle size shortens the path length of this diffusion process, improves mass transfer, and provides better efficiency [9].

Figure 2. Smaller particle sizes increase efficiency and result in a wider range of flow rates [9].

3. Effect of particle size on the separation efficiency, speed and resolution of chiral separations

3.1. Relationship between particle size, and column efficiency

Particle size is known to be the mean diameter of the spherical support employed in column packing [6]. This physical dimension significantly impacts HPLC column performance [9]. A decrease in particle size increases peak efficiencies (Figure 2). This is based on the resolution equation (Eq. (1)) which comprise of three terms: selectivity, retention capacity, and efficiency [10]. The components of an analytical method alter each of these terms. In particular, the column’s particle size affects the efficiency factor from the equation [10]. Efficiency is a qualitative term used to measure the number of theoretical plates in a column. Put simply, as particle size is lowered, efficiency increases, and more resolution is achieved [10].

\[ R_s = \frac{2(t_{R2} - t_{R1})}{(w_{b1} + w_{b2})} \]  

(1)
Burns et al. observed a nearly linear correlation between the width of the particle size distribution of commercially available HPLC particles and the minimum reduced plate height, the van Deemter equation (VDE) A-term and the minimum reduced separation impedance [10, 11]. Column efficiency in HPLC is influenced by a number of factors such as particle size, flow rate and degree of cross-linkage of gels [12]. Particle size distribution (PSD) of packing materials is also considered one of the important factors [12]. It has been empirically found that column efficiency is improved by narrowing the PSD [11, 12]. Because the greatest the achievable plate height is, the more effective the PSD has on the separation efficiency [12]. On one side, column efficiency or plate number is dependent on particle size, and the pore size controls the surface area where retention is controlled primarily by the surface area [9–12].

3.2. The impact of the pore size of silica gel on the CSPs

Retention is directly related to surface area; therefore, the use of large-pore columns is not desirable when small-pore columns can be used [13]. Selection of the pore size is based on providing easy access for the molecules to the pores in the column [10–12]. Consequently, the higher surface area associated with small pore-columns is preferred mainly because the analytes are small enough to pass through the pores [12, 13].

The performance of the CSP increases with pore size when the pore diameter is large enough for the penetration of macromolecules [13]. Specific surface area and, therefore, the number of silanol groups on the surface of the silica gel, decreases with increasing of pore size; consequently, the bound amount of the chiral selector depends on the pore size of silica matrix [13]. The particle size of silica gel has a major effect on the performance of the column, increasing the particle size from 3 to 10 μ decreased the theoretical plate number [13]. A reduction in particle size can lead to more compact and stable packing and thus better column efficiency.

3.3. Chromatographic effects of varying particle size and size distributions

In the late 1960s, Horvath and coworkers introduced columns packed with rigid pellicular particles (40–50 μ) applicable under high pressures [14]. The thin porous coat allowed for robust solute mass transfer through the packing, hence, improving column efficiency. However, pellicular particles had a drawback of low sample capacity [16]. In the 1970s, large porous/pellicular particles were reduced down to smaller porous particles of 10 μ to eliminate the drawbacks of pellicular materials [16]. However, particles of silica smaller than 40 μ have demonstrated some difficulties with packing reproducibility [17]. Irregular shapes of microporous particles were used throughout the 1970s until the spherical material was obtained and improved. In the 1980s, 5 μ became the standard particle diameter, and in the early 1990s, 3–3.5 μ particle diameters were also commercially available [16, 17]. The latter demonstrated 30–50% faster analysis times and higher efficiencies compared to 5 μ. Methods can be easily transferred from 5 μ to similar 3 stationary phases [14–17] (Figure 3) [15].
Figure 3. The reduction of particle packing throughout the years in hope for the shortest fastest chromatogram [16].

4. Small-particle (sub-2-μm) columns for high efficiency and speed

Separation efficiency is inversely proportional to the stationary phase diameter [17]. Stationary phase manufacturers have reduced particles for packing down to micron-sized [17]. If packing materials could be further reduced in the future down to the nanometre scale, the band dispersion would consequently be reduced further by 6 magnitude orders [17, 18]. As a consequence, in 2004, the first available porous silica with small particle size was commercialised (1.7 μ), which allowed better resolution compared to the current 5 or 3.5 μ [18]. Several column suppliers now offer columns packed with particles in the range of 1.5–2 μ [18]. The term sub-2 micron, including particles of 2 μ, is used in this work for the sake of clarity [19]. Different works, dealing with drug and metabolites analysis, bioanalytical as well as environmental separations, compared columns packed with 5 μ and sub-2 micron supports and demonstrated that the latter clearly reduced the analysis time with comparable efficiency [20]. However, the quest towards the use of nanomaterials in chromatography has encountered serious challenges such as extremely high back pressures and problems associated with frits [20, 21].

The high back pressure is induced by the friction of the mobile phase percolating through the sub-2 μm particles stationary phase, generating heat that can be detrimental to the separations [22]. Studies suggest that reducing column internal diameter (i.d.) minimised frictional heating effect from the radial temperature gradient [22, 23]. This is due to fast heat dissipation within such a narrow-bore column [23]. As column length is proportional to the particle size, shortening the columns lead to fast separation with sub-2 μm particles [23].
As shown in Figure 4, sub-2 micron particles are noted to be highly efficient and hence, the column length can be shortened while maintaining resolution in a shorter analysis time [23]. To achieve fast separations, short columns and high flow rates are necessary. In such columns, it can be practically difficult to achieve axially and radially homogenous beds [24]. It seems more logical to use smaller diameter columns, such as 3 mm i.d. when using sub-2 micron particles [23, 24]. This significantly reduces the flow rate required to achieve optimum efficiency, which in turn minimises the extra-column dispersion caused by the tubing [25]. Higher linear velocities can be achieved at lower flow rates, with much lower pressure drops in the tubing [25].

If the main goal is reducing analysis time, an increase in the flow rate above the optimum rate allows for robust separations, while maintaining resolution due to the small particles lower mass transfer resistance [24]. On the other side, if the primary goal is higher resolution, maintaining column length can increase resolution with a subsequent increase in analysis time [24]. Particle size reduction has more effect than column length, gradient time, or flow-rate to improve peak capacity in gradient mode [26]. However, these small particles can generate a high bp incompatible with conventional instrumentation [27]. It is evident that a reduction in particle size reduces backpressure because of the inverse square relationship between them [27]. As a result, new HPLC instrumentation such as ultra-high performance liquid chromatography (UHPLC) has been developed to handle elevated pressures above 400 bar [27]. However, employing shorter columns with smaller particles (i.e. sub-2 micron) have the ability to combine high resolution without exceeding the pressure limit of 400 bar associated with conventional HPLC [23–27].

4.1. Van Deemter analysis of sub-2 micron CSPs

According to VDE, the A- and C-terms are directly proportional to the particle size [28]. Therefore, the use of smaller particles provides a decrease of the plate height together with a flatter profile of the right branch of the van Deemter curve [28]. A reduction in column i.d. results in less chromatographic dilution and, consequently, increased concentration of the injected sample on LC system [25]. The chromatographic dilution increases proportionally

![Figure 4](image.png)

Figure 4. A reduction in particle size results in an increase in column efficiency, a wider range of flow rates is applicable for small particles [23].
with the square of the column radius and with the square root of column length [29]. Thus, a reduction in column diameter results in a significantly lower dilution factor, thereby increasing the concentration in the eluted peak [29]. Downscaling the column used in an analytical method should result in an almost 4000-fold gain in sensitivity [28]. With the reduction in particle size, column efficiency improved further allowing a reduction in column length. Shorter columns are now in vogue [28]. Not only does a shorter column provide faster separations but also solvent use is reduced [28]. Columns of 50 mm in length now provide plate counts formerly obtained on 15- and 25-cm columns packed with larger particles [25, 28, 29]. The sub-2-μm columns have struck the fancy of those who wish to decrease their analysis times by shortening the column length and to those who want to have greater plate count by using longer columns, albeit at the higher pressure [25, 28, 29].

The equation demonstrated the obvious advantage of using small particles to decrease plate height [30]. The chromatographic separating power of HPLC is dependent upon the selectivity of the mobile/stationary phase and the efficiency of the column [12, 13]. The column efficiency is dependent upon multiple factors most importantly: the column length and the packing particle size together with the mobile phase velocity [22]. At a fixed velocity, the column efficiency increases in direct proportion to the column length [24]. At the minimum of the plate height versus velocity curve, the column efficiency increases in inverse proportion with the particle size [22, 24].

As seen in Figure 5, van Deemter equation describes that efficiency varies with the linear velocity, and the nature of the second and third terms of the equation indicates a minimum value for plate height (HETP) [31]. In the third term of van Deemter equation, the particle size is squared, and so the curve is steeper for larger particles at high linear velocities [31]. The A term depends

![Particle Size and Flow Rate van Deemter Equation](image)

**Figure 5.** Van Deemter equation describes that efficiency varies with the linear velocity, and the second and third terms indicates a minimum value for HETP [31].
on both the quality of the column packing and the contribution values of the coefficients A, B and C for the different columns, can be accounted for by the minor contribution of several effects (packing characteristics and the combined effects of frictional heating and high pressure) on the velocity-dependence of the plate height. B- and C-terms of the equation depend on analyte retention [32]. The B-term is expected to increase with analyte retention as more time is available for diffusion to take place in the mobile phase [32]. According to the theory, smaller particles should perform lower plate heights and higher optimum linear velocity [31, 32].

The dependence of the third term (C-term), is considered to represent mainly the resistance to mass transfer in the mobile phase, on the square of the particle size translates into a substantial decrease in the plate height with smaller particles, especially at high linear velocities [33]. Small particle diameters induce an increase in efficiency, optimal velocity and mass transfer. Sub-2 micron particles packed into shorter column permit shorter analysis time along with using less solvent without compromising the resolution between closely eluting peaks [34]. Because the H-u curves are flatter (lower C term) for the smaller particle diameters, they also allow conducting separations at linear velocities higher than the optimum without significant loss of efficiency [34]. Using small diameter packing material reduces eddy diffusion and mass transfer resistance in the mobile phase [28, 33]. Van Deemter realised a correlation between increasing peak efficiency and a reduction in particle size [28, 34].

Optimization of efficiency and analysis time can be useful but generally leads to a large increase in the analysis time [35]. Instead, tuning the column length together with the stationary phase morphology (e.g. particle size) can result in a better compromise between the plate count and analysis time [36, 37]. Column length and plate count are related through the height equivalent to one theoretical plate with the relationship between the theoretical plate and mobile phase velocity described by VDE as the sum of different band-broadening contributions [34–36].

Based on VDE, the solution for enhancing chromatographic performance is to use shorter columns with small particle diameters (i.e. sub-2 micron particles) to induce a simultaneous improvement in efficiency, optimal velocity and mass transfer. The use of these sub-2 micron materials in LC is examined, including their applications in normal-phase LC, reversed-phase LC. In this study, the possibilities and restrictions of chromatographic separations obtained with 5 cm long bore columns packed with sub-2 micron particles is presented. Performance of columns immobilised on silica gel with different internal diameters and different lengths will be briefly mentioned to provide an overview of the miniaturisation of HPLC column technology.

5. Performance of CHIRALPAK IG-U®: In terms of enantioselectivity and solvent versatility

In recent years, column miniaturisation has been investigated and tested in order to achieve highly sensitive chromatography [38]. The miniaturised columns are better for handling minute and/or dilute samples, especially in an area such as forensic science and sport drug trails [38]. The idea of miniaturisation is to provide higher sensitivity and peak capacity than standard columns with minimal dead volume for small sample amounts [39]. Narrow-bore
columns are used on a conventional HPLC system [24]. The sub-2 micron silica-based stationary phases have established themselves as an effective analytical tool in achiral applications, but in the field of chiral separations, the technology related to the development of sub-2 micron CSPs is still not used in the market on conventional HPLC [40–43].

Daicel group recently commercialised a sub-2 micron 5 cm column applicable for use on conventional HPLC [44]. The design is based on the simple way of reducing solvent usage by using a shorter column. A more dynamic saving in solvent usage is made by reducing the i.d. of the column, together with an appropriate scaling down in the flow rate [44]. Separation efficiencies are also recovered by reducing particle size down to sub-2 micron; because columns packed with sub-2 micron particles offer advantages over the more traditional systems containing 3 and 5 micron particles by allowing operation at higher flow rates without compromising efficiency [45]. Consequently, this results in shorter analysis times and a reduction in solvent consumption, together with associated improvements in resolving power, sensitivity and peak capacity [45].

Ghanem et al. [46] investigated the impact of reducing the three VDE parameters (Length, i.d. and particle size) on separation and efficiency via the transition from conventional CHIRALPAK IG® to the sub-2 micron CHIRALPAK IG-U®. The effects of miniaturising the three column parameters (i.d., length and particle size) five times from CHIRALPAK IG® (250 mm length, 4.6 mm i.d., and 5 mm particle size) to CHIRALPAK IG-U® (50 mm length, 3 mm i.d., and 1.6 μm or sub-2 micron particle size) using similar CSP amylose tris (3-chloro-5-methylphenylcarbamate) for the enantioselective separation of racemates under normal standard, non-standard organic phase, and reversed-phase chromatographic conditions are discussed below [47].

Pore size influences several factors such as: the retention factor, the separation factor, and the resolution of racemates was also examined. CHIRALPAK IG-U® has been tested in normal-phase mode chromatographic separation consisting of n-hexane/ethanol screened from 90:10 to 10:90 v/v at 1 mL/min flow rate on CHIRALPAK IG-U® at fixed UV detection 245 nm. Out of the twenty-eight compounds tested, eleven compounds (Naftopidil, Naproxen, Indoprofen, Cizolitrine, Carprofen, Miconazole, Nomifensine, Tocainide, Propafenone, Flavanone, and 6-Hydroxyflavanone) were partially, or baseline separated under either 90:10 or 80:20 v/v n-hexane/ethanol mobile phase. Substituting ethanol (EtOH) with 2-propanol (2-PrOH) resulted in the separation of only seven compounds (Naftopidil, Carprofen, Sulconazole, Propafenone, Flavanone, 6-Hydroxyflavanone, and 1-Acenaphthenol) under either 90:10, 80:20, 70:30 or 60:40 v/v n-hexane/2-PrOH. Regarding resolution (Rs) and separation factor (α), EtOH in mobile phase system showed better results than 2-PrOH where (Naproxen, Indoprofen, Miconazole, Nomifensine, and Tocainide) were all separated when 2-PrOH was replaced by n-hexane (See Figure 6 for examples). This is mainly because the mobile phase travels easily in large spaces between particles and hence particle size affect permeability; smaller particles can be packed closer together, thus, using ethanol resulted in the best separation with the highest resolution and separation factor [46, 47].

CHIRALPAK IG-U® showed great performance in different solvents such as non-standard solvents namely dichloromethane, tetrahydrofuran and Methyl tert Butyl Ether [46]. The
addition of non-standard solvents in mobile phase composition enhanced resolution and separation in several tested racemates for example, in case of Tocainide, Rs 1.44 and α 1.39 in standard solvents namely n-hexane/EtOH 90:10 v/v were enhanced to Rs 1.89 and α 1.66 when using non-standard solvent in mobile phase composition (n-hexane/DCM/EtOH 50/50/0.2 v/v/v) (Figure 7) [47]. Of interest, compound 1-phenyl-2,2,2-trifluoroethanol which was not resolved under any standard solvents’ combination investigated, was baseline separated under non-standard organic solvent (MtBE/EtOH 40/60% v/v) with Rs 1.55 and α 1.78. Similarly, compounds Aminogluthethimide and α-Methyl DOPA were only separated under non-standard organic mobile phase composition (MtBE/EtOH 40:60 v/v) and (n-hexane/DCM 85%/15% v/v) [47]. The chiral recognition of sub 2-micron column CHIRALPAK IG-U® is like that of CHIRALPAK IG® where polarity is playing a role. Another reason might be the stereo environment of the chiral cavities in amylose derivatives which might be favorable in the presence of ethanol. Other researchers have speculated that the configuration of the chiral cavities in the amylose tris (3,5-dimethylphenyl carbamate) is determined by the composition of mobile phase in normal phase mode while the configuration of reversed phase mode remains unchanged. It is of essential to note that the chiral recognition is due to different factors such as hydrogen bonding, π-π interactions and the chiral cavities of CSPs with specific configuration responsible for bonding of varying magnitude between the stationary phase and enantiomers [46, 47]. CHIRALPAK IG-U® was investigated under reversed-phase conditions including ACN and H₂O ranging from 10 to 90% (v/v) [46]. The resolution and separation factors were enhanced in several compounds compared to other separations.
achieved under standard and non-standard organic solvents. For example, few separations were achieved for compounds (Naftopidil, Miconazole, Sulconazole, Aminoglutethimide, Tocainide, Propafenone, Flavanone, and 6-Hydroxyflavanone). Compound Naftopidil was baseline separated with a Rs of 2.56 and α 1.62 under (ACN/H₂O/TEA 80:20:0.1% v/v/v) (Figure 8) [47]. Similarly, in case of compound Aminoglutethimide; Rs 1.90 and α 1.34 were superior to other separations achieved under standard and non-standard organic solvents. Compound 6-Hydroxyflavanone which was separated under standard and non-standard solvents, was separated under reversed phase condition (ACN/H₂O/TEA 40:60:0.1% v/v/v) with superior Rs 2.39 and α 2.71 (Figure 8) [47].

According to the following results and VDE Ghanem et al. [46, 47] and VDE, all three parameters (length, i.d., and particle size) reduced in the transition from conventional CHIRALPAK IC® to the sub-2 micron CHIRALPAK IG-U® resulted in an enhanced separation and resolution (Figure 9). However, Practical difficulties that one can expect following the sub-2-micron particle approach are twofold: one is inherently related to the decrease of column permeability

Figure 8. Naftopidil was baseline separated with a Rs of 2.56 and α 1.62 under reversed phase solvent composition (ACN/H₂O/TEA 80:20:0.1% v/v/v). Aminoglutethimide with Rs 1.90 and α 1.34 were superior to other separations achieved under standard and non-standard organic solvents. Compound 6-Hydroxyflavanone which was separated under standard and non-standard solvents, was separated under reversed phase condition (ACN/H₂O/TEA 40:60:0.1% v/v/v) with superior Rs 2.39 and α 2.71.

Figure 9. CHIRALPAK IG-U® (left side) shows enhanced separation and resolution in shorter time of 2 minutes compared to 20 minutes using the CHIRALPAK IC® (right side).
that accompanies the particle size reduction; the other one is associated to the adaptation of the surface modification chemistry of classical CSPs to smaller particles [21–26].

The column permeability reduction is linked to the increase in pressure that is proportional to the inverse of the particle diameter squared: thus, reducing the particle diameter by a factor of 3 will result in a ninefold increase in the column back pressure [48]. As a consequence, depending on the column length and eluents viscosity, the full potential of high-speed separations can only be exploited on chromatographic hardware that can withstand elevated pressures (UHPLC) [49]. An additional complication may arise from the pronounced propensity of the smaller particles to aggregate during synthetic steps leading to a final stationary phase with non-optimal performances primarily regarding permeability and/or efficiency [18]. Mechanical resistance and long-term stability of the packed bed are also of significant concern when high flow (and hence high pressure) applications are planned [18].

This area needs considerable attention as solvent efficient narrow-bore columns have already become mainstream for ‘greener’ chromatography [50]. For ultrafast separations, the sub-2-μm totally porous particles provide better solutions [18, 48]. Thus it can be a viable option to achieve ultrafast separations with slightly lower efficiency, but without a large investment in ultrahigh-pressure instruments [49].

6. Future perspectives

Instruments have been trying to follow the footpath of column developments [51]. The life cycle for instrument development is much longer than what is required for new packings and columns [52]. An area that has been delaying further improvements in column efficiency is the instrument contribution to band dispersion associated with HPLC and UHPLC instruments and their column-instrument interface designs [53]. Integration of column hardware and instrument connections are essential to eliminate dead volumes, much like what has been achieved in some nano and chip instruments [53]. The area of frit and end fitting design needs attention since the column packing where the separation takes place should be located at or near the injector device and the detector measurement device [52, 53]. This may necessitate a new column design that not only cuts down on this extra-column volume but can handle higher pressures associated with smaller particles [53].

Reducing the column i.d. is the first of several critical steps in miniaturising a LC system. Extra column peak broadening must be reduced accordingly to preserve optimal performance [54]. Excessive extra column band-broadening causes considerable loss of separation efficiency and, thereby, sensitivity. Connection tubing should be kept as short and especially as narrow as possible to minimise extra column band broadening and result in an acceptable increase in back pressure [35, 54]. Making connections with silica capillaries can be a challenge to less-experienced users and often has been considered the most difficult part of setting up a nano-liquid chromatography (nano-LC) system [55]. LC system implies that all system components should be downscaled, including column, connecting tubing, connections, injector, and the interface to the detector [55]. Nano-LC columns typically require flow rates of 500 nL/min or less [35]. Achieving reproducible flow and gradient formation requires dedicated approaches.
Ideally, the flow should not be split and used directly from the pump as it is used in standard HPLC [35]. These nano-LC systems are often called splitless systems and are commercially available [53]. The direct flow systems can be divided into two groups: the ‘solvent refill’ systems and the ‘continuous flow’ systems [53]. Commonly, these systems also are capable of operating at UHPLC pressures. Whereas UHPLC is mostly used to increase throughput in standard-bore LC, its use in nano LC is mainly aimed at improving separation efficiency through the use of longer nano-LC columns packed with 2-μm (or smaller) particles [53].

Further miniaturisation of standard UHPLC instrumentation is possible [50]. Microfluidics has proven a great success as an alternate approach to achieve analytical separations [48]. Such downsizing of the LC experiment would undoubtedly require a major redesign in the column and instrumentation [51, 54]. The use of miniaturised instruments would result in a significant solvent, bench-space, and sample savings, and with mass spectrometry would allow even better interfacing [35, 53–55]. Chip-based LC systems have been extensively investigated, and a limited number of instruments have already been commercialised [55]. However, the adoption rate for commercial instruments has been somewhat slow and, compared to regular analytical columns, in microfluidics column efficiencies are not as high as expected [54]. The packing of microparticles within narrow channels is difficult, and one of the reasons for low column efficiency in microfluidics-based column systems [18, 35, 50–55].

### 7. Conclusion

Chromatographic technique development has always strived towards higher efficiency and more rapid resolution in diverse areas such as clinical, pharmaceutical and toxicology analysis, as well as enantioselective separation, to reduce costs and enhance throughput. Conventional HPLC, could not fully satisfy these requirements due to the relatively low efficiency and lengthy analysis time. Several approaches have been undertaken to achieve these goals, such as increasing flow rates and shortening the column length by using monolithic columns. However, these approaches may result in low phase ratio and low capacity factor. One promising approach is to use smaller size silica particle (less than 2 μm, as compared to conventional 3 and 5 μm size column packing materials. This is motivated by VDE that shows an inversely proportional relationship between the separation efficiency and particle size. Therefore, nano-or sub-micron size supporting materials may be promising to improve separation efficiency.

### Abbreviations

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<tr>
<th>Abbreviation</th>
<th>Definition</th>
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<tr>
<td>CSP</td>
<td>chiral stationary phase</td>
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<td>HETP</td>
<td>plate height</td>
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<td>HPLC</td>
<td>high-performance liquid chromatography</td>
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<td>i.d.</td>
<td>internal diameter</td>
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LC    liquid chromatography
Nano-LC  nano-liquid chromatography
PSD    particle size distribution
SP     stationary phase
UHPLC  ultra-high performance liquid chromatography
VDE    Van Deemter equation

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