GE Healthcare

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

Instructions

Sephacryl™

Contents

1. Introduction

To obtain good resolution in gel filtration, it is important that the column is well packed.

An air bubble or small disturbance in the medium bed will cause the sample zone to broaden. This broadening is amplified as the zone migrates down the column. The result is broader peaks and lost resolution.

With traditional packing methods you often get good results. However, a disadvantage is that the medium becomes most tightly packed at the bottom of the column, instead of at the top.

GE Healthcare has developed an improved packing method which results in increased resolution. A short version of the packing method follows.

1. Insert an adaptor at the bottom of the column.
2. Pour the medium into the column and pack the column in 2 steps.
3. Insert the bottom piece or a second adaptor at the top.
4. Turn the column upside-down.

The sample will be applied in the most tightly packed zone of the medium, now at the top of the column. The result will be improved resolution.

We recommend you to follow this procedure since it has been shown to give the best results.

1.1 Equipment needed

	Laboratory scale		**Process scale**
Pump	P-1, P-50, ÄKTAdesign 100 pump (P-901) or ÄKTAprime system		≈1800 ml/h
Column*	XK 16/40	XK 26/40	XK 50/60
	XK 16/70	XK 26/70	XK 50/100
	XK 16/100	XK 26/100	
Cross-sectional area of the column	2.0 cm^2	5.3 cm^2	19.6 cm^2
Adaptor	AK 16	AK 26	AK 50
Packing reservoir	RK 16/26	RK 16/26	RK 50

* The first number in the column name refers to the inner diameter of the column in mm. The second number refers to the lenght of the column i cm. C and TricornTM columns can also be used. The C columns must be used with the appropriate thermostat jacket. For preparative chromatography we recommend column XK 26/70.

Measuring cylinder, Large beaker, Buffer**, Glass rod, Small spoon or plastics spatula, (Pasteur pipette)Technical data

2. To prepare the medium suspension

1. Determine the desired packed bed volume by multiplying the cross-sectional area of the column (see table above) by the desired bed height.
2. Gently shake the bottle of Sephacryl™ HR to make an even slurry.
3. Measure out the required volume of medium slurry, 1.5 x the desired packed medium volume***, using a measuring cylinder and pour it into a beaker.
4. Dilute the medium suspension with eluent buffer to 2 x the desired packed medium volume.
5. Stir with a glass rod to make a homogeneous suspension**** free from aggregates. Never use a magnetic stirrer.

3. To pack the column

Note: Columns may be packed using either one adaptor and a bottom piece, or two adaptors. The packing methods for these two arrangements differ only in point 10 and 13.

Pack the column at the temperature at which it will be used.

1. Make sure the column is not damaged and that all parts are really clean. It is of special importance that the nets, net fasteners and glass tube are not damaged.
2. Attach the packing reservoir tightly (don't forget the sealing ring) and mount the column vertically on a stand.
3. Wet the adaptor by drawing water through it, making sure no air bubbles are trapped under the net. This is best done by submerging the plunger in a beaker of water and attaching the tubing to a pump (Fig.1) or a syringe. Close the tubing with a stopper when all air bubbles have been removed.
4. Insert the adaptor at the bottom of the column far enough to give the desired bed height.
5. Wet the column glass tube with eluent leaving a few centimeters of fluid in the bottom. Make sure the net is completely free from air bubbles.

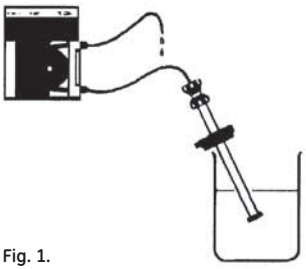


Fig. 1.

** The buffer may be degassed, but it is usually not necessary.

*** The required volume of settled medium is about 1.1 x the desired packed medium volume.

**** The medium suspension may be degassed, but it is usually not necessary.

6. Resuspend the medium and pour the well-mixed medium suspension carefully down the wall of the column using a glass rod (Fig. 2). Pour all the medium in one operation. Fill the reservoir to the top with buffer.
7. Screw on the reservoir cap tightly. Connect it to the pump. Open the outlet (Fig. 3).
8. Pack the column in two steps using the flow rates given in the table below. Please note that the recommendations are for aqueous buffers at room teperature.

   If other conditions are used please consult the column instruction manual for pressure rating. Pack the medium in STEP 1 for 2 hours or until the medium has reached a constant height. Then increase the flow rate to the value listed for STEP 2 and pack for 60 minutes.
9. Stop the pump and close the outlet. Remove the packing reservoir. This is most easily done by first removing the column from the stand and then unscrewing the reservoir over a sink (Fig. 4). For larger columns it may be easier to use a siphon.

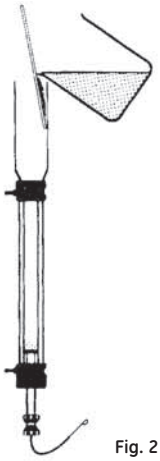


Fig. 2

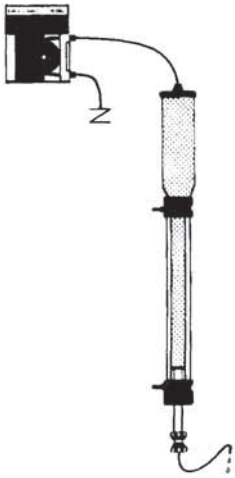


Fig. 3.

Recommended packing flow rates (ml/h) with aqueous buffers at room temperature

	Sephacryl S-100 HR S-200 HR		Sephacryl S-300 HR S-500 HR		Sephacryl S-400 HR	
Column	STEP 1	STEP 2	STEP 1	STEP 2	STEP 1	STEP 2
XK 16/40	60	150	60	190	60	250
XK 16/70	60	110	60	140	60	180
XK 16/100	60	100	60	120	60	160
XK 26/40	150	410	240	490	240	650
XK 26/70	150	300	240	360	240	480
XK 26/100	150	270	180	320	240	430
XK 50/60	600	1150	600	1400	600	1800
XK 50/100	500	800	600	950	600	1300

10. **Using one adaptor and a bottom piece.** Remove excess medium carefully with a small spoon or a plastic spatula. The bed surface should be about 4–5 mm below the end of the glass tube. When the bottom piece is inserted in point 13, it will be pressed about 5 mm into the medium. If there is not enough medium in the column it will be necessary to use an adaptor (see below) or to repack the column with excess medium.

    Using two adaptors. Remove excess medium by gently stirring the top of the bed with a glass rod and removing the suspended medium with a Pasteur pipette.

    Remove enough medium so that the plunger will be visible below the end piece.
11. Mount the column vertically on the stand and fill the column to the top with buffer.
12. Wet the bottom piece (or a second adaptor) a desribed above (3).
13 **Using one adaptor and a bottom piece.** Take up the slack on the O-ring adjusting nut, and tighten one half turn. Screw the bottom piece several turns into the column, making sure that no air bubbles are trapped under the net; see Fig. 5.

    Remove the stopper from the bottom piece tubing. Note: do not open the outlet on the adaptor at the bottom of the column. Tighten the O-ring adjusting nut half a turn more before screwing the bottom piece itself completely into place. Close the bottom piece outlet, with the stopper, again.

    Using two adaptors. Insert the second adaptor carefully so that no air bubbles are trapped under the net, as shown for the insertion of the bottom piece in Fig. 5.

    Remove the stopper from the second adaptor tubing (not from the adaptor at the bottom of the column). Bring the adaptor down into the column and make sure that there are no air bubbles under the net. Bring the adaptor to the medium surface and then a further 5 mm into the medium. Tighten the O-ring above the plunger and lock the adaptor in this position. Close the adaptor tubing with a stopper.

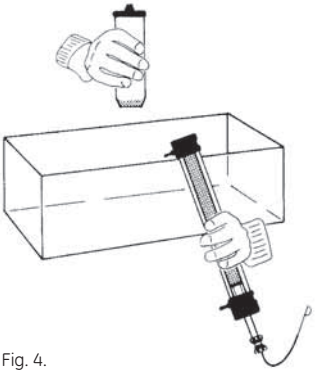


Fig. 4.

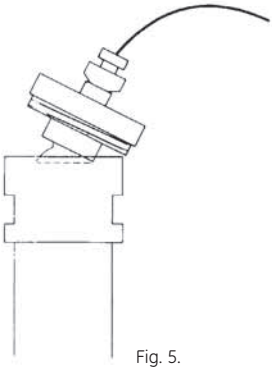


Fig. 5.

14. Run the pump to remove the air from the pump tubing.
15 Remove the stopper from the first adaptor (Fig.6a). If there is an air bubble in the tubing, remove it by opening the upper outlet for a few seconds. Connect the tubing from the first adaptor to the pump or a valve (Fig.6a).
16. Turn the column upside-down (Fig. 6) or use it with upward flow.
17. When running the column, do not exceed the flow rate given for STEP 1 in the table above.
18. Equilibrate the column with two bed volumes of start buffer. A larger volume may be required with detergent solutions.

Provided that the packing instruction was followed, you will now have a column with excellent separation capability. In almost all cases you can use the column directly.

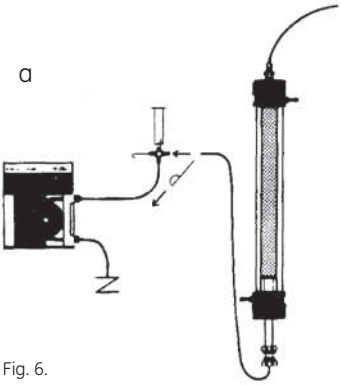


Fig. 6.

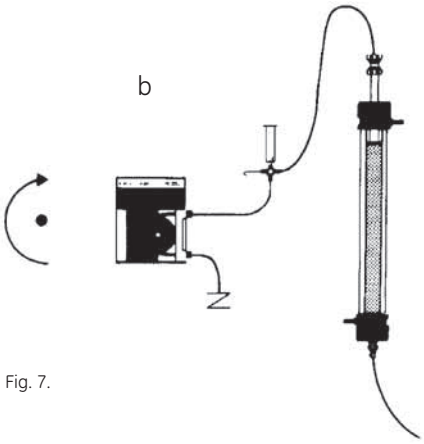


Fig. 7.

4. Further information

To check the quality of the column packing it is recommended to do an efficiency test to determine the theoretical plate number and peak symmetry.

Determination of plate number

1. Prepare sample of acetone 5–10 mg/ml in distilled water or your buffer.
2. Use the test conditions given for the appropriate column in the table below.

Test conditions	Column XK 16	XK 26	XK 50
Sample volume (ml)	200	500	500
Flow rate (ml/h)	60	150	400
Chart speed (cm/h)	30	30	12
Detection (nm)	280	280	280

3. Calculate the plate number (N) according to the formula

$$N=5.54\ (V_e/W_h)^2 \times 1000/L$$

N=Plate number per metre

V_e=Peak elution volume (ml)

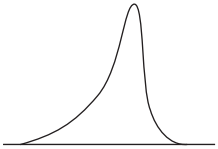
W_h=Peak width at half peak height (ml)

L=Length of column, bed height, (mm)

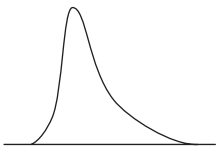
A plate number of 9 000 per meter or more, which corresponds to a reduced plate height of 2.4, is often achieved.

Peak symmetry

For advanced packing the flow rate in STEP 2 can be adjusted depending on the shape of the acetone peak.



When repacking decrease the flow rate in step 2 by 5–20%



When repacking increase the flow rate in step 2 by 5–20%

5. Medium characteristics

	S-100 HR	S-200 HR	S-300 HR	S-400 HR	S-500 HR
Useful fractionation range (MW)					
globular proteins	1×10^3–1×10^5	5×10^3–2.5×10^5	1×10^4–1.5×10^6	2×10^4–8×10^6	
dextrans		1×10^3–8×10^4	2×10^3–4×10^5	1×10^4–2×10^6	4×10^4–2×10^7
DNA exlusion limit (base pairs)		30	118	271	1078
Bead form	Spherical, diameter 25–75 µm in wet form				
Bead structure	Allyl dextran and N,N'-methylene bisacrylamide				
Chemical stability	Stable to all commonly used buffers: 0.2 M NaOH, 0.1 M HCl, 1 M acetic acid, 8 M urea, 6 M guanidine HCl, 1% SDS, 2 M NaCl, 24% ethanol, 30% propanol, 30% acetonitrile (tested at 40°C for 7 days)				
pH stability*					
Long term	3–11				
Short term	2–13				
Physical stability	Negligible volume variation due to changes in pH or ionic streght				
Antimicrobial agent	20% ethanol				
Package sizes	150 ml, 750 ml and 10 l				

* The ranges given are estimates on based on our knowledge and experience. Please note the following:
pH stability, long term, refers to the pH interval where the medium 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 of regeneration, cleaning-in-place and sanitization.

6. Ordering information

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

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