#T01

EPROGEN INC

Understanding MICRA® *NPS*®

NPS and Fast Chromatography

MICRA® *NPS*® is a breakthrough in fast HPLC. *NPS* is ultra-pure, highly uniform non porous silica spheres which provide the LC chromatographer greatly improved mass transfer and lower detection limits. Coupled with enhanced stability and dramatically reduced solvent usage, *NPS* is the ideal column to meet the ever increasing demands placed on today's analytical labs - Improved pro-

NPS an excellent support for separations in < 3 minutes

Fast HPLC

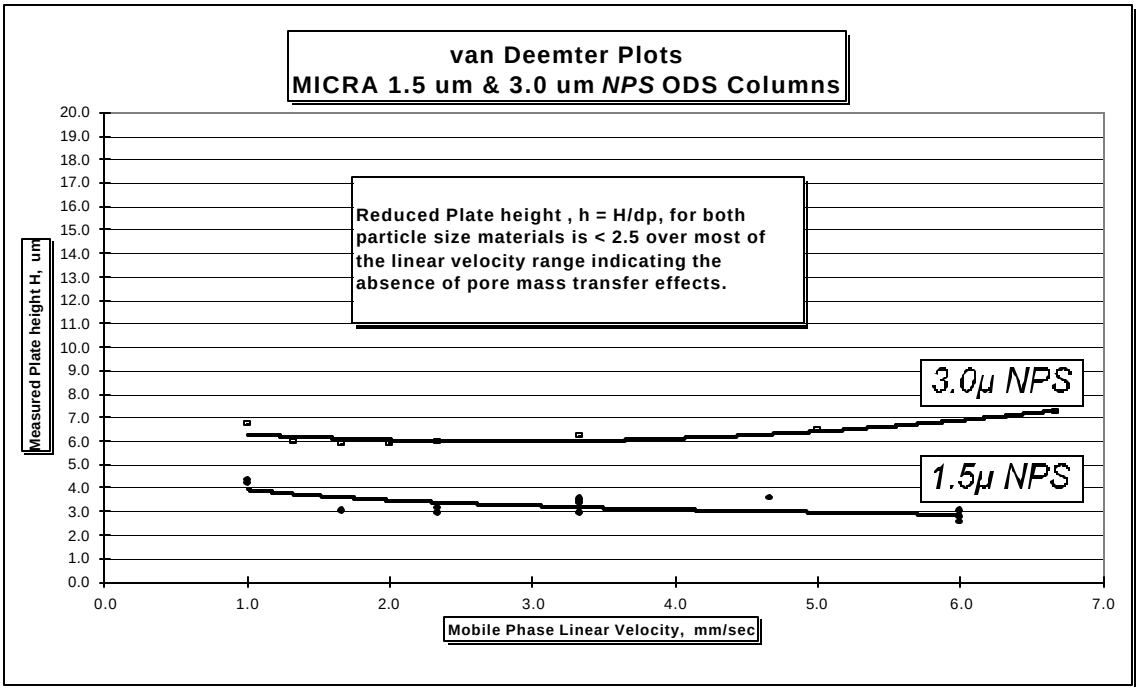
Eliminating pore diffusion and the use of small, highly uniform particles maximizes speed and

RESOLUTION

The essential goal of fast chromatography is to be able to substantially reduce analysis time while still maintaining good resolution of the components of interest. While there are several ways to improve speed, the most widely used means have centered on using shorter columns packed with smaller particles and increasing flow rate. These methods are preferred because they do not alter the selectivity (α) nor the retention factor (**k**) of the components. This means that the analyte resolution (R_s) will change only with changes in column efficiency (**N**).

$$R_s = (1/4)(\alpha - 1/\alpha)(k/k+1)(\sqrt{N})$$

Since column efficiency is directly linked to the column length (**L**) and particle size (d_p) by the equations (**H = L/N**) and ($N \mu 1/d_p$) respectively, the ideal case for fast chromatography is to use short columns packed with small particles to maximize speed while maintaining column efficiency. Column efficiency, however, can be further enhanced by minimizing mass transfer effects associated with the support material. The use of nonporous particles essentially eliminates the pore diffusion (**B & C**) terms of the van Deemter equation (**H = A + B/u + Cu**). As shown in the van Deemter plots above, columns packed with 1.5µ and 3.0µ *NPS* particles exhibit very high efficiencies that are insensitive to changes in flow rate or linear velocity, **u**. MICRA *NPS* represents the ideal HPLC column support for fast separations at the highest resolution possible.



MICRA *NPS* and Fast Chromatography

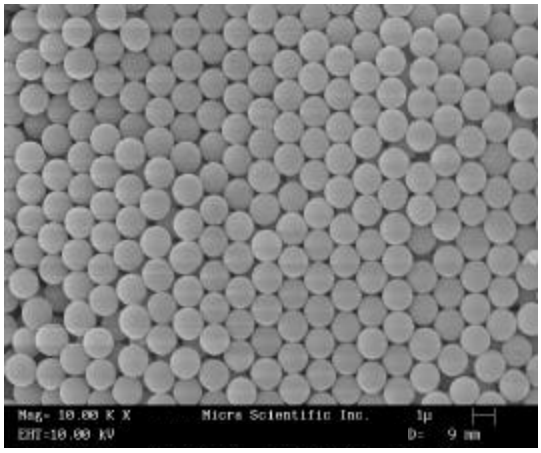
Retention Factor, k and Speed

Eliminating Porosity can speed up HPLC analysis by as much as a factor of 10 while still maintaining high resolution and sensitivity!

Eliminating pores also reduces the available surface area for C-18 bonding substantially. For a given diameter of highly uniform nonporous silica particles, the surface area (a_{NPS}) available for bonding is calculated from the density (ρ) and particle diameter (d_p) of the silica.

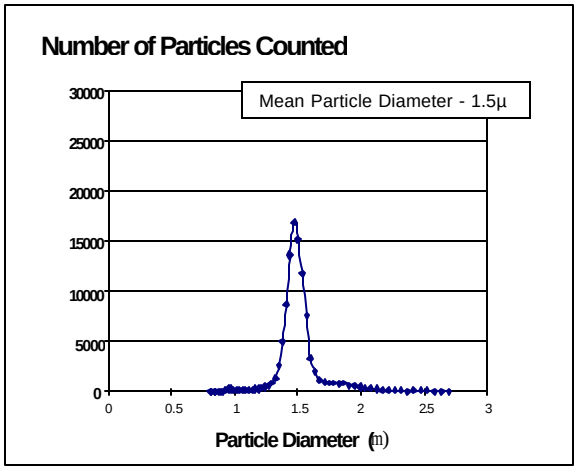
$$a_{NPS} = 6/\rho\, d_p$$

For *NPS* we typically measure a density of 2.1 ± 0.1 g/mL. For a 1.5µ *NPS* material the surface area then is expected to be 1.9 m^2/g and is what we typically observe. This can then be compared to a typical porous support surface area of around 200 m^2/g and a silica density of 0.4 g/mL.



Typical SEM Photograph of 1.5µ *NPS*

Lower Surface Area of *NPS* leads to lower modifier usage. A total savings of



Particle Size Distribution
MICRA *NPS* 1.5µ

column, V_m, is a sum of the interstitial and pore volumes. These volumes are roughly equal for a typical porous support. Eliminating the porosity eliminates 50% of the available liquid volume in a packed column.

$$V_{m,NPS} \approx 1/2\, V_{m,porous}$$

NPS has 5 times the density and 1/100 the surface area of porous supports. For the same separation and mobile phase composition, *NPS* will exhibit capacity factors, **k**, roughly 10 times smaller compared to a typical porous silica;

$$\Rightarrow \quad k_{NPS} \approx 1/10\, k_{porous}$$

The net effect is that analytes will elute about ten times faster on a nonporous support compared to a porous support for the same mobile phase composition.

In reversed phase separations, the equation below can be used to compare the expected values of **k** for the two supports.

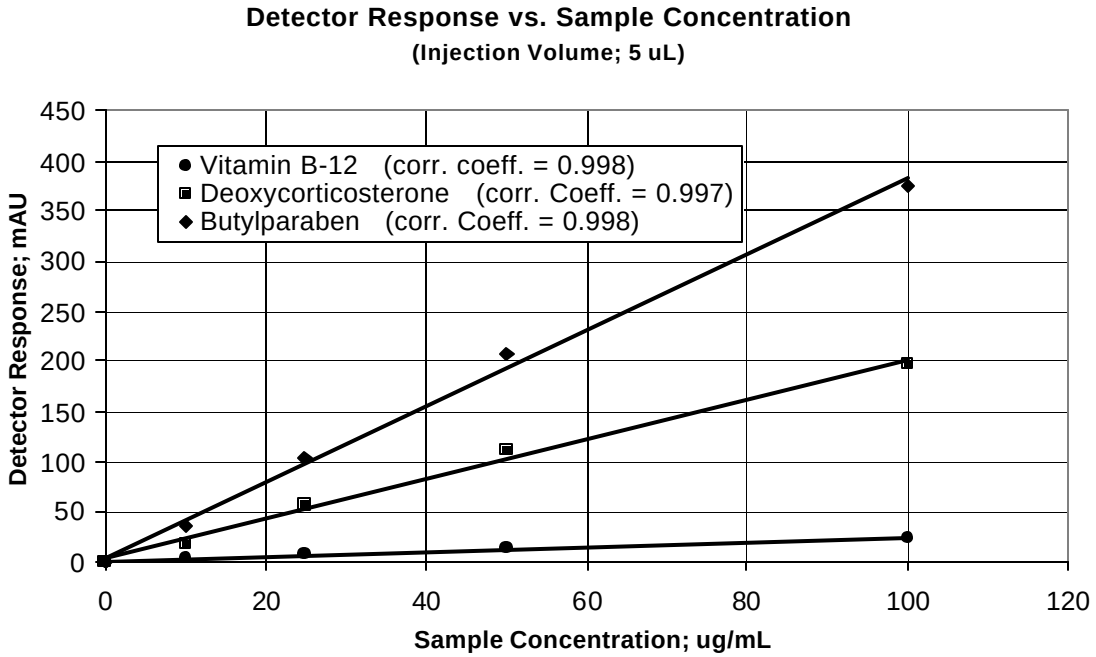
$$k = KV_s/V_m$$

Here, the distribution coefficient (**K**), the support volume (V_s) and the mobile phase concentration (V_m) contribute to the distribution of solute between the stationary surface and moving liquid. It is assumed here that the pores themselves do not change the solute-solvent equilibrium to any significant degree. The surface area of the support is, therefore, very representative of the support contribution to the equilibrium of the column (i.e. $V_s \propto a_s$).
The available volume for liquid in a packed

Optimizing k on *NPS*

The optimum **k** for HPLC separations is between 2 - 10. A peak eluting at 30 minutes [at 1 mL/min] on a 4.6 x 250 mm column packed with porous particles [$k \approx 10$] will elute in < 1 minute on a 4.6 x 33 mm *NPS* column [$k \approx 1$]. Reducing the strength of the mobile phase by a factor of 2 - 3, using 1/3 less modifier compared to a porous column, will convert the 30 minute assay [$k \approx 10$] into a 2 minute assay [$k \approx 3$] on a MICRA *NPS* column with excellent resolution.

A 30 minute assay using a porous support can be converted into a 2 minute assay using ***NPS*** by simply reducing the modifier content of the mobile phase by a factor of 3.



MICRA *NPS* has sufficient capacity for routine HPLC analysis.

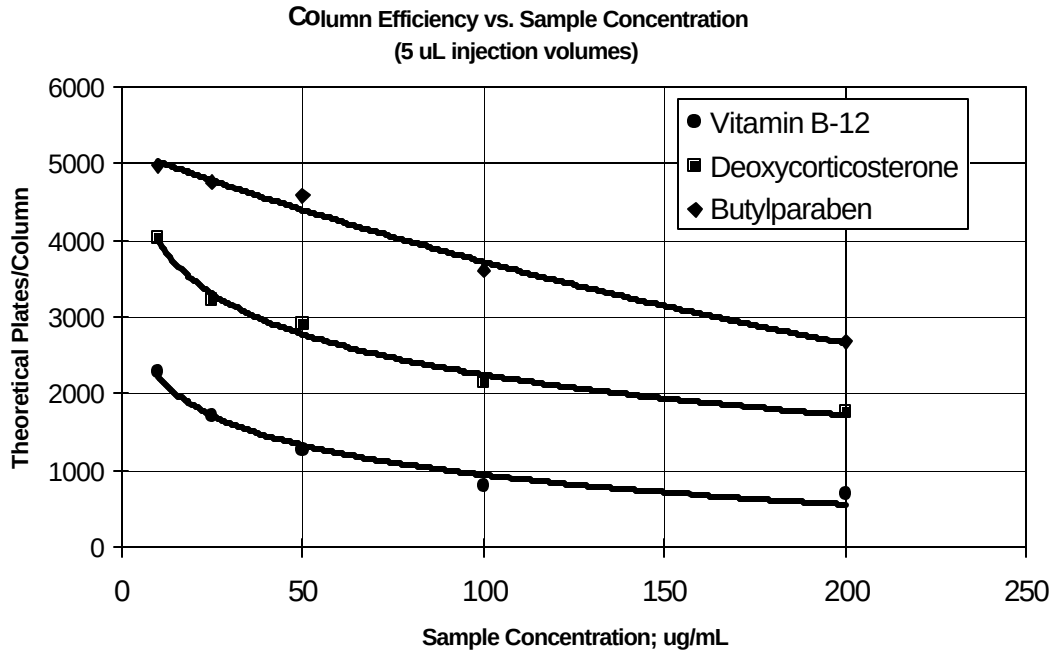
Excellent results are obtained for sample

Column Capacity

From the viewpoint of Loadability, it is obvious that using nonporous supports will result in an inherent lower overall capacity. The critical issues, though, are detection limits and sensitivity and not absolute capacity. The use of shorter columns with nonporous particles directly creates increased mass sensitivity. More sample is detected in less eluted volume allowing for injections of less sample in smaller volumes. Moreover, most HPLC assays (using UV-Vis detection) can easily be run with sample concentrations =100 µg/mL and a typical HPLC autosampler can reproducibly inject 1-100 µL making the total sample loading range only around 0.01-10 µg. As the above graph shows, at these sample loading levels, *NPS* has more than enough capacity to work well under routine analytical conditions, even for 4.6 x 33 mm column. It is the lower limit of detection that is critical to HPLC analysis, not the upper limit.

Detection Limits of 0.01 - 10 µg are easily obtained!

MICRA *NPS* exhibits good performance on small and large molecules.



MICRA *NPS* and Fast Chromatography

Think small

Think fast

Think *NPS*®

Short Columns have small total liquid volumes. Weaker eluting sample solvents are important for obtaining high efficiency at high analysis speeds .

For **MICRA *NPS*,** injection volumes of ≤ 5 µL are best when samples are dissolved in the mobile phase.

Injection Volumes up to 50 µL are possible but require weak eluting sample solvents.

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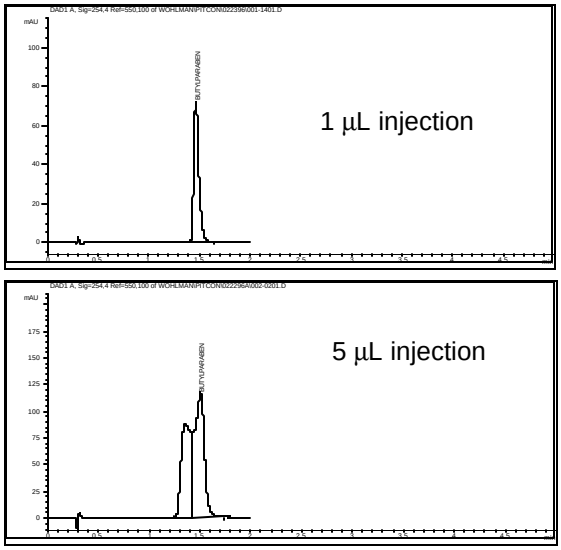
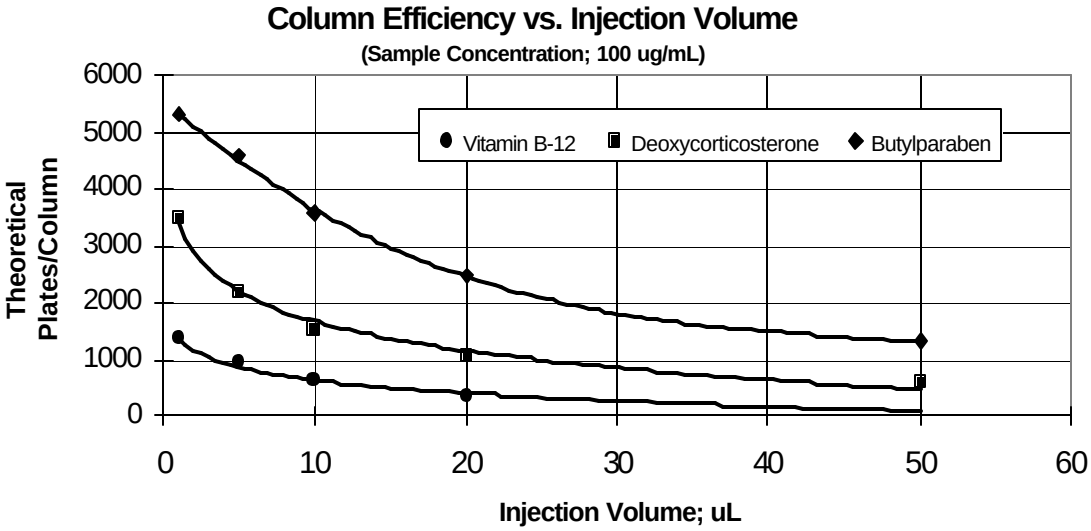
Injection Volume

Using short columns requires the use of smaller injection volumes (≤20 µL) to minimize dispersion associated with the sample. A 4.6 x 33 mm column has an empty column volume of 550 µL. After packing, the available volume for a nonporous packing is roughly 150 µL (300 µL for a porous packing), making sample solvent mismatch an important issue. The top figure at the right shows this effect for injection of 1 µL & 5 µL of a 100 µg/mL Butylparaben sample dissolved in 100% ACN on a 1.5µ *NPS* 4.6 x 33 mm column. The 5 µL injection shows clear signs of peak splitting associated with solvent mismatch.



100 µg/mL Butylparaben injection in



For samples dissolved in mobile phase we performed a complementary study to the detector response data [top page 3]. Here we plotted column efficiency (**N**) as a function of sample concentration at constant (5 µL) injection volume [bottom page 3] and, as in the graph above, a function of injection volume at constant sample concentration. For short columns, best results will be obtained for samples dissolved in mobile phase or weaker eluting power solvents. Injection volumes of ≤ 10 µL and sample concentrations of ≤ 100 µg/mL will minimize the loss in column efficiency. For short columns, the weaker the sample solvent the higher the injection volume possible.