Technical Review

Chiral separations − efficient, fast and productive

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Introduction

Chromatographic analysis and purification of optically active compounds are still areas with large potential for improvement. In the analytical field, chiral stationary phases (CSP) with better performance giving enhanced resolution and shorter analysis time are desirable. Within recent years the Kromasil group has launched two new polysaccharide CSP's: Kromasil® AmyCoat™ which is based on a tris-(3,5-dimethylphenyl) carbamoyl amylose selector, and Kromasil® CelluCoat™, which is based on a tris-(3,5-dimethylphenyl) carbamoyl cellulose selector.

The stationary phase

The in-house developed wide pore silica is specially designed to minimize the amount of achiral interactions with the silica surface, while maintaining the mechanical strength of Kromasil silica. This mechanical strength allows operating the columns without pressure restriction within the standard HPLC pressure range (≤400 bar).

The amylose and cellulose selectors are well known for their ability to resolve a broad range of racemates. This unique coating technology ensures homogenous distribution of the selector and an optimal thickness − both important to generate a high-performing, yet stable, product.

Highly efficient 3 μm particles

Kromasil AmyCoat and CelluCoat are available in 3, 5, 10 and 25 μm particle sizes. The smallest particle size, 3 μm, gives high efficiency, and consequently a high resolution. Table 1 illustrates the similarity in chiral recognition capability between Kromasil AmyCoat 3 μm and 5 μm. The higher resolution obtained using Kromasil AmyCoat 3 μm is a result of the higher plate count achieved with a smaller particle size.

For difficult separations reducing the particle size could make the crucial difference between achieving baseline separation or not, as illustrated in figure 2.

High speed chromatography

The mechanical strength of Kromasil AmyCoat and CelluCoat allow the columns to be operated at high flow rates. High flow rate combined with short column length provides very short analysis time. Figure 3 a shows high speed chromatography with baseline

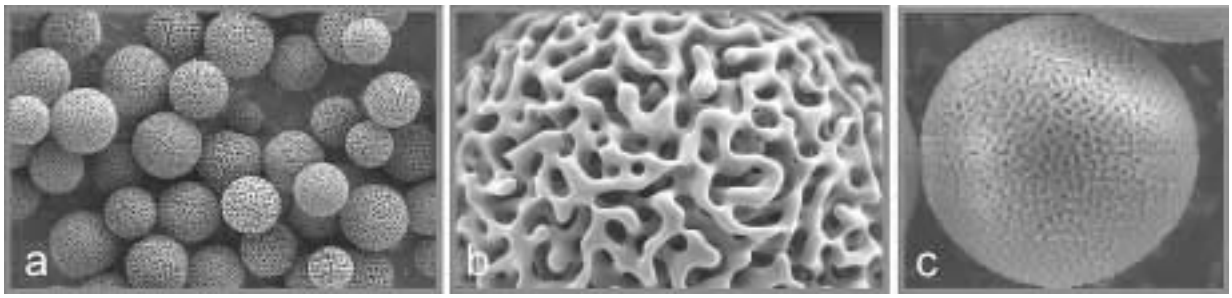

Figure 1. FE-SEM pictures. a and b: pictures of uncoated Kromasil wide pore silica used for AmyCoat and CelluCoat; c: picture of CelluCoat

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Table 1. Selectivity and resolution comparison of Kromasil AmyCoat 3 µm and 5 µm. Column size: 4.6×150 mm

Racemate	AmyCoat 3 µm		AmyCoat 5 µm		Mobile Phase	Flow rate [mL/min]
	α	R_s	α	R_s		
Benzoin	1.3	6.5	1.3	4.4	heptane/2−propanol (90/10)	1
Bucetin	1.8	8.2	1.7	5.8	heptane/2−propanol (90/10)	2
Trifluoroanthrylethanol	1.4	6.4	1.4	4.2	heptane/2−propanol (90/10)	1
Hexobarbital	1.4	4.7	1.4	3.2	heptane/2−propanol (90/10)	1
Oxamniquine	1.2	3.2	1.2	2.3	heptane/2−propanol/DEA (90/10/0.1)	0.8
Alprenolol	1.6	5.3	1.7	4.4	heptane/2−propanol/DEA (90/10/0.1)	1
Metoprolol	1.5	3.2	1.4	2.0	methanol/DEA (100/0.1)	0.5

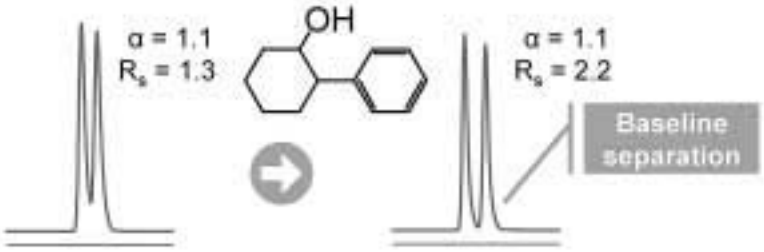


Figure 2. Separation of 2-phenyl-1-cyclohexanol in heptane/2-propanol (95/5), detection UV @ 220 nm, temp. 25℃, flow rate 1 ml/min, column size: 4.6×150 mm

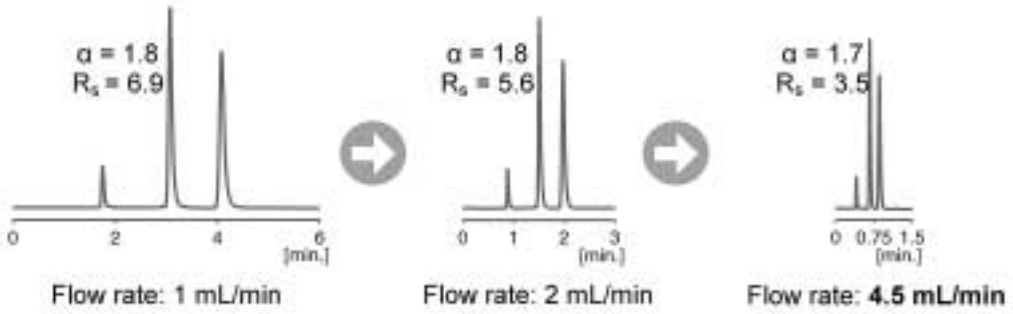


Figure 3a. Separation of Troger's base in heptane/2-propanol/DEA (90/10/0.1), detection UV @ 220 nm, temp. 22℃, column: Kromasil AmyCoat, 3 µm, 4.6×150 mm.

separation in less than 1 minute. Since the van Deemter plot, figure 3 b, is more flat for the smaller particle size, Kromasil AmyCoat 3 µm should be the first choice when running at elevated flow rates.

Mechanical stability

Mechanical strength is an important product lifetime parameter, and makes it possible to pack columns at higher pressure, to obtain higher efficiency. Kromasil AmyCoat and Kromasil CelluCoat are based on a mechanically strong spherical silica matrix, withstanding repeated cycles of packing in columns at high pressure.

Stable performance

It is most desirable to be able to run the compatible normal, po-

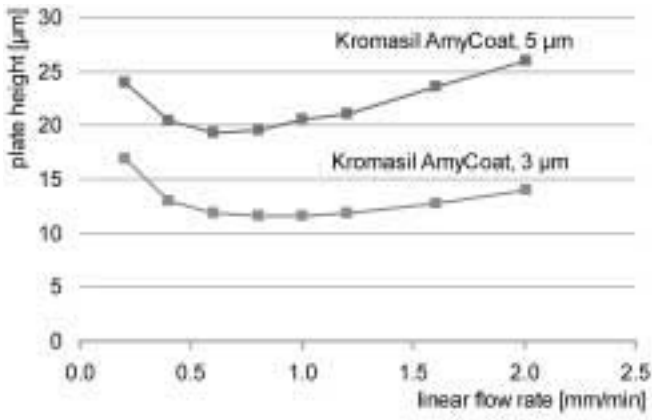


Figure 3b. van Deemter plots for Kromasil AmyCoat 3 and 5 µm.

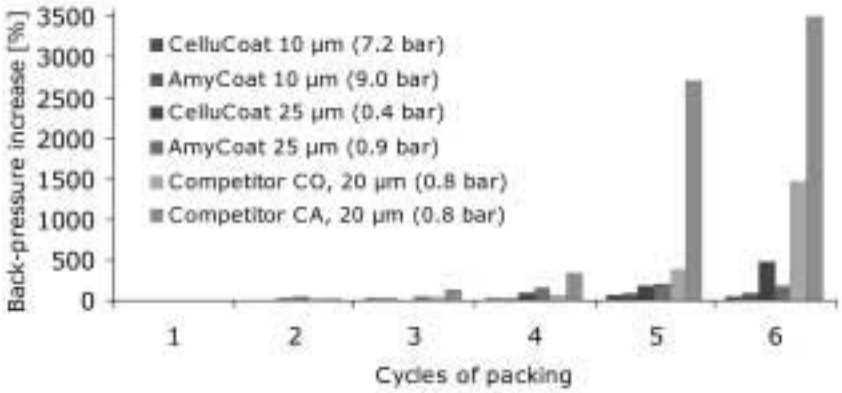
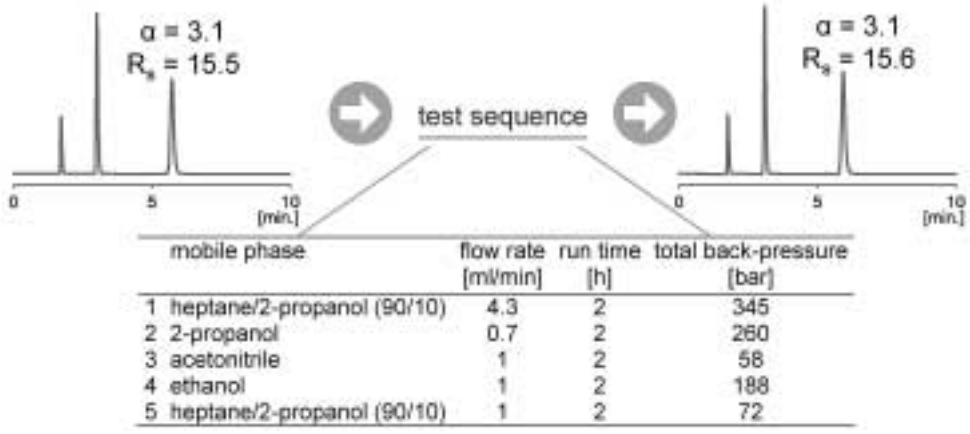


Figure 4. The test shown above was designed to exert greater than normal mechanical stress on the chiral stationary phases, and is performed at a packing pressure above the maximum 50 bar recommended by the manufacturer of competitor CA and competitor CO.



	mobile phase	flow rate [ml/min]	run time [h]	total back-pressure [bar]
1	heptane/2-propanol (90/10)	4.3	2	345
2	2-propanol	0.7	2	260
3	acetonitrile	1	2	58
4	ethanol	1	2	188
5	heptane/2-propanol (90/10)	1	2	72

Figure 5. Maintained efficiency after extended use. Separation of trans-stilbene oxide in heptane/2-propanol (90/10), detection UV @ 229 nm, temp. 25℃, column Kromasil AmyCoat, 3 µm, 4.6×150 mm

lar organic, and reversed mobile phases without irreversibly damaging the stationary phase. Switching between compatible normal to polar organic mobile phases will not lead to any degradation in performance. By using Kromasil AmyCoat and CelluCoat there are no need for solvent dedicated columns. In order to test the stability of Kromasil AmyCoat, in this sense, the chromatographic performance was evaluated before and after high flow rate conditions. As shown in figure 5, the column efficiency was maintained even after the harsh conditions of the test sequence.

Scalability

Depending on the purpose of the chiral separation method the optimum particle size of the CSP varies. Small particles (3 µm and 5 µm) should be applied in analytical scale work, and larger particles should be used when going to preparative scale.

Since particle sizes from 3 µm to 10 µm gives identical selectivity, it will be easy to scale up both Kromasil AmyCoat and CelluCoat, while maintaining excellent performance. Figure 6 shows the separation of trans-Stilbene oxide on Kromasil CelluCoat 3 µm, 5 µm, and 10 µm.

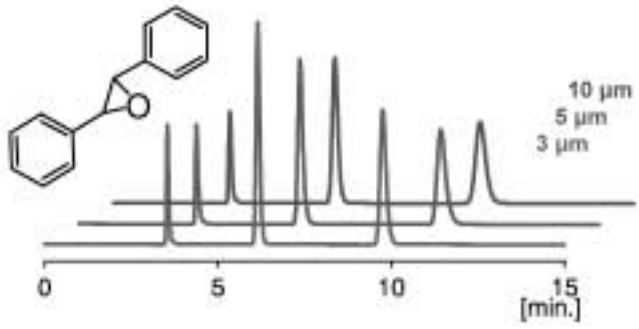


Figure 6. Consistent retention and selectivity independent of particle size. Separation of trans-stilbene oxide in heptane/2-propanol (90/10), detection UV @ 220 nm, flow rate 0.5 ml/min, column Kromasil CelluCoat 3, 5, and 10 µm, 4.6×150 mm

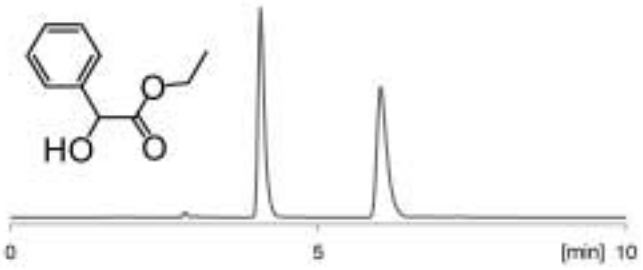


Figure 7a. Analytical injection. Solute: Ethyl mandelate 9 mg/ml in mobile phase. Injection: 5 µl. Column: Kromasil CelluCoat 3 µm, 4.6×150 mm. Flow rate: 1.0 ml/min. Mobile phase: heptane/2-propanol (90/10). Detection: UV @230 nm.

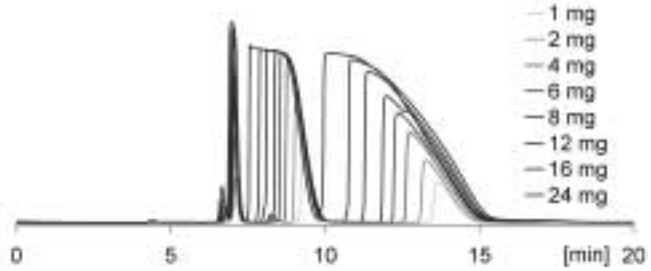


Figure 7b. Overloaded injections. Solute: Ethyl mandelate 140 mg/ml in mobile phase. Column: Kromasil CelluCoat 10 µm, 4.6×250 mm. Flow rate: 0.7 ml/min. Mobile phase:heptane/2-propanol (90/10). Detection: UV @ 254 nm.

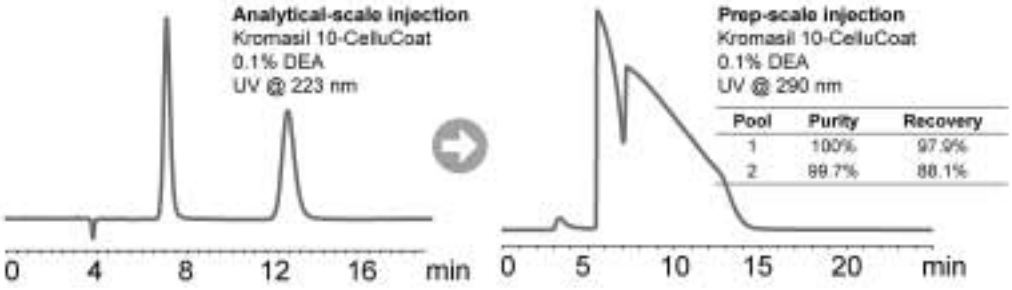


Figure 8 a. Separation with additive, 0.1% DEA

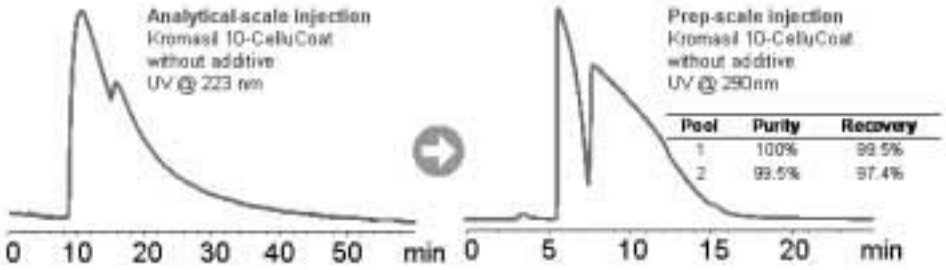


Figure 8 b. Separation without additive

Semi-preparative chiral separations

Important aspects in preparative chiral separations are productivity, loadability, selectivity and, solubility. Figure 7 a and 7 b illustrate a semi-preparative application of Ethyl mandelate on Kromasil 10-CelluCoat. The analytical chromatogram was run on a 3 µm CelluCoat column and since an identical manufacturing technology is used for all particle sizes the results could easily be translated to a column packed with 10 µm particles.

Additives in the mobile phase

In preparative chromatography additives are undesirable since they complicate the solvent recovery process and they could also reduce the stability of the enantiomers in the mobile phase, particularly during evaporation. Screening without additives with analytical injections should however be made with careful considerations. A study performed on the separation of Metoprolol clearly indicated, using analytical injections, that 0.1% DEA was needed in the mobile phase. However, at overloaded conditions no additive was needed in the mobile phase or in the injection solvent, as can be seen very clearly from the fraction analysis presented in Figure 8 a and 8 b. This behaviour could be explained by the fact that the injected substance itself will quickly saturate any acidic sites available on the surface, and thus “self-buffering” the phase at overload conditions.

Conclusion

Kromasil AmyCoat and CelluCoat are two fully back-integrated chiral stationary phases from Kromasil. The specially designed silica offers a high mechanical stability, which allows columns to be operated at pressures up to 400 bar.

The amylose and cellulose selectors are well-known for their ability to resolve a broad range of racemates.