Halo Columns: New Generation Technology for High Speed Liquid Chromatography

Imran Ali[1,*], Vinay D. Gaitonde[2], and **Anders Grahn[3]**

[1]Department of Chemistry, Jamia Millia Islamia (Central University), New Delhi—110025, India; [2]Prochrome India, A/2,Varsha Milan, Sahar Road, Andheri East, Mumbai—400999, India; and [3]Biotech AB, Box 133, 439 23 Onsala, Sweden

Abstract

Fast speed and high sample loading and the pressing demands of industries and researchers are compelling scientists and manufacturers to explore the new horizons in column technology. Recently, superficially porous silica particle columns are manufactured with some salient features such as super fast speed, sharp peaks, good sample loading, and low backpressure. The commercially available columns are Halo (Advanced Material Technology, Wilmington, DE), Express (Supelco, Bellefonte, PA), and Proshell 120 (Agilent, Santa Clara, CA). Halo columns are of C_8, C_{18}, RP Amide, and HILIC types with 2.7 µm over all diameters, 0.5 µm porous thick layers containing 90 Å as pore diameter, and 150 m^2/g surface area. These columns have been used for fast separation of low molecular weight compounds with some exception for large molecules such as protein, peptides, and DNA. The present article describes the importance of these state-of-the-art superficially porous silica particles based columns with special emphasis on Halo columns. The different aspects of these columns such as structures, mechanism of separations, applications, and comparison, with conventional columns have been discussed.

Introduction

To reduce the costs and increase sample throughput, the speed of analyses is becoming increasingly important in many application areas of high-performance liquid chromatography (HPLC), especially in the pharmaceutical industries, biological, and environmental sciences. It is due to strong economic pressure from pharmaceutical, food, and chemical industries. Besides, the challenges of proteomic, genomic, and metabolic research require super fast speed and efficient columns. High speed analyses without loss in separating power can be achieved by reduction of particle size of the column. A twofold reduction in particle size can lead to a 1.4 times (square root of 2) increase in column efficiency while backpressure increases four times. Therefore, a compromise on particle size, column length, and pressure is a subject of extensive research (1). Accordingly, column technology is evolving quickly with the development of finer packing particles. During the last decade, people focused on the use of short columns with smaller particles than 3.0 µm to decrease analysis time (2–4). The columns of sub-2.0 µm particles are operated at very high backpressures (~ 15,000 psi) for expected efficiency, which requires costly ultra-high-pressure liquid chromatography (UPLC) instruments. Besides, smaller particles lead to more tightly packed columns, inducing high backpressure. Hence, it is not possible to achieve all UPLC goals like high speed, reliability, precision, and sensitivity with the new breed of ultra-fast HPLC columns. In addition to these facts, small particle based columns need rigorous filtration of mobile phase to avoid blockage of 0.5 µm frits of the column. The development of monolith rods was also introduced to overcome the difficulty of producing stable beds of packing particles and high speed (5,6). However, these materials are less versatile than the packed particle columns. Therefore, scientists attempted more and explored new types of silica particles. Recently, the special type of silica particles [i.e., superficially porous particles (shell particles)] were prepared for super fast speed (reducing run time by 70% or more) and has been introduced into the market. The columns packed with these particle are available with various manufacturers by the name of Halo (Advanced Material Technology, Wilmington, DE), Express (Supelco, Bellefonte, PA), and Proshell 120 (Agilent, Santa Clara, CA). Incorporation of these innovative technologies has lead to a reduction in analytical time and the consumption of high-cost HPLC reagents. Besides, loading capacity is quite good and gives an indication of future perspectives of large scale separations. These overall developments influenced the cost benefits aspect of these sophisticated analytical technologies. A thorough search of literature was carried out, and only very few papers are available on Express and no papers on Proshell 120 columns. On the other hand, a good amount of work has been done on Halo columns. In view of these facts, attempts have been made to describe the state-of-the-art Halo columns.

Structure of Halo columns

Basically, the credit of developing of superficially porous silica gel goes to Kirkland, who in 2000 (7) developed smaller particles with 5.0 µm overall diameter including a 0.25-µm thick porous layer with 30.0 nm pores for the separation of macromolecules. This is to take an advantage of the smaller diffusion distances for these low diffusivity solutes. The superficially porous silica gel particles were prepared by Fused Core Technology, which is a trademark of Advanced Materials Technology. The columns packed with this material are called Halo columns. A C_{18} bonded phase yielded reduced plate heights of < 2.5, which is similar to values for totally porous packing. Later on, the same author (8) developed C_8 and C_{18} particles with overall diameter of 2.7 µm with a porous layer 0.5 µm thick containing 90 Å as pore diameter. The layer on these particles represents 75% of the volume of the whole, potentially limiting the earlier problems of sample capacity. Presently, the commercial C_8, C_{18}, RP Amide, and HILIC Halo silica-based columns of different dimensions are manufactured by Advanced Materials Technology. The SEM photographs of superficially porous particles at different magnifications scales are shown in Figure 1 (7,9). Besides, Figure 2 (9) indicates the cross-section of

* Author to whom correspondence should be addressed: e-mail drimran_ali@yahoo.com.

superficially porous particles indicating overall diameter of 2.7 µm with a porous layer of 0.5 µm thick having 90 Å pore size.

Normally, superficially porous particles have only a 0.5-µm diffusion path as compared to 1.5-µm diffusion path of a 3.0 µm totally porous particle. Due to this, the performance of superficially porous particles becomes more apparent when separating larger molecules and operating at faster mobile phase flow rates. Generally, superficially porous particles columns specifications comprise ultra pure Type B silica of 150 m^2/g surface area. It is densely bonded phase with maximized endcaping by C_{18} (octadecyldimethylsilane) or C_8 (octyldimethylsilane) capable to work at a pH range of 2.0–9.0 at a maximum pressure of 9,000 psi. Halo, Express, and Proshell 120 columns are faster than columns packed with 3.0 µm particles but are just as rugged and easy to use as columns containing 5.0 µm particles.

Mechanism of separation

It is well-known that the van Deemter equation describes the dependence of column efficiency on linear velocity of the mobile phase. This equation may be written as given below:

$$H = A + B/\mu + C\mu$$

The C term is directly proportional to the mobile phase linear velocity and, therefore, is important to consider during designing stationary phases for fast HPLC. Superficially porous particle columns are more efficient than columns packed with 5.0 or 3.5 µm particles and can be run at higher mobile phase linear velocity. A comparison of Halo columns with 2.5 and 5.0 µm has been shown in Figure 3 (9), indicating a good relation between plate height and mobile phase velocity. Superficially porous particles columns reduced the diffusional mass transfer path by one-third compared to 2.5 µm particles due to 0.5 µm porous shells, resulting in sharp peaks. The thin porous shell is responsible for excellent mass transfer (kinetic) of solutes, which allows analytes to rapidly enter and exit the porous structure for interaction with the stationary phase. Due to this, high mobile phase velocities can be used for very fast separations. As discussed previously, Halo columns are made of ultra-pure reagents and Type B silica gel without metal contamination, which minimizes silanol groups interactions. Therefore, the peak shapes of bases and acids are sharp on these columns. Besides, column-to-column reproducibility is also excellent due to the elimination of secondary retention of solutes from metal contamination or silanol interaction.

Gritti et al. (10) reported good performance of Halo columns due to low eddy diffusion (consistent with a narrow particle size distribution) and smaller axial diffusion term (because of lower internal porosity of the material). Nevertheless, mass transfer terms of these particles were reported to be higher than expected (11). Furthermore, Gritti et al. (10) concluded that the external porosity of Halo columns was unusually large with much less permeability than expected and not consistent with the Kozeny-Carman correlation. This might be due to a very narrow particle size distribution and high degree of roughness (α = 5%) of superficially porous material. Fortunately, high external porosity leads to a permeability that was comparable to that of 3.0-µm particles. For low molecular weight compounds, a reduced HETP between 1.5 and 2.0 was achieved for reduced velocities between 1.5 and 5 mm/s. These authors (10) attempted to describe the separation mechanism of Halo particles by comparing separation profiles with conventional silica gel B (3.0 µm size) columns. The authors described that van Deemter parameters were empirical, but their experimental values confirmed that the eddy dispersion and axial diffusion of naphthalene were smaller on

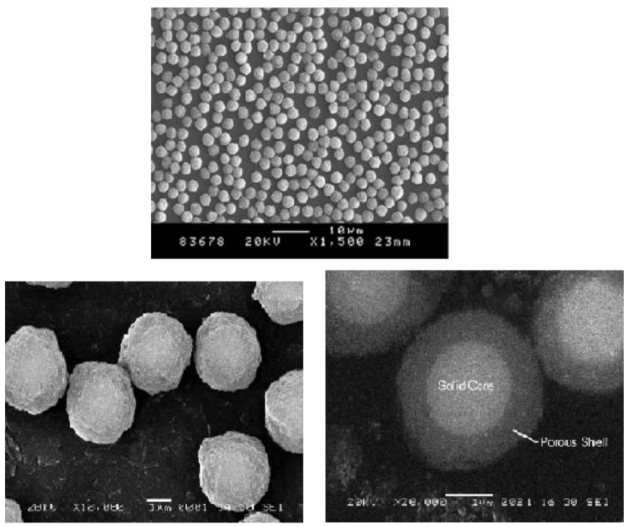

Figure 1. SEM images of superficially porous silica particles at different magnifications (7,9).

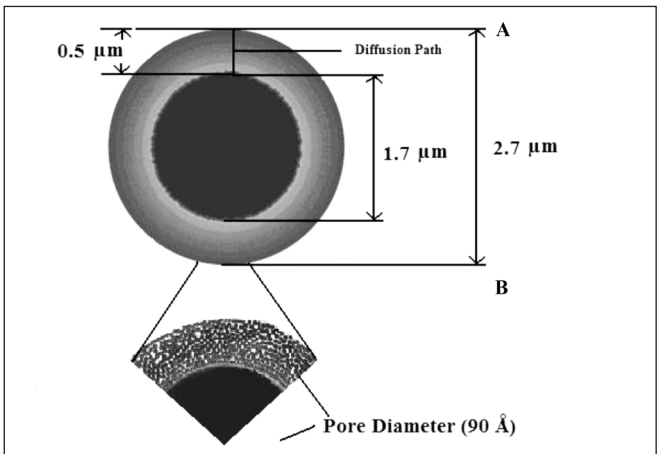

Figure 2. The cross sections and internal structures of superficially porous particles (9).

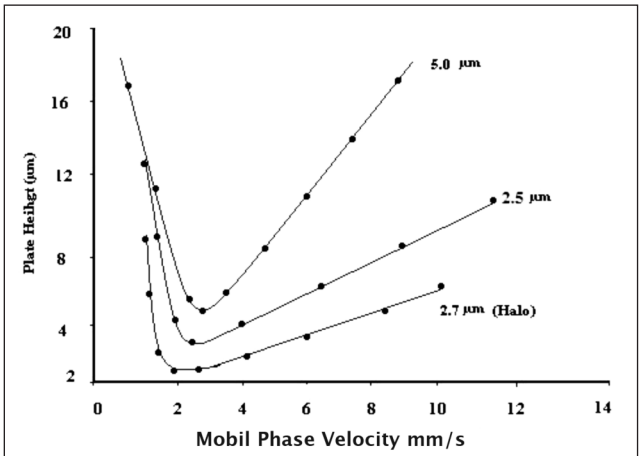

Figure 3. A comparison of Halo and other conventional columns for van Deemter plots (9).

Halo than on silica B-packed column. The experimental data rather suggested that trans-particle mass transfer resistance was not the major contribution to the mass transfer resistances for low molecular weight compounds. Surprisingly, there were two unexpected explanations to high efficiency of Halo columns. Firstly, the difference in the values of B terms was expected based on its internal porosity being about half that of the totally porous material. Therefore, for a given superficial flow velocity, the hold up time in Halo column was shorter, and axial diffusion took place for a shorter time in comparison to silica gel B. Secondly, a fit of HETP data to van Deemter equation gave a 25% larger A value for silica gel B columns than for Halo. Even though the beds of Halo columns are less densely packed, the eddy dispersion terms of low molecular weight compounds are smaller on these columns. Figure 4 indicates the reduced HETP versus reduced velocity of insulin and the difference of the values of the C terms of two columns. The slope of high velocity for Halo column is much lower than for other columns, as expected from a theoretical point of view.

Cavazzini et al. (12) concluded from their work that Halo columns showed good performance and proved to be advantageous for super fast analyses of low and moderate molecular weight compounds. This was due to the combination of a 25% lower axial diffusion term (due to the solid core in the particle) and a 20% lower eddy dispersion term (due to a narrow particle size distribution) in the HETP equation. Although, the shell structure did not provide any advantage for low molecular weight compounds (mass transfer kinetics of low molecular weight compounds), it led to faster kinetics for high molecular weight compounds and allowed markedly improved performance at high flow rates. For compounds of high molecular weight and low diffusivities (i.e. proteins, peptides, DNA, etc.), the mass transfer kinetics is fast, and the C term of Halo columns is about half that of a column packed with totally porous silica particles. The experimental results of Cavazzini and co-workers (12) were in good agreement with the predictions of a theoretical model.

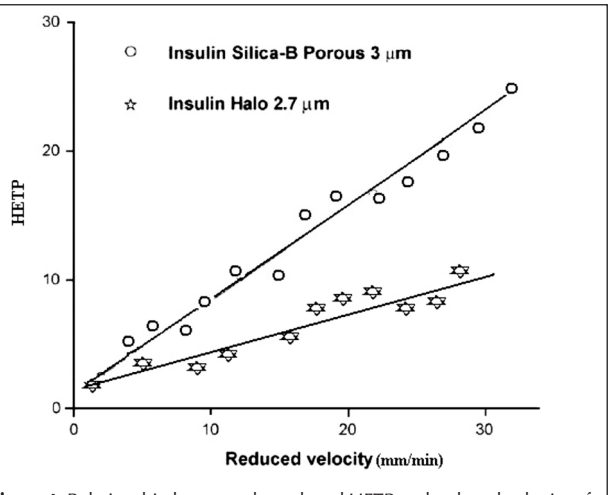

Figure 4. Relationship between the reduced HETP and reduced velocity of insulin (5.8 kDa) (10).

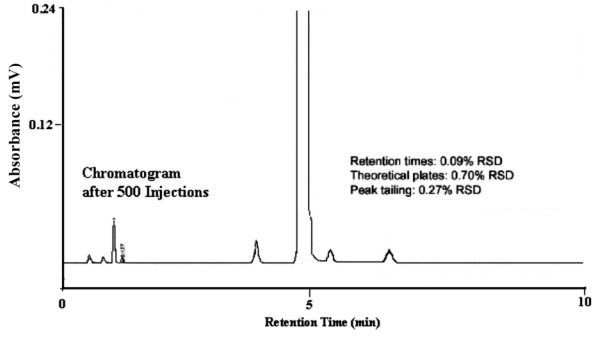

Figure 5. The stability test after 500 injections. Column: 100 × 4.6 mm fused-core C_{18}; Mobile phase: acetonitrile–water (70:30, v/v); Flow rate: 2.0 mL/min; Temperature: 35°C, Pressure: 260 bar (13).

Applications

As mentioned previously, superficially porous silica gel particles-based Halo columns have been used by many workers for the separation and identification of some compounds. These are more useful for delivering over 50% more separating power (theoretical plates) than columns of the same length packed with 3.5 µm totally porous particles (9). These new breed columns have twice the number of theoretical plate and resolving ability with generating low backpressure. The unretained solutes eluted quickly from Halo columns as compared to total porous particle columns. These columns provide rapid separations with > 2,00,000 theoretical plates/meter. High efficiency is due to narrow particles size distribution and high particle density. The short diffusion paths of the thin porous crust and reduced backpressure allowed mobile phase velocities at the plate height minimum for highest efficiency. The surface area (150 m^2/g for shell particles in Halo columns) is suitable for separating small molecules with good sample loading capacities. DeStefano et al. (13) described the stability test of Halo column as shown in Figure 5. The reported percentage standard deviations (0.09–0.70) for many sample loadings indicated a good stability of these new generation columns.

The commercial available Halo columns are of C_8, C_{18}, RP Amide, and HILIC types. All these columns have their own specific features and applications. Not much work has been done on these phases; however, the reported literature is included and discussed in this

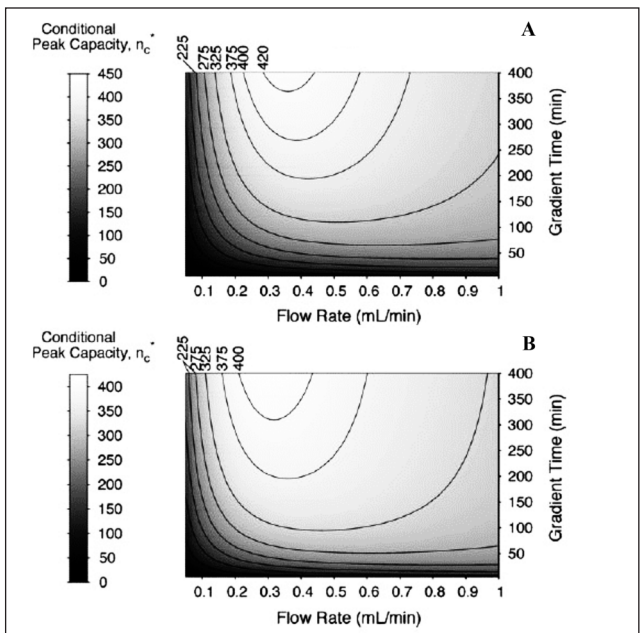

Figure 6. The representation of peak capacity surfaces for 15.0 cm long Halo columns at (A) 20°C and (B) 60°C, respectively (16).

article. Kaczmarski and Guiochon (14) applied the general rate, lumped pore diffusion, transport dispersive and equilibrium dispersive models of chromatography to the columns packed with shell particles. All the four models predicted the similar elution band profiles in all modalities of chromatography. HETP equation of columns packed with shell particles contains a term accounting for the contribution of the internal mass transfer resistance. Analysis of this equation indicates that the internal mass transfer resistance depends mainly on the value of the effective diffusivity for compounds with a large molecular size such as proteins, peptides, DNA, etc. A decrease in the thickness of the porous shell results in a proportional decrease in HETP and an increase in the column efficiency. This increase is important for proteins and peptides but negligible for small molecules. As per the authors, if the shell thickness was less than about half the particle radius, HETP decreased linearly with decreasing shell thickness. For Halo particles, HETP for peptides and proteins was approximately half that of fully porous particles, but there was no difference in HETP for small molecules. These predictions were in agreement with the experimental data published previously (15). Furthermore, Kaczmarski and Guiochon (14) predicted that numerical calculations of band profiles under non-linear conditions demonstrated the use of shell particles providing an important improvement in the separations of proteins or other compounds with large molecular weights. It may be due to slow kinetics of internal mass transfers because of the reported particle diameter and the shell thickness. The applications of all these four types of Halo columns are described in the following sections.

Halo C_{18} Column

Destefano et al. (13) described the utility of Halo C_{18} columns with sample loading and packed bed stability. The compounds separated were naphthalene, virginiamycin, pesticides, and explosives. The authors achieved the separations of pesticides and explosives in 0.7 and 3.5 min, respectively. The superior mass transfer (kinetic) properties of the fused-core particles resulted in improved separation efficiency at higher mobile phase velocities, especially for > 600 molecular weight solutes. Cavazzini et al. (12) reported the analyses of peptides of moderate molecular weights and small proteins on the Halo C_{18} column. The authors described the potential advantages of these particles due to shorter average path length. Marchetti and Guiochon (16) reported the use of Halo C columns of 5.0 and 15.0 cm length for the separation of tryptic digests of myoglobin and bovine serum albumin. The authors discussed the influences of temperature, mobile phase velocity, and pressure. According to the authors, the reduced mobile phase velocity decreased rapidly with increasing molecular weight of the sample due to the correlative decrease in the molecular diffusivity of these compounds. Hence, the authors could not reduce velocities exceeding 10.0 mL/min for anthracene or other low molecular weight analytes. However, a reduced velocity of 20.0 and 30.0 can easily be reached with kallidin (decapeptide with MW = 1212 g) and insulin, respectively, at the same actual flow velocity. Therefore, Halo columns can be used successfully to separate peptide mixtures because the size exclusion effect was negligible below 2.0 kDa. As per the authors, it was possible to obtain fast separations of peptide mixtures exhibiting a high separation power as illustrated by peak capacities between 100 and up to nearly 500 (Figure 6). Marchetti et al. (17) described the separation of the tryptic digests of myoglobin and bovine serum albumin in the gradient elution mode using TFA 0.1% in pure water and 01.% TFA in acetonitrile as the mobile phases on Halo C_{18} columns (50 × 4.6 mm and 150 × 4.6 mm). Similarly, the capability of Halo C_{18} column can be assessed from Figure 7, indicating the isocratic base line separations of eight component mixture of pesticides within 0.7 min (~ 40 s) (13). A good gradient separation of fourteen explosives on Halo C_{18} column has been reported in 3.5 min.

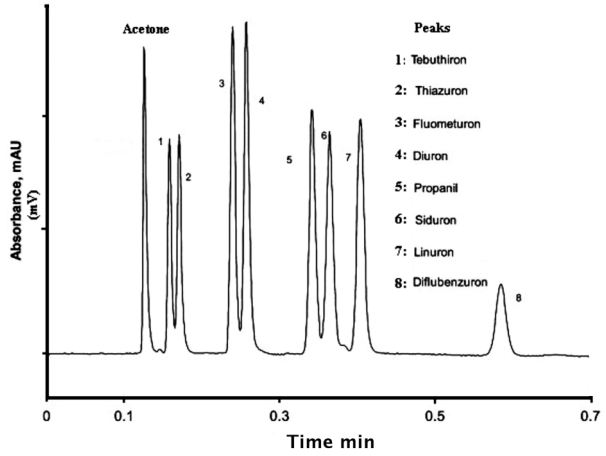

Figure 7. Separation of pesticides on fused-core particles column C_{18} (50 × 4.6 mm), Mobile phase: acetonitrile–water (45:55, v/v); Flow rate: 4.0 mL/min; Temperature: 60°C; Pressure: 230 bar; Detection: UV 245 nm (13).

Halo C_8 column

Another model of Halo column available is of C_8 silica gel, but few applications are available on this. The applications include the separations of naphthalene, lorazeopam, and virginiamycin molecules on a 50 × 4.6 mm dimension column. The mobile phases used were different combinations of acetonitrile and water and acetonitrile and phosphate buffers. Besides, a mixture of uracil, phenol, 4-chloro-1-nitrobenzene, and naphthalene were resolved with sharp peaks on Halo C_8 (50 × 2.1 mm) column using acetonitrile–water (50:50, v/v) as the mobile phase. In other applications, a mixture of acids and bases was separated base line within 2.0 min (Figure 8), indicating a good resolution (9). A mobile phase of methanol–phosphate buffer (pH 2.5) (55:45, v/v) was found suitable for good separations of uracil, phthalic acid, 2-fluorobenzoic acid, 3-nitrobenzoic acid, fluorobenzoic acid, and *m*-toluoic acid. DeStefano et al. (13) described reduced plate height for virginiamycin on Halo C_8 using acetonitrile–phosphate buffer (pH 3.0) (35:65, v/v).

Halo RP Amide Column

Sometimes, C_8 and C_{18} phases fail to give sharp peaks for certain acidic and basic compounds and under such situations Halo RP Amide columns are the best choice. Basically, Halo RP Amide columns are well-suited for the separation of highly water-soluble

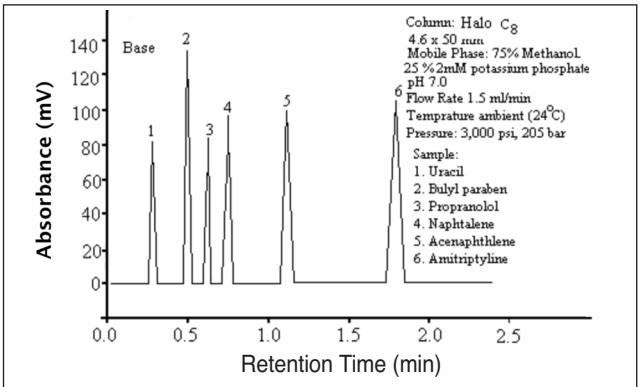

Figure 8. Separations of basic compounds on Halo C_8 column. (9).

compounds that require high aqueous mobile phases in which the polar amide phases are fully wetted. The structure of RP Amide silica is shown in Figure 9, indicating proprietary bonding chemistry, which makes for excellent stability and long column life. These phases should not be confused with other amide embedded phases that exhibit weak hydrolytic stability. The mobile phases used were mixtures of water and organic solvents making this stationary phase suitable for mass spectrometry (MS) detector, too. The mechanism of the separation is not known, but resolution on these phases is influenced by hydrophobic interactions with the alkyl chain and hydrogen bondings with the embedded amide group. The compounds having hydrogen bond donor features can be more retained on RP Amide phase. Figure 10 shows an application of the separation of benzyl alcohol, benzylbenzoate, *N,N*-dimethylaniline, 2-chlorophenol, butyl paraben, 3-ethylphenol, and 4-chloro-3-nitroanisole on RP Amide and C_{18} columns, indicating that 2-chlorophenol, 3-ethylphenol, and butyl paraben are more strongly retained on the RP Amide than Halo C_{18}. Generally, the order of retention on these phases is acids > bases > neutral molecules. Similarly, the separations of 2-nitroaniline, 4-bromoacetanilide, 2,2'-biphenol, and benzyl benzoate was achieved on the RP Amide column.

Halo HILIC column

Generally, in these columns silica gel is bare or have small organic moieties to increase wetability, which is called hydropHILIC chromatography (HILIC), a method developed in 1990. In this modality, a hydrophobic (mostly organic) mobile phase is used, resulting in an increase of retention with hydropHILIC solutes (18). Although the use of this modality was limited, it has suddenly increased over the last few years for analyzing polar compounds in complex mixtures. Besides, the coupling of liquid chromatography with MS has increased its applications as the mobile phases used (partly aqueous eluents high in acetonitrile) are compatible with MS detector. HILIC is a complimentary to reversed-phase chromatography and is an especially attractive tool for the separation of compounds of high polarity (water soluble). Such types of molecules may be separated well on HILIC columns. Hemström and Irgun (19) reviewed HILIC chromatography and discussed the separation mechanism and applications. Pesek and Matyska (20) also compared the application of HILIC chromatography with other phases and found former modality suitable for certain compounds.

Still the mechanism of retention in HILIC column is not known but appears to be a combination of hydropHILIC interactions, ion-exchanges, and some reversed-phase retentions. The aqueous layer on HILIC particles promotes interactions with polar solutes. The strongest and weakest mobile phases have high concentration of water and organic solvents, and hence, both acidic and basic compounds often produce highly symmetrical peak shapes that are normally superior to those found in reversed-phase chromatography. The greatest retention for basic and acidic analytes is found using about 70% acetonitrile in acidic mobile phases. The organic solvents used in HILIC chromatography are acetonitrile and methanol with the exception of tetrahydrofuran, acetone, acetonitrile, isopropanol, ethanol, and methanol. Buffers of about 10–20 mM concentrations may be used as mobile phase with HILIC columns. Phosphate buffers are not recommended due to their poor solubilities in high organic mobile phases and incompatibility with MS detection. On the other hand, volatile ammonium formate and ammonium acetate buffers up to 5–20 mM concentrations and 5.0 pH can be used for separating both acidic and basic compounds with MS detection. Grumbach et al. (21) used HILIC columns for the analysis of acetylcholine, choline, and choline-trimethyl-d_9 molecules.

Recently, McCalley (22) investigated sample capacity, column efficiency, and variation in flow rates on Halo HILIC columns for separation of basic compounds. The efficiencies of 1,00,000 theoretical plates were obtained. The authors reported that shorter columns offered a possibility of fast analysis of bases. The separation of phenol, 2-naphthalenesulfonic acid, *p*-xylenesulfonic acid, caffeine, nortriptyline, diphenhydramine, benzylamine, and procainamide on Halo HILIC columns of different sizes, using acetonitrile–0.1 M ammonium formate of pH 3.0 (85:15, v/v) as mobile phase, is shown in Figure 11, indicating good peak shapes. The other applications of Halo and Express columns are summarized in Table I.

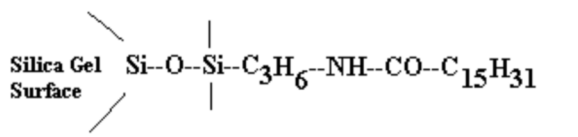

Figure 9. The chemical structure of RP Amide superficially porous silica gel.

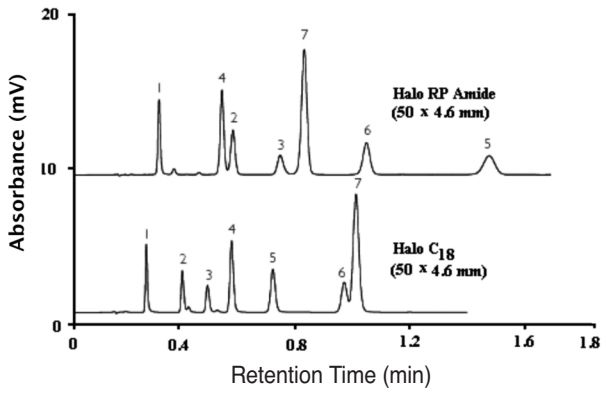

Figure 10. A comparison of the separations of 1, benzyl alcohol; 2, 2-chlorophenol; 3, ethylphenol; 4, benzylbenzoate; 5, butyl paraben; 6, 4-chloro-3-nitroanisole; and 7, *N,N*-dimethylaniline molecules on Halo C_{18} and Halo RP Amide columns, respectively. Mobile phase: acetonitrile–20mM potassium phosphate buffer (pH = 7.0) (50:50, v/v); Flow rate: 2.0 mL/min (9).

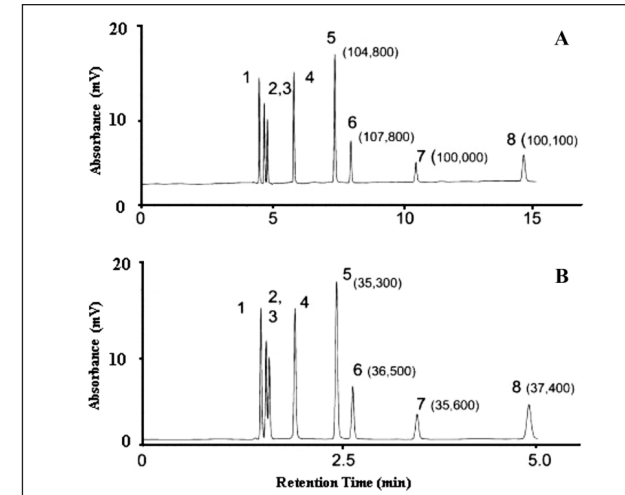

Figure 11. Separations of 1, phenol; 2, 2-naphthalenesulfonic acid; 3, p-xylenesulfonic acid; 4: caffeine; 5, Nortriptyline; 6, diphenhydramine; 7, benzylamine; and 8, procainamide on (A) Halo C_{18} (3.0 × 15.0 cm) and (B) Halo C_{18} (1.0 cm × 15.0 cm) columns, respectively (22).

Table I. The Applications of Halo and Express Columns

Compounds	Mobile Phases	Flow Rates	Columns	Refs.
Polystyrene standards	THF	0.2 mL/min	Halo C_{18}	26
Bradykinin kallidin	ACN, water, and TFA	1.0 mL/min	Halo C_{18}	12
Naphthalene, anthracene	ACN and water	1.0 mL/min	Halo C_{18}	10
Lys-bradykinin brady kinin	ACN, water, and TFA	1.0 mL/min	Halo C_{18}	10
Myoglobin bovin SA	ACN, water, and TFA	0.5 mL/min	Halo C_{18}	17
Naphthalene, insulin, bovin serum albumin	ACN, water, and TFA	0.01–3.0 mL/min	Halo C_{18}	23
Phenol and caffeine	MeOH-water	1.0 mL/min	Halo C_{18}	24
Insulin and lysozyme	ACN, water, and TFA	1.0 mL/min	Halo C_{18}	24
Kallidin bradykinin	ACN, water, and TFA	1.0 mL/min	Halo C_{18}	16
Phenol, 2-naphthalene, sulfonic acid, xylene sulfonic acid, caffeine, nertriptylene, diphenhydramine, benzyl amine, and procanamide	ACN/0.1 M ammonium, formate, pH 3	1.0 mL/min	Halo C_{18}	22
Uracil, acetophenone, benzene, toleuene, and naphthalene	ACN and water	0.4 mL/min	Halo C_{18} and Express	25

Table II. The Physico-Chemical Properties of the Five Columns Given by the Manufacturer, Column Permeability, and Retention Factors of Naphthalene, Naphtho[2,3-a]pyrene, Insulin, and Bovine Serum Albumin (23)

	Neat silica Halo 2.7 µm	Zorbax 3.5 µm	Luna 3.0 µm	Luna 5.0 µm	Atlantis 3.0 mm
Particle size (µm)	2.70	3.5	3.08	5.0	3.0
Pore diameter (Å)	90	80	93	100	101
Surface area (m2/g)	150	180	426	400	315
	Halo C_{18}	Zorbax Extend C_{18}	Luna C_{18}	Luna C_{18}	Atlantis C_{18}
Bonded phase analysis					
Total carbon (%)	??	12	17.77	17.5	12.71
Surface coverage 3.50 (µmol/m^2)	??	3.24	3.	1.75	
Endcapping	Yes??	Yes, double endcapping	Yes, C1	Yes, C1	Yes, C1
Packed column analysis					
Serial number	USFHOO1289	USKC002882	380692-5	233512	W40541T 15
Dimension (mm × mm)	4.6 × 150	4.6 × 150	4.6 × 150	4.6 × 150	4.6 × 150
Total porosity*	0.506	0.515	0.645	0.630	0.695
External porosity†	0.423	0.426	0.383	0.372	0.380
Particle porosity	0.144‡, 0.192§	0.155§	0.425	0.411	0.508
Column permeability					
Koseny-Carmann constant**	270	200	190	210	170
Retention factors††					
Naphthalene	1.04	1.23	1.22	1.42	1.11
Naphtho[2,3-α]pyrene	2.45	3.56	1.92	2.46	1.94
Insulin	0.22	0.14	0.27	0.66	1.46
Bovine serum albumin	–0.19	–0.20	–0.43	–0.42	–0.47

* Measured by pycnometry (THF-CH_2Cl_2).
† Measured by inverse size exclusion chromatography (polystyrene standards).
‡ Particles porosity including the volume of the solid core of the Halo particle.
§ Particles porosity omitting the volume of the solid core (only the volume of the porous shell is considered).
** Calculated from the pressure drop using pure acetonitrile and the SEM average particle size.
†† Measured at a flow rate of 1.0 mL/min and taking the hold-up time t_0 as given by the pycnometric measurements.

Comparison with conventional columns

An interesting, often unrecognized additional advantage of shell particle based columns is that unretained solutes (t_0) are more quickly eluted in comparison to totally porous particles of the same size. This increases the separation speed by reducing the dead time of the column about half that for comparable columns of totally porous particles. To ascertain the speeds, efficiencies, selectivities, stabilities, and reproducibilities of Halo columns, it is important to compare them with other conventional columns of comparable particle sizes. Some workers attempted to compare Halo and Express columns with other conventional columns of small particle sizes and their findings are discussed herein. The group of Professor G. Guiochon at University of Tennessee carried out a remarkable work on the comparison studies of Halo columns with other conventional columns. These authors (10) compared the performances of Halo columns with that of a column packed with 3.0 µm fully porous silica B by separating naphthalene and anthracene as model compounds. The C term measured with Halo columns was similar or even larger than that of fully porous silica particles. The external roughness of the Halo particles was suspected to generate an enhanced film mass transfer resistance with this new material, canceling out the benefit of shortening the diffusion path in the particle. The same group (23) compared the performance of five different C_{18} bonded silica gel columns (i.e., 3.0 and 5.0 µm Luna, 3.0 µm Atlantis, 3.5 µm Zorbax, and 2.7 µm Halo). The physico-chemical properties of these columns are given in Table II, indicating various properties for different columns. Halo and Zorbax have irregular morphology of the external surface while the surfaces of Luna and Atlantis particles are smooth. The authors separated naphthalene, insulin, and bovine serum albumin as model compounds on these columns at 0.010 to 3.0 mL/min flow rates. The C terms of these columns were calculated and given in Table III. The data shows 2.5 times higher values for Halo columns. Halo columns performed the best separation of low molecular weight compound naphthalene (minimum reduced HETP, 1.4) but were not as good as the Atlantis or Luna columns for large molecular weight compound insulin. As per the authors, it was most likely due to roughness of the external surface of Halo and Zorbax particles limiting the performance of these columns at high flow rates, generating an unusually high film mass transfer resistance. Furthermore, Gritti and Guiochon (11) studied the comparative performances of Halo columns with RP C_{18} columns packed with 5.0 and 3.0 µm

totally porous silica B particles by measuring reduced HETPs of naphtho(2,3-a)pyrene. Halo C_{18} particles exhibited high HETP at elevated linear velocities, particularly at high temperatures (310 and 323 K). A comparison of HETP data measured for naphtho(2,3-a)pyrene and for bovine serum albumin demonstrated that this behavior was related not only to the mass transfer kinetics in the stationary phase but also to some unexpected variation of the eddy diffusion with linear velocity at high temperatures. As per the authors, the coupling theory of eddy diffusion proposed by Giddings failed to describe the data obtained for Halo column. The explanation did not reside in the width of the particle size distribution but more likely in the roughness of the external surface of the Halo particles. It was demonstrated that this was not only due to smaller effective diffusion coefficient but also the roughness of the surface of the Halo particles. In this series of studies, these authors (24) compared the loading capacities of Halo C_{18} and a Luna C_{18} column for low and high molecular weight compounds. The former column was found to be superior for loading capacity in comparison to the latter one. This fact is supported by the point that Halo columns gave remarkable performance under both isocratic (10) and gradient elution (17) conditions.

Table III. A Comparison Between the Theoretical and Experimental C Terms for the Five Columns Regarding the Reduced HETPs of Naptho[2,3-a]pyrene (23)

	Halo 2.7 µm	Zorbax 3.5 µm	Luna 3.0 µm	Luna 5.0 µm	Atlantis 3.0 µm
D_{eff}/D_m	0.81	0.70	0.92	0.92	1.18
Parking method particle surface morphology (SEM)	Rugose	Rugose	Smooth	Smooth	Smooth
Theoretical C term	0.0327	0.0686	0.0446	0.0491	0.0346
Experimental C term	0.0751	0.1661	0.0529	0.0588	0.0373
Relative diff. in C term (%)	+130	+142	+18	+20	+08

Table IV. A Comparison of Column Back Pressure and Peak Efficiencies of Acetophenone and Naphthalene on Different Columns*

	Back Pressure		Efficiency	
	(psi)	(bar)	Acetophenone	Naphthalene
Acquity	6531	450	15,885	20,464
Zorbax	5551	382	13,805	16,659
Express	3078	212	12,377	16,575
Thermo	5461	376	14,019	17,192
Halo	2806	193	12,236	17,920

*Using MeCN-H_2O (50:50, v/v) as mobile phase (25).

Table V. A Comparison of Back Pressure and Peak Efficiencies of Acetophenone and Naphthalene on Three Halo Columns Couples at Different Flow Rates*

Flow Rate (mL/min)	Back Pressure		Efficiency	
	(psi)	(bar)	Acetophenone	Naphthalene
1.00	4322	298	84,300	80,730
1.25	5090	351	87,850	88,380
1.50	6352	438	87,550	92,750
1.75	7411	511	86,850	92,600
1.95	8412	580	88,550	89,100

*Using MeCN–H_2O (50:50, v/v) as mobile phase (25).

Cunliffe and Maloney (25) compared the performance of columns packed with superficially porous silica gel particles (i.e., Halo C_{18} and Express C_{18}) with sub-2.0 µm silica particles of Waters Acquity C_{18} and Thermo Scientific Hypersil Gold. The authors carried out a good comparison of these columns by considering various aspects. The reproducibilities of these columns for five compounds are shown in Figure 12, and authors observed that Thermo column was the least retentive (k = 4.3) with a 6% relative standard deviation (RSD) followed by the Ascentis Express (k = 4.8 and RSD = 1%). The Halo and Acquity columns had the most similar k values (i.e., 5.3 and 5.2 with 1–3% RSD), suggesting that Halo and Acquity columns had closer hydrophobic retentivity. The Zorbax column was the most retentive with a k value of 7.0 but suffering with high backpressure. It is clear from this that, with the exception of the Thermo column, column-to-column reproducibility was satisfactory with RSD of 1–3%. The peak efficiencies of these columns for acetophenone and naphthalene are given in Table IV, indicating that Halo column was 77–88% as efficient as the Acquity column but at only 43% of the backpressure. Similar results were obtained on Acsentis Express columns with 78–81% as efficient as the Acquity columns at 47% of the backpressure. Furthermore, these columns were also compared for peak capacities, and it was observed that maximum peak capacity was with Halo columns. The Knox plots were also constructed for these columns using naphthalene in order to compare columns independently of the particle size. Findings are given in Figure 13A, indicating low reduced height plates (hmin < 2.0). Similarly, van Deemter plots (Figure 13B) demonstrated that Halo column did not exhibit any difference in shape or slope to that of the sub-2.0 µm columns, indicating a good efficiency and, hence, could be operated at high flow rates. The column backpressure was also measured at varying flow rates as shown in Figure 14, indicating much lower backpressures of Halo column than sub-2.0 µm columns, in spite of smaller size. These authors also evaluated peak efficiencies of acetophenone and naphthalene on the coupled columns at different flow rates and internal diameters. The results are given in Figure 15 and Table V, indicating that 80,000–95,000 plates can be readily achieved by coupling three 15.0 cm columns together. The backpressure of the coupled columns did not exceed 400 bar at ≤ 1.25 mL/min, enabling the use of traditional HPLC instruments. The flow rates of 2.0 mL/min could be used to achieve faster separations and greater column efficiency.

Attempts have been made to compare the column lengths for separation capacities, and Halo columns were found to have higher resolution capabilities than 2.2, 3.0, 3.5, and 5.0 µm totally porous silica gel particles based columns. Besides, the loss in resolution versus flow rate was also compared, and the results indicated zero resolution loss at 0.35 mL/min for Halo column in comparison to the other columns (2.2, 3.0, 3.5, and 5.0 µm totally porous silica gel particles). Furthermore, it was observed that the loss in resolution was at 0.4 mL/min with columns having 1.7 and 1.8 µm particles but with backpressure problems (9). In addition to this, a comparison of Halo columns with different columns of varying particle sizes, in terms of relative backpressure, was also carried out, and it was found that the relative backpressure of Halo column was lower than 1.7, 1.8, and 2.2 µm particles (9).

Recently, DeStefano et al. (13) described a comparison of Halo columns with other conventional columns. Their findings are summarized herein. Figure 16 indicates the effect of particle sizes with respect to plate heights at various mobile phase velocities for naphthalene. Normally, as per chromatographic theory, the plate heights for 50 × 4.6 mm columns are increasingly smaller for particle size

ranging from 5.0 to 1.8 µm, but the plate height for Halo columns is lower than a column even packed with 1.8 µm totally porous particles. Furthermore, a relationship of reduced plate height (plate heights H divided by particle diameters, dp) and mobile phase velocities indicated that 3.0 and 3.5 µm particles produced reduced plate height minima of about two for small molecules. On the other hand, Halo column has a plate height minimum of about 1.5 µm.

Normally, it is very difficult to pack homogeneous packed beds columns of small particle sizes (2.0 µm or below). But good efficiency of Halo columns may be due to very narrow particle size distribution. Figure 17 shows that standard deviation for Halo column is 5–6% while it is about 19% for 3.0 µm totally porous particles. Besides, narrow size particle distribution of Halo particles, coupled with higher density of this material (30–70% more dense compared to totally porous particles depending on porosity), allowed the formation of homogeneous packed beds. Therefore, the resulting fine packed bed homogeneity probably leads to a good efficiency of Halo columns. Figure 18 indicates a comparison of plate number, pressure, and plates/bar for Halo and other columns of smaller particle sizes. This clearly shows an advantage for Halo column in terms of available plates as a function of pressure requirements. As discussed previously, the mass transfer is better in Halo columns where solutes diffuse more quickly in and out of the porous structure for interaction with the stationary phase. For smaller molecules (e.g., MW < 600) the effect is not dominant. The sample loading capacities of Halo particles are quite good in comparison to total porous particles. Almost same sample loading was observed with 150 and 440 m^2/g surface areas of Halo column and columns packed with total porous particles. These results indicated the capabilities of Halo columns to operate in situations where measurements of both major and minor components are required in the same separation.

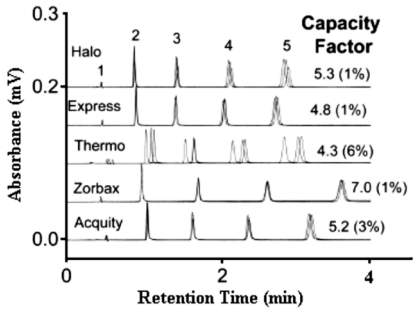

Figure 12. A comparison of the performance of five columns with the reproducibilities for 1, uracil; 2, acetophenone; 3, benzene; 4, toluene; and 5, naphthalene using water–acetonitrile (50:50, v/v) as mobile phase (25).

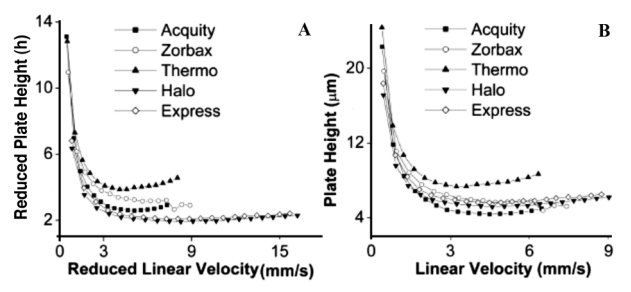

Figure 13. The plots of (A) Knox and (B) van Deemter for five columns (100 × 2.1 mm) using naphthalene as model compound. Mobile phase: water–acetonitrile (50:50, v/v) (25).

Conclusions

A comparison of the applications and other features of shell and totally porous particle based columns indicated that Fused Core Technology Halo columns are the effective stationary phases in the separation science with good efficiency, reduced plate heights of about 1.4 for small molecules. The separation speed is faster than of sub 2.0-µm columns with about one half of the pressure drop. The outer porous shell results in superior mass transfer kinetics and better efficiency at high mobile phase velocities. These columns have been used for the separations and identifications of small to moderate molecular weight compounds with the exception of large molecular analytes. Furthermore, Halo columns can be used with conventional HPLC instrument and are time and cost-saving in nature. The quite good loading capacities of these columns may prove semi-preparative in nature, especially for those compounds having high internal mass transfer resistance. These columns are stable up to 12,000 psi pressure with short retention times. Furthermore, a comparison of superficially porous silica gel columns (Halo, Express, and poroshell 120) indicated better efficiency of Halo columns.

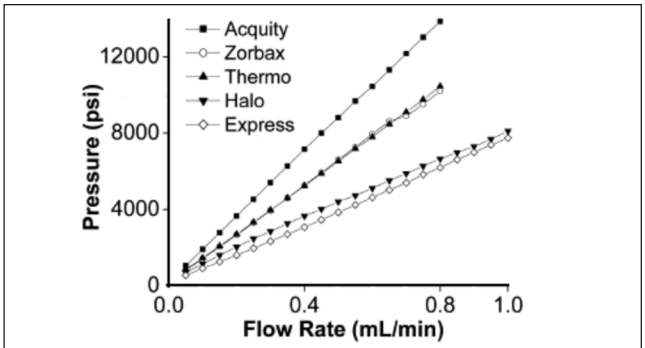

Figure 14. A comparison of relationships between pressure and flow rates for five columns (100 × 2.1 mm). Mobile phase: water–acetonitrile (50:50, v/v) (25).

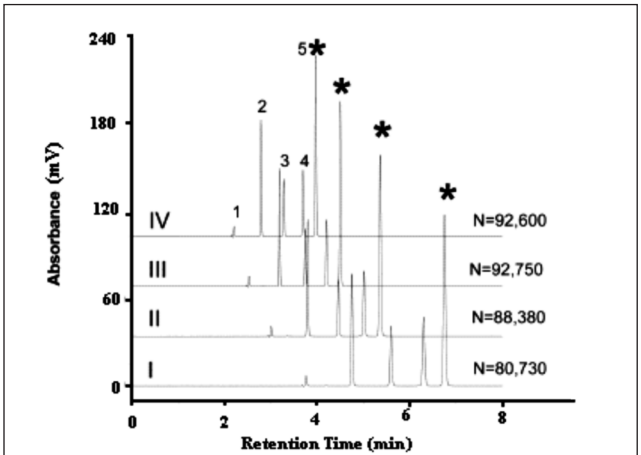

Figure 15. A comparison of three coupled (150 × 4.6 mm) Halo C18 columns (450 mm total length) at four different flow rates: (I) 1.00, (II) 1.25, (III) 1.50, and (IV) 1.75 mL/min. Samples 1, uracil; 2, acetophenone; 3, benzene; 4, toluene; and 5, naphthalene. Mobile phase: acetonitrile–water (80:20, v/v) (25).

Moreover, Halo columns are available in different four modes (C_8, C_{18}, RP Amide, and HILIC) having wide range of applications. Briefly, the future of Halo and other shell particles based columns is bright for fast, economic, and reproducible separations.

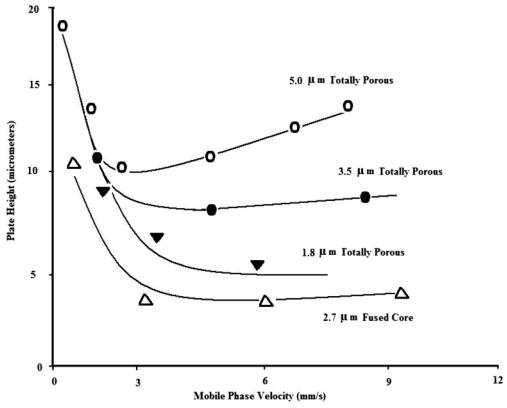

Figure 16. Effect of particle types on the retention of naphthalene on totally porous and fused silica gel-based columns (50 × 4.6 mm). Mobile phase: acetonitrile–water (60:40, v/v) (13).

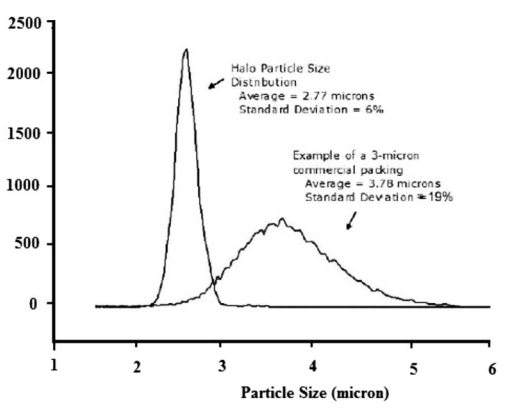

Figure 17. A comparison of particle size distribution of 2.7-µm fused core and 3.0 µm totally porous particles (13).

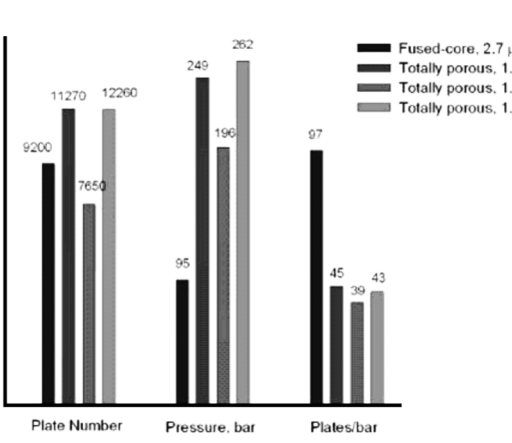

Figure 18. A comparison of plate number, pressure and plates/bar for sub-2 µm (totally porous) and 2.7-µm fused-core particles (13).

References

1. J.N. Done, G.J. Kennedy, and J.H. Knox. Revolution in liquid chromatography. *Nature* **237:** 77–81 (1972).
2. R.E. Majors. Anatomy of an LC column from the beginning to modern day. *LC–GC* **24:** 248–266 (2006).
3. J.W. Thompson, J.S. Mellors, J.W. Eschelbach, and J.W. Jorgenson. Recent advances in ultra-high pressure liquid chromatography. *LC–GC* **24:** 16–21 (2006).
4. J.J. Kirkland. Ultra-fast reversed-phase high-performance liquid chromatographic separations: An overview. *J. Chromatogr. Sci.* **38:** 535–544 (2000).
5. S.D. Rogers and J.G. Dorsey. Chromatographic silanol activity test procedures: The quest for a universal test. *J. Chromatogr. A* **892:** 57–65 (2000).
6. N. Tanaka, H. Kobayashi, N. Ishizuka, H. Minakuchi, K. Nakanishi, K. Hosoya, and T. Ikegami. Monolithic silica columns for high-efficiency chromatographic separations. *J. Chromatogr. A* **965:** 35–49 (2002).
7. J.J. Kirkland, F.A. Truszkowski, C.H. Dilks, and G.S. Engel. Superficially porous silica microspheres for fast high-performance liquid chromatography of macromolecules. *J. Chromatogr. A* **890:** 3–13 (2000).
8. J.J. Kirkland, T.J. Langlois and J.J. DeStefano, Fused core particles for HPLC columns. *Am. Lab.* **39:** 18–21 (2007).
9. Guide to ultra fast HPLC, Quick tips for converting conventional reversed phase HPLC separations to ultra fast separations, MAC-MOD Analytical, Inc. 103 Commons Court, Chadds Ford, PA 19317, USA, 2007, www.mac-mod.com.
10. F. Gritti, A. Cavazzini, N. Marchetti, and G. Guiochon. Comparison between the efficiencies of columns packed with fully and partially porous C18 bonded silica materials. *J. Chromatogr. A* **1157:** 289–303 (2007).
11. F. Gritti and G. Guiochon. Unusual behavior of the height equivalent to a theoretical plate of a new porous shell stationary phase at high temperatures. *J. Chromatogr. A* **1169:** 125–138 (2007).
12. A. Cavazzini, F. Gritti, K. Kaczmarski, N. Marchetti, and G. Guiochon. Mass-transfer kinetics in a shell packing material for chromatography, *Anal. Chem.* **79:** 5972–5979 (2007).
13. J.J. DeStefano, T.J. Langlois, and J.J. Kirkland. Characteristics of superficially porous silica particles for fast HPLC: Some performance comparisons with sub-2-µm particles. *J. Chromatogr. Sci.* **46:** 254–260 (2008).
14. K. Kaczmarski and G. Guiochon, Modeling of the mass-transfer kinetics in chromatographic columns packed with shell and pellicular particles, *Anal. Chem.* **79:** 4648–4656 (2007).
15. X. Wang, W.E. Barber, and P.W. Carr, A practical approach to maximizing peak capacity by using long columns packed with pellicular stationary phases for proteomic research. *J. Chromatogr.* A **1107:** 139–151 (2006).
16. N. Marchetti and G. Guiochon. High peak capacity separations of peptides in reversed-phase gradient elution liquid chromatography on columns packed with porous shell particles. *J. Chromatogr. A* **1176:** 206–216 (2007).
17. N. Marchetti, A. Cavazzini, F. Gritti, and G. Guiochon, Gradient elution separation and peak capacity of columns packed with porous shell particles. *J. Chromatogr. A* **1163:** 203–211 (2007).
18. A.J. Alpert. HydropHILIC-interaction chromatography for the separation of peptides, nucleic acids and other polar compounds, *J. Chromatogr. A* **499:** 177–196 (1990).
19. P. Hemström and K. Irgun. HydropHILIC interaction chromatography. *J. Sep. Sci.* **29:** 1784–1821(2006).
20. J. Pesek and M.T. Matyska, A Comparison of two separation modes: HILIC and aqueous normal phase chromatography, *LC-GC* **25:** 480–490 (2007).
21. E.S. Grumbach, M.D. Wagrowski-Diehl, J.R. Mazzeo, B. Alden, and P.C. Iraneta. HydropHILIC interaction chromatography using silica columns for the retention of polar analytes and enhanced ESI-MS sensitivity, *LC–GC* **22:** 1010–1023 (2004).
22. D.V. McCalley. Evaluation of the properties of a superficially porous silica stationary phase in hydropHILIC interaction chromatography, *J. Chromatogr. A* **1193:** 85–91 (2008).
23. F. Gritti and G. Guiochon. Comparative study of the performance of columns packed with several new fine silica particles. Would the external roughness of the particles affect column properties? *J. Chromatogr. A* **1166:** 30–46 (2007).
24. F. Gritti and G. Guiochon. Comparison between the loading capacities of columns packed with partially and totally porous fine particles. What is the effective surface area available for adsorption? *J. Chromatogr. A* **1176:** 107–122 (2007).
25. J.M. Cunliffe and T.D. Maloney. Fused-core particle technology as an alternative to sub-2 µm particles to achieve high separation efficiency with low back-pressure. *J. Sep. Sci.* **30:** 3104–3109 (2007).
26. Z. Bayram-Hahn, B.A. Grimes, A.M. Lind, R. Skudas, K.K. Unger, A. Galarneau, J. Iapichella, and F. Fajula. Pore structural characteristics, size exclusion properties and column performance of two mesoporous amorphous silicas and their pseudomorphically transformed MCM-41 type derivatives. *J. Sep. Sci.* **30:** 3089–3103 (2007).

Manuscript received October 30, 2008;
revision received November 30, 2008.