BioSepra® Trisacryl® GF05

Trisacryl® GF05 M, LS

Size Exclusion Chromatography Sorbents

Trisacryl® GF05 sorbent is composed of a highly hydrophilic copolymer designed for medium pressure gel filtration chromatography.

Trisacryl® GF05 is designed for desalting and for the separation of small molecules.

Trisacryl® GF05 provides a wide range of advantages over existing gels :

- High separation efficiency.
- Large desalting capacity, up to 33% of the gel volume.
- Small particle size (40-80 µm for M grade) with narrow bead size distribution for laboratory use.
- Large particle size (80-160 µm for LS grade) for fractionation at high flow with low pressure, designed for industrial scale.
- Ready and easy to use.
- High flow rates.
- Non biodegradable.
- Highly stable in acidic media.

These characteristics allow Trisacryl® GF05 sorbents to perform chromatographic separations quickly and with great selectivity under medium pressure, sterile conditions, and in the absence of any non-specific interactions with the matrix.

Chemical composition and structure.

Trisacryl® GF05 is a highly hydrophilic copolymer formed by the copolymerization of N-acryloyl-2-amino-2-hydroxymethyl-1,3-propanediol, and a hydroxylated acrylic bifunctional monomer. See chemical structure in Figure 1.

The molecule features a high degree of hydrophilicity contributed by a secondary amide group, and by the presence of three primary hydroxymethyl groups per repeating unit.

Figure 1: Chemical structure of Trisacryl GF05 sorbents.

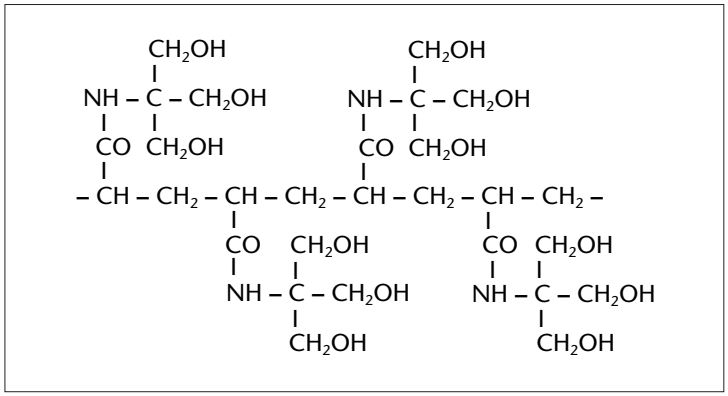


Table 1. Main properties of Trisacryl GF05 sorbents.

Particle size	M grade: 40 - 80 µm LS grade: 80 - 160 µm
Exclusion limit	3,000 dt*
Linear fractionation range	200 - 2,500 dt
Resolution power	2,500 plates/m
Desalting capacity	33 % gel volume
Pressure resistance	up to 3 bar (44 psi)
Stability to detergents and dissociating agents	Excellent
Thermal stability	Up to 121°C
pH stability	1 - 11

* Calculated on the basis of a standard experimental curve.

Porosity.

The modification of relative concentrations of the monomer and the cross-linking agent allow precise control of Trisacryl® GF05 porosity.

The exclusion limit of Trisacryl® GF05 (determined using oligonucleotides) is 3,000 dt.

Close control over the polymerization reaction guarantees not only a regular porosity inside each bead, but also excellent reproducibility from lot to lot.

Chemical stability.

Trisacryl® GF05 is insoluble in chromatographic solvents commonly used in biochemistry (8 M urea, 6 M guanidine), as well as in detergents (Triton X-100, SDS, lubrol, ...).

Trisacryl® GF05 is resistant to acid treatments, making it an ideal matrix for peptide separations which require the use of acetic or hydrochloric acids. The matrix is not modified by incubation in the presence of 1 M HCl for several hours. However, Trisacryl® GF05 is sensitive to strong alkaline agents such as sodium hydroxide. Sensitivity to alkaline medium is minimal in 0.1 M NaOH at +4°C for a maximum of one hour. If the NaOH concentration or the temperature are increased, the sorbent undergoes progressive hydrolysis of amide groups to give carboxyl groups. This will introduce some ion exchange properties.

Thermal stability.

Trisacryl® GF05 is stable at high temperature (up to +121°C). It can be sterilized by autoclaving without undergoing any change in its chromatographic properties. However, the operation must be performed in buffered conditions, pH 7 in the absence of oxidizing agents.

Mechanical stability.

Due to its chemical composition which imparts rigidity, Trisacryl® GF05 is resistant to pressures up to 2-3 bar. This exclusive property permits elevated flow rates.

Resistance to micro-organisms.

Due to its synthetic matrix, Trisacryl® GF05 is resistant to microbial and enzymatic degradation.

Fractionation range.

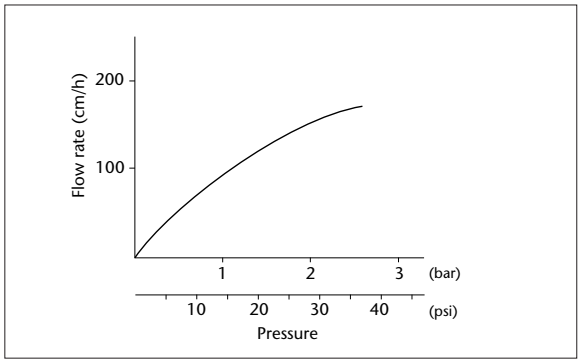
The effective fractionation range for Trisacryl® GF05 is between 200 and 2,500 dt. This was determined with globular proteins below 1000 Kd and extrapolates up to the exclusion limit.

Resolution power.

The resolution power of the M grade of Trisacryl® GF05 (expressed by the number of theoretical plates per meter of column under normal running conditions) is relatively high — about 2,500 plates per meter or HETP (height equivalent to a theoretical plate) = 0.4 mm (value obtained using a 40 cm column for a molecule eluted in the total available gel volume).

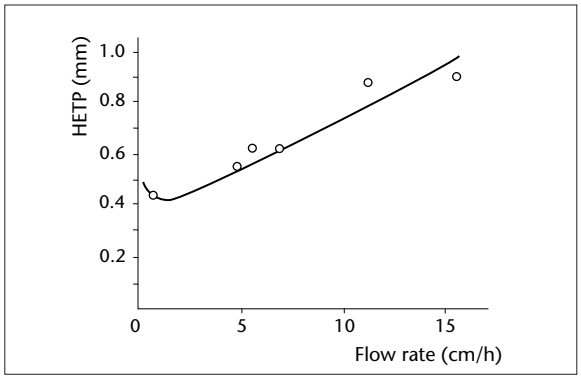
The resolution power of Trisacryl® GF05 depends on the flow rate and varies with the molecular size of the product used. The flow rate needed to obtain optimal separation ranges from 2 to 3 cm/h. Because the height equivalent of a theoretical plate (HETP) increases very slowly as a function of flow rate (2-3 times less rapidly than with soft sorbents), Trisacryl® GF05 provides excellent resolution power for proteins (see Figure 3).

Figure 2. Flow rate variation of a Trisacryl GF05 M column as a function of pressure.



Column dimensions : 1.4 cm I.D. x 10 cm. The column was packed under pressure from 0 to 2 bar; Flow rates were measured under pressures from 2 to 0 bar.

Figure 3. HETP variation of Trisacryl GF05 M (height equivalent to a theoretical plate) as a function of the flow rate.



Column: 1.6 cm I.D. x 36 cm. Sample : 5 mg NAD (nicotinamide adenine dinucleotide, MW 700) in 1 ml buffer; Buffer: 0.1 M phosphate buffer, pH 7.4 containing 0.5 M NaCl; Temperature: +20°C.

Applications

Desalting on Trisacryl® GF05.

Trisacryl® GF05 is particularly suitable to rapid desalting of large solution volumes.

- Pressure resistance allows high flow rates.
- Provides a low dilution factor (about 1.05-1.1).
- Provides large desalting capacity (up to 33% gel volume).

These desalting properties are illustrated by the results obtained in the use of Trisacryl® GF05 for human plasma fractionation: 17 L of human plasma were run through a 60 L Trisacryl® GF05 column (44 cm I.D. x 40 cm) and desalted within 1.5 hour. Linear flow rate was 35 cm/h, corresponding to a volumetric flow rate of 36 L/h. The volume of desalted plasma was 18 L, corresponding to a dilution factor of 1.059. Similar results are illustrated in Figure 4.

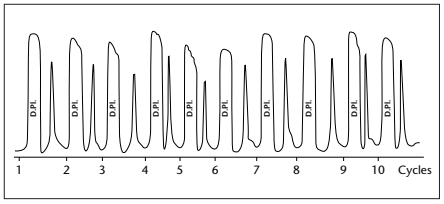
Trisacryl® GF05 can be applied to several other "desalting" operations such as removal of small aromatic molecules, separation of fluorochromes after protein labelling, elimination of detergents in protein solutions, separations of sugars, elimination of peptides.

Trisacryl® GF05 has an appreciable time advantage over dialysis for desalting. Desalting by gel chromatography is presently the only available method for removing salts from very labile biological substances.

Specially designed for preparative chromatography, Trisacryl® GF05 LS offers increased flow rate, while retaining the properties of Trisacryl® GF05 M.

Flow rates over 80 cm/h are commonly used for this type of gel in installations using columns of 50 to 200 L, with backpressures less than 1 bar (see Figure 4).

Figure 4. Steps from an automatic desalting of human plasma on a Trisacryl GF05 LS column.

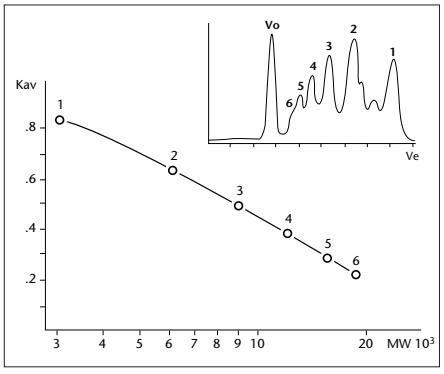


Column dimensions: 44 cm I.D. x 40 cm (60 L of gel); Buffer: 0.05 M Tris-HCl, pH 8.4; Flow rate: 80 L/h; Sample volume: 12.5 L; Desalting time per cycle: 75 min (including equilibration time). Volume/h: 1.3. Pressure: 0.7 bar (average). D.Pl. = desalting plasma fraction. (Courtesy of Mr. J. Saint-Blancard, CTSA Clamart, France).

Determination of the molecular weight of an unknown molecule.

The measurement of molecular weight can be performed by comparing the Kav of the studied molecule to the Kav relative to standard molecular markers obtained on Trisacryl® GF05 selectivity curve (see Figure 5). In this case, it is important to notice that the selectivity curve must be determined using molecules from the same species as the studied molecule. For example, the standard molecules must be performed with known oligosaccharides if the studied molecule is a sugar.

Figure 5. Selectivity curve determined using a mixture of 5'-TMP oligomers on Trisacryl GF05.



The range of molecular weight exploited ranges between 306 (monomer) and 1836 (hexamer). Column: 1.6 cm I.D. x 40 cm; Sample volume: 1 ml; Buffer: 0.05 M Tris-HCl, pH 7.4 containing 0.17 M sodium chloride; Linear flow rate: 5 cm/h. The insert represents the complete chromatogram obtained.

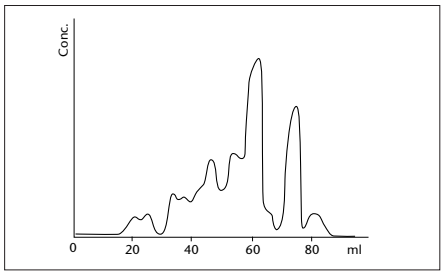
Fractionation and purification of biological molecules on Trisacryl® GF05.

Trisacryl® GF05 permits the separation of a variety of complex mixtures (see Figure 6) such as :

- Polypeptides
- Oligonucleotides
- Oligosaccharides
- Small proteins
- Polynucleotides
- Small water-soluble polymers

The best resolution is obtained with a sample volume between 0.5 and 4% of gel volume.

FIgure 6 : Fractionation of peptides obtained from trypsin hydrolysis of bovine thyroglobulin.



Buffer: 0.1 M KH_2PO_4, 0.15 M KCl, pH 7.4; Sample: 1 ml. U.V; Detection: 280 nm; Flow rate: 11.5 cm/h; Column: 1.6 x 45 cm; Experiment time: 8 hours.

References

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Ordering Information

Product	Cat. No.	Size
Trisacryl GF 05 M	25914-060	100 ml
	25914-037	1 L
	25914-045	10 L
Trisacryl GF 05 LS	25916-040	100 ml
	25916-016	1 L
	25916-032	10 L

PALL Life Sciences

New York - USA
+1 516.484.5400 phone
+1 516.801.9548 fax
pharmafilter@pall.com

Portsmouth - Europe
+44 (0)23 9230 3303 phone
+44 (0)23 9230 2506 fax
BioPharmUK@europe.pall.com

Cergy - France
Pall BioSepra
+33 (0)1 34 20 78 00 phone
+33 (0)1 34 20 78 78 fax
bioseprainfo@pall.com

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