Abstract

Due to the internal pressures of HPLC and UHPLC columns, cold drawn stainless steel tubing is the material of choice. When stainless steel tubing is cold drawn, the physical reduction of the inner diameter of the bore deforms the inner surface, which subsequently generates a very rough surface \( (Ra > 1000 \text{ nm}) \). The surface roughness is in the order of \( \mu \text{m} \). If you were to pack HPLC columns with this tubing the surface roughness would interfere with the best quality along the wall and result in broad peaks and asymmetrical peak shape. The inside of the column can be treated mechanically or chemically in order to provide a smoother surface and increase the column efficiency. Stainless steel column tubing from four different manufacturers were analysed and compared. The surface roughness of the columns measured covered a wide range from \( Ra = 424 \text{ nm} \) to \( Ra = 2110 \text{ nm} \). This inner diameter surface roughness variation can be attributed to the various post-processing surface finishing methods used to reduce the surface roughness.

For the best column performance however, we have developed a range of glass lined tubing whose surface characteristics cannot be achieved by treating steel tubing \( (Ra = 105 \text{ nm}) \). Inner diameter dimensions of glass lined columns also exhibit extremely tight tolerances. In fact, they are tighter than what is achievable by stainless steel tubing alone. An additional benefit of glass lined column hardware is the suppression of non-specific interactions between chelating sample components and the metal surface.

Fundamental Difference in the Manufacturing of Stainless Steel and Glass Tubing

Stainless steel forms a protective oxide layer on its surface. While the metal is ductile, the oxide layer is not. Seamless stainless steel tubing is manufactured in a cold drawing process where a larger diameter piece of steel is drawn through a number of dies in order to reduce the dimensions to the required values. During this process the oxide layer crumbles and forms a very rough inner surface. Glass is heated to its softening point and then drawn at a certain speed to achieve a smoother surface finish. Glass does not have the problem caused by the oxide layer and any imperfections in the surface are further reduced during the drawing process.

Surface Roughness and Chromatographic Performance

It has been described \( (J.J. \text{ Kirkland, J.J. DeStefano, The art and science of forming packed analytical HPLC columns, J.Chrom. (2006)}) \) that surface roughness has a large impact on ability to achieve a well packed column. Manufacturers of column hardware will usually claim the Ra values for their tubing.

The difference in performance between a column packed into standard stainless steel tubing and into polished tubing is profound.

Surface Analysis of Commercial HPLC Tubing

HPLC tubing from some leading HPLC suppliers were cut open, sectioned and thoroughly cleaned in an ultrasonic bath before being analysed using optical microscopy and the Bruker Contour surface measurement instrument. The resulting surface topography plots and the microscopy images are shown below together with the measured Ra value. The Ra value is the arithmetic average of the deviation of the surface from a mean value taken from measurement points. In this case all Ra values are quoted in \( \text{nm} (10^{-9} \text{ m}) \).

Example A: Example B:

![Surface Analysis of Commercial HPLC Tubing](image)

Measured Ra = 1675 nm Measured Ra = 509 nm

Example C: Example D:

![Surface Analysis of Commercial HPLC Tubing](image)

Measured Ra = 422 nm Measured Ra = 735 nm

Surface Analysis of Glass Lined Tubing (GLT®)

SGE Analytical Science GLT® has a far superior finish to all samples of polished stainless steel. The measured Ra value was 62 nm which is almost seven times better than the best of the stainless steel samples. Because the surface finish is inherent in the manufacturing process and does not rely on an additional post-processing step, the quality of the tubing is highly consistent between batches. An additional advantage of GLT is that the glass lining prevents any non-specific interactions between the metal surface and the sample.

Inertness of Glass

Many functional groups in pharmaceuticals or bio-molecules can form undesired non-specific interactions with metal surfaces. Non-specific binding can lead to peak broadening and reduced sensitivity and in more extreme cases to sample loss and increased carryover. Some examples for “difficult” analytes would be phosphopeptides or metal chelating drugs such as the tetracycline group of antibiotics.

The performance test was performed with N-hydroxypyridine-2-on, the active part of the anti-fungal drug ciclopirox and a very strong probe for metal interactions.

![Surface Analysis of Glass Lined Tubing](image)

The tailing factors for the different columns were:

\[ Tf = 1.2 \] for the GLT column
\[ Tf = 1.6 \] for the SS column
\[ Tf = 2.8 \] for column A
\[ Tf = 4 \] for column B

The results for columns A and B were also influenced by the different stationary phase of the respective manufacturer.

Conclusions

We have shown that GLT has at most only 1/3 of the surface roughness than polished stainless steel. The reduced surface roughness facilitates an improved packing quality and leads to a better chromatographic performance.

The second advantage of GLT is that the sample does not come in contact with a metal surface. Many pharmaceutical active compounds and biological samples contain groups that interact strongly with transition metals such as iron, chromium or nickel. Suppressing these interactions results in a better peak shape and higher sensitivity.

Finally, the low surface roughness of glass is an intrinsic property of the material and does not require a secondary step of surface treatment. It is therefore highly reproducible.

For further information visit: [www.sge.com/protecolbioinertuhplc](http://www.sge.com/protecolbioinertuhplc)

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