MINIMIZATION OF EXTRA COLUMN EFFECTS IN CAPILLARY AND NANO-FLOW LIQUID CHROMATOGRAPHY

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INTRODUCTION

In Proteomic research samples can be very complex. A mammalian cell produces about 2000 proteins which when enzymatically digested produce 10s of thousands of fragments. The analysis is further complicated by a large variation of expression level spanning 5 orders of magnitude, where generally proteins of interest are present at the lower end of the concentration scale. The main objective in developing a suitable nano-LC separation system is to reduce extra-column band broadening which degrades the resolution of the column.

FACTORS REDUCING CHROMATOGRAPHIC PERFORMANCE

- Void Volumes
- Non-Specific Interactions
- Disruption of the Laminar Flow

VOID VOLUMES CAUSED BY THE COLUMN

a) Packing quality: The density of the packing is determined by the packing process (slurry composition, packing pressure and duration) but is also influenced by the "wall effect" (particles at the column wall are less densely packed than the column centre). A bad packed column can settle during use generating a void on the column inlet.

Due to the wall effect a 100mm column with 3µm particles will give a better resolution than a 150mm column packed with 5µm particles.

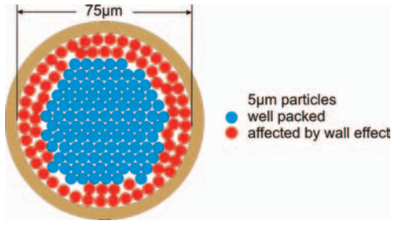

Figure 1. Illustration of the wall effect on the example of 5µm particles in a 75µm ID column

b) Deactivation of all wetted surfaces: This includes use of highly end-capped packing material, chemical deactivation of the column wall and the inner bore surfaces of all connection capillaries and avoiding ferrous metal in the frit material.

c) Minimizing volume and exposed surface of the frit: A frit made of woven fibres has a lower internal volume and smaller exposed surface area than sinter-metal frits.

ProteCol™ COLUMN DESIGN

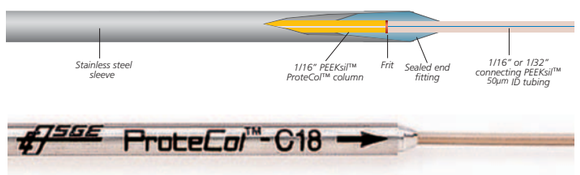

Figure 2. Integrated design of ProteCol™ columns

- High quality packing material
- Integrated connection capillaries
- PEEKsil™ (fused silica lined PEEK™) for column body and connection tubing
- All surfaces are deactivated: Silanol groups on the fused silica are chemically deactivated; the stainless steel fibres of the frit are gold plated and covalently deactivated

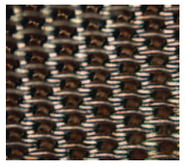

Figure 3. Microscopic picture of the gold-plated frit material

BAND BROADENING CAUSED BY CONNECTIONS AND CONNECTION TUBING

Laminar flow disruption can be minimized by the wall smoothness on a microscopic level reducing the amount of micro turbulence.

Lowest possible tolerances on concentricity of tubing: good alignment of the bores (figure 4).

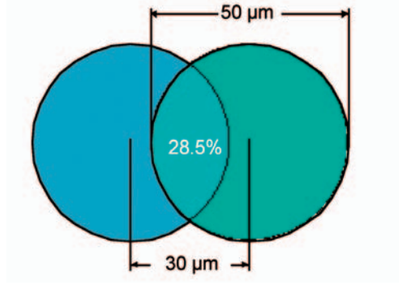

Figure 4. Worst possible alignment of 50µm ID capillaries with ±15µm tolerance on concentricity

REDUCTION OF VOID VOLUMES:

Square tubing ends allow zero-volume butt connections (figure 5)

Integrated design minimizes the number of connections and reduces tubing length.

Use of low volume accessories like in-line filters (figure 6), splitters and unions.

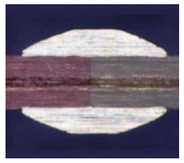

Figure 5. Zero-volume butt connection

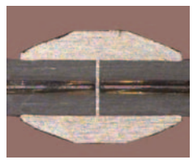

Figure 6. Low volume in-line filter and column ends

EFFECT OF VOID VOLUME

Any additional volume increases the dwell time, the time the sample spends in the system and with it the time it has to diffuse (figure 7).

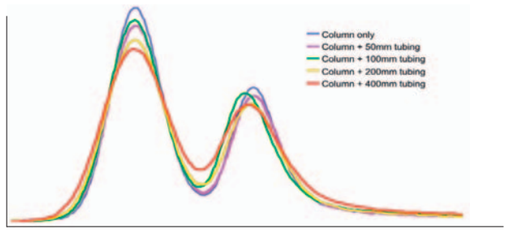

Figure 7. Effect of additional tubing on the peak resolution

EFFECT OF ADDITIONAL CONNECTIONS

Due to a unavoidable tolerance in the concentricity of capillary tubing each connection introduces a disturbance in the laminar flow. Furthermore, user made connections can be non-ideal by leaving a small gap between the connecting capillaries. (figure 8)

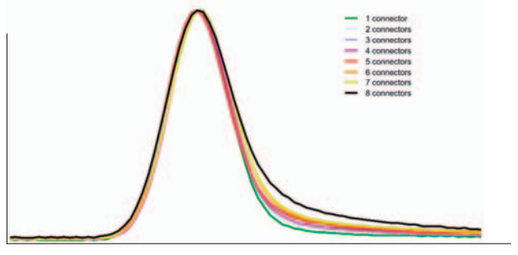

Figure 8. Effect of the number of connections on peak broadening

ProteCol™ RANGE OF PRODUCTS

The aim is to provide a complete system of columns and accessories with the minimization of band broadening as the highest priority.

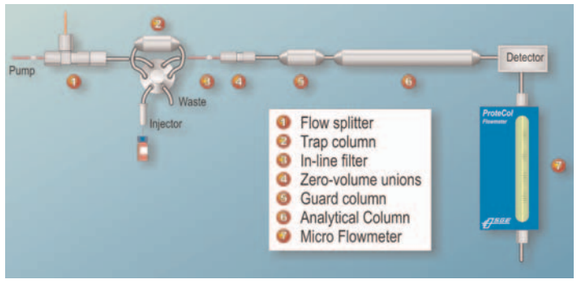

Figure 9. Schematic overview of the ProteCol system

APPLICATION EXAMPLE: TRYPTIC DIGEST OF A MONOCLONAL ANTIBODY

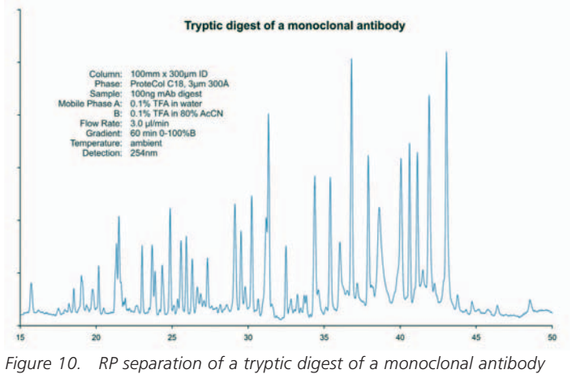

Figure 10. RP separation of a tryptic digest of a monoclonal antibody

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