Chiral separation – Efficient, fast and productive

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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

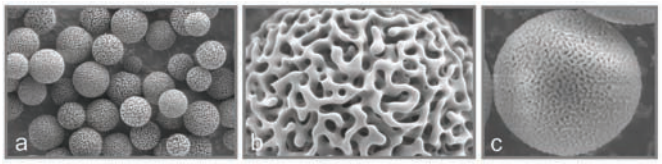


Figure 1. FE-SEM pictures. a and b: pictures of uncoated Kromasil wide pore silica used for AmyCoat and CelluCoat; c: picture of CelluCoat

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 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.

High efficient 3 µm particles

Kromasil AmyCoat and CelluCoat are available in 3, 5, 10 and, 25 µm particle sizes. The small 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.

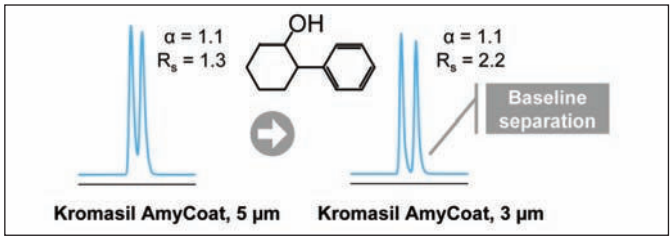
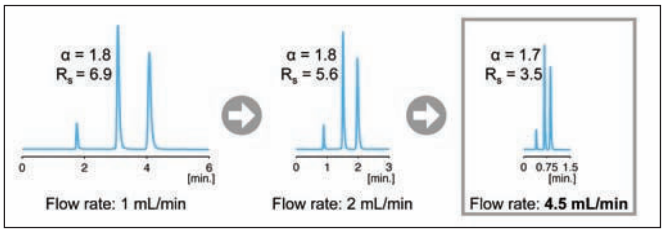


Figure 2. Separation of 2-phenyl-1-cyclohexanol in heptane/2-propanol (95/5), detection UV @ 220 nm, temp. 25°C, flow rate 1 ml/min, column size: 4.6 x 150 mm

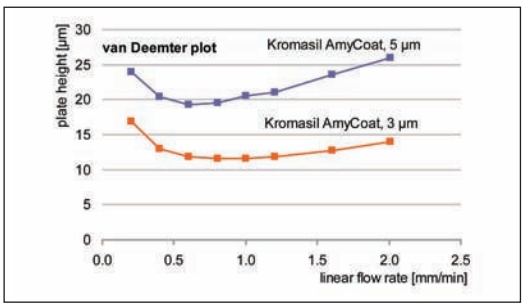
High speed chromatography

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



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

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

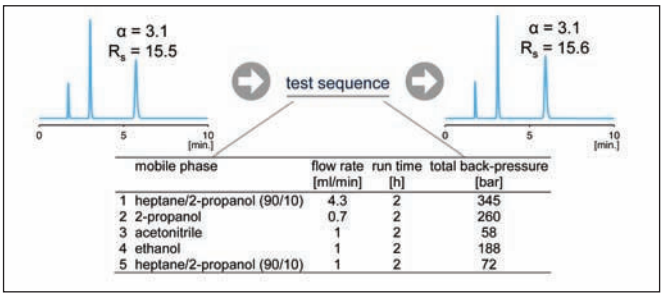


Racemate	AmyCoat 3 µm α	AmyCoat 3 µm R_s	AmyCoat 5 µm α	AmyCoat 5 µm R_s	Mobile Phase	Flow rate [mL/min]
Benzoin	1.3	6.5	1.3	4.4	heptane/2-propanol (90/10)	1
Bucetin	1.8	8.2	1.7	5.8	heptane/2-propanol (90/10)	2
Trifluoroanthrylethanol	1.4	6.4	1.4	4.2	heptane/2-propanol (90/10)	1
Hexobarbital	1.4	4.7	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

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

Stable performance

It is most desirable to be able to run the compatible normal, polar organic and, reversed mobile phases without irreversibly damage 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 case, the chromatographic performance was evaluated before and after high flow rate conditions. As shown in figure 4, the column efficiency was maintained even after the harsh conditions of the test sequence.



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

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 used when going to preparative scale.

When particle sizes from 3 µm to 10 µm giving identical selectivity, Kromasil AmyCoat and CelluCoat make it easy to scale up while retaining excellent performance. Figure 5 shows the separation of trans-Stilbene oxide on Kromasil CelluCoat 3 µm, 5 µm, and 10 µm.

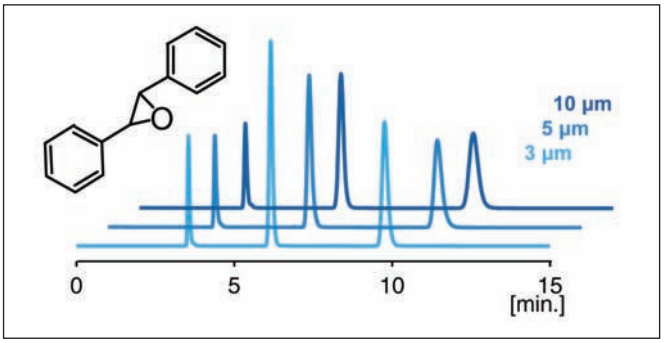


Figure 5. Consistent retention and selectivity independent of particle size. Separation of trans-stilbene oxide in heptane/2-propanol (90/10), detection UV @ 220 nm, flow rate 0.5 ml/min, column Kromasil CelluCoat 3, 5 and, 10 µm, 4.6 x 150 mm

Semi-preparative chiral separations

Important aspects in preparative chiral separations are productivity, loadability, selectivity and, solubility. Figure 6a and 6b illustrate a semi-preparative application of Ethylmandelate 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.

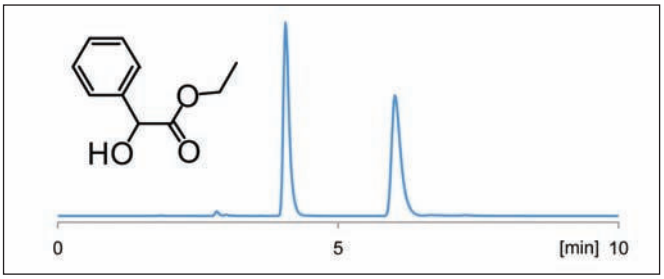


Figure 6a. Analytical injection. Solute: Ethylmandelate 9 mg/ml in mobile phase. Injection: 5 µl. Column: Kromasil CelluCoat 3 µm, 4.6 x 150 mm. Flow rate: 1.0 ml/min. Mobile phase: heptane/2-propanol (90/10). Detection: UV @ 230 nm.

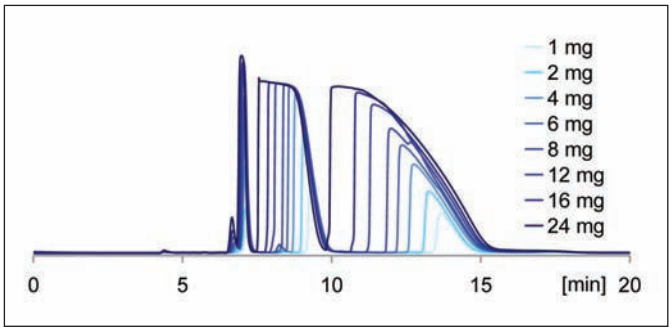


Figure 6b. Overloaded injections. Solute: Ethylmandelate 140 mg/ml in mobile phase. Column: Kromasil CelluCoat 10µm, 4.6 x 250 mm. Flow rate: 0.7 ml/min. Mobile phase: heptane/2-propanol (90/10). Detection: UV @ 254 nm.

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 7a and 7b. This behaviour could be explained by the substance buffering the phase itself at overload conditions.

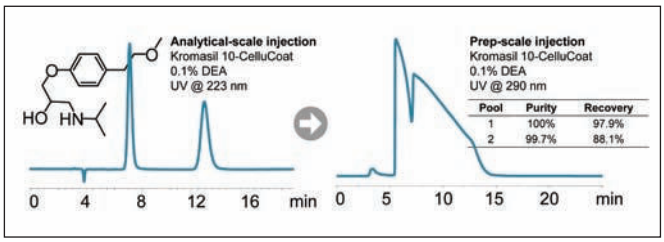


Figure 7a. Separation with additive, 0.1 % DEA

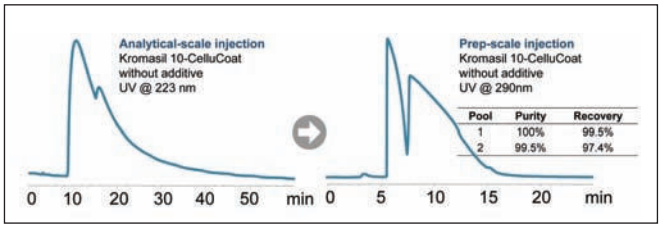


Figure 7b. Separation without additive

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-selector are well known for their ability to resolve a broad range of racemates.