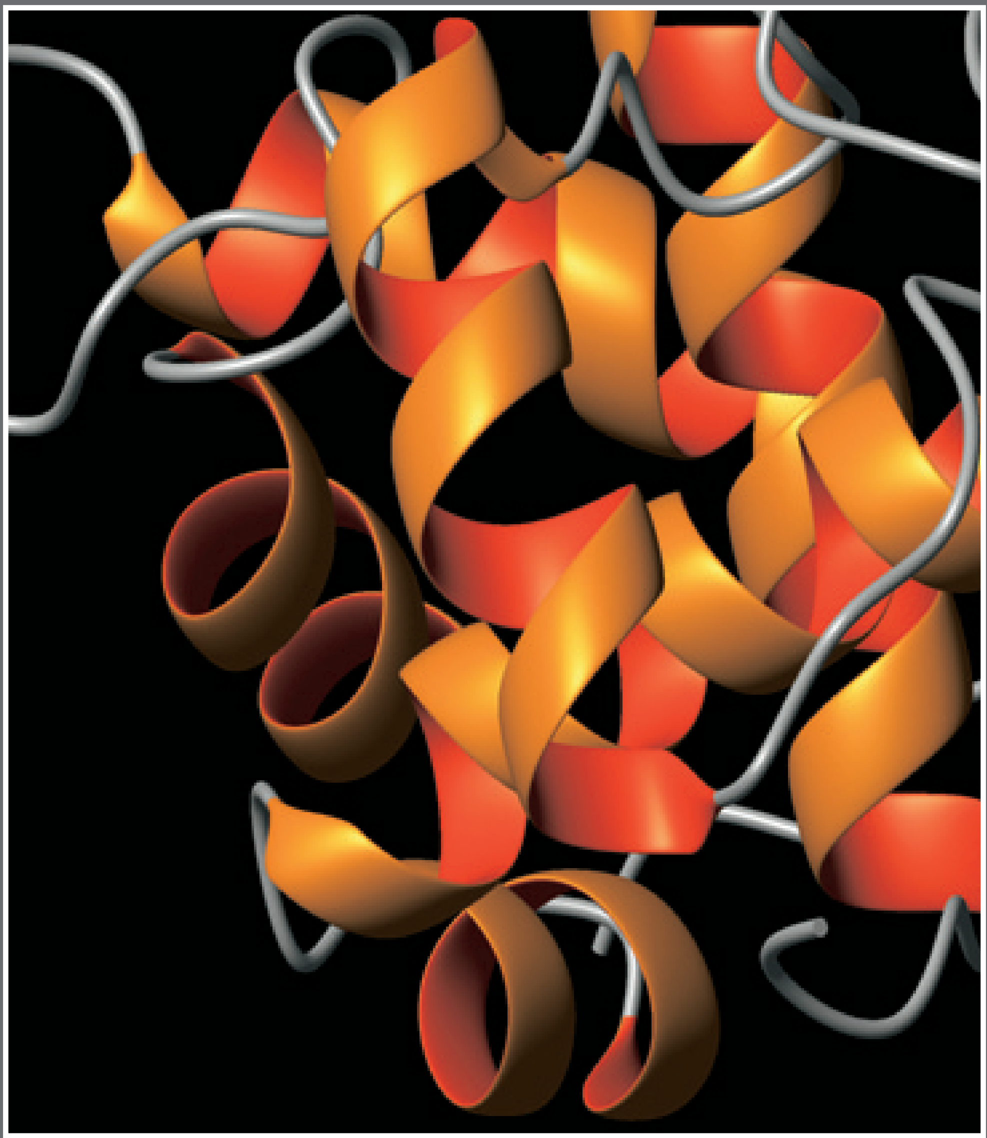
Kromasil 300 Å
– for your protein separations



Kromasil®

The way to peak performance
in liquid chromatography

Kromasil 300 Å – protein separations from analytical to process scale

Kromasil 300 Å is designed to be the perfect choice for proteins and biomolecules larger than 8–10 kD. A 300 Å material with a narrow pore size distribution ensures a good mass transfer for molecules in this range, resulting in narrow peaks and no size-exclusion effects.

Figures 1 and 2 show FE-SEM studies of Kromasil 300 Å, indicating a very regular pore structure, with no voids or dense clusters.

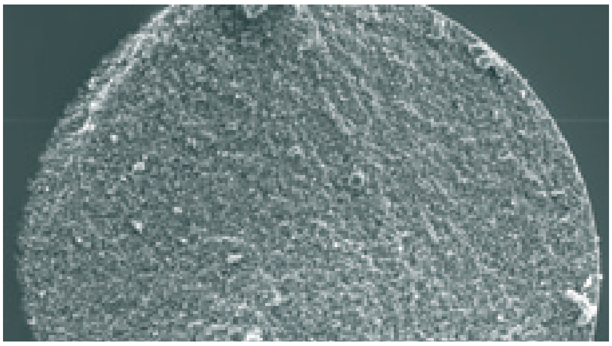


Figure 1 | FE-SEM picture of a cut through a Kromasil 300 Å particle at 5,000 × magnification.

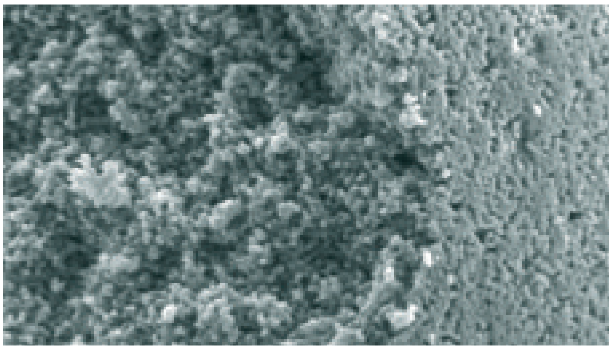


Figure 2 | FE-SEM picture of a cut through a Kromasil 300 Å particle at 35,000 × magnification, showing both the outer surface and the fracture through the particle.

Mechanical stability

Kromasil 300 Å is perfectly spherical, with regular pore structure, and a surface area and pore volume providing high loadability and mechanical stability. The high mechanical stability is especially important when packing large diameter columns with dynamic axial compression (DAC). Figures 3 – 4 show the result of a comparison of mechanical stability between Kromasil 300 Å and competitor "V" 300 Å C4.

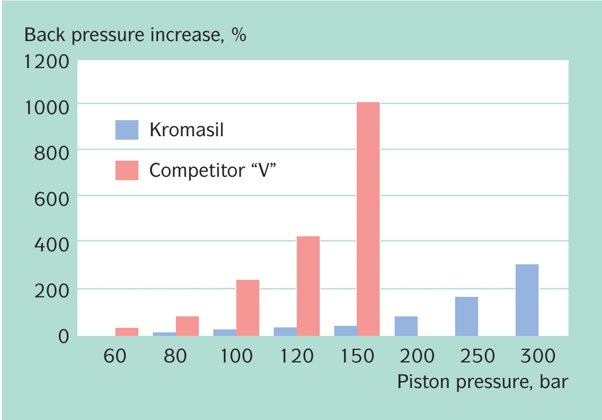


Figure 3 | Back pressure increase after compression in a 50 mm ID DAC column, with a bed length of 25 mm. The back pressure increase is relative to the pressure at 40 bar piston pressure. The study of competitor "V" had to be terminated at 150 bar due to formation of fines.

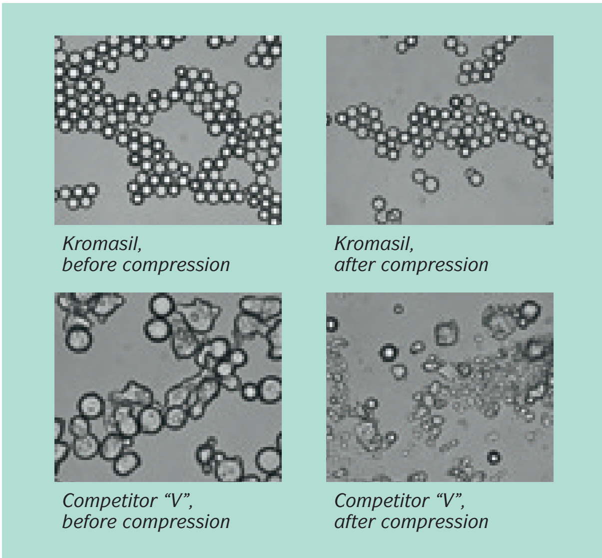


Figure 4 | Light microscope images of Kromasil and competitor "V", before and after compression in the DAC column shown in figure 3. Note that Kromasil was compressed up to 300 bar, while competitor "V" was compressed only up to 150 bar piston pressure.

Chemical stability

The chemical stability is together with mechanical stability the most important factor for determining the lifetime of your column or packing material. At low pH the bonded phase can be hydrolyzed, resulting in a less hydrophobic surface, and reduced retention times for lipophilic compounds. At higher pH the silica matrix itself can be dissolved, and both silica and bonded phase are lost, causing void formation. This process results in changed retention times and poor peak shape.

The chemical stability for Kromasil C4 and competitor “V” C4 was tested at low, neutral and high pH using conditions giving accelerated breakdown.

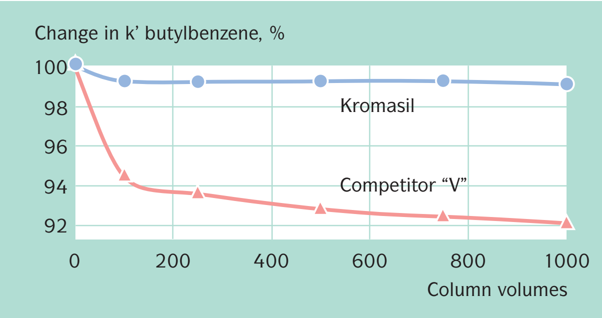


Figure 5 | Chemical stability at low pH.

Conditions: Mobile phase: ACN/H_2O/TFA = 50/50/1 Flow rate: 2 ml/min. Temperature: 20 °C

Material	Leakage of Si at: neutral pH	high pH
Competitor “V”	42 ppm	total dissolution
Kromasil	2 ppm	50 ppm

Table 1 | Chemical stability at neutral and high pH. Columns were purged, and the chemical stability was monitored by analyzing the concentration of silicon in the effluent using AAS.

Conditions, neutral pH test: Mobile phase: ACN/0.25 M Na_2HPO_4 = 20/80, 1000 column volumes. Flow rate: 1 ml/min. Temperature: 60 °C

Conditions, high pH test: Mobile phase: n-propanol/0.1 M NaOH = 50/50, 10 column volumes. Flow rate: 1 ml/min. Temperature: 22 °C

Chromatographic properties

Kromasil 300 Å is designed and manufactured to exhibit a surface chemistry similar to the well-known Kromasil 100 Å silica. This ensures excellent peak shape and resolution for acidic, neutral and basic molecules. Kromasil 300 Å shows symmetrical and narrow peaks even for proteins and other demanding molecules, as shown in figures 6 and 7.

Tryptic digest of BSA

A common test for RP packings aimed for separation of biological material is to run a tryptic digest of BSA. The digest contains fragments of various sizes, and the separation of these into individual peaks is a good evidence of the power of resolution (figure 8).

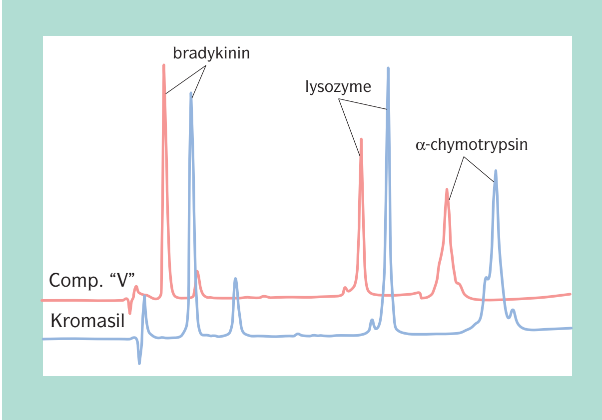


Figure 6 | Peptide and protein separation for Kromasil KR300-5-C4 and competitor “V” 300 Å 5 µm C4.

Conditions: Column: 4.6 mm × 250 mm Mobile phase: A: ACN/H_2O/TFA = 5/95/0.1 B: ACN/H_2O/TFA = 90/10/0.1 Gradient: 25% – 75% ACN in 25 min. Flow rate: 1.0 ml/min. Temperature: 20 °C Detection: UV at 220 nm

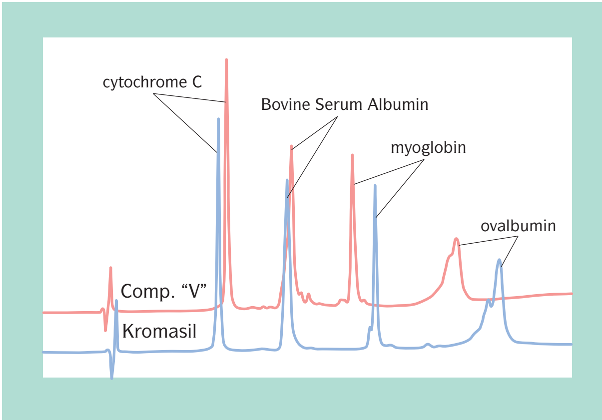


Figure 7 | Protein separation for Kromasil KR300-5-C4 and competitor “V” 300 Å 5 µm C4.

Conditions: Column: 4.6 mm × 250 mm Mobile phase: A: ACN/H_2O/TFA = 5/95/0.1 B: ACN/H_2O/TFA = 90/10/0.1 Gradient: 30% – 70% ACN in 30 min. Flow rate: 1.0 ml/min. Temperature: 20 °C Detection: UV at 220 nm

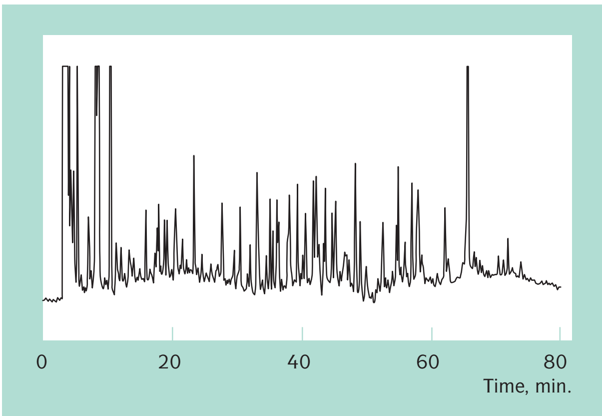


Figure 8 | Tryptic digest of bovine serum albumin (BSA).

Conditions: Column: 4.6 mm × 250 mm, Kromasil KR300-5-C4 Mobile phase: A: ACN/H_2O/TFA = 4/96/0.085 B: ACN/H_2O/TFA = 90/10/0.1 Gradient: 4% ACN for 5 min, 4% – 40% ACN in 75 min. Flow rate: 1.0 ml/min. Detection: UV at 215 nm Temperature: 22 °C

Cover figure prepared with MOLMOL (Koradi et al., 1996, J Mol Graphics 14, 51).