

Sephacryl S-100 High Resolution
Sephacryl S-200 High Resolution
Sephacryl S-300 High Resolution
Sephacryl S-400 High Resolution
Sephacryl S-500 High Resolution

INSTRUCTIONS

Sephacryl™ High Resolution chromatography media is a highly versatile gel filtration media that offers a wide range of fractionation capabilities. The hydrophilic, rigid matrix of Sephacryl minimizes non-specific adsorption while maximizing recoveries. Characteristics of the different types of Sephacryl media are listed in Appendix C, Table 1.

A well-packed column is critical to achieving good resolution in gel filtration chromatography. The following column packing method has been developed by Amersham Biosciences to provide optimal conditions for high resolution.

The instructions that follow are based upon packing Sephacryl gel filtration media in the recommended XK 16/70 column. Flow rates are given in specific volumetric values, with reference to the linear flow rate. To modify these instructions for a column with different dimensions, refer to Appendix A.

Detailed information on the technique of gel filtration can be founded in "Gel Filtration; Principles and Methods" from Amersham Biosciences.

Material needed

Sephacryl HR

Column XK 16/70

Packing Reservoir RK 16/26

Pump (Peristaltic Pump P-1 or High Precision Pump P-500)

Valve (LV-3 or LV-4)

Graduated cylinder or beaker

Large beaker

Glass rod, syringe, 5 ml

Small spoon or plastic spatula

Buffer

Preparing the gel suspension

1. Equilibrate all material to room temperature.
2. Gently shake the bottle of Sephadex HR to make an even slurry.
3. Pour the 150 ml of gel slurry into a graduated cylinder or beaker. Dilute the gel slurry with buffer to 200 ml.
4. Stir with a glass rod to make a homogeneous suspension free from aggregates. DO NOT use a magnetic stirrer.

Assembling the column

Details of the column parts can be found in the instructions supplied with the column. Before packing ensure that all parts, particularly the nets, net fasteners and glass tube, are clean and intact.

1. Attach the packing reservoir firmly to the column.

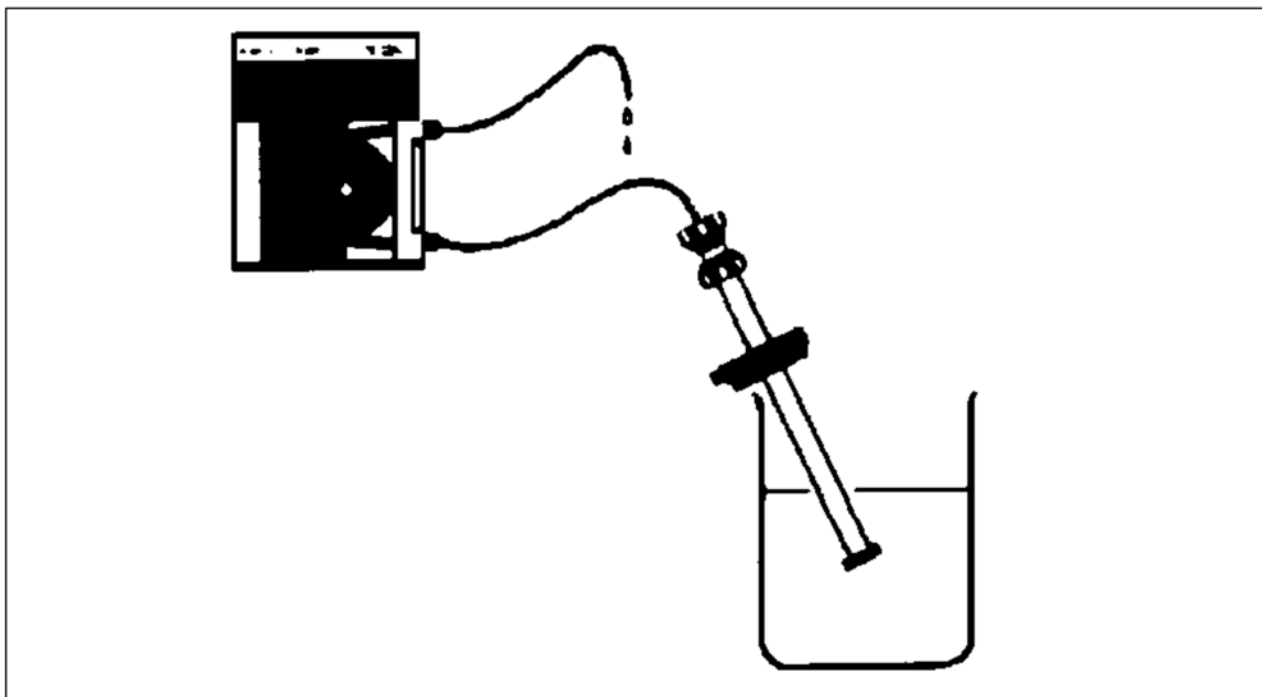


Fig. 1.

2. Connect the adaptor to a pump or syringe. Submerge the plunger end in buffer and fill the adaptor using the pump or syringe (Fig. 1). Ensure that there are no air bubbles trapped under the net. Close the tubing with a stopper. Insert and tighten the adaptor 2 cm from the bottom of the column.
4. Flush the column with buffer, leaving a few ml at the bottom. Mount the column vertically on a laboratory stand.

Packing the column

These instructions are for packing Sephacryl gel filtration media in the recommended XK 16/70 column. Flow rates are given in specific volumetric values, with reference to the linear flow rate. To modify these instructions for a column with different dimensions, refer to Appendix A.

1. Resuspend and pour the gel slurry into the column in one continuous motion. Pouring down a glass rod held against the wall of the column helps prevent the introduction of air bubbles. Fill the reservoir to the top with buffer. Screw on the reservoir top tightly, and connect it to the pump. Open the column outlet (Fig. 2.)

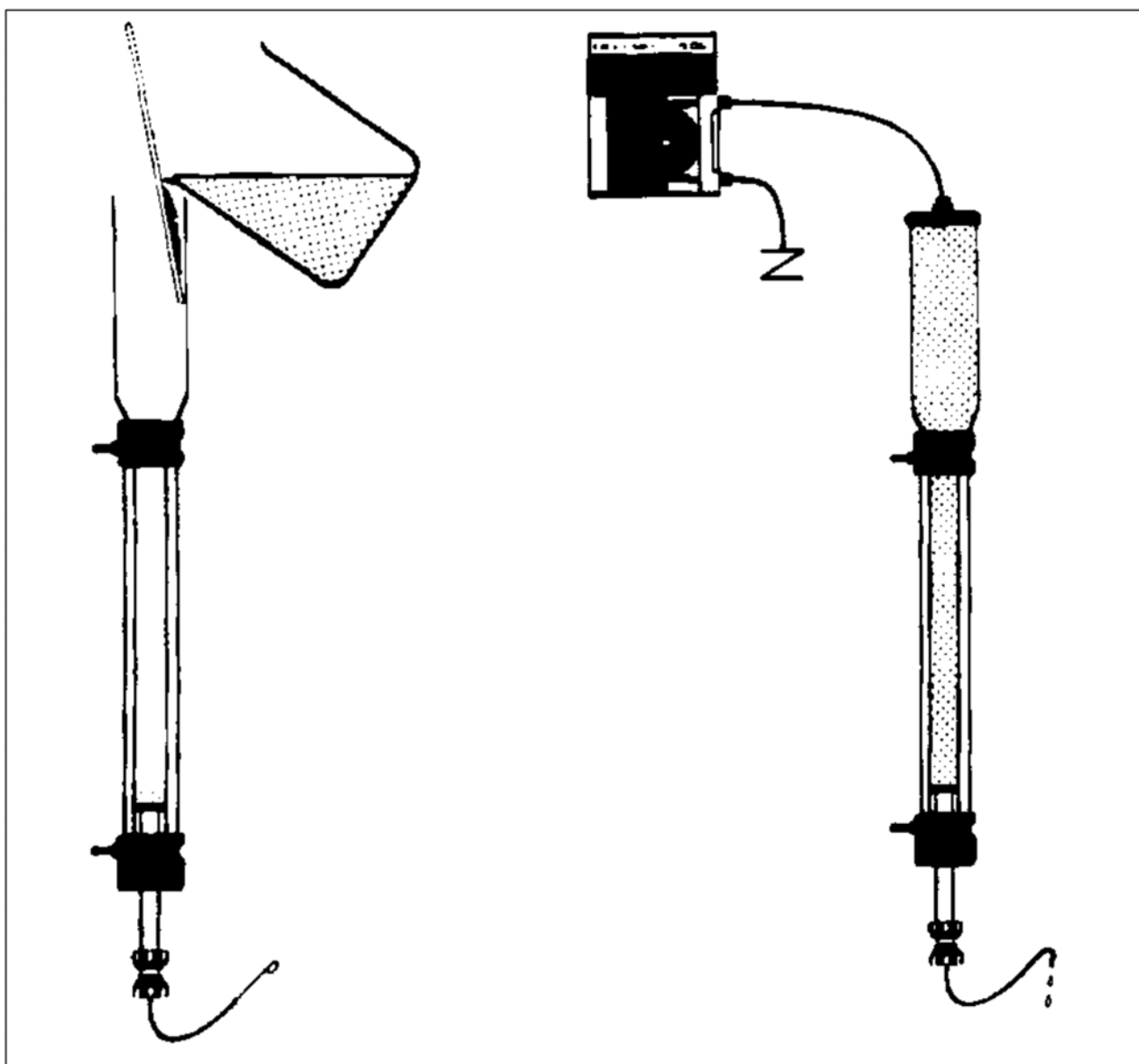


Fig. 2.

2. Pack the column in the following two steps:
STEP 1: Pack the column at 1 ml/min for 2 hours, or until the bed height is constant.
STEP 2: Increase the flow rate to the appropriate value from the table below and continue packing for 1 hour.

Sephacryl Type	STEP 1	STEP 2
Sephacryl S-100 HR	1 ml/min	1.8 ml/min
Sephacryl S-200 HR	1 ml/min	1.8 ml/min
Sephacryl S-300 HR	1 ml/min	2.3 ml/min
Sephacryl S-400 HR	1 ml/min	3.0 ml/min
Sephacryl S-500 HR	1 ml/min	2.3 ml/min

3. Stop the pump and close the column outlet. Disconnect the pump. Take the column from the stand and remove the packing reservoir over a sink. Using a small spoon or plastic spatula, carefully remove gel from the top of the gel bed until the surface is approximately 4-5 mm below the end of the glass tube.

Note: If there is not enough gel in the column it will have to be re-packed from the beginning using additional gel.

4. Remount the column vertically on the stand and fill to the top with buffer.

Final column assembly

1. Wet the column bottom piece by drawing water through it. Make sure there are no air bubbles trapped under the net and close the tubing with a stopper (Fig. 3).
2. Tighten the bottom piece O-ring adjusting nut one-half turn. Insert the bottom piece at angle to avoid trapping air and screw it several turns onto the column.
3. Remove the stopper from the end-piece tubing.

Note: DO NOT open the outlet on the adaptor at the bottom of the column.

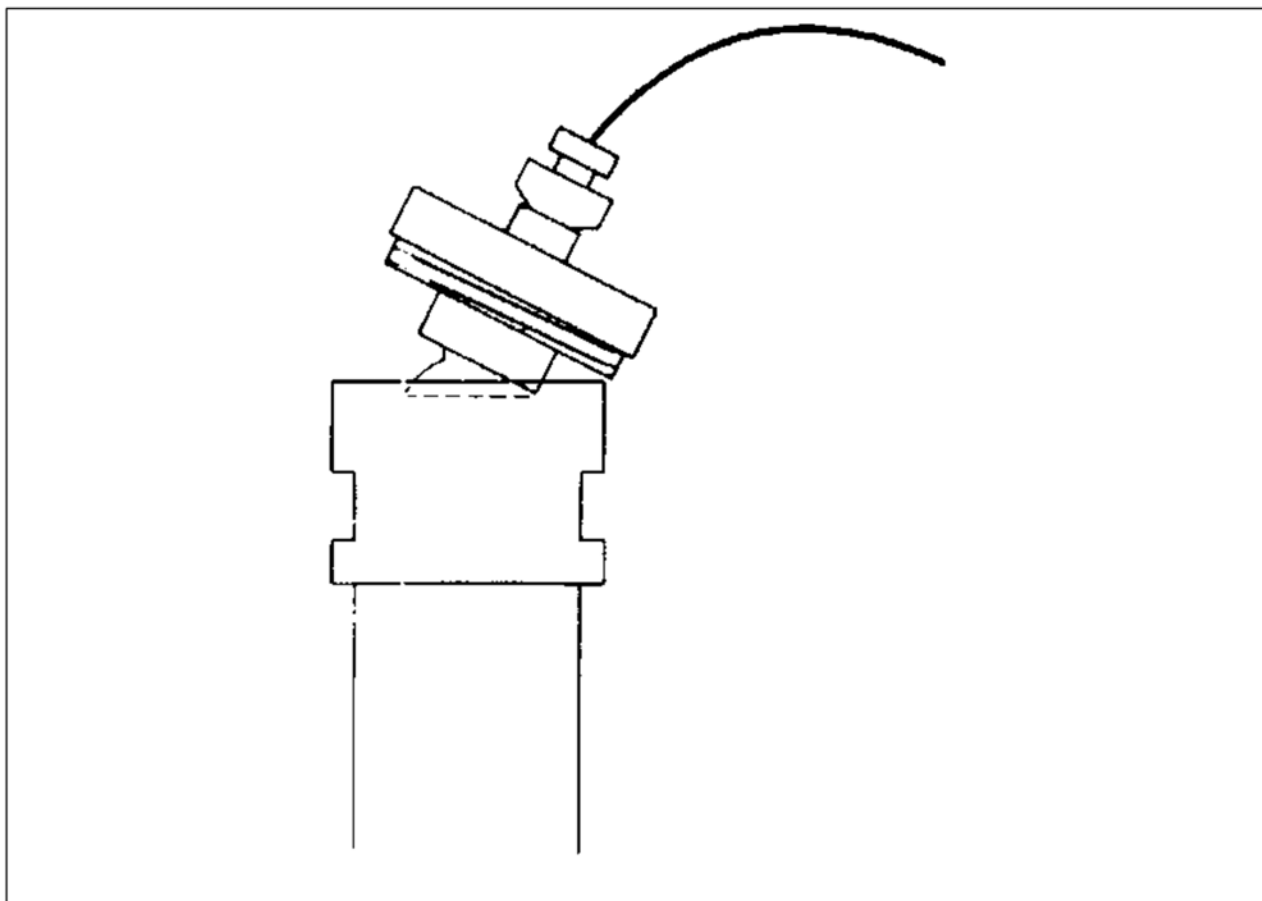


Fig. 3.

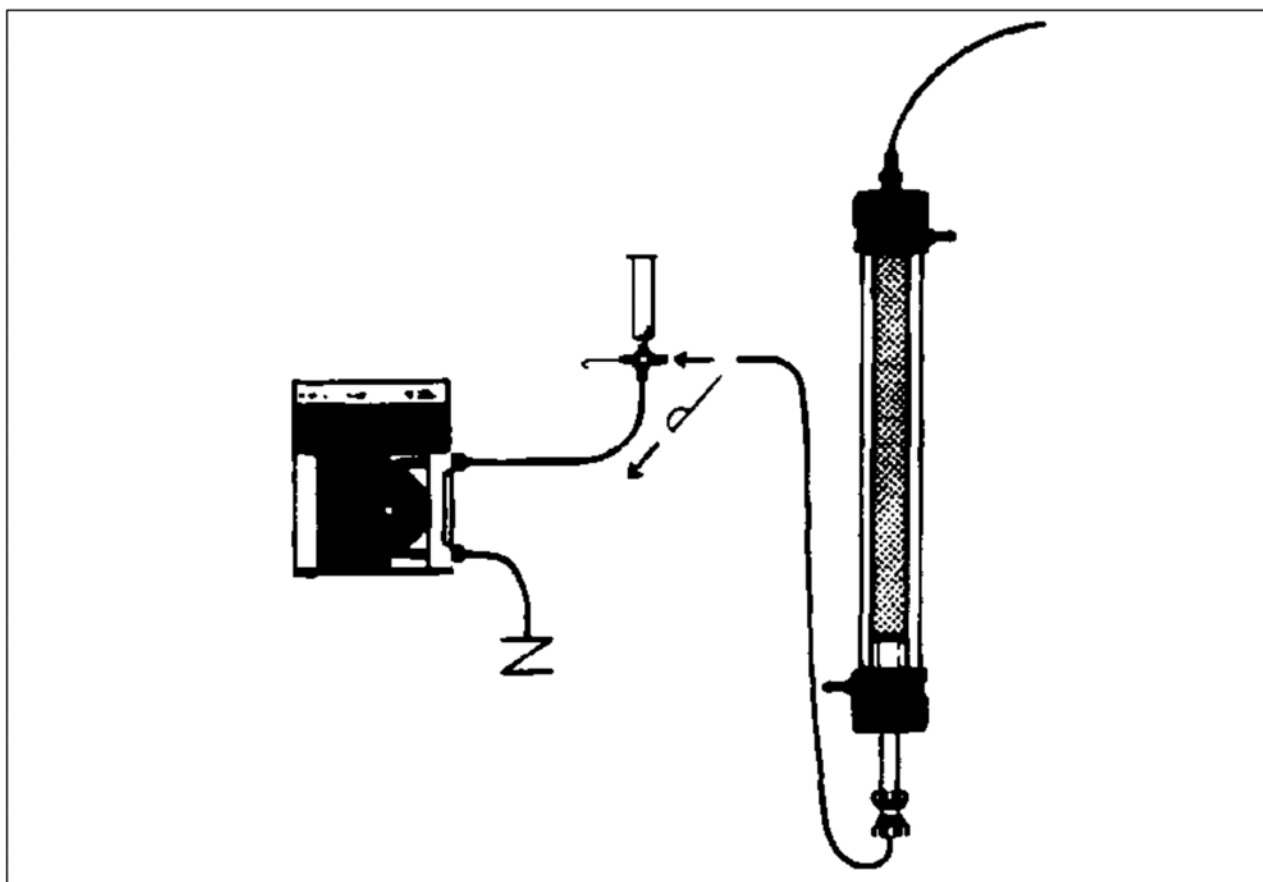


Fig. 4.

4. Tighten the O-ring adjusting nut an additional one-half turn, and then screw the bottom piece completely into place. The end piece should be pressed a distance of approximately 5 mm into the packed gel. Replace the stopper.
5. Connect the sample application valve to the pump. Run the pump to remove all air in the tubing up to the valve
6. Remove the stopper from the adaptor tubing. If there are any air bubbles in the tubing below the adaptor, remove the stopper from the end piece tubing and allow buffer to run through the line for a few seconds. Connect the tubing from the adaptor to the sample application valve (Fig. 4.)

7. Turn the column upside-down or run it with upward flow.

For most applications the column is now ready for use. For extremely difficult separations requiring maximum performance the column packing should be checked using the testing procedure described in Appendix B.

Equilibration

Equilibrate the column with 200 ml of running buffer. A larger volume may be required if detergent solutions are used. Do not exceed the maximum flow rate from Step 2 of column packing when equilibrating or running the column.

Buffers

Buffer composition does not directly influence the resolution which can be obtained in gel filtration chromatography and buffers can be chosen to match the requirements of the sample. However, an ionic strength equivalent to 0.15 M NaCl or greater is recommended to avoid ionic interactions with the gel matrix.

To ensure long column life, all buffers should be centrifuged or filtered (0.45 μm) before use.

Samples

The sample volume should be in the range of 1-4 ml (1-4% of the total bed volume). To ensure long column life, samples should be centrifuged or filtered (0.45 μm) before use.

Flow rates

A recommended flow rate is 0.17-0.83 ml/min (5-25 cm/hour) for the XK 16/70 column. The lower the flow rate, the better the

resolution. In a cold room at 4 °C, aqueous buffers approximately double in viscosity compared to room temperature, and flow rates should be lowered accordingly.

Re-equilibration

To re-equilibrate the column between samples, wash with 200-300 ml of elution buffer.

Cleaning-in-place (CIP)

To remove precipitated material, wash the column in the reversed flow direction with 50-100 ml of 0.2-0.5 M NaOH or a solution of a non-ionic detergent at a flow rate at 0.5 ml/min (15-20 cm/hour). The total contact time with the cleaning solution should be 1-2 hours. After washing, always re-equilibrate the column before re-use.

Sanitization

Sanitization reduces microbial contamination of the gel bed to a minimum. To sanitize, wash with 0.1-0.5 M NaOH at room temperature for a contact time of 30-60 minutes. For the XK 16/70 column, set the flow rate at approximately 1.2 ml/min (35 cm/hour). Re-equilibrate the column with sterile buffer before use.

Storage

Unopened media can be stored at +4 °C to +30 °C. Packed columns and used gel should be stored in 20% ethanol at +4 °C.

Appendix A

Converting to columns of different dimensions

Flow rates

Flow rates quoted in this instruction are for an XK 16/70 column. To convert flow rates for columns of different dimensions:

1. Divide the volumetric flow rates (ml/min) quoted by a factor of 2 (the cross-sectional area in cm² of the XK 16/70) to give the linear flow rate in cm/min.
2. Maintain the same linear flow rate and calculate the new volumetric flow rate according to the cross-sectional area of the specific column to be used

$$\text{Linear flow rate} = \frac{\text{Volumetric flow rate}}{\text{Column cross-sectional area}}$$

Volumes

Volumes (buffers, gradients, etc.) quoted in this instruction are for an XK 16/70 column that has a bed volume of 100 ml (bed height x cross-sectional area). To convert volumes for columns of different dimensions, increase or decrease in proportion to the new column bed volume.

$$\text{New volume} = \text{Old volume} \times \frac{\text{New bed volume}}{\text{Old bed volume}}$$

Appendix B;

Testing the packed column

To check the efficiency of the column packing, determine the theoretical plate number and peak symmetry. If the column is packed according to the instructions described above typical values should be:

Efficiency: $N > 9,000$ theoretical plates per meter

Peak symmetry: $A_s = 0.80 - 1.50$

Solutions required

Distilled water

Sample: acetone 2% (v/v) in distilled water

1. Establish a flow rate of 1 ml/min (i.e., a linear flow rate of 30 cm/hour) through the packed XK 16/70 column with 20% ethanol. Set the monitor at 280 nm at an absorbance of 1.0 AUFS for a 10 mm path length cell. Run the chart recorder at a minimum of 10 cm/min. Zero the monitor and chart recorder.
2. Inject 200 μ l acetone (100 μ l per cm^2 cross-sectional area) onto the column at a flow rate of 1 ml/min (i.e., a linear flow rate of 30 cm/hour).
Record the absorbance from time of injection until the acetone peak has been detected and the monitor signal has returned to baseline. If the chart recorder tracing of the acetone peak is not at least 70% of the full scale chart deflection, increase the sensitivity of the monitor setting accordingly and re-run.

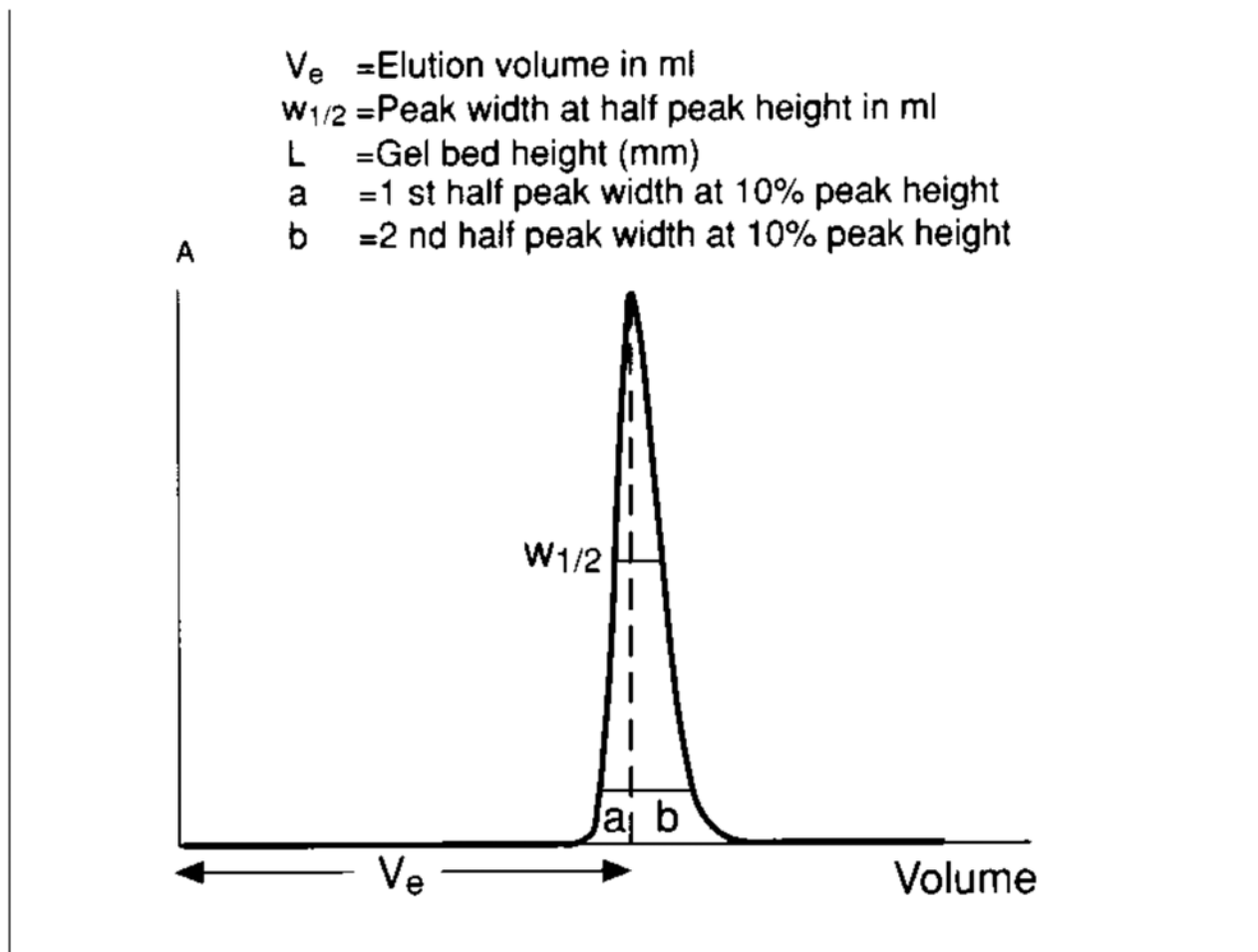
3. Calculate the column efficiency (plate number)
Referring to the figure below, calculate the column efficiency (N) as follows:

$$N = 5.54 (V_e/W_{1/2})^2 \times (1000/L)$$

For the XK 16/70, the bed height (L) can be measured by adding 24 mm to the visible bed height.

Referring to the figure below, calculate the symmetry factor by the formula:

$$A_s = b/a$$



Troubleshooting

If N is $<9,000$ and $A_s < 0.7$, repack the column with 0.1% TWEEN 20 in the gel suspension.

If N is $<9,000$ and $A_s > 1.3$, repack the column with 0.1% TWEEN 20 in the gel suspension and increase the flow rate in Packing Step 2.

If N is $>9,000$ and $A_s < 0.7$, repack the column with 0.1% TWEEN 20 in the gel suspension.

If N is $>9,000$ and $A_s > 1.3$, repack the column with 0.1% TWEEN 20 in the gel suspension and decrease the flow rate in Packing Step 2.

Appendix C;

Tables

Table 1. Gel characteristics

Fractionation range (globular proteins)- M_r	1x10 ³ -1x10 ⁵ (Sephacryl S-100 HR) 5x10 ³ -2.5x10 ⁵ (Sephacryl S-200 HR) 1x10 ⁴ -1.5x10 ⁶ (Sephacryl S-300 HR) 2x10 ⁴ -8x10 ⁶ (Sephacryl S-400 HR)
Fractionation range (dextrans)- M_r	1x10 ³ -8x10 ⁴ (Sephacryl S-200 HR) 2x10 ³ -4x10 ⁵ (Sephacryl S-300 HR) 1x10 ⁴ -2x10 ⁶ (Sephacryl S-400 HR) 4x10 ⁴ -2x10 ⁷ (Sephacryl S-500 HR)
DNA exclusion limit (base pairs)	30 (Sephacryl S-200 HR) 118 (Sephacryl S-300 HR) 271 (Sephacryl S-400 HR) 1078 (Sephacryl S-500 HR)
Bead structure	Allyl dextran and N,N'-methylene bisacrylamide
Bead size	25-75 μ m (mean 47 μ m)
Maximum operating pressure	0.2 MPa (2 bar, 28 psi)
Maximum linear flow rate	60 cm/hour
Recommended volumetric flow rate*	0.3-1.2 ml/min (XK 16/70)
Recommended linear flow rate*	10-35 cm/hour
Chemical stability	All commonly used buffers, 0.2 M NaOH, 0.2 M HCl, 1 M acetic acid, 8 M urea, 6 M guanidine HCl, 1% SDS, 24% ethanol, 30% propanol, 30% acetonitrile

pH stability (long term)**	3-11
pH stability (short term)**	2-13
Autoclavable	at 121 °C, pH 7 for 30 min.

*At room temperature in aqueous buffer.

** pH stability, long term refers to the pH interval where the gel is stable over a long period of time without adverse effects on its subsequent chromatographic performance.

pH stability, short term refers to the pH interval for regeneration, cleaning-in-place and sanitization procedures. All ranges given are estimates based on our knowledge and experience.

Ordering Information

Description	Pack size	Code No.
Sephacryl S-100 HR	150 ml	17-0612-10
Sephacryl S-100 HR	750 ml	17-0612-01
Sephacryl S-200 HR	150 ml	17-0584-10
Sephacryl S-200 HR	750 ml	17-0584-01
Sephacryl S-300 HR	150 ml	17-0599-10
Sephacryl S-300 HR	750 ml	17-0599-01
Sephacryl S-400 HR	150 ml	17-0609-10
Sephacryl S-400 HR	750 ml	17-0609-01
Sephacryl S-500 HR	150 ml	17-0613-10
Sephacryl S-500 HR	750 ml	17-0613-01

Related Products

Column XK 16/70	18-8775-01
Packing reservoir RK 16/26	18-8793-01
Valve LV-3	19-0016-01
Valve LV-4	19-0017-01
Peristaltic Pump P-1 (220 V)	19-4610-02
Peristaltic Pump P-1 (110 V)	19-4611-02
High Precision Pump P-500	18-1003-65
Gel Filtration; Principles and Methods	18-1022-18