

Sephadex® G-25 media and pre-packed columns

Data File

Desalting/buffer exchange and gel filtration

- Reproducible desalting and buffer exchange in minutes with 90%–100% recovery
- Convenient pre-packed columns are simple to use with minimal equipment
- Pre-packed columns give optimal results on automated systems
- Pack sizes available for all scales
- Easy to scale up
- High chemical stability for cleaning-in-place and sanitization
- Sephadex G-25 media are also BioProcess Media and carry comprehensive technical and regulatory support for pharmaceutical production

Introduction

Gel filtration has occupied a key position in the purification of sensitive biomolecules in both the lab and production hall for the past 35 years. In addition to its widespread usefulness, gel filtration is reliable and simple to perform. Minimal equipment is required and good separations and yields are easy to obtain. A wide range of reliable gel filtration media is available from Pharmacia Biotech.

Sephadex G-25 is one of the most useful and widely documented of these media. Continuous development work has led to a steady expansion of the range of products based on the original gel. Today, gel filtration products based on Sephadex G-25 form a family of media and pre-packed columns for fundamental separation tasks in the modern life science research laboratory as well as in the industrial manufacture of biopharmaceuticals.



Sephadex® G-25 gel filtration media form the basis of a range of products for essential group separation applications at laboratory and industrial scales.

Sephadex G-25 media are members of the BioProcess family and carry comprehensive technical and regulatory support for production applications. The availability of this information can speed up the validation process, resulting in shortened time to market when registering a new biopharmaceutical.

Sephadex G-25 *Bead structure*

Sephadex is a bead-formed gel prepared by cross-linking dextran with epichlorohydrin. It is supplied in its dry form. The gel swells in aqueous solutions (see Table 1). Different types of Sephadex differ in their degree of cross-linking and hence in their degree of swelling and their molecular fractionation range. Sephadex G-25 is one of eight different G-types ranging from G-10 for small molecules to G-200 for large molecules.

Table 1. Dry and hydrated bead sizes of Sephadex G-25.

Product	Dry bead size μm	Hydrated median diameter μm^*	Diameter ratio hydrated/dry
Sephadex G-25 Coarse	100–300	320	1.62
Sephadex G-25 Medium	50–150	140	1.61
Sephadex G-25 Fine	20–80	88	1.56
Sephadex G-25 Superfine	20–50	52	1.57

* Average values from three product batches of each grade.

In addition, Sephadex G-25 is available in four different particle size grades (Coarse, Medium, Fine and Superfine). These four grades form the basis of a range of gel filtration products, discussed below.

Separation principle

Gel filtration separates molecules according to their relative sizes. In Sephadex, the degree of cross-linking of the dextran determines the extent to which macromolecules can permeate the beads. Large molecules are totally excluded while smaller sized molecules enter the beads to varying extents according to their different sizes. Large molecules thus leave the column first followed by smaller molecules in the order of their decreasing size.

Sephadex G-25 has a fractionation range for globular proteins of 1000–5000 molecular weight.

The principles of gel filtration and the structures of Sephadex and other gel filtration media are explained in more detail in the handbook “Gel filtration – Principles and Methods”, which is available from Pharmacia Biotech.

Uses of gel filtration

There are two distinct ways of applying gel filtration in a separation scheme.

“Group separation” separates the components of a sample into two groups, e.g. high molecular weight substances, which are excluded from the gel and thus elute first, from lower molecular weight substances that enter the pores and thus elute later.

“Fractionation”, on the other hand, separates molecules of different sizes and can thus be used to fractionate complex samples into individual components.

The separation range of Sephadex G-25 makes it suitable for group separation work such as the removal of low molecular weight contaminants from molecules larger than about 5000 molecular weight.

The effect of particle size

Coarse and Medium grades are preferred for group separations at process scale where high flow rates and low operating pressures are required. The Fine and Superfine grades are for routine laboratory work including preparative separations. The finer beads of the Fine and Superfine grades give shorter diffusion distances. This gives high efficiencies at high flow rates.

Examples shown later illustrate several of these applications.

The particle size of Sephadex is usually reported as dry diameter in μm (see Table 1). However, as the media are swollen before use, it is the wet bead diameter that is of practical importance when choosing the correct type of equipment to use. Table 1 therefore gives the hydrated median diameter of the four grades of Sephadex G-25 swollen in 0.15 M sodium chloride. The diameter ratio hydrated/dry is also given.

Note that the degree of swelling in organic solvents is not the same as in aqueous solutions, and must be determined by experiment for each solvent system. For routine use in organic solvents, lipophilic Sephadex LH-20 or Sephadex LH-60 is recommended.

Stability

The mechanical strength and pH stability of Sephadex gel filtration media depend on the degree of cross-linking.

Sephadex G-25 is one of the more rigid of the family and has a working pH range of 2–13*. In trials, Sephadex G-25 has been exposed to 0.1 M HCl for 1–2 hours and 0.02 M HCl for 6 months without affecting its chromatographic properties. Leakage studies have also shown that Sephadex G-25 withstands long term exposure to NaOH. It may be safely stored in 0.01 M NaOH without affecting its performance. 20% ethanol may also be used for storage. For cleaning-in-place and sanitization, 60–90 minutes exposure to 0.2 M NaOH followed by flushing with water or buffer is recommended. This procedure can be used for hundreds of cleaning cycles.

Sephadex media can be autoclaved in their wet form (pH 7.0) at 120 °C for 30 minutes.

The rigidity of the matrix means that Sephadex G-25 can be used at relatively high flow rates for rapid separations. Please refer to the applications section for details.

Batch-to-batch reproducibility

Consistent quality and performance from batch-to-batch is important for all separation media, but it assumes extra significance when the media are routinely used in industrial processes. Quality control data gathered from nearly 200 production batches of Sephadex G-25 Coarse grade show outstanding batch-to-batch consistency for more than 30 years (Table 2).

* Working pH is the pH at which the medium can be used routinely without adverse effects to the matrix.

Working with Sephadex G-25

Column dimensions

In gel filtration, column length is important for resolution and column diameter determines loading capacity. In fractionation work, long columns (30–100 cm) and small sample volumes give the best resolution. For group separations, however, short (5–30 cm), wide columns give excellent results at high sample loadings.

Column packing

As Sephadex is supplied as a dry powder, it must be swollen in buffer before packing in the column.

Laboratory columns are simply packed by pouring the swollen G-25 slurry into the column and using a pump to pack the gel bed at as high a flow rate as possible without deforming the beads. The result is an evenly packed stable gel with consistently good separation performance.

Packing process scale columns requires modified techniques but nevertheless gives excellent results and consistent performance. Well-proven methods for a range of large scale column types both with and without adjustable adaptors are readily available upon request.

Cleaning a packed column

When a column has been in use for some time, it may be necessary to remove precipitated proteins or other contaminants that have built up on the gel bed. Columns packed with Sephadex G-25 may be cleaned with 2 column volumes of 0.2 M NaOH or a non-ionic detergent solution (60–90 minutes exposure). The frequency of cleaning will depend on the nature of the sample material and should be worked out on a case-by-case basis.

Table 2. Quality control data demonstrate the outstanding batch-to-batch consistency of Sephadex G-25 Coarse over thirty years.

Batch no.	Year of production	Water regain ml/g	Particle size distr. Vol. % 100–300 µm	Non-specific adsorption – % recovery of cytochrome C
(Limits)	–	(2.3–2.7)	(min. 90)	(min. 85)
1	1963	2.52	–	–
40	1970	2.50	96	88
80	1974	2.43	99.8	93
115	1979	2.53	94.0	92
150	1985	2.50	96.3	90
175	1989	2.48	96.0	94
180	1994	2.43	96.1	96

Scale-up

Scaling up a separation based on Sephadex G-25 to pilot level and then into routine production is straightforward and trouble-free. Well known examples of successful commercial applications include buffer exchange in processes for removing endotoxins from albumin (see applications section) and as a preparative step during the production of vaccines.

Gel filtration products based on Sephadex G-25

Laboratory and process scale media packs

These are the most flexible and economical way to use Sephadex G-25 on a regular basis. Each of the four grades is available in a range of pack sizes (see Ordering information).

Mini-packs

Sephadex G-25 Medium grade is available in a Mini-pack size of 25 g. Mini-packs offer users a quick and affordable opportunity to try this grade of Sephadex G-25 in their applications.

Step-by-step instructions for optimal packing and running are included in each pack.

Pre-packed columns

Table 3 lists four column types pre-packed with Sephadex G-25 media and gives some basic column properties. Their fundamental characteristics are summarized below.

Pre-packed Disposable Columns PD-10

PD-10 columns are available in boxes of 30 columns, each containing 8.5 ml Sephadex G-25 Medium. They can desalt sample volumes of up to 2.5 ml with high recovery and low dilution. In use, the column is suspended over a test tube and equilibrated with buffer. The sample is then applied

and the high molecular weight fraction is eluted in the void volume by gravity flow. The separation takes about 5 minutes. PD-10 columns are inexpensive and can be disposed of after use to eliminate sample cross-contamination.

HiTrap Desalting

HiTrap® Desalting is a medical grade plastic column pre-packed with 5 ml Sephadex G-25 Superfine. Its low back-pressure enables fast separations using either a syringe, a peristaltic pump or a liquid chromatography system. Recommended flow rates are high (up to 10 ml/min) with a maximum of 15 ml/min, and with separation times of about 1–2 minutes. The recommended sample volume for each column is up to 1.5 ml and can be scaled up to 7.5 ml by connecting up to five columns in series.

Fast Desalting HR 10/10

Fast Desalting HR 10/10 is high performance column specially designed for fast and convenient group separations on FPLC® System. It comprises a high resolution HR 10/10 glass column pre-packed with 8 ml Sephadex G-25 Superfine. Fast Desalting HR 10/10 can be used at flow rates up to 6 ml/min, giving a separation time of 1–2 minutes and is an ideal column for rapid, routine group separations in automated systems.

Fast Desalting PC 3.2/10

Fast Desalting PC 3.2/10 is optimised for use on the micropreparative scale with SMART® chromatography system. It is packed with Sephadex G-25 Superfine, but the narrow column diameter gives a much smaller bed volume (0.8 ml) which is ideal for smaller samples. The column is made of glass and fluoroplastics with a titanium filter. The maximum recommended flow rate is 0.9 ml/min and the operational pressure limit is 1.2 bar. Typical separation times are 1–2 minutes. Fast Desalting PC 3.2/10 is also ideal for routine use in automated separation schemes, especially when recovery of limited samples is critical.

Table 3. Pre-packed columns containing Sephadex G-25.

Pre-packed column	G-25 grade	Bed vol.	Rec. flow rate range	Equipment	Max. sample vol.
PD-10	Medium	8.5 ml	gravity flow	stand alone	2.5 ml
HiTrap Desalting	Superfine	5 ml	1–10 ml/min	syringe/pump	1.5 ml
Fast Desalting HR 10/10	Superfine	8 ml	2–6 ml/min	standard LC equipment	2.4 ml
Fast Desalting PC 3.2/10	Superfine	0.80 ml	0.01–0.9 ml/min	SMART System or HPLC	0.2 ml

The new Column Holder 10 cm now allows this column to be run on high performance liquid chromatography systems (HPLC), including microbore HPLC.

Applications of Sephadex G-25 media and pre-packed columns

As noted earlier, group separation of biomolecules is the most widely used application of Sephadex G-25. With a fractionation range for globular proteins of 1000–5000 molecular weight, this ensures group separations of proteins/peptides larger than 5000 from molecules with a molecular weight less than 1000.

Buffer exchange and desalting are common examples of group separations. Buffer exchange is frequently necessary when the buffer composition of the sample must be changed between chromatography steps. Desalting a sample is often needed before ion exchange and after hydrophobic interaction or affinity chromatography.

At small scale, gel filtration with Sephadex G-25 has replaced much slower and more cumbersome dialysis. Simple-to-use pre-packed columns such as the PD-10 and HiTrap Desalting are now popular tools for desalting or buffer exchange. Figure 1 shows the removal of NaCl from albumin on a PD-10 column (Sephadex G-25 Medium).

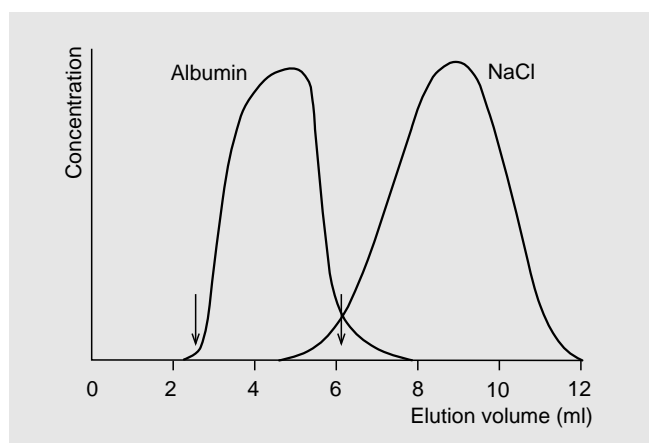


Fig. 1. Removal of NaCl from albumin solution. A PD-10 column containing Sephadex G-25 Medium was equilibrated with distilled water. The sample contained human serum albumin (25 mg) dissolved in NaCl solution (0.5 M, 2.5 ml). Yield of albumin in 3.5 ml after sample application (between arrows) was 95.3%. The salt content was 2.0% of that originally present.

Figure 2 illustrates the scalability of Sephadex G-25 for a similar application. Here a production scale 75 liter stainless steel column was used for the buffer exchange step in the the production of albumin and IgG from human plasma.

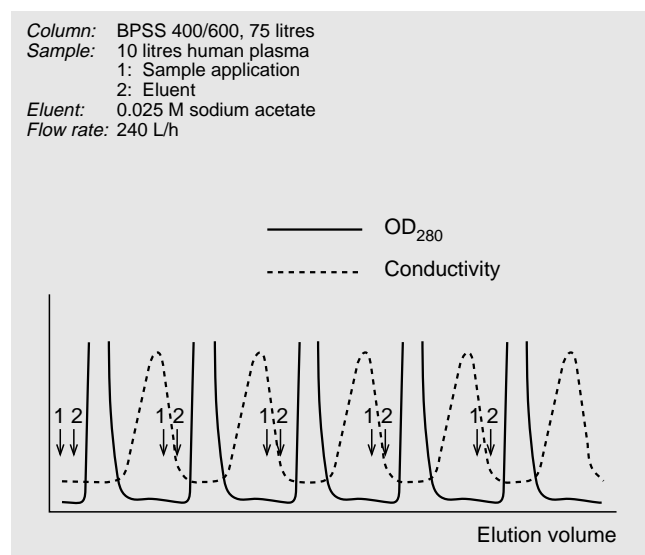


Fig. 2. Chromatogram of the buffer exchange step on Sephadex G-25 Coarse during the production of albumin and IgG from human plasma.

Figure 3 shows process data from a buffer exchange step on Sephadex G-25 Coarse included in a process for removal of endotoxins and/or ethanol from albumin. The high throughput of Sephadex G-25 Coarse grade resulted in a rapid and efficient separation despite the high concentration of protein in the feedstock.

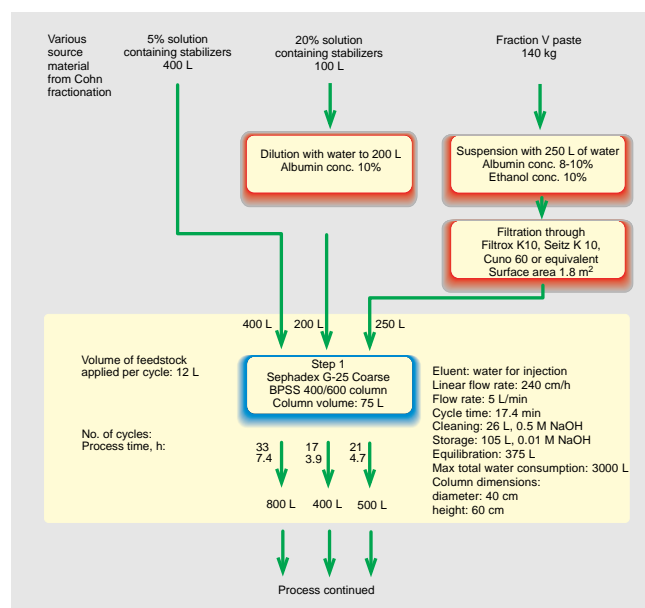


Fig. 3. Process data of a buffer exchange step on Sephadex G-25 Coarse from a production scale process to remove endotoxins and/or ethanol from albumin.

HiTrap Desalting columns can easily be attached to other columns for on-line buffer exchange as shown in Fig. 4b. The pH of mouse monoclonal IgG eluted from a HiTrap Protein G affinity column was adjusted by connecting a HiTrap Desalting column in series. Figure 4a shows the original separation without the extra desalting step. The yield obtained was 18% higher when the HiTrap Desalting column was used for on-line desalting (Fig. 4b).

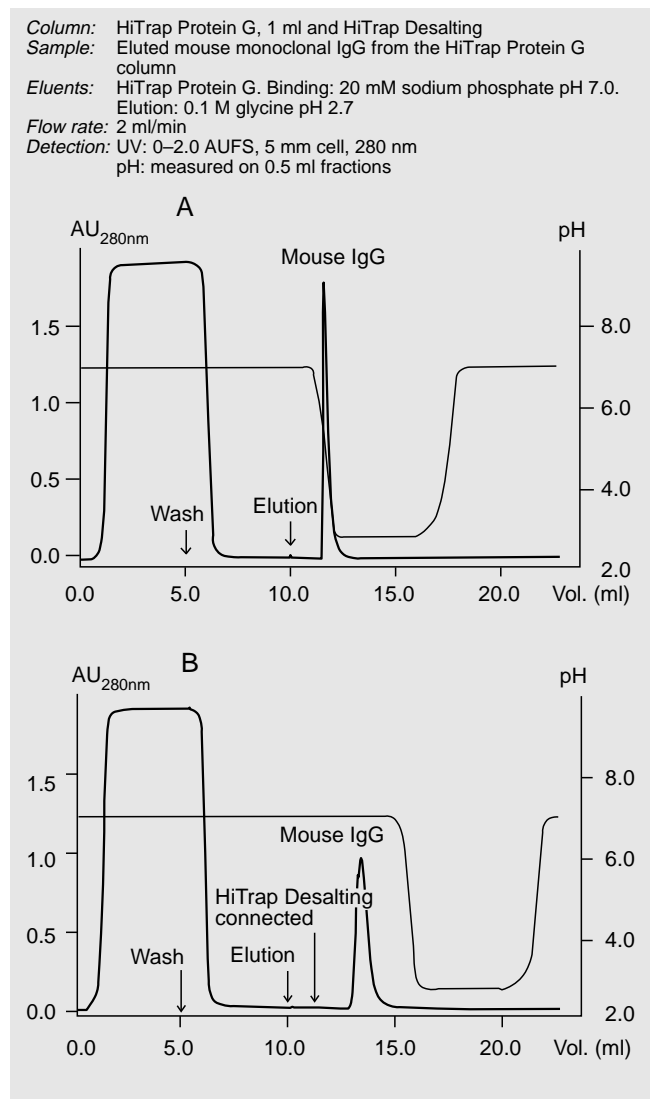


Fig. 4. On-line buffer exchange with HiTrap Desalting (Sephadex G-25 Superfine) improves the yield from a HiTrap Protein G affinity purification.

A further group separation that illustrates the usefulness of HiTrap Desalting is shown in Fig. 5. During coupling of IgG to NHS-activated Sepharose (HiTrap NHS-activated) N-Hydroxy Succinimide (NHS) is released. NHS shows strong absorbance at 280 nm. To measure the coupling yield NHS must be removed. In this example, the column was operated using a syringe.

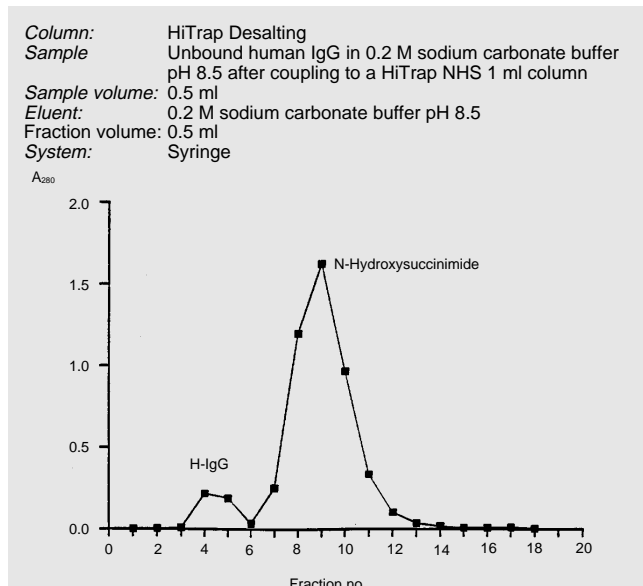


Fig 5. HiTrap Desalting operated with a syringe to remove unwanted components following a coupling reaction.

Finally, Figure 6 shows a high resolution group separation. Here free ¹²⁵I was removed from labelled insulin on a Fast Desalting HR 10/10 column run on FPLC System.

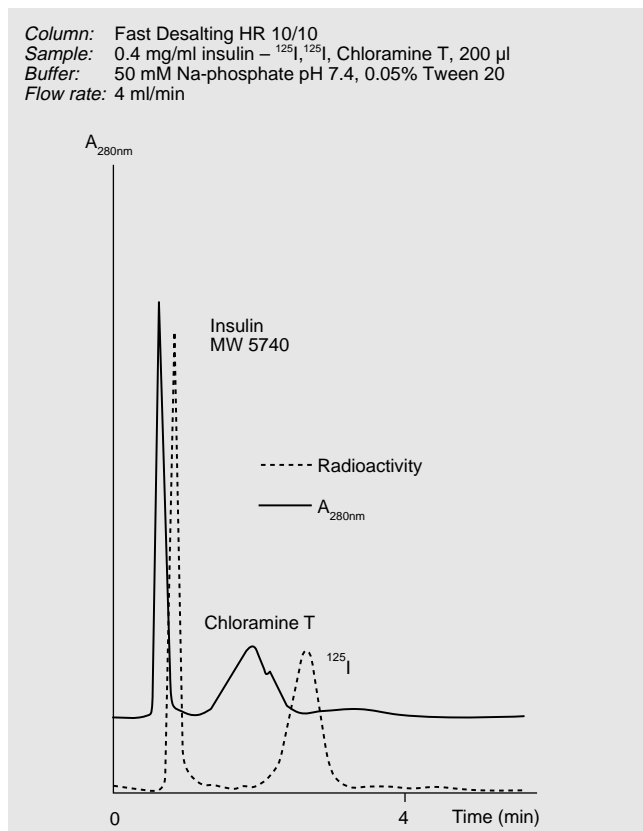


Fig. 6. Removal of free ¹²⁵I from labelled insulin on a Fast Desalting HR 10/10 column (Sephadex G-25 Superfine) run on FPLC System.

Ordering information

Product	Quantity	Code No.
Sephadex G-25 Coarse	100 g	17-0034-01
Sephadex G-25 Coarse	500 g	17-0034-02
Sephadex G-25 Coarse	5 kg	17-0034-03
Sephadex G-25 Coarse	40 kg	17-0034-07
Sephadex G-25 Medium	25 g*	17-0033-10
Sephadex G-25 Medium	100 g	17-0033-01
Sephadex G-25 Medium	500 g	17-0033-02
Sephadex G-25 Medium	5 kg	17-0033-03
Sephadex G-25 Medium	40 kg	17-0033-07
Sephadex G-25 Fine	100 g	17-0032-01
Sephadex G-25 Fine	500 g	17-0032-02
Sephadex G-25 Fine	5 kg	17-0032-03
Sephadex G-25 Fine	40 kg	17-0032-07
Sephadex G-25 Superfine	100 g	17-0031-01
Sephadex G-25 Superfine	500 g	17-0031-02
Sephadex G-25 Superfine	5 kg	17-0031-03

* Mini-pack size.

Pre-packed columns

Pre-packed column	Quantity	Code No.
Pre-packed Disposable Columns PD-10	30	17-0851-01
HiTrap Desalting (includes Luer adaptors, tubing connectors and domed nuts)	5 × 5 ml	17-1408-01
Fast Desalting Column HR 10/10	1	17-0591-01
Fast Desalting PC 3.2/10	1	17-0774-01

Large scale columns

Pharmacia Biotech has a large range of columns for scale up and commercial production. Contact your local representative for more information.

Further information

More details of the gel filtration technique can be found in the Pharmacia Biotech handbook "Gel filtration – Principles and Methods" code no. 18-1022-18. Further product and application information is available in the Pharmacia Biotech general and process scale catalogues. Contact your local office for your own copies.

