Kromasil 300Å for macromolecular analysis and separation

Kromasil®

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AKZO NOBEL

RP separation of peptides and proteins

RP separations of macromolecules such as polypeptides and proteins are most often carried out by using shallow gradients. The reason for this is that they are desorbed from the stationary phase in a very narrow window of the mobile phase composition. This behavior is often referred to as a pronounced on/off mechanism type of chromatography. Moreover, macromolecules, due to their molecular size is also subject to an adsorption type, in contrast to a partition type, chromatography. The adsorption type interaction is a pronounced hydrophobic interaction and is mostly dependent on the actual function, the outermost layer, facing the mobile phase. In addition, additives to the mobile phase, such as TFA, are also used to suppress other types of interactions.

Due to these facts bonded phases with altered hydrophobicity offers somewhat different, but not totally different selectivities.

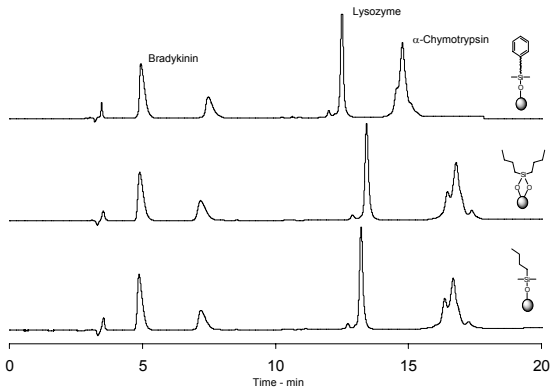

Figure 1 Separation of Bradykinin, Lysozyme and α-Chymotrypsin on Butyl-, Di-butyl- and Phenyl functionalized KR 300Å 5 µm material. Mobile phase: (A) H2O/ACN/TFA 90/10/0.1 (B) H2O/ACN/TFA 5/95/0.1 Gradient 25% - 75% B in 25 min Detection: UV@220 nm Flow rate: 1 ml/min Temperature: 20°C

Chromatographic examples

Figure 1 shows the separation of peptides/proteins on three differently functionalized Kromasil 300Å materials. Only small differences are observed in the behavior of the analytes, despite substantial differences in sorbent functionality. This behavior is generally observed, due to the complexity and multiplicity in the sorbent-analyte interactions. This renders separate interactions, *e.g.* π-π-interactions, not clearly observable.

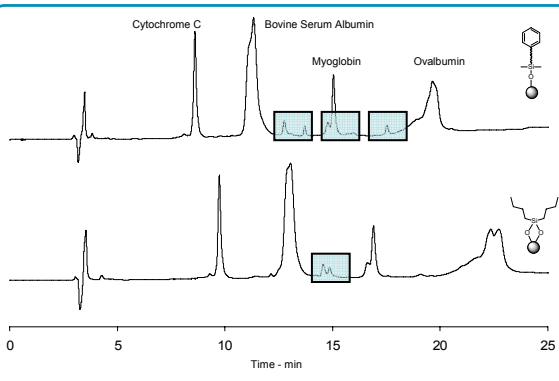

Figure 2 Separation of Cytochrome c, Bovine Serum Albumin, Myoglobin and Ovalbumin on Di-butyl- and Phenyl functionalized KR 300Å 5 µm material. Mobile phase: (A) H2O/ACN/TFA 90/10/0.1(B) H2O/ACN/TFA 5/95/0.1 Gradient 30% -70% B in 30 min Detection: UV@220 nm Flow rate: 1 ml/min Temperature: 20°C

Figure 2 shows the separation of four proteins on Di-butyl and Phenyl functionalized Kromasil 300Å materials. As can be seen small, but important, differences are observed in the chromatographic behavior. Using the phenyl-modified Kromasil 300Å material provides an increased resolution of the impurity profile in this case. (For example: The Phenyl phase seems to be able to separate one impurity component from the ovalbumin peak and to separate two of the impurities in the BSA peak.)

The characteristics of Kromasil 300Å silica based sorbents

Chromatographic separation of proteins and peptides are fully dependent on the base materials, the matrix, used for this purpose and also on the actual matrix modification, the chemical function. Silica has been used for decades, both within analytical and preparative chromatography, for small molecules and is now finding its use also for larger macromolecules.

Kromasil 300 Å is designed to be the perfect choice for proteins and biomolecules larger than 8–10 kD and ranging up to approximately 100 kD.

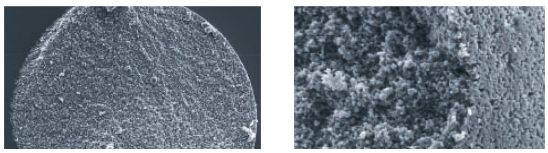

Figure 3 FE-SEM pictures of Kromasil 300Å, Left: picture of a cut through a Kromasil 300 Å particle at 5,000 X magnification. Right: FE-SEM picture of a cut through a Kromasil 300 Å particle at 35,000 X magnification, showing both the outer surface and the fracture through the particle.

Figure 3 shows FE-SEM images of Kromasil 300 Å, indicating a very regular pore structure with no voids or dense clusters, which ensures a good mass transfer of macromolecules.

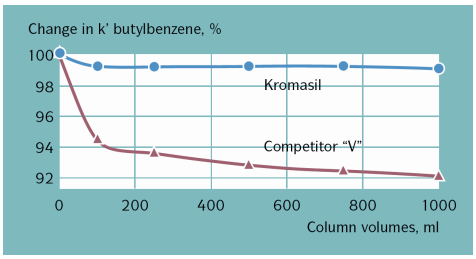

Figure 4 Chemical stability at low pH. Conditions: Mobile phase: ACN/H2O/TFA = 50/50/1 Flow rate: 2 ml/min. Temperature: 20°C

Figure 4 shows the change in retention factor for Kromasil 300 C4 and a competitor material when subjected to an acidic mobile phase. The retention factor is a measure of the chemical stability of a phase as the bonded phase can be hydrolysed at low pH. Hydrolysis of the bonded phase is accompanied by a shortening of the retention time for hydrophobic analytes. Figure 4 shows that the retention factor for the Kromasil 300 C4 material is practicaly the same throughout the test, in contrast to the competitor material.

Conclusions

Kromasil 300 Å products are the perfect choice for your macromolecular separations. The wide variety of accessible chemical functions (modifications) in combination with the possibility to adjust the chromatographic separation parameters, *i.e.* organic component, pH and gradient slope, due to chemical and overall product stability, make Kromasil 300 Å products the ideal tool for protein separations.