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Bestdex LH-20 Dextran Resin

Instruction for use

Contents

1. Introduction

Bestdex LH-20 is modified with hydroxypropyl on the basis of dextran gel Bestdex G-25.The introduction of hydroxypropyl makes the resin both hydrophilic and lipophilic. Organic solvents can be used as mobile phase. It is suitable for separation and purification of active ingredients in traditional Chinese medicine as well as fine purification of antibiotics and chemical drugs. The resin combines the characteristics of gel filtration, distribution chromatography, and adsorption chromatography to separate molecules with very similar structures.

2.Technical characteristics

Appearance	White to off white spherical powder
Matrix	Dextran gel
Particle size(dry)+	30~120μm
Average particle size(dry)++	75μm
Average particle size(wet)	Large difference in different solutions
Exclusion limit	4~5KD
Max. Linear flow velocity	700cm/h
Max. Pressure	0.3MPa
Chemical stability	Stable in water and most organic solvents. It's unstable below pH2, and it's also unstable to strong oxidants.
pH Stability	2~13
Working temperature	2~40℃
Recommended Linear flow velocity	60cm/h

+Particle size is normally distributed, and particles within this range account for more than 85% of the total.

++ There will be differences in different solutions, this data is the particle size in PBS

List of swelling ratio of Bestdex LH-20 in different solvents:

Solvent	Swelling ratio	Solvent	Swelling ratio
Dimethyl sulfoxide	4.4~4.6	Propanol	3.7~4.0
Pyridine	4.2~4.4	Ethanol	3.6~3.9
Water	4.0~4.4	Isobutanol	3.6~3.9
Dimethylformamide	4.0~4.4	Formamide	3.6~3.9
Methanol	3.9~4.3	Methylene chloride	3.6~3.9
Dichloroethylene	3.8~4.1	Butanol	3.5~3.8
Chloroform	3.8~4.1	Isopropanol	3.3~3.6
Tetrahydrofuran	3.3~3.6	Acetone	2.4~2.6
Acetonitrile	2.2~2.4	/	/

Note：The swelling ratio is the volume(mL)per gram of dry powder after swelling.

3. Method of chromatographic

The resin is provided as a dry powder and must be swollen before use.

3.1 Swelling

- Calculate the required amount of Bestdex LH-20 dry powder according to the volume of the chromatography column.

  Dry powder(g) =(CV×1.15)÷ swelling ratio.
- Pour the resin into the corresponding solvent of 5 times the weight of dry powder and stir it a little, swelling generally takes more than 4h (note that during the swelling process, the use of magnetic stirrer will cause the resin particles to rupture so do not use a magnetic stirrer to stir). After swelling at normal temperature, it is best to degassing under negative pressure.
- After swelling is completed (the swelling at high temperature needs to be cooled to room temperature), remove part of the supernatant to make the volume of the sedimentation gel account for 50% to 75% of the total volume, and mix well for use.

3.2 Column packing

- ✶ **Note: Since Bestdex LH-20 is often used in organic solvents, please make sure that the chromatography column you use can tolerate the corresponding organic solvents. High concentration of organic solvent will cause the laboratory BXK column shell to rupture. During the packing process, be careful not to let the organic solvent contact the laboratory BXK column shell.**
- ✶ **When using dichloromethane or chloroform to pack the column, the resin will float on the surface of the liquid. Use double column head to load columns from bottom to top.**
- Use a liquid consistent with the swelling fluid as the packing buffer.
- Take a cleaned BXK column (BXK series columns with diameters ranging from 1cm to 30cm can satisfy different scale chromatography applications). Purge the bubbles trapped at the end-piece net by draining some packing solution through the column outlet. Leave about 1cm water at the

bottom of the column and close the bottom outlet. Adjust the column so that it is perpendicular to the ground.

- Slowly pour the slurry into the column at one time (use a packing reservoir if necessary). Do not bring any air bubbles into the column.

  Packing reservoir: Empty glasstube with same diameter as the BXK column.

- Fill the remainder of the column with packing solution. Connect the packing reservoir to the chromatography system, open the flow velocity, drain the bubbles in the hose, close the flow velocity, and tighten the top cover of the packing reservoir.

  Note: This operation is only applicable to BXK 50 and below chromatographic columns.

✧ After pouring, stir well again with stirrer, and then wash the resin particles on the inner wall of the column from top to bottom with the packing solution, and let the resin settle naturally until there is about 1cm of clarifying solution on the suspension. Mount the adapter and connect the adapter to the chromatography system or peristaltic pump. Lower the adapter to descend to contact with the clarifying solution and tighten the sealing ring after it is fully immersed in the clarifying solution. With the outlet of the top piece is opened, slowly move the adapter down until all bubbles are drained.

Note: This operation is only applicable to BXK 100 and above columns. Flushing the inner wall reduces the resin particles sticking between the seal ring and the column wall, avoiding the risk of leakage.

- Set the packing flow velocity. Due to the high rigidity of Bestdex LH-20 resin, flow velocity and pressure are positively correlated. Since packing pressure lower than 3bar will hardly damage resin, it is recommended to pack column at highest possible flow.
- Open the column bottom plug and start the peristaltic pump or chromatography system at above-set flow velocity. Make sure internal column pressure is lower than the max pressure of column. Thus, appropriately reduce the flow velocity when pressure is too high during column packing.
- When the resin is fully gravity-settled, keep it for more than 30 minutes, mark the resin surface, stop the pump.
- Remove the packing reservoir (if any), mount the adaptor, lower the adaptor to about 0.5cm above the resin surface, and continue to press the column using the above flow velocity until the bed is completely consolidated, mark the consolidated bed height.
- Stop the pump, open top plug, close the bottom plug, loosen the O-ring seal slightly, press the adaptor to about 0.3cm below the marked position, tighten the O-ring seal, close adaptor stop plug, and complete the column packing.

3.3 Evaluation of Packing

- The packing quality of chromatographic column can be confirmed by column efficiency measurement and evaluation.The tests are required after the column packing, during the column working life and when the separation and purification performance weakens. The method usually relies on the

height equivalent to a theoretical plate(HETP) and the asymmetry factor(As).

- Acetone or NaCl solution can be used as sample for the testing. Sample solution and mobile phase can be prepared according to the following table.

	Acetone method	NaCl method
Sample	1.0%(v/v)acetone in water	0.8M NaCl in water
Sample volume	1.0%CV	1.0%CV
Mobile phase	Water	0.4M NaCl in water
Flow velocity	30cm/h	30cm/h
Monitor	UV280 nm	Conductivity

- Method for measuring HETP and As:

  Use UV curve or the conductivity curve to calculate the height equivalent of theoretical plate (HETP), number of theoretical plates(N) and the asymmetry (As):

  HETP=L/N

  $N=5.54(V_R/W_h)^2$

  Note: V_R = retention volume

  W_h = half-peak width

  L = column height

  N = the number of theoretical plates

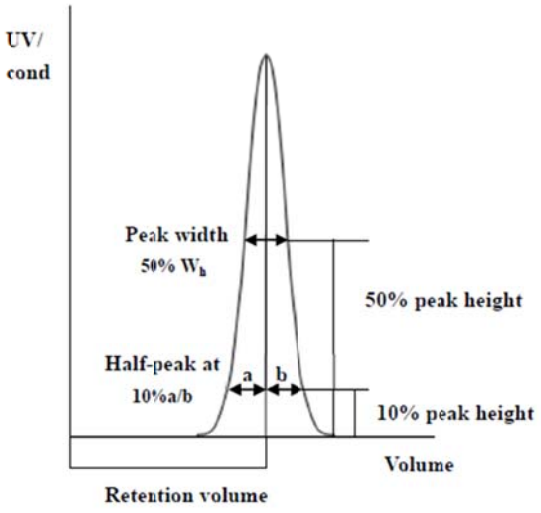
  (The units of V_R and W_h should be the same)

  As=b/a

  Note:

  a= 1st half peak width at 10% of peak height

  b= 2nd half peak width at 10% of peak height



- Evaluation the column packing

  As a guideline, if the value of HETP is more than 3 times the average particle size(d_{50}) of the resin and the As is between 0.8~1.8, the column is very efficient. The unsatisfactory results should be analyzed and the column should be repacked.

3.4 Chromatographic method

- Buffer selection: Bestdex LH-20 can be run in various aqueous or organic phases. The mobile phase is selected according to the nature of the species being separated and the principle of distribution chromatography. A mixed solution of ethyl acetate, methanol, and water is often used. Adjust the ratio of each substance in the mobile phase according to the peak time of the target.
- Flow velocity: Take the column height of 80cm as an example, the generally recommended flow velocity is about 30 ~ 90cm / h, the greater the column height, the slower the flow velocity.
- Sample preparation: In order to prevent blocking of the column, the sample needs to be filtered by microporous membrane of 0.45μm before loading.
- Equilibration: The chromatographic column is washed with the equilibrium buffer solution. The pH and conductivity of the buffer solution to be exported are consistent with that of the buffer

solution before entering the chromatographic column, which means that the chromatographic column is balanced. Generally, 2~5CV are required. If the solvent is changed, note the swelling properties of the medium in the new solvent and adjust the position of the adapter accordingly.

- Sampling: The sample is loaded onto the chromatography column through the loading ring and other devices of the chromatography system. The volume of the loading sample is different according to the molecular size of the object and impurities or the separation method. The loading volume is usually 1~2% of the column volume.
- Rinse: According to the composition of the sample, set the elution flow velocity (1-10cm /h is recommended) for elution, and the smaller the flow velocity, the better the separation effect.
- Regeneration: 2~3CV volumes of the chromatographic column were washed with a balanced buffer solution.
- Rebalancing: After rinsing with equilibration buffer, the second sample can be loaded and repeated.

4.Influencing factors of separation effect

The separation effect of Bestdex LH-20 gel resin is affected by various principles:

- Gel filtration: If there is no interaction between the target and the resin, the separation is based on the principle of molecular sieve. The choice of buffer mainly considers the solubility of the target. The influencing factors of solubility refer to the influencing factors of gel filtration. Factors such as column efficiency, flow velocity and sample loading were considered.
- Distribution chromatography: According to the principle of similar compatibility, the target can be reasonably distributed in the mobile phase and the stationary phase by adjusting the polarity of the mobile phase.
- Adsorption: The small amount of ethoxy group and hydroxypropyl group contained in the cross-linking agent can bind the biomolecule through the hydrophobic effect. The hydroxyl group of the hydroxypropyl group can bind the biomolecule through the action of hydrogen bonding. The above two aspects can be considered and adopt corresponding measures if binding occurs.

5. Cleaning-in-place (CIP)

Bestdex LH-20 series resin may decrease the column efficiency after using for a period of time, and the separation effect becomes poor. The following process can be used for cleaning and regeneration.

- Rinse 2CV with distilled water
- Flush 1CV with 1M NaCl
- Rinse 1CV with 0.2M NaOH
- Rinse 4CV with distilled water

6. Sterilization

The swollen Bestdex LH-20 can be autoclaved at 121℃ for 20 minutes or treated with 0.5M NaOH for 30~60 minutes to reduce the risk of microbial contamination.

7. Storage

The dry powder Bestdex LH-20 is air-tightly stored in a cool and dry place to prevent moisture absorption. The swollen Bestdex LH-20 should be stored in 20% ethanol and sealed at 2-30℃ after use, in order to prevent ethanol volatilization and microbial growth, it is recommended to replace the storage solution every 3 months, and the effect is better when stored at 2-8℃.

8. Disposal and Recycling

Bestdex LH-20 is very difficult to degrade in nature, incineration is recommended to protect the environment.

9. Order information

Product	Code No.	Pack size
Bestdex LH-20	AG121305	25g
	AG121307	100g
	AG121311	500g
	AG121312	1kg
	AG121313	5kg
	AG121316	10kg
	AG121314	25kg
	AG121712	1L