

Superdex 30, 75 & 200 prep grade BioProcess™ Media

Data File Gel Filtration Media

Superdex prep grade is a preparative gel filtration medium with a unique composite matrix of dextran and agarose. This matrix combines the excellent gel filtration properties of cross-linked dextran (Sephadex) with the physical and chemical stabilities of highly cross-linked agarose, to produce a separation medium with outstanding selectivity and high resolution. In addition, its low non-specific interaction permits high recovery of biological material. Together these properties make Superdex prep grade the first choice gel filtration media for all applications from laboratory to process scale.

Superdex prep grade is available in three selectivities:

Superdex 30 prep grade has unique selectivity for molecules in the molecular weight range up to 10 000 MW, such as peptides, oligosaccharides and small proteins.

Superdex 75 prep grade is specially recommended for the purification of recombinant proteins.

Superdex 200 prep grade is recommended for the purification of monoclonal antibodies.

- Unique selectivity gives unmatched resolution
- Excellent flow properties
- High chemical stability
- High productivity
- Easy to scale-up

Media characteristics

Superdex prep grade is produced by the covalent binding of dextran to highly cross-linked porous agarose beads. Figure 2 shows a hypothetical view of a section through a bead of Superdex prep grade.

Unique selectivity gives unmatched resolution

Superdex 30 prep grade has a selectivity between that of Sephadex G-25 and G-50, while Superdex 75 prep grade and 200 prep grade have



Fig. 1. Superdex gel filtration media.

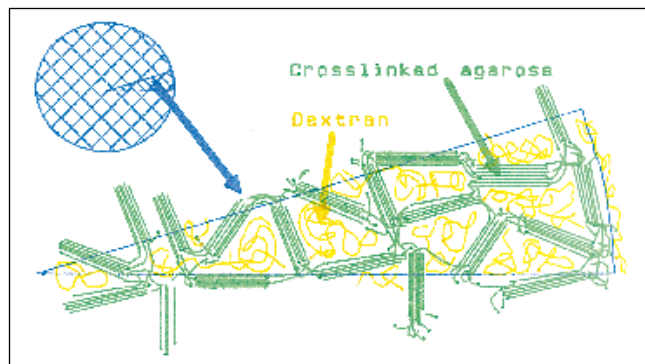


Fig. 2. Hypothetical view of a section through a bead from Superdex. Superdex prep grade has a mean particle size of 34 μm .

selectivities similar to those of Sephadex G-75 and Sephadex G-200 respectively. The high selectivity of Superdex prep grade, shown in Fig. 3, ensures unmatched resolution in the molecular weight ranges: up to 10 000 MW for Superdex 30 prep grade, 3 000–70 000 MW for Superdex 75 prep grade and 30 000–600 000 MW for Superdex 200 prep grade. Figs. 4 a–c show separations of test substances on all three media.

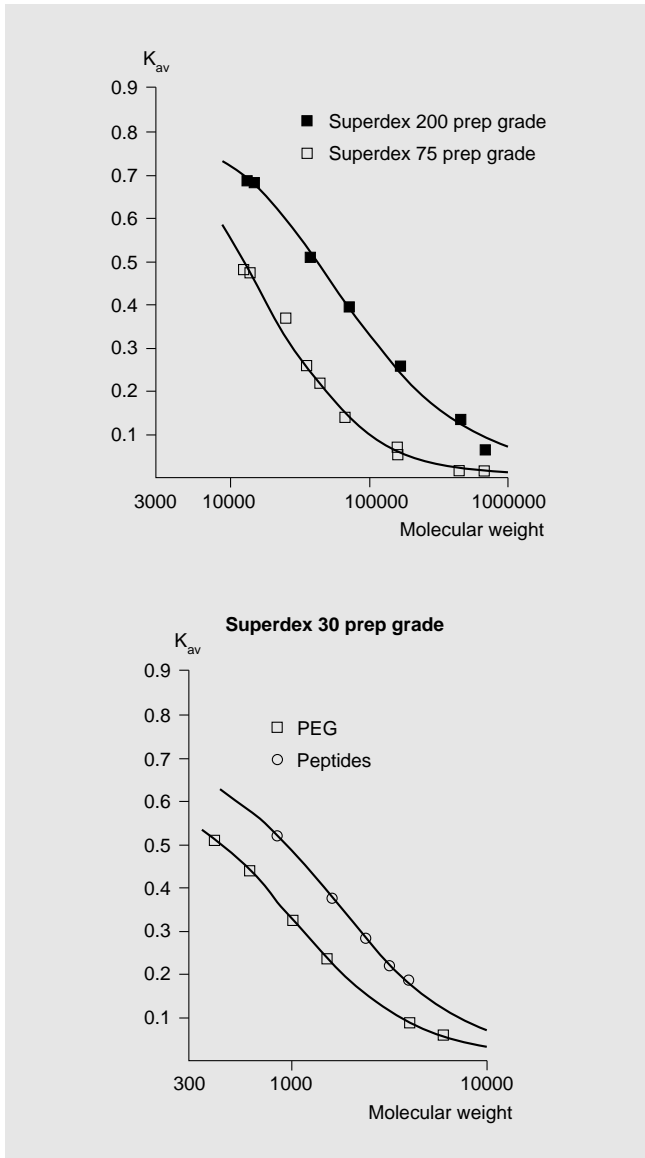


Fig. 3. Selectivity curves for Superdex prep grade.

Excellent flow properties

The carefully chosen particle size, 34 μm , in combination with the narrow particle size distribution of Superdex prep grade media gives good separation performance without creating high back pressure (Fig. 5).

High chemical stability

Separation media used in industrial applications and preparative work must have high chemical stability to withstand the harsh treatments used for cleaning and sanitization.

Extensive studies performed on Superdex prep grade media have demonstrated their high chemical stability (2, 3).

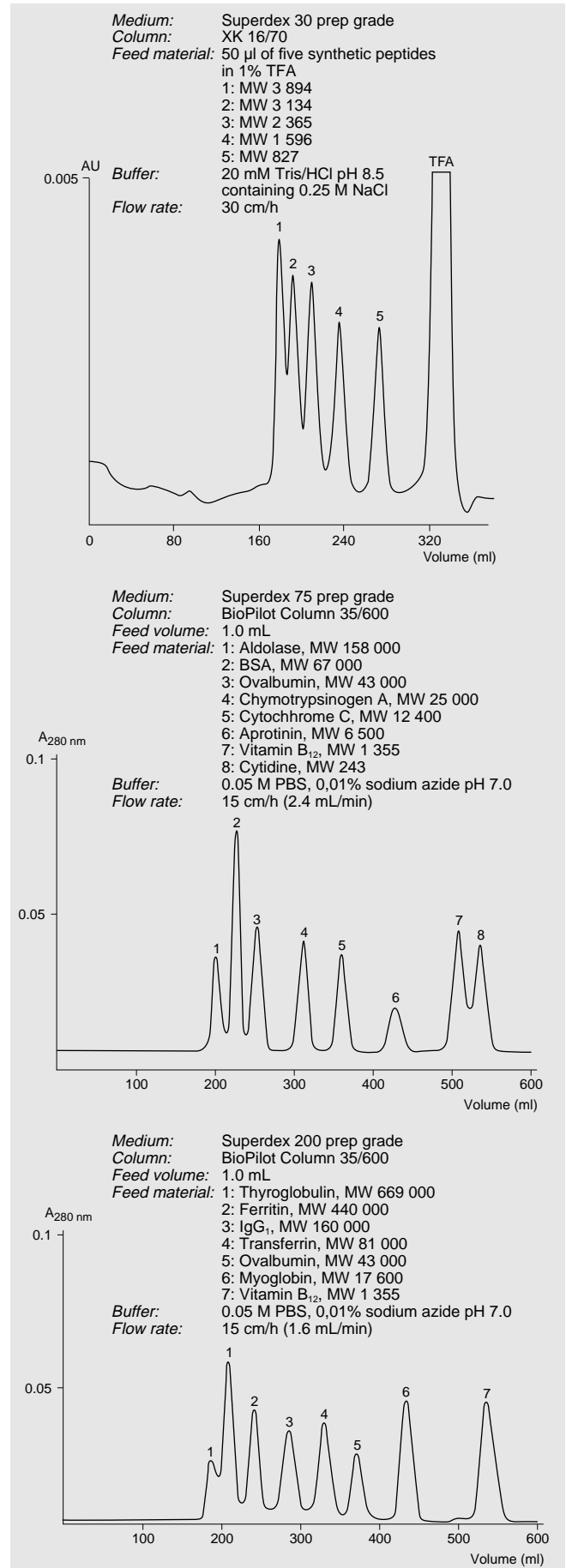


Fig. 4. Separation of test substances on Superdex 30, 75 and 200 prep grade.

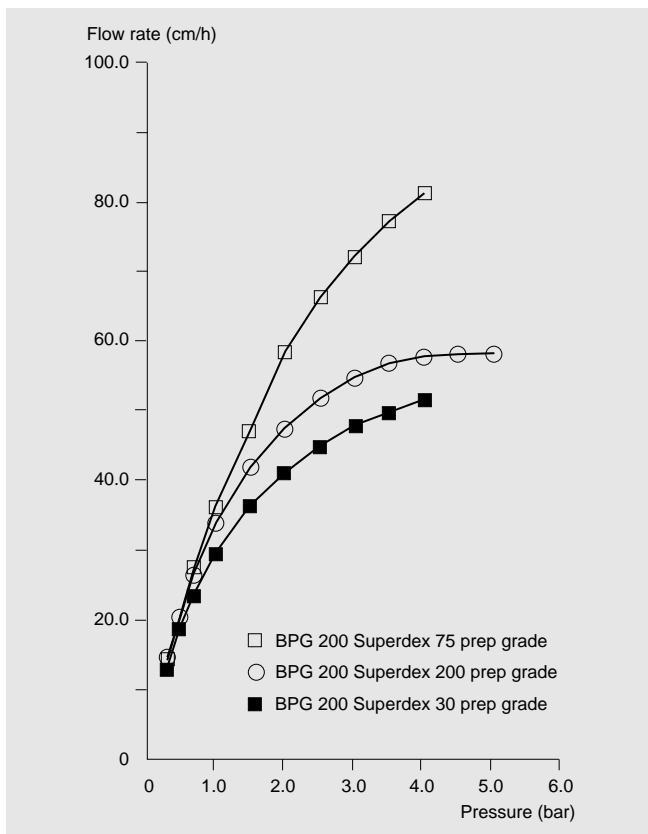


Fig. 5. Pressure flow rate curves in BPG 200 glass column, bed height 60 cm.

In one of these studies the media were subjected to more than 300 h, accumulated exposure, to 0.1 M HCl and 1 M NaOH. During this prolonged exposure the K_{av} values for the media did not change significantly. See Fig 6.

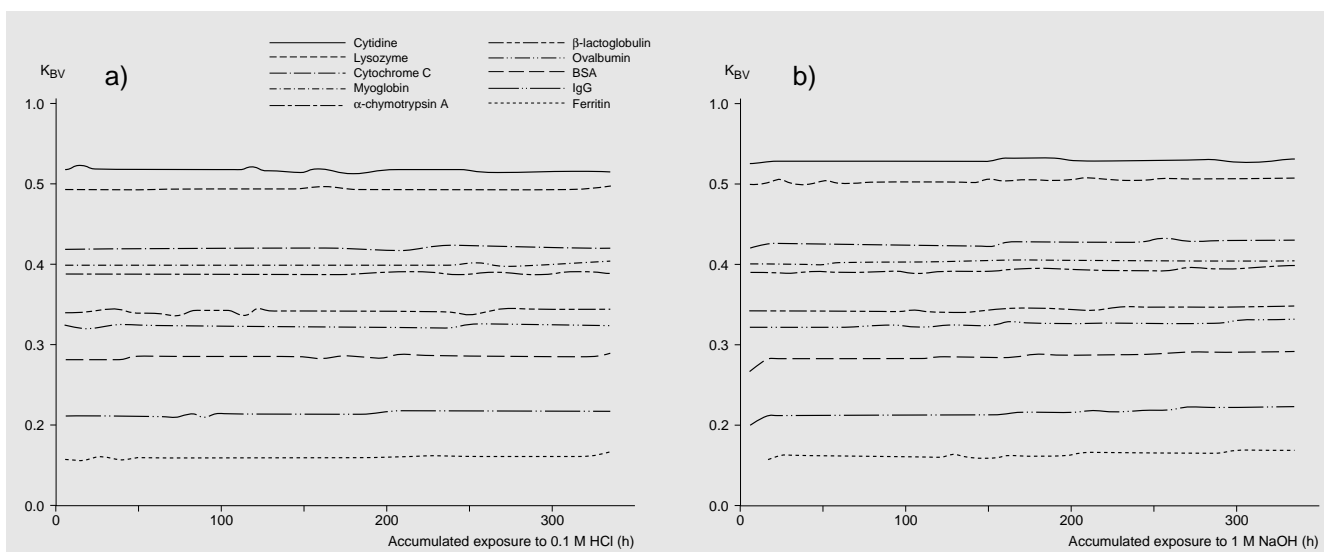


Fig. 6. Performance of Superdex 200 prep grade measured as K_{av} values of a protein mixture after repeated treatment with a) 0.1 M HCl and b) 1 M NaOH.

Table 1. Media characteristics.

Separation range (MW)	up to 10 000
Superdex 30 prep grade	3 000–70 000
Superdex 75 prep grade	10 000–600 000
Superdex 200 prep grade	34 μ m
Mean particle size	24–44 μ m >75%
Bead size range	composite of cross-linked agarose and dextran, spherical
Bead structure	Solutions in which the media are stable
	all commonly used buffers
	1 M acetic acid
	8 M urea
	6 M guanidine hydrochloride
	30% isopropanol
	30% acetonitrile
	70% ethanol
	1 M sodium hydroxide
	0.1 M hydrochloric acid
pH stability	
working range	3–12
cleaning-in-place	1–14
Autoclavability	at 120 °C, pH 7 for 30 min

In aqueous solutions, Superdex prep grade media are stable over a wide pH range. Except for strong oxidizing agents, Superdex prep grade media can tolerate all buffers and cleaning agents commonly used in chromatography. See Table 1 for detailed information.

Low non-specific interaction

In process chromatography, where high-value products are regularly handled, it is important to avoid undesired interactions such as protein-protein interactions and non-specific interactions with the chromatographic media.

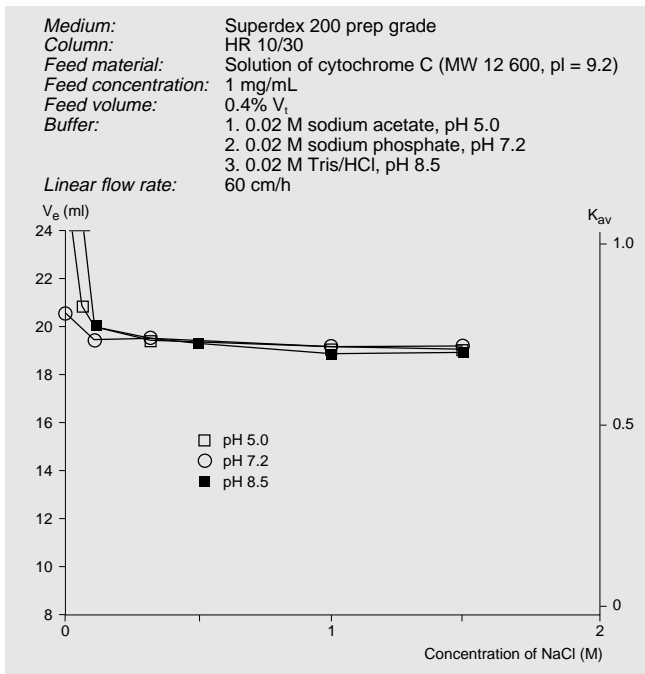


Fig. 7 a. Retention volume of cytochrome C on Superdex 200 prep grade at different pH values.

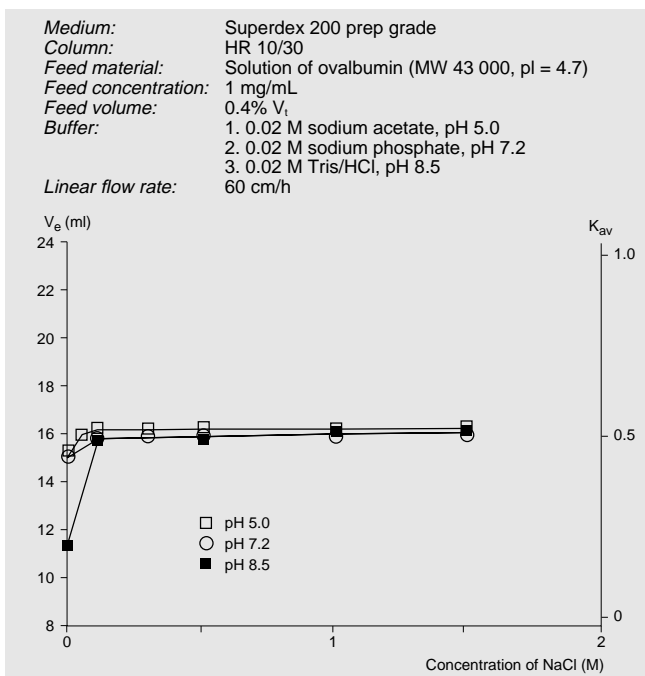


Fig. 7 b. Retention volume of ovalbumin on Superdex 200 prep grade at different pH values.

Superdex prep grade media were tested within the ionic strength interval corresponding to 0.15–1.5 M NaCl at pH 5, pH 7.2 and pH 8.5 with cytochrome C (pI ≈ 9.2) and ovalbumin (pI ≈ 4.7). The results show that there are no significant changes in the elution volumes of these proteins. In Figs. 7 a–b the retention volumes (V_e) of these two proteins, cytochrome C and ovalbumin, on Superdex 200 prep grade are

plotted as a function of increasing NaCl concentration in three different buffer systems. The test shows that Superdex prep grade media are well suited to process chromatography due to their low non-specific interaction.

However, to minimize any effects from undesired interactions we recommend using an eluent with an ionic strength corresponding to 0.15–1.5 M NaCl.

Operation

Process optimization

Gel filtration is widely used in process chromatography, particularly for polishing of the final product. In addition to removal of product aggregates, the technique also allows the transfer of product to formulation buffer.

When optimizing a gel filtration step to achieve maximum productivity, the following parameters need careful consideration:

- feed concentration
- flow rate
- feed volume

High productivity

The excellent flow properties and unique selectivity of Superdex prep grade media allow separation conditions to be optimized for maximum productivity. However, in any chromatographic process there is always a balance between resolution and productivity. Figs. 8 a–f show the influence of feed concentration, flow rate and feed volume on resolution on Superdex prep grade. Figs. 8 a–c pertain to Superdex 30 prep grade and Figs. 8 d–f pertain to Superdex 200 prep grade.

To illustrate how feed concentration, flow rate, and/or feed volume influence the balance between resolution and productivity we have separated IgG and transferrin on Superdex 200 prep grade, Fig. 8 d. The example shows that a feed concentration in the range 24–155 mg/ml does not affect resolution. High sample concentration does however, reduce resolution, but this effect is less at higher linear flow rates, Fig. 8 e. Feed volume influences resolution the most, Fig 8 f. From the results it can be seen that it is advantageous to use a high feed concentration, a high linear flow and to adjust feed volume to obtain the required resolution. Each case, however, has to be optimized individually.

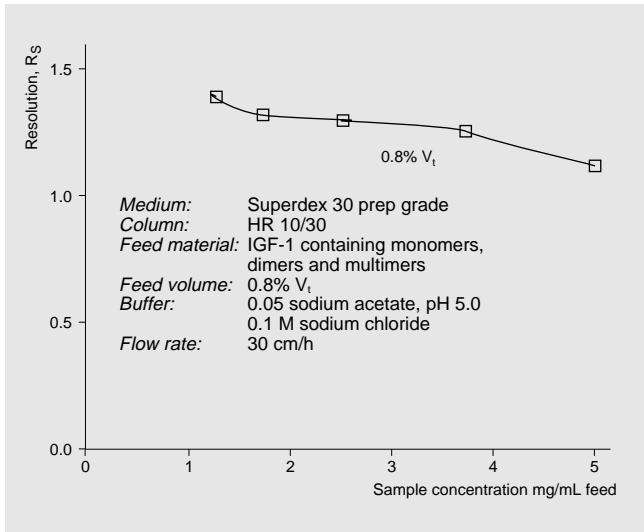


Fig. 8 a. Influence of feed concentration on the resolution of IGF-1 and multimers on Superdex 30 prep grade.

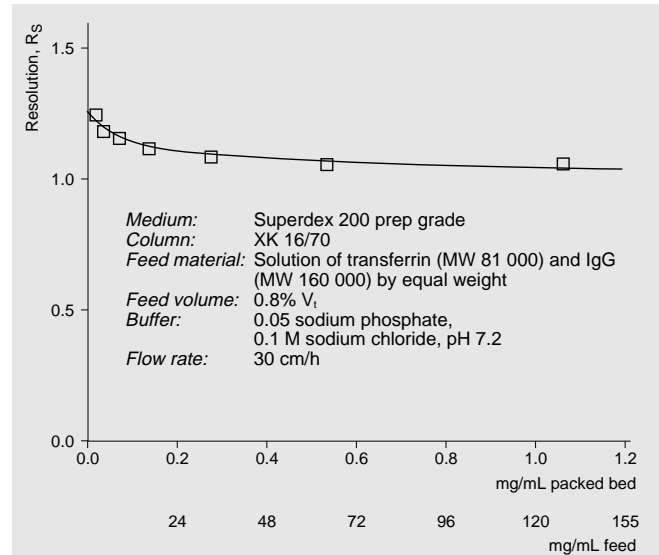


Fig. 8 d. Influence of feed concentration on the resolution of transferrin and IgG on Superdex 200 prep grade.

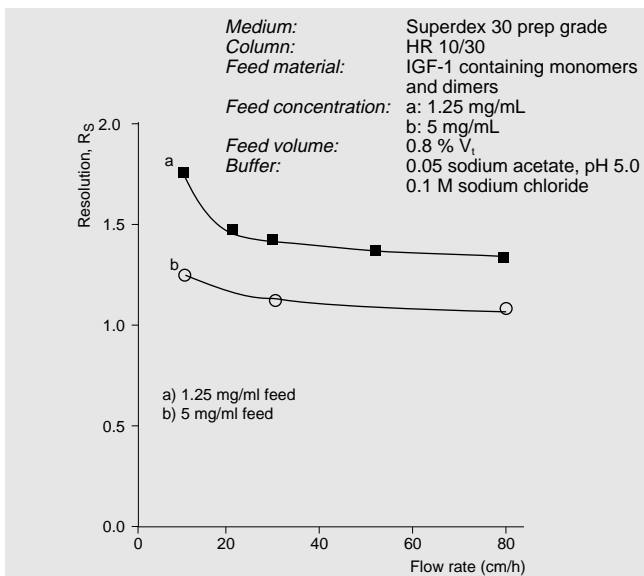


Fig. 8 b. Influence of flow rate on the resolution of IGF-1 and multimers on Superdex 30 prep grade.

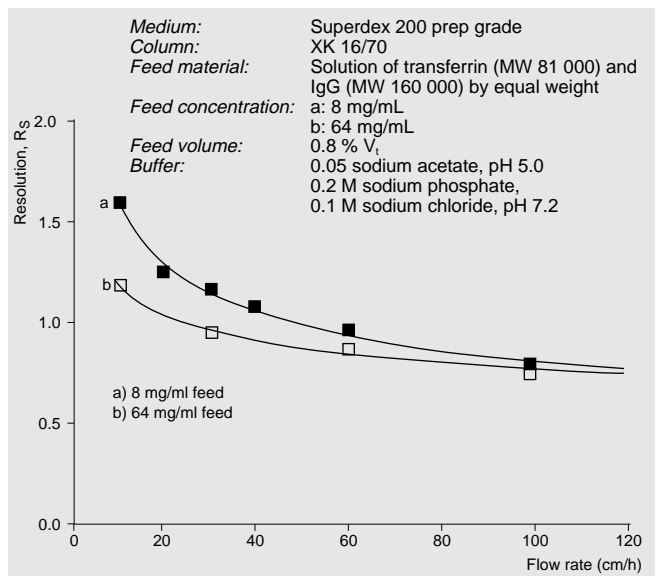


Fig. 8 e. Influence of flow rate on the resolution of transferrin and IgG on Superdex 200 prep grade.

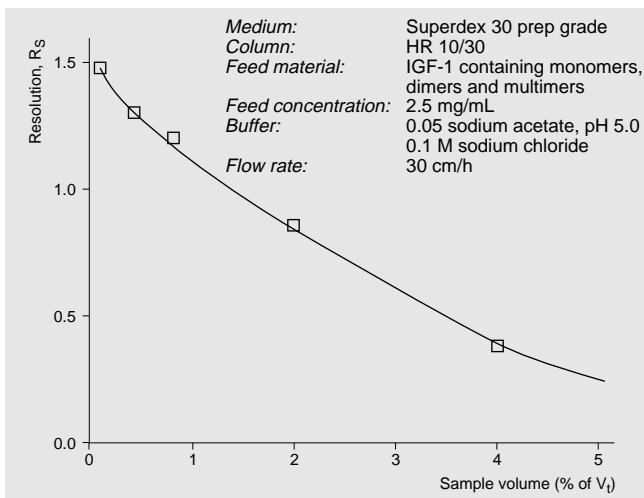


Fig. 8 c. Influence of feed volume on the resolution of IGF-1 and multimers on Superdex 30 prep grade.

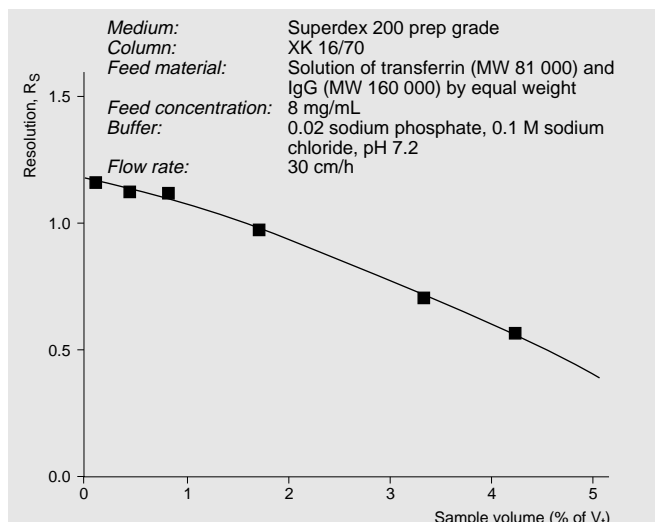


Fig. 8 f. Influence of feed volume on the resolution of transferrin and IgG on Superdex 200 prep grade.

Easy to scale up

Superdex prep grade media are designed, developed and tested for the industrial user. They can be packed efficiently and run optimally in Pharmacia Biotech large scale columns.

An example of a large scale purification is shown in the Applications section.

Recommended columns

Table 2 lists the recommended Pharmacia Biotech columns for Superdex prep grade media.

Table 2. Recommended columns for Superdex prep grade media.

Column	Rec. bed height	Bed volume
XK 26/70	60 cm	320 mL
XK 26/100	90 cm	480 mL
BPG 100/950	60 cm	4.8 L
BPG 200/950	60 cm	18.9 L
BPG 300/950	60 cm	42.4 L

Applications

Superdex 30 prep grade is a new gel filtration medium for the purification of small molecules, such as low molecular weight proteins, recombinant and synthetic peptides, and oligosaccharides in preparative and process scale applications.

As an example we show Superdex 30 prep grade used in the final polishing of Epidermal Growth Factor (EGF) (9). The previous steps were hydrophobic interaction chromatography with Phenyl Sepharose 6 Fast Flow (high sub), and ion exchange chromatography with Q Sepharose High Performance. The total protein recovery after the gel filtration step was 81% at pilot scale and 74% at process scale, see Fig. 9.

Superdex 75 prep grade is very well suited for the purification of recombinant and other proteins in the molecular weight range 3 000–70 000 MW (10). Contaminants such as degraded peptides, differently folded forms, multimers and the fusion protein can easily be separated from the protein of interest, see Fig 10.

Large scale purification

The use of Superdex 200 prep grade considerably improves the purification of monoclonal antibodies.

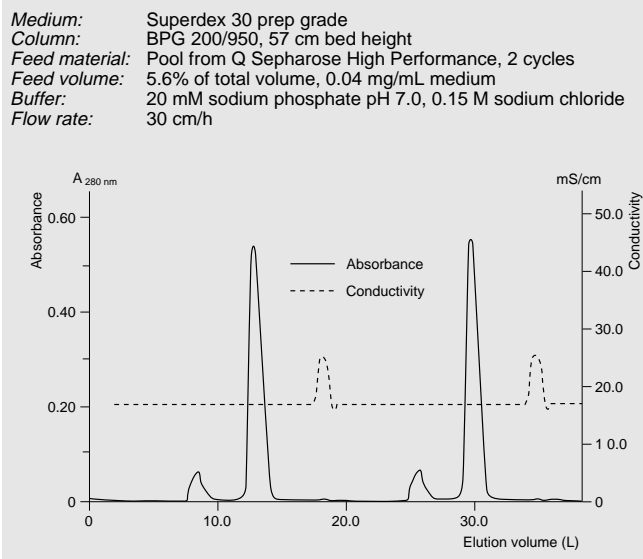


Fig. 9. Final polishing of EGF (MW 6 000) with Superdex 30 prep grade.

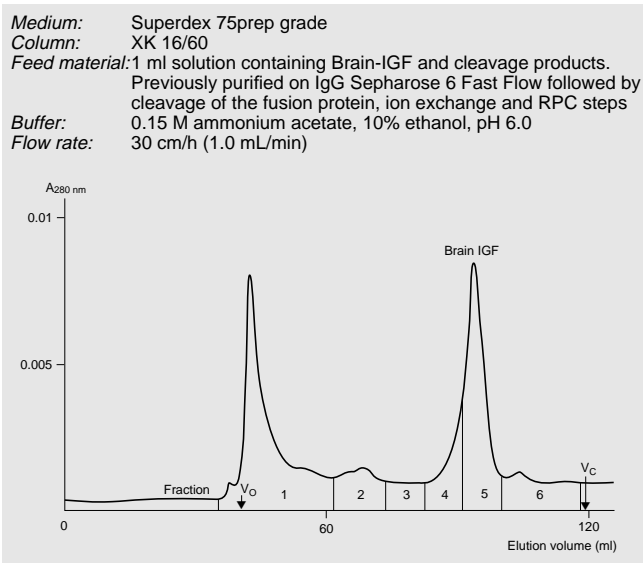


Fig. 10. Final polishing of Brain IGF (7.3 kDa) on Superdex 75 prep grade.

In this example (Fig. 11) Superdex 200 prep grade is used in the final polishing step for the purification of monoclonal IgG1, anti gp 120, intended for intravenous administration in clinical studies in the treatment of AIDS. The complete purification scheme is shown in Fig. 12. The sample volume in the gel filtration step was as large as 8.5% of the bed volume and yet a purity of 98% was obtained with a yield of 92%. (Determined with SDS-PAGE using PhastSystem and PhastImage).

All chromatographic experiments were performed on BioPilot System.

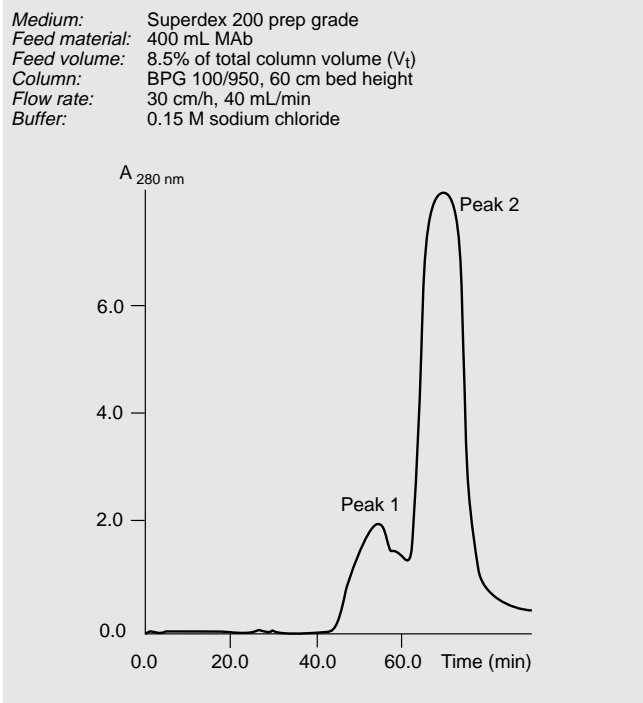


Fig. 11. Large scale purification of mouse IgG₁ on Superdex 200 prep grade.

Cleaning-in-place

The chemical stability of Superdex prep grade media permits the use of effective cleaning-in-place (CIP) protocols which help to ensure a longer column life and good process economy. Repeated separation cycles tend to cause a build up of contaminants and specific CIP protocols should therefore be developed as part of the routine separation process.

Table 3 gives an example of effective cleaning-in place protocols.

Table 3. Cleaning-in-place and sanitization protocols.

Purpose	Procedure
To remove hydrophobic proteins or lipoproteins	Wash the column with one column volume of 0.5 M NaOH at 20 cm/h with reversed direction of flow.
To remove lipid and very hydrophobic proteins	Wash the column with two column volumes of 70% ethanol or 30% isopropanol at 10 cm/h with reversed direction of flow.
Sanitization	Expose the column to 0.5 M NaOH for 30–60 minutes at room temperature.
After treatment with sodium hydroxide, ethanol, isopropanol or acetonitrile wash the column with water prior to re-equilibration with buffer.	

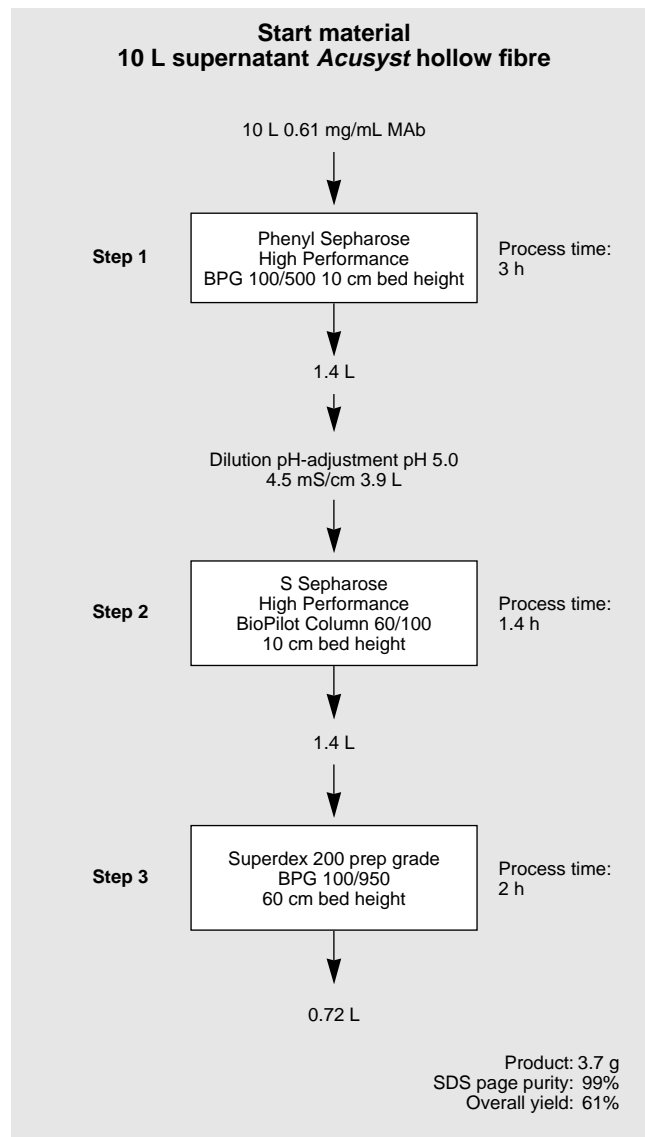


Fig. 12. Purification scheme for the large scale purification of mouse IgG₁ anti gp120. (In collaboration with B. Wahren, *et al.* of the National Bacteriological Laboratory (SBL), Department of Virology, Sweden.)

Sanitization

Sanitization is the use of chemical agents to inactivate microbial contaminants in the form of vegetative cells; it also helps maintain a high level of process hygiene and process economy.

An example of an effective sanitization protocol is given in Table 3 (8).

Storage

Before storing a packed column, first sanitize and then equilibrate the column with 20% ethanol as a bacteriostat.

Ordering information

Superdex prep grade media are supplied preswollen in 20% ethanol.

Superdex 75 prep grade and Superdex 200 prep grade are also available prepacked in HiLoad and BioPilot Columns. These products offer convenience, reproducibility and simplicity of use. For further information ask for brochures on HiLoad Columns and BioPilot Columns.

Product	Pack size	Code No.
Superdex 30 prep grade	150 mL	17-0905-01
Superdex 30 prep grade	1 L	17-0905-03
Superdex 30 prep grade	5 L	17-0905-04
Superdex 75 prep grade	1 L	17-1044-02
Superdex 75 prep grade	5 L	17-1044-04
Superdex 200 prep grade	1 L	17-1043-02
Superdex 200 prep grade	5 L	17-1043-04
HiLoad Columns		
HiLoad 16/60		
Superdex 75 prep grade		17-1068-01
HiLoad 26/60		
Superdex 75 prep grade		17-1070-01
HiLoad 16/60		
Superdex 200 prep grade		17-1069-01
HiLoad 26/60		
Superdex 200 prep grade		17-1071-01
BioPilot Columns		
Superdex 75 prep grade 35/600		17-1041-01
Superdex 75 prep grade 60/600		17-1042-01
Superdex 200 prep grade 35/600		17-1045-01
Superdex 200 prep grade 60/600		17-1046-01

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References

1. Chemical, physical and chromatographic properties of Superdex 75 prep grade and Superdex 200 prep grade gel filtration media. *J. Chromatogr.* 537 (1991) 17-32, Kägedal, L., *et al.*
2. Chemical properties of and solute-support interactions with the gel filtration medium Superdex 75 prep grade. *J. Chromatogr.* 514 (1990) 137-146, Johansson, B.-L., *et al.*
3. Stability of Superdex 75 prep grade and Superdex 200 prep grade under different chromatographic conditions. *J. Chromatogr.* 547 (1991) 21-30. Drevin, I., Johansson, B.-L.
4. Characterization of two new gel filtration media. Chromatographic and Physico-Chemical properties. Eighth International Symposium on HPLC of Proteins, Peptides and Polynucleotides. Copenhagen, Denmark. Oct 31-Nov 2, 1988, poster 101, Engström, B., *et al.*
5. A method for the recovery of monoclonal antibodies (MAB) from a hollow fibre bioreactor system. The Engineering Foundation Conference on "Recovery of biological products V". St Petersburg, Florida, USA. May 1990. Pettersson, T., *et al.*
6. Preparative gel filtration goes high performance. Downstream 7, p. 1, Pharmacia LKB Biotechnology AB.
7. Data File 3020 "BioPilot Column, Superdex 75 prep grade BioPilot Column, Superdex 200 prep grade".
8. Inactivation of microbial contamination in chromatographic separation media using sodium hydroxide. Tenth Congress of the International Society of Blood Transfusion. London, England. July 10-15, 1988. Adner, J. H. *et al.*
9. Development and a scale up study of a chromatographic downstream process for the purification of recombinant EGF. Ninth International Biotechnology Symposium and Exposition. Harnessing Biotechnology for the 21st Century. Crystal City, Virginia, USA, August 1992. Daniels, A. I., Pettersson, T., Pharmacia BioProcess Technology AB, Scandella, C., Chiron Corp.
10. Large scale affinity purification of human IGF-1 from culture media *E. coli*. *Bio/Technology* 5, (1987) 379-382. Moks, T., *et al.*