

Constant Flow Packing

- Gel: Q Sepharose Fast Flow
 - Desired bed height $L = 20$ cm
 - $d_{50} = 90$ μm
- Column BPG 200 / 500
 - 23 μm nets ($< 1/3 d_{50}$)
 - I. D. = 20 cm
 - $A_c = 314$ ml/cm
 - $L_{\text{max}} = 38$ cm
 - $V_{c \text{ max}} = 38 \text{ cm} \times 314 \text{ ml/cm} = 11.9\text{L}$

Constant Flow Packing

- Slurry Calculations
 - Desired bed height $L = 20$ cm
 - Compression Factor $CF = 1.15$
 - Packed column volume
$$V_c = 20 \text{ cm} \times 314 \text{ ml/cm} = 6.28 \text{ L}$$
 - Settled gel volume required
$$V_{gs} = V_c \times CF = 6.28 \times 1.15 = 7.22 \text{ L}$$



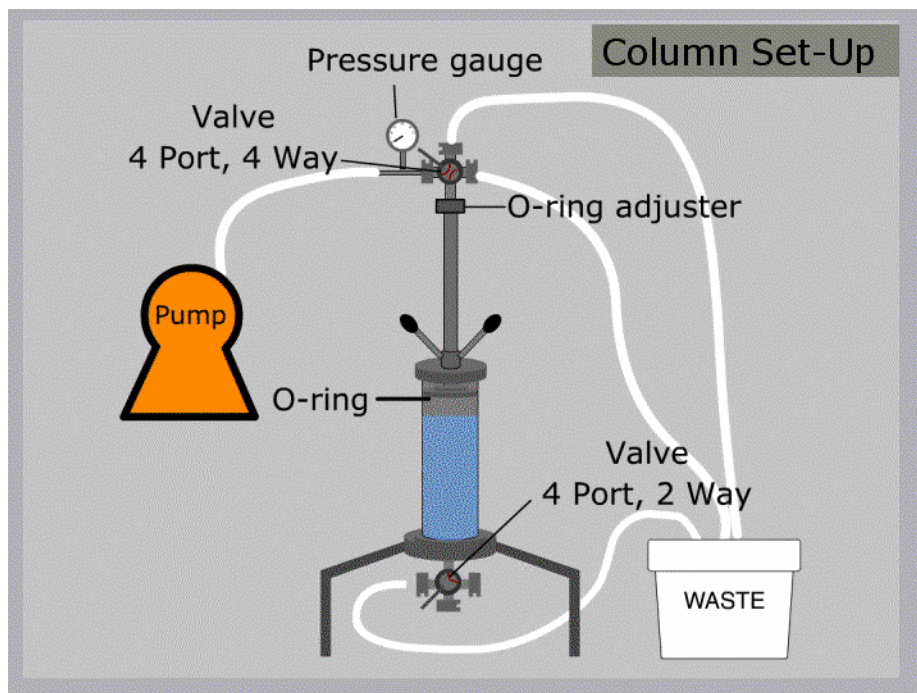
Constant Flow Packing

- Slurry Preparation

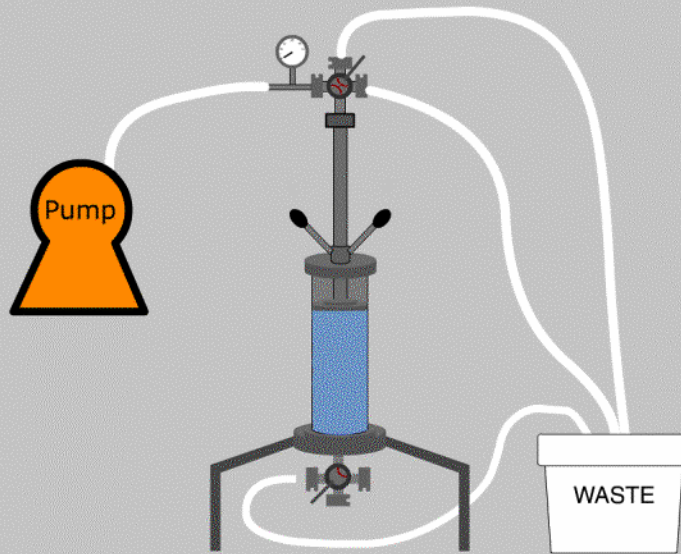
- $V_{c \text{ max}} = 11.9 \text{ L} = \text{Max slurry volume}$
- $V_{\text{gs}} = 7.22 \text{ L}$
- $\text{Slurry \%} = 7.22 / 11.9 = 61\%$
- $\text{Slurry volume} = V_{\text{gs}} / \text{Slurry \%} = 7.22 \text{ L} / 0.61 = 11.9 \text{ L}$

Notes:

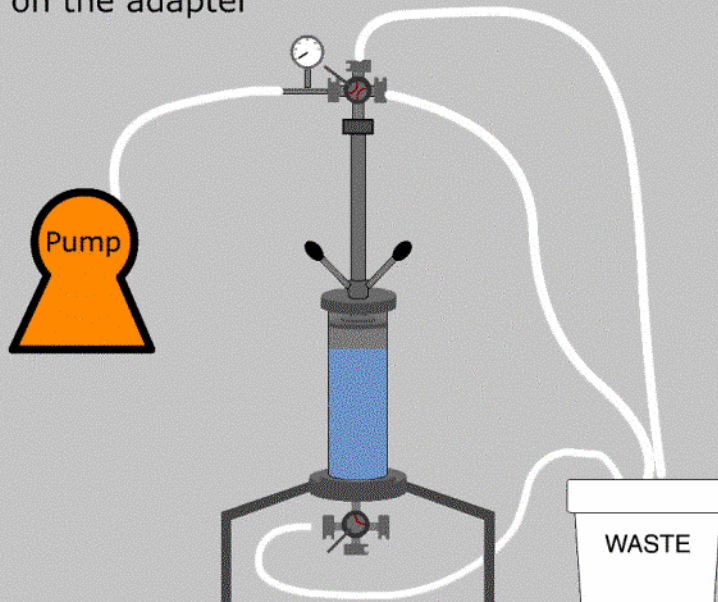
- 1) A packing reservoir or longer column tube would be required to accommodate any slurry % below 61%.
- 2) Q Sepharose FF is prepared as a slurry in dH_2O
- 3) Prepare at least $5 \times V_c \text{ dH}_2\text{O}$ for packing ($5 \times 6.28 \sim 32\text{L}$)



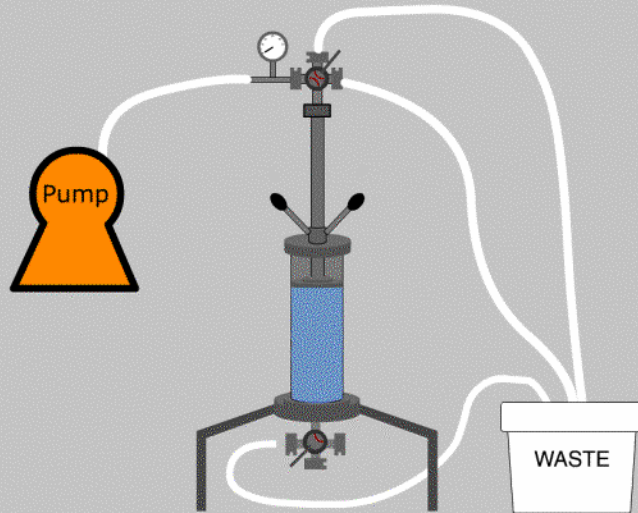
Lower adapter to slurry



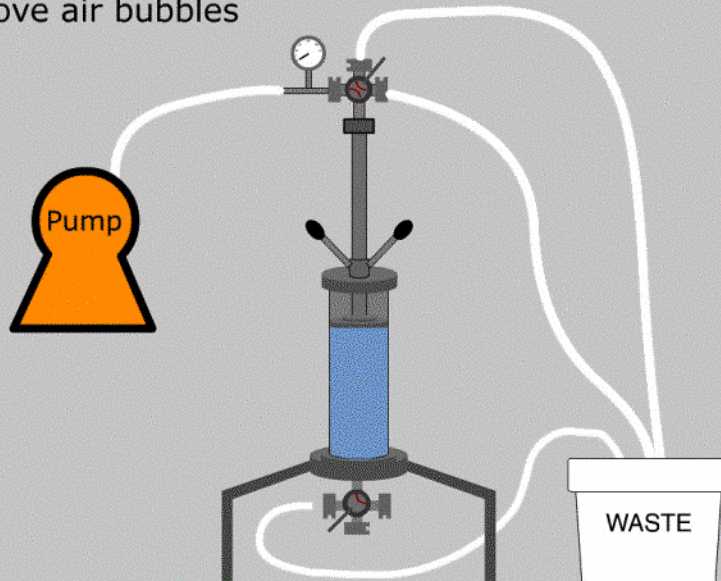
Bolt on the adapter



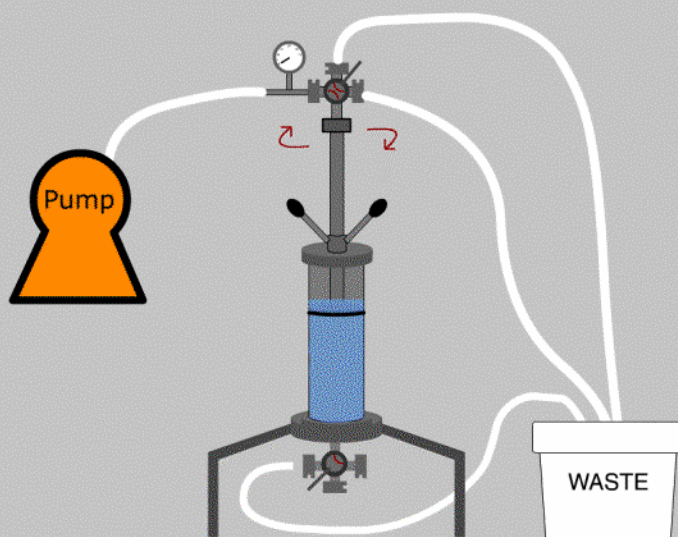
Jiggle the adapter to clear bubbles



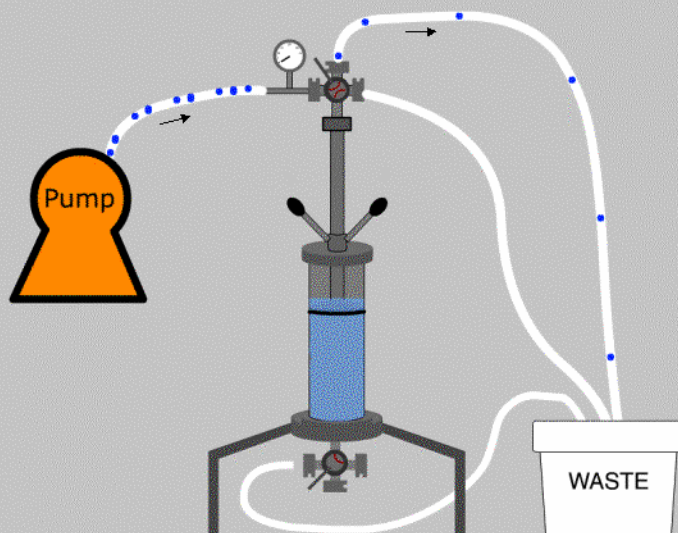
Moving the adapter up and down quickly will help remove air bubbles



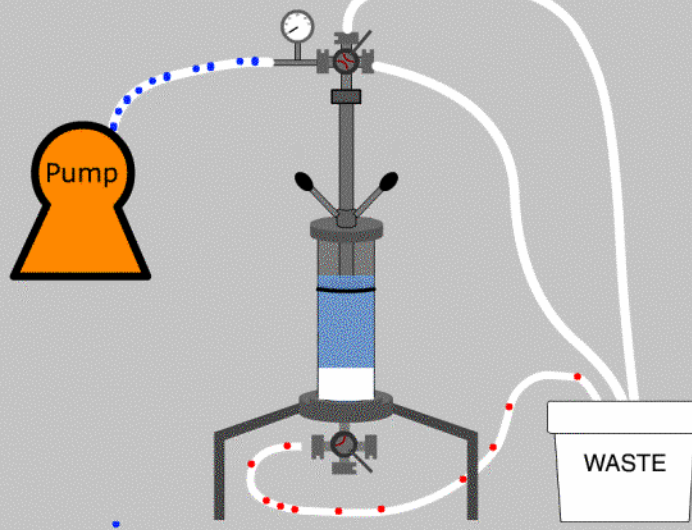
Tighten O-ring



Purge the pump and feed line



Check the flow rate every 2 minutes for the first 5 minutes of the pack. Adjust as necessary.



Constant Flow Packing

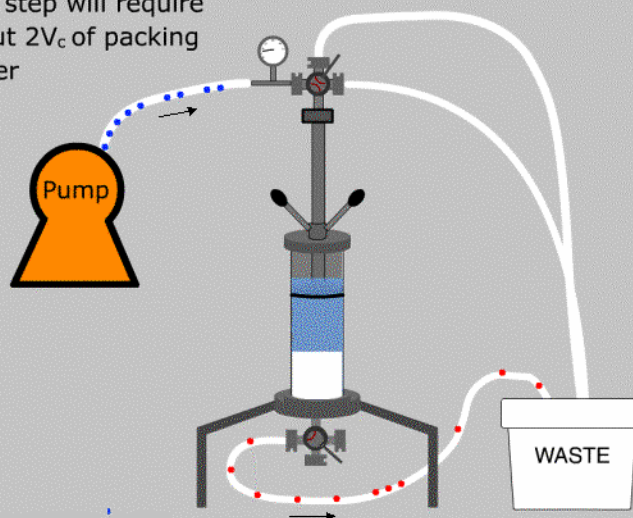
- 2 steps
- Step 1
 - Low flow to form the bed
 - Requires ca. $2 V_c$
 - Continue flow until bed height is stable for 2 to 3 minutes and/or the pressure has stabilized at this low low rate.
 - Lower the adapter to the bed surface

Constant Flow Packing

- 2 steps
- Step 1
- Step 2
 - Higher flow to achieve desired compression
 - Continue flow until bed height is stable for 2 to 3 minutes and/or the pressure has stabilized at this low low rate.
 - Lower the adapter to the bed surface

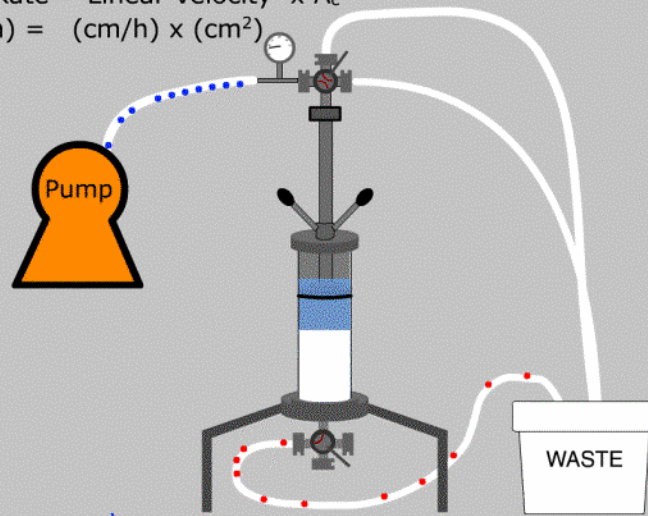
Step 1

This step will require about $2V_c$ of packing buffer



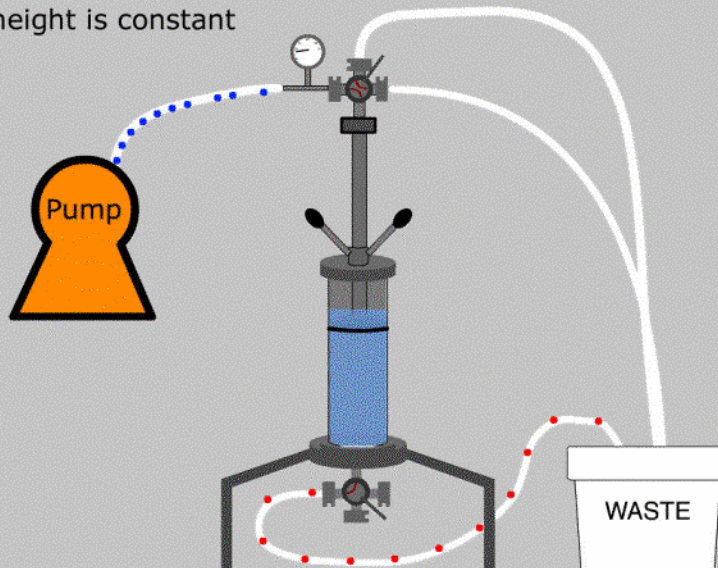
Step 1

$$\text{Flow Rate} = \text{Linear Velocity} \times A_c$$
$$(\text{ml/h}) = (\text{cm/h}) \times (\text{cm}^2)$$



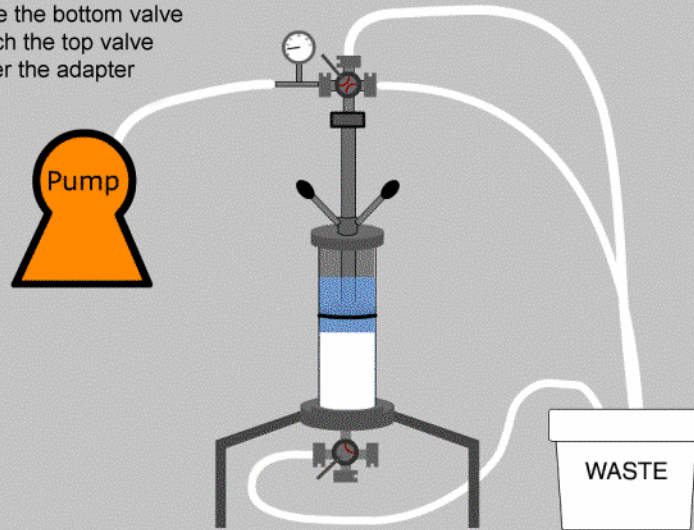
Step 1

Pressure is constant
Bed height is constant

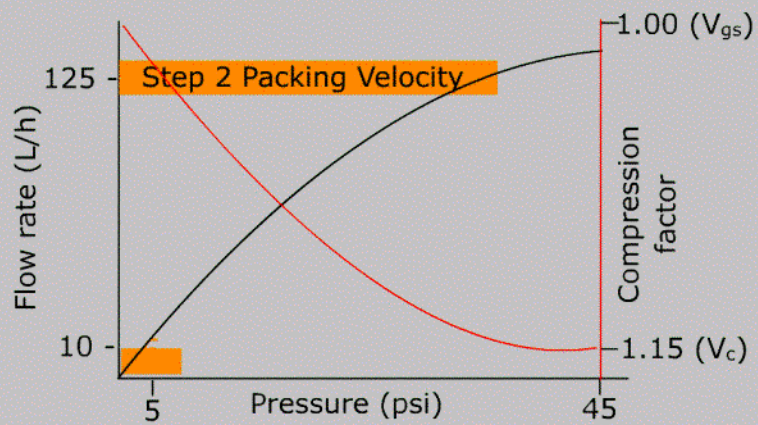


Step 1

Stop the pump
Close the bottom valve
Switch the top valve
Lower the adapter

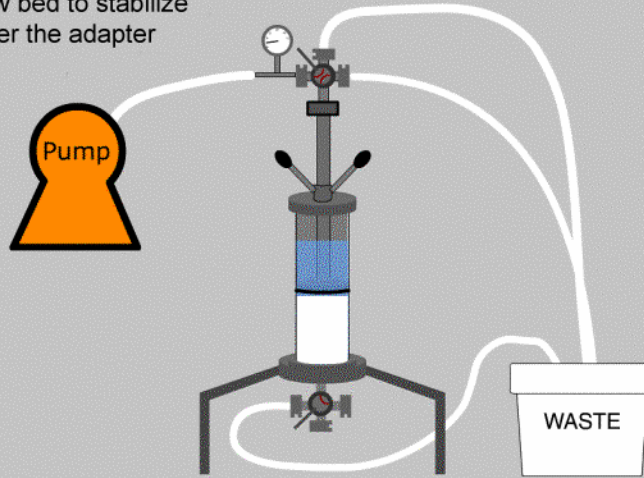


Pressure flow curve Q Sepharose Fast Flow
BPG 200, $L \cong 20$ cm in water at room temp.



Step 2

Restart at higher flow rate
Allow bed to stabilize
Lower the adapter



Setting the adapter must be done quickly

Step 2

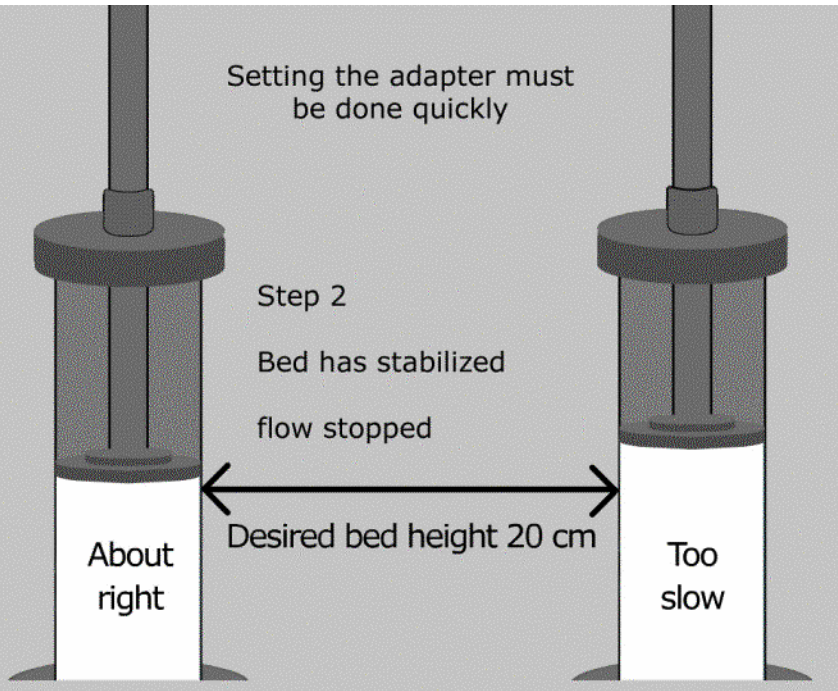
Bed has stabilized

flow stopped

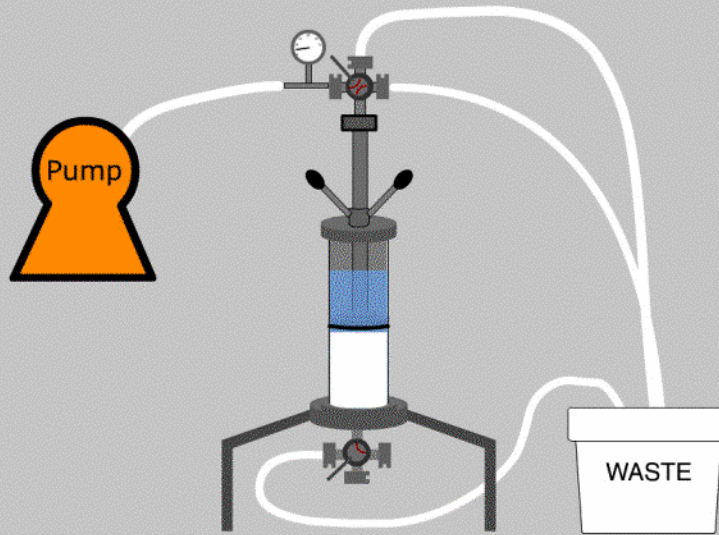
About right

Desired bed height 20 cm

Too slow



Step 3
Repeat Step 2



Q Sepharose FF in a BPG 200 Column

