Preparing G-50 desalting columns

- use sephadex G-50 fine from GE
- The media swells to about 10 times it's weight. Calculate how much volume you will need and weigh media accordingly. In the small Bio-rad columns, the volume should be around the narrowing of the tube, or about 1.9 ml of media.
- Prepare a blocking reagent such as milk or BSA solution in buffer such as HBS (milk is probably better) and add to media in access. Allow to swell - gentle stirring is fine but don't use magnetic stirrers.
- Prepare dye solution (it is better to make fresh, or filter the dye if it is more than a few days old):
    - 1 mg Orange G
    - 10 mg Blue Dextran
    - Add 1 ml HBS (or the buffer the media is in)
    - filter through spin filters (not spin concentrators!)
- Pack the column and wash blocking reagent with buffer (HBS - in my experience it is better than water because water can cause streakiness).
- Calibrate volume by addition of dye solution:
    - Apply 100 µl of dye solution
    - Apply 1 ml of buffer (if the blue has not escaped the column fully, keep adding buffer in 100 µl increments until all blue is gone and track the total volume used)
    - All of the Blue Dextran should elute while the Orange G should remain in the column.
    - If not all Blue Dextran eluted - remove media
    - If Orange G eluted or was close to elute - add media
- Wash with water
- To store - wash with water supplemented with azide. Cap both sides or use foil for the top side and a cap for the bottom side.