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# Comparison of the kinetic performance and retentivity of sub-2 μm core–shell, hybrid and conventional bare silica phases in hydrophilic interaction chromatography

James C. Heaton, David V. McCalley*

*Centre for Research in Biosciences, University of the West of England, Frenchay, Bristol BS16 1QY, UK*

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ABSTRACT

The separation performance and retention properties of four sub-2 μm underivatised silica materials were evaluated in the hydrophilic interaction chromatography (HILIC) mode. These included an organic/inorganic hybrid silica, conventional silica, narrow particle size distribution silica and a core–shell silica. Van Deemter characterisation was performed using conditions to give high retention factors ($k$ = 5.5–6.0) with 10 cm columns to limit the contribution of extra-column dispersion. The core–shell 1.6 μm bare silica (Cortecs) was shown to be kinetically superior to fully porous particle types. Little column-to-column variation in the reduced *b*-coefficient was observed for the test analytes as corroborated by arrested elution experiments. However, the reduced *b*-coefficient was shown to be different between analytes, e.g. cytosine versus nortriptyline. It is speculated that the nature of the retention mechanism (hydrophilic versus ionic retention) and solute physiochemical properties perhaps influence the *b*-coefficient. Maxwell-Effective Medium Theory (EMT) applied to results for a wider range of solutes indicated that the intra-particle diffusion ($D_{part}$) behaviour for individual compounds is broadly similar irrespective of the particle morphology in HILIC. Finally, the impact of varying buffer concentration for a test mix showed that retention and peak shape varied considerably between different silicas. High efficiency separations can be achieved for hydrophilic and basic solutes using a combination of sub-2 μm core shell bare silica particles and appropriate buffer concentrations.

## 1. Introduction

Hydrophilic interaction liquid chromatography (HILIC) is becoming increasingly popular for the separation of polar or ionised solutes. In HILIC, retention is thought to occur principally by partitioning between the hydro-organic mobile phase (> 70% ACN v/v) and a water-rich layer which is held at the stationary phase surface [1]. Additionally, adsorption and ionic processes contribute to the retention mechanism, due to specific interactions between solute and stationary phase. Many stationary phase types are now available for HILIC. Bare silica is popular, and can be a useful alternative to reversed-phase (RP) for the separation of basic pharmaceuticals [2]. One of the major drawbacks of RP is the overload of ionised basic compounds resulting in poor peak shape [3]. Using bare silica in HILIC, symmetrical peak shapes can be obtained for some charged bases with higher sample loads [2] regardless of the particle morphology [4]. Furthermore, the high concentrations of organic solvents used in HILIC mobile phases make the technique very suitable for use with detection techniques such as electrospray ionisation-mass spectrometry and charged aerosol detection (CAD).

Modern sub-3 μm superficially porous particles with relatively thick shells have been shown as a useful adaptation of earlier pellicular materials [5,6], which suffered from low sample capacity due to their thin shells. These new materials can generate almost the same efficiency as fully porous sub-2 μm particles but at lower pressure drops [7]. The lowest reduced plate heights have been obtained from wider-bore 4.6 mm columns rather than the more convenient 2.1 mm ID format [8]. However, the superior kinetic performance of shell compared with totally porous particles of the same diameter has still not been fully explained [9,10]. Shell particles can be manufactured with a very narrow particle size distribution [11]. While this factor per se may not be the reason for the substantially lower eddy dispersion observed for shell particles, it is possible that it improves the packing properties of these materials [9]. The roughness of some types of shell particle may

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* Corresponding author.
*E-mail address:* david.mccalley@uwe.ac.uk (D.V. McCalley).

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also improve the packed bed stability and homogeneity [12]. Also, the superior thermal conductivity of shell particles assists in radial heat dissipation. Otherwise, frictional heating can adversely affect the performance at high flow [10]. This factor explains why even 4.6 mm columns of shell particles can be used under some conditions. Furthermore, axial dispersion has been shown to be reduced in reversed-phase shell columns due to restricted diffusion from the presence of a solid-core [13–15]. Another development in particle synthesis has been that of fully porous hybrid organo-silica phases, giving rise to pH stable ($_w^w$pH 2–10, possibly $_w^w$pH 1–12 for short periods), sub-2 μm particles for ultra-high pressure chromatography (600–1000 bar) [16]. In contrast, a practical limitation of conventional silica-based shell materials (which are not currently available based on this hybrid technology), is their reduced stability at higher pH (>7, or >8 for short periods) making them less flexible for method development. This is even more important in HILIC, since the hydrolytic stability offered by chemical modification is absent for bare silica phases.

The aim of this study was to compare the kinetic and retention characteristics of different sub-2 μm bare silica columns in 2.1 mm formats (HSS, TITAN, Cortecs, BEH) in the HILIC mode. These represent a wide range of recently available materials comprising conventional, narrow particle size distribution, core–shell and inorganic/organic hybrid silica particles. Bare silica is less suitable for the analysis of strongly acidic compounds due to electrostatic repulsion from ionised silanol groups, thus our study has concentrated instead on neutral and basic solutes [17]. Kinetic performance was assessed by evaluating the plate count versus flow of each phase and supporting the observations with data from arrested elution experiments. Arrested elution experiments also allowed for further characterisation of the intrinsic column performance with respect to the reduced *b*-coefficient. Results were also examined using kinetic plot representation of data which employs non-reduced van Deemter coefficients which are of relevance to the practitioner. In order to highlight differences in the retention mechanism of each of the investigated phases, variation in buffer concentration at fixed organic concentration was also performed.

## 2. Experimental

### 2.1. Chemicals and reagents

HPLC grade acetonitrile (ACN) and tetrahydrofuran (THF) were purchased from Fisher Scientific (Loughborough, UK). Ammonium formate, formic acid, toluene, benzylamine, diphenhydramine, phenylephrine, uracil, 5-(hydroxymethyl)uridine, cytosine, nortriptyline, propranolol and procainamide were all obtained from Sigma–Aldrich (Poole, UK). A polystyrene calibration kit (MW 575–2,851,000) for inverse size exclusion chromatography (ISEC) was obtained from Varian Inc./Agilent Technologies (Stockport, UK). Water at 18.2 mΩ was from a Purite Onedeo purifier (Thame, UK). Mobile phases were prepared as pre-mixed solutions as shown in Table 1. Note for nortriptyline, the buffer concentration was varied to allow maintenance of high *k* without using less than 5% water in the mobile phase, which is a common lower limit often employed in HILIC. The peak shape for nortriptyline was excellent on all columns under the conditions in Table 1 (see below).

### 2.2. Apparatus and methodology

Flow rate versus plate height experiments and Cox plot analysis (*k* versus $1/[M^+]$) were performed on a Waters Acquity Classic Ultra Pressure Liquid Chromatograph (UPLC, Waters Corp., Milford, MA, USA). The instrument included a binary solvent manager, sample manager, diode array detector (DAD, 80 Hz data collection) equipped with a 500 nL flow cell and was operated using Empower 2 software. The $V_{\text{ext}}$ of the UPLC system was approximately 12.0 μL and the extra-column variance ($\sigma^2$) of the UPLC at 0.4 mL/min was around 3.5 $\mu L^2$, measured using similar mobile phases as in Table 1. Peak parking experiments were performed using a model 1290 Infinity ultra-high pressure liquid chromatograph (UHPLC, Agilent Technologies, Walbronn, Germany) operated using Chemstation software. This instrument included a binary pump, column compartment, autosampler and DAD (0.6 μL flow cell). The $V_{\text{ext}}$ of the Agilent system was 13.6 μL and the extra-column variance ($\sigma^2$) around 5.0 $\mu L^2$ at 0.4 mL/min. Arrested elution experiments were performed (0.4 mL/min flow) using a 6-port, 2-position dual column switching valve, which allowed the measurement column to be held under pressure while an identical dummy column of the same particle size and dimensions was used in place during switching times. The arrested elution times used were 0, 2, 5, 10, 20, 30 and 60 min. Column hold-up volumes ($V_{\text{m}}$) were determined using toluene in the injected sample which was dissolved in the exact mobile phase in each case. Columns were maintained at 30 °C in the column compartment throughout all experiments. All efficiency and extra-column peak measurements were made at $5\sigma$ (4.4% peak height). Corrections for extra-column effects at each flow rate were obtained using a zero-volume connector. However, due to the high *k* used for the construction of each van Deemter curve and the length of the columns used, these corrections were very small. Detector settings were 270 nm for cytosine and procainamide, 240 nm for nortriptyline and 210 nm for diphenhydramine, propranolol, benzylamine and phenylephrine on both chromatographic systems. 1.0 μL (full loop on Acquity UPLC) injections of 10 ppm solutions dissolved in the exact mobile phase were used in all experiments except for benzylamine and phenylephrine which were at 50 ppm. The bare silica columns (all 100 × 2.1 mm ID) used were BEH HILIC (1.7 μm particle size, hybrid silica), Prototype HSS HILIC (1.8 μm particle size, conventional silica), Cortecs HILIC (1.6 μm particle size, shell particles with $\rho = 0.7$) from Waters Corp. (Milford, USA) and Prototype TITAN HILIC (1.9 μm particle, narrow particle size distribution) from Supelco (Bellefonte, PA, USA). The column external porosity was measured by ISEC using a series of polystyrene standards dissolved in and eluted with pure THF at a flow rate of 0.1 mL/min. van Deemter curves were constructed using flow rates from 0.05 to 1.4 mL/min (17 measurements) for the TITAN, BEH and HSS columns and from 0.05 to 1.2 mL/min (15 measurements) for the Cortecs columns, due to the increased pressure resulting from the smaller particle size. Diffusion coefficients were determined experimentally by the Taylor-Aris open tubular method using a flow rate of 0.1 mL/min and temperature controlled water bath at 30 °C. The tubing used was PEEK with an internal diameter of 0.05277 cm, length of 303.1 cm, and coil diameter 22 cm. The Taylor-Aris measurements were verified using thiourea at 25 °C (Experiment $1.32 \times 10^{-5}$ cm$^2$/s, literature $D_{\text{m}} = 1.33 \times 10^{-5}$ cm$^2$/s [18,19]). Use of a longer tube did not appreciably affect the results. All measurements were performed at least in duplicate and were averaged.

## 3. Results and discussion

### 3.1. Porosity comparison of different bare silica packed beds

Fig. 1 shows the inverse size exclusion chromatography (ISEC) plots for the four bare silica columns using a series of polystyrene standards eluted with neat THF. The external porosity ($\varepsilon_e$) was determined by extrapolation of the linear portion of the curve to zero MW. The total porosity ($\varepsilon_T$) of each column was determined from the elution volume of toluene in the HILIC mobile phase.