Overview
This poster describes the development of a new substrate particle for HPLC, based on the mathematical concept of scalable fractals.

Introduction
The analysis of proteins and peptides is a challenge in HPLC for a variety of reasons, namely adsorption and mass transfer. A suitable stationary phase must be chosen for the analytes being separated to avoid irreversible adsorption onto the surface and prevent carry over between runs. Additionally, conventional silica particles typically have pore sizes in the region of 100 Å or less which are too small to allow access to the pore structure, especially when analyzing large proteins. One solution is to increase the pore diameter, however this leads to significant mass transfer problems, particularly at higher flow rates, as one analyte molecule may spend a lot of time within the pore structure as another simply passes through the column.

Recent research [1] has found that modifying the Stöber process by using tetraethyl orthosilicate (TEOS) as the silica precursor with the presence of a hydrophilic polymer and cationic surfactant leads to the production of monodisperse, porous silica microspheres. If TEOS is replaced with 3-mercaptopropyltrimethoxysilane (MPTMS), particles in the region of 5 µm are produced with a single layer of nanospheres approximately 200 nm in diameter coating the surface. These are described as sphere on sphere (SOS) particles.

This design of particle leads to the possibility of fractal like structures. The concept of fractals was first introduced in the 17th century by the mathematician Leibniz, who was investigating the possibility of recursive self-similarity. The first pictorial representations of a fractal shape is given by Koch curve, the construction of which is shown in Figure 1.

FIGURE 1. An example of a scalable fractal structure. The shape if made by replacing all straight lines with the primary shape (a), and keep repeating this process ad infinitum. For the final shape no matter what scale we view it at it will always have the same structure.

(a)

(b)

(c)
In terms of applying this science to real world scenarios, the concept of fractals has been applied to porous structures: however there is a limitation in transferring the concept of fractals from a purely mathematical perspective to the real world. In a mathematically generated fractal there is a degree of self similarity upon dilation, thus the same structures are visible at different length scales, however, with a real structure this degree of self similarity in general does not exist and so it is necessary to define two or more length scales that will allow for the concept of self-similarity. It is possible to use the concept of a fractional dimension and apply this to determine the dimensionality of the porous structure, which has been done with silica porous structures, resulting in a dimension between 1.7 and 2.7 dependent on the manufacturing process. This calculation uses a box or tiling method, with a definition of the fractional dimension being:

\[
\ln(n_m) = \ln(k) - \left(\frac{d_f}{2}\right)\ln(\sigma)
\]

Equation 1

Where \(\sigma\) is the effective cross-sectional area of each particle, \(k\) is a proportionality constant, thus a plot of the concentration of the particle versus the effective cross-sectional area will give the fractal dimension.

Methods

Sample Preparation

The reaction is based on a modified Stöber reaction and typically has PVA, poly(vinyl alcohol), (0.25 g, 5 wt%) and CTAB, (3-glycidoxy propyl) trimethoxy silane, cetyltrimethylammonium bromide, (0.1 g, 2 wt%) dissolved in 5 g water. (The concentrations of polymer and surfactant in this article were referred to the amount of water.) To this solution, 8 mL methanol was added while stirring. As-purchased ammonium hydroxide solution was diluted with water to 5.6% and 2 ml of this solution was added into the reaction mixture. After stirring vigorously for 15 min, 0.5 mL MPTMS (3-mercaptopropyl) methyltrimethoxysilane) was added drop-wise over a 30 second period. The reaction was stirred for 24 hours at room temperature. The resulting silica microspheres were collected by centrifuging the suspension. These are described as sphere on sphere and cationic surfactant leads to the production of monodisperse, porous silica microspheres. If TEOS is used it will increase the pore diameter, however this leads to a decrease in the fractal dimension and apply this to determine the sectional area will give the fractal dimension.

Results

Effect of varying experimental parameters

Figure 3 shows the effects of altering some of the experimental parameters.

FIGURE 3. Some of the different morphologies obtained when varying an experimental parameter.

- A. Standard SOS particle
- B. Effect of stirrer speed
- C. Effect of altering the alkalinity
- D. Altering the original polymer concentration

Variations to the original recipe were investigated to determine how these parameters would affect the final morphology of the silica particle.
Bonding

Typically, 1.0 g of the calcined SOS particles were dispersed in 7.5 mL toluene, 0.5 g butyl(chloro)dimethyl silane (C4) and 0.1 g imidazole and stirrer bar was added, vessel sealed and placed into the reactor. The reaction was micro waved for 20 minutes at 110 °C, at a power setting of 200 W. Resultant particles were thoroughly washed with toluene, methanol, 50% aqueous methanol, then methanol. End-capping was performed using the same method with 0.5 g 1-(trimethylsilyl)imidazole in place of C4 and the omission of imidazole.

Chromatography

Figure 4 shows two chromatograms obtained with a commercially available solid core phase and the new SOS phase. It can be seen that the SOS material has a comparable performance to the commercially available phase that has been designed for larger molecules.

Experimental conditions: mobile phase A. Water + 0.2% TFA, B. Acetonitrile + 0.2% TFA, Gradient; 30-75% B in 10 minutes, 400 μL/min, 50 °C.

A – Commercially available solid core C4, 100 x 2.1 mm.
B – SOS material, 100 x 2.1 mm.

Conclusion

We have introduced the concept of fractal chromatography which has aided the understanding of the structure of a new particle. Using a modified Stöber reaction it has been demonstrated that a fractal type structure can be manufactured, and the effects of a series of variables on this morphology has been investigated including:

- Effect of stirrer speed
- Effect of polymer reagent concentration
- Effect of altering the alkalinity

The particle substrate has been bonded and compared with a commercially available solid core phase. The resultant chromatography obtained is comparable for a range of protein molecular weight.

References