FAST PERFORMANCE LIQUID CHROMATOGRAPHY (FPLC)

Before one attempts to use the FPLC one should carefully read the books supplied by Pharmacia. These are well written book which cover the subjects of ion chromatography, gel filtration and general use of the machine. These books cover everything one needs to know to operate the FPLC. The one thing it does not stress, however, is be constantly cautious when using the FPLC and do not forget to do the little things (i.e. close the flow valve).

After reading these books and familiarizing yourself with the FPLC the best thing to do is just use the machine. It will take practice to do everything correctly, but that is the only way. Good luck and remember don't
forget to do the little things first.

Materials/Reagents

TEN_0 buffer (20mM tris pH 7.4, 0.1mM EDTA)
TEN_{1000} buffer (20mM tris pH 7.4, 0.1mM EDTA, 1M NaCl)

Procedure

1) TEN_0 buffer should be flowing into pump A and TEN_{1000} buffer into pump B.
2) The fraction collector should be filled with the desired tubes and the valve/outlet adjusted to the proper height. The fraction collector should be positioned so that the tube is directly over the first collection tube.
3) The loading column should be unscrewed from the first valve, top should be washed thoroughly, and the plunger pushed down.
4) Add the sample to be separated into the loading column and put on the top again.
5) Screw in the bottom of the loading column back to the original position (position 6 on valve 1).
6) Hold the loading column in an upright position and push buffer through the column using valve position 1.2 and a rate of 1-10ml/min. Stop the pump once the sample has been pushed to the top and a tiny bit is pushed through the top outlet tube. (there should be no air in the loading column between the plunger and top of the column.)
7) Invert the loading column, fix the column using a clamp, and screw in the top outlet tube into its original valve position (position 2 on valve 1). (any small bubbles should now be at the plunger, opposite to the outlet tube.)

8) Using a syringe, push water or buffer through position 4 on valve 1 until you see your sample coming out of the waste outlet in position 3 of valve 1. (this is to remove any air bubbles, preventing them from getting into the columns.)
9) Calculate the amount of time it will take for your sample to be loaded based on the volume of your sample in the loading column and the rate at which the FPLC loads your sample for that particular column. For example, the DEAE load rate is 2mL/min. If you have 10mL of sample, this will take 5 minutes to load.
10) In the program for your desired column, change the first non-zero time point (the time point indicating the end of loading your sample on the column) to the time calculated in step 9.
11) Once it has been changed, proceed to make any adjustments to the gradients that you wish. Hit end when everything is done.
12) On the fraction collector, change the fraction size to the volume you wish to collect at. Hit "Frac", then your volumn, and then "store".
13) Finally, on the FPLC, type in the number of the program you wish to run, and hit "do/run".

Troubleshooting/Critical Parameters

Be sure to prevent air bubbles from getting into tubes when connecting the loading column. Always make "wet" connections, pouring buffer into connections before screwing tubes in.
See the manual for "check codes" and other errors.

The DEAE column uses large tube for elution and collection volume of 25mL.
Resource Q uses small tubes for elution with 7mL collection volume.
Mono Q and Mono S uses small tubes with 0.5-1mL collection volumes.

Programming the FPLC

The FPLC has several parameters you can adjust: Concentration of each pump, flow rate (ml/min), UV chart recorder rate (cm/min, currently not in use), port set, and valve position. In manual mode, you can set these parameters for a one step procedure. When programming, you set these parameters for each step of your program, as measured by time.

Using manual:

1) Hit "manual".

2) Type in desired concentration of the "B" pump and hit "do/run". If the desired valve is the default, nothing has to be done.
3) Hit "step forward" to get to the next parameter. Type in the desired flow rate and hit "do/run".
4) Hit "step forward" or "step backward" to set each desired parameter by typing in the value followed by the "do/run" button.
5) The FPLC will begin executing your parameter as soon as you hit the "do/run" button. To stop manual control, hit the "end" button.

Programming:

1) Hit "method file".
2) Hit "step forward" until you see "program method 0".
3) Type in the number of program you want to program or modify and hit "do/run".
4) The first number in the program is the time point and the second number on the far right hand side is the value of that parameter. First enter the time point for the parameter (0 for the first step) followed by "do/run" and then the value of the parameter followed by "do/run".
5) The next parameter screen should appear. If the time is the same, simply hit "do/run" and enter the value as described before. If a particular parameter is not changed relative to the previous time point, it can be skipped over by first hitting "do/run" to indicate the time point, and then "step forward" or "step backward" to skip ahead to another parameter to be changed.
6) Once all parameters for the particular time point have been made, type in the next time valve for the next step (when the far left "time" number is flashing) and hit "do/run".
7) Enter in the parameters for the time point as described previously.
8) When finished, hit "end".
9) To make a buffer gradient, in the program, for the beginning of the gradient, indicate the starting percentage of pump B at the desired time point. Then, at the gradient's end time point, indicate the final pump B percentage. The FPLC will automatically make a linear gradient of pump A and B when you have two different percentage values for pump B at two different time points.

Changing a program:

1) To change a value of a step (such as the time or flow rate), hit "method file" and then step forward to "program method". Enter the number of the program you want to modify, and hit "do/run".
2) Hit "step forward" to get to the step you want to modify.
3) Hit "change".

4) The time number will flash. Enter in a new time. If it is the same, just hit "do/run". (note, if you change the time of the first parameter for a time point, it will change the time for all the parameters in the same step/time point. It will also keep the relative amounts of time between that time point and all subsequent time points.)
5) Next, the value of the parameter will flash. Enter in a new value and hit "do/run".
6) To add a step, simply go to the part of the program into which you want to insert a step, hit "insert" and type in the time and value.
7) To delete a step, go to the step to be deleted and hit "delete".

Reference:

The FPLC manual by Pharmacia.

Submitted by:

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