

## Column Cation Separation

Resin Type: (Biorad resin AG 50W-X8 100-200 mesh)

### DO ALL SAMPLES IN BATCH SHEET ORDER

Before beginning the separation, make sure that there are at least 1000 ml of 1.2 N HCl, 500 ml of 3 N HCl, and 1000 ml 6 N HCl mixed. Use the labeled mixing bottles and a clean (acid washed and NANOPURE RINSED) graduated cylinder to mix more if needed.

Drain the columns prior to use. Make sure that the columns have been cleaned! Gather together 4 waste bottles and place them under the columns. Prepare the columns by running through 10 ml of 1.2 N HCl and making sure that there are no air bubbles in the column. Water expands the resin and causes air bubbles, leading to uneven elution. Run the acid through, shutting the valve just as the acid level reaches the top of the resin. Set a clean, yellow reservoir next to each column. These are numbered to correspond with each numbered column.

Place four clean 240 ml beakers on the counter and label them for the **Be fraction** of the first four samples on the batch sheet (**use blue, Be**). Cover them with watch glasses.

Remove the beakers containing the first four samples from hot plate and place a watch glass over each. Place them on the column bases in the column hood. If the weather is humid, the sample may begin to appear moist after several minutes as it absorbs moisture. Add an **orange** label with the sample name and “**Al**” to each of the four beakers now containing the sample; it will be reused to hold the Al fraction. Make these labels small enough that they will fit on a 15 ml test tube. Transfer the existing orange mylar label to the column that will be used for the sample. Record the column IDs on the batch sheet.

Add several drops of 1.2 N HCl to all four samples. The samples should redissolve easily. Swish the sample around the beaker trying to get all of the cake dissolved. Use the pipette to assist in the mixing. Add another few drops of 1.2 if the sample is not fully dissolved.

When the sample is fully dissolved, use a pipette to remove it from the beaker. Place the several drops of acid which contain the sample directly onto the resin (avoid the sides of the column). **DO THIS SLOWLY TO AVOID DISTURBING THE RESIN.** Open the valve long enough that the sample drops are drawn into the resin. Close the valve. Add 1 to 2 ml more ml of 1.2 N HCl to the beaker, swirl, use the pipette to rinse down the walls and transfer this liquid to the column. Run-in as above. Add 2 ml of clean 1.2 acid directly to the column to rinse the column walls and run in. Dispose of the pipette as Be waste and move the beaker with the orange label out of the way behind the column covered with its watch glass. **REPEAT FOR THE OTHER THREE SAMPLES.**

Place a balance under each column and place the waste bottle on each balance. Adjust height of column. **TARE THE BALANCE.** Use the squirt bottle to drip about 5 ml of 1.2 N HCl into each column. Drip the acid down the sides of the column to buffer the acid's impact on the resin. Put the yellow reservoir on the column. Use the squirt bottle

to gently add more acid. After the column is filled with acid, use the large bottle to fill the reservoir to 90 ml of 1.2 N HCl. Open the valve to start flow at one drop per second.

Collect the first 25 g in the waste bottle. Turn off valve. Rinse the tube with 1.2N HCl into bottle then remove the eluent bottle and replace it with the Be fraction beaker. ADJUST COLUMN HEIGHT to prevent splash. Do this for all columns before proceeding.

TARE THE BALANCE. Carefully open valve. Collect the next 60 ml (the Be fraction) in the 240 ml Teflon container with the blue label. Turn off valve when the column has fully drained. Do this for all columns. Set the beaker containing the Be fraction onto the hot plate and replace with the orange labeled beaker. MAKE SURE COLUMN HEIGHT IS ADJUSTED LOW ENOUGH TO PREVENT SPLASH.

Add 60 ml of 3 N HCl to the reservoir, slowly at first so as not to disturb the resin.

Carefully open valve. Collect the 60 ml (the Al fraction) dropwise in the 240 ml Teflon container with the orange label. Turn off valve after the column is empty and put the Teflon container on the hotplate to dry down at a sub boiling temperature (275 setting).

To clean up the columns for next time, add 100 ml of 6N acid to the reservoirs. Turn on valves and run this acid into the waste bottle. Lower the face of the hood when using 6N. Turn off the valve after acid has finished flowing through the column. Dispose of this acid in the large waste container. Add 100 ml DI water to each reservoir, turn on valve and flush to the acid waste bottle. Dispose of this rinse in the sink. Attach a red "clean" label to the column.

Remove the yellow reservoir. Rinse into the waste container with 1% Nitric from the squirt bottle. Rinse well with NANOPURE. Replace in clean box.

REPEAT PROCEDURE FOR THE OTHER SET OF FOUR SAMPLES.

ALWAYS LEAVE THE COLUMNS CLEAN AND MARKED WITH A RED TAPE "C". Leave columns in NANOPURE WATER.