GE Healthcare
Life Sciences

Slurry and Buffer Preparation

Slurry Calculations

The desired packed column volume (V_c) is calculated from the following equation where Ac is the surface area of the column and L is the desired packed bed height in cm.

$V_c = A_c \times L$

The volume of gravity-settled media needed to properly pack the column is then determined by the following equation in which CF is the compression factor for the media.

Required volume of gravity settled media for packing (V_{gs}) = $V_c \times CF$

The value of CF for your particular media can be determined from the data obtained by constructing a pressure flow curve.

Slurry Measurements

If desired, the media can be gravity-settled overnight and storage buffer removed by decanting and replacement with an approximately equal volume of packing buffer. The container is then shaken or stirred with a paddle until the contents are homogenous. A calibrated container such as a 100 ml graduated cylinder should immediately be filled with a representative sample from the media container and allowed to sit undisturbed overnight or until the media has completely settled. The % of media in the slurry (% slurry) can then be read directly from the graduations on the cylinder. Alternatively, a 10 ml calibrated syringe barrel can be fitted with a frit (such as that supplied with the empty PD-10 columns) that will retain the media within the syringe. A calibrated pipette is used to place 10 ml of the homogeneous slurry into the syringe, which is held in an upright position until all liquid has drained off (15-20 min). The % slurry concentration can then be determined by the volume of drained media remaining in the syringe minus the volume occupied by the frit.

$$\frac{\text{Volume of drained gel} - \text{volume of frit}}{10} \times 100 = \text{\% Slurry Concentration}$$

The volume of slurry you will need to remove from the container to give you the desired amount of gravity settled media (V_{gs}) to pack your column is determined by the equation:

$$\frac{V_{gs} \times 100}{\text{\% slurry in container}} = \text{Slurry volume needed from container } (SV_c)$$

The following equation is then used to adjust the slurry to the desired % concentration for packing:

$$\frac{V_{gs} \times 100}{\text{\% slurry in container}} = \text{Slurry volume needed for packing } (SV_p)$$

$SV_p - SV_c$ = Volume of packing buffer to add to SVc before packing

Slurry Handling

The slurry should be mixed by shaking in a closed container, or stirring with a rod or paddle. A magnetic stir bar should never be used, as it will physically damage the media by grinding the beads against the surface of the container. Defining the media is usually not necessary, but can be done by repeatedly settling the media, decanting the buffer and reslurrying the media in the desired packing buffer. Removal of the storage solution or buffer exchange can be done by the same procedure. However, it is faster to pour the slurry into the column, drain off the storage solution or buffer present, and replace it with the desired packing buffer. The slurry should be temperature equilibrated in the packing location if significantly different than that of the preparation room.

Packing Buffer Preparation

Buffers should be prepared in a convenient location using distilled water and filtered if necessary. The buffer should be temperature equilibrated in the packing location before use. Degassing is usually not necessary if made a day in advance. The amount of packing buffer depends on the packing method used. In flow packing or performing a pressure flow curve, the buffer can be recycled through the pump and column minimizing buffer requirements to 3-4 times the packed column volume. Pressure packing does not allow continual recycling and may require 5-6 times the column volume of buffer. For pack-in-place methods, no more then 3 column volumes of buffer are needed to prepare the slurry and fill the column. The suction and axial compression packs require only buffer to prepare the slurry and remove air from the bed supports. Extra buffer will be needed for the pressure vessel if buffer instead of water is used to fill the hydraulic chamber during axial compression packs,

Test Buffer and Sample Preparation

Testing buffer should be prepared using distilled water and filtered if necessary. All test solutions should be temperature equilibrated in the packing location before use. Degassing is usually not necessary. Required volume of testing buffer is dependant on whether preequilibration of the column is to be done, how many tests are to be run, and whether the test buffer is to be recycled. Preequilibration may require as many as 3 packed column volumes of buffer with approximately one column volume needed per test without recycling. The amount of sample prepared must allow initial purging of the pump and hosing between the

sample container and column application valve in addition to the volume to be applied to the column per test. Care should be taken to avoid precipitation of sample components during preparation.

Pressure Flow Curves

Preparing a Pressure Flow Curve

A pressure flow curve using the chosen media, column and hardware should be prepared at approximately the desired bed height with buffer viscosity and temperature conditions chosen for packing. Assuming your system is not hardware limited, the information obtained from this data curve will allow appropriate selection of the optimal flow velocity for flow and suction packing methods, or pressure for constant pressure packing. The compression factor (CF) obtained under these packing conditions is also extracted from this curve, allowing calculation of the amount of media needed to obtain the desired bed height with good accuracy and reproducibility. Information regarding the CF of your media is also critical for hydraulic and suction packs, such that final adapter position based on media volume can be utilized as one goal in a successful pack. With the pack-in-place method where adapter position is fixed, a known CF allows the correct amount of media to be delivered into the column, thus removing an important variable in developing your packing methodology.

A pressure flow curve is performed first using only packing buffer in the column to establish the pressure contribution of the system and hardware. This will be subtracted from the pressure flow curve with the media in the column to obtain the pressure flow curve representative of the packed bed only. The amount of media used is approximated based on the desired bed height and the homogeneous slurry added to the prepared column to gravity-settle overnight. The bed height of the settled media, Lgs, is critical for determining the CF and should be accurately determined and plotted against zero flow velocity. When Lgs > 30 cm, physical compression of the media at the bottom of the gravity-settled bed may produce an artificially low Lgs value which should not be used for CF calculations. In this case, the slurry in the column is adjusted to a known volume, SVp, and the % slurry determined by settling a sample of the slurry in a 100 ml graduated cylinder. The Lgs is then accurately calculated as follows:

$$\frac{SV_p \times 100}{\%\ \text{Slurry} \times A_c} = L_{gs}$$

The media is then reslurried and the adapter bolted in place. Air is purged from the column and system lines and a flow rate of ~50 cm/hr established to settle the media again. When the bed has stabilized, the flow rate, pressure and bed height is recorded. The flow (or pressure) is then increased in increments appropriate to the media type, and flow rate, pressure and bed height recorded after bed stabilization at each step until the pressure reaches a limiting value. If the limitation is not due to media stability, the optimal flow rate and CF for packing will not be achievable by your system. However, the information from a pressure flow curve is still quite useful for appraising the capabilities of your system, making media calculations, and establishing expectations.

Interpreting a Pressure Flow Curve

To remove hardware contributions and obtain the pressure drop across the packed bed, pressure values obtained from a pressure flow curve generated with the "empty" column should first be subtracted from pressure values recorded with the media-filled column. Flow rates are then plotted against pressure with the resulting curve becoming parabolic to the pressure axis at highest flow rates attainable based on media pressure limitations. This provides a guideline for the maximal flow velocity, V_{crit} that should not be exceeded in your packing protocol due to physical deformation of the bead-shaped media. Recommended final step flow packing rates for which a stable, quality pack can be obtained are generally 80-90% of V_{crit}. From the pressure flow curve data, the bed height at the selected packing flow rate can be obtained. The compression factor for use in media calculations is obtained by dividing the bed height of the gravity-settled media by the bed height at the selected packing flow rate.

imagination at work

GE Healthcare Bio-Sciences AB, Björkgatan 30, 751 84 Uppsala, Sweden.

29-0013-51 AB 10/2011