APPLICATION BRIEF 22005

Using CX-1 pH gradient buffers for developing salt gradient methods

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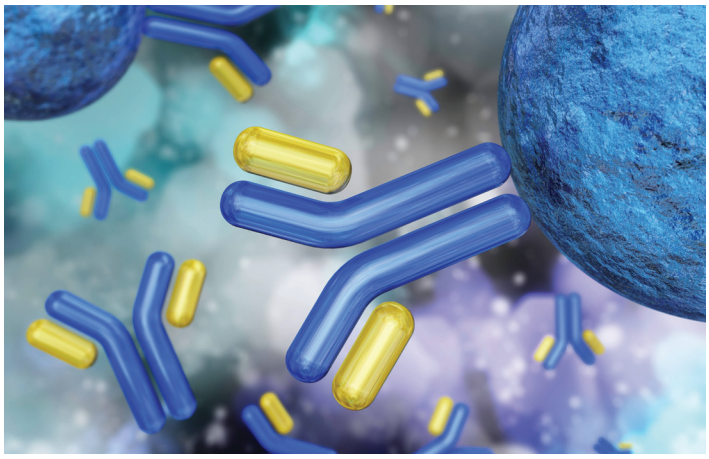
Introduction

The traditional method for ion exchange chromatography uses an increasing gradient of salt at a fixed pH. This is easy to understand and implement for routine work. However, development can be very tedious because the optimum pH is not known in advance, and it takes repeated buffer preparations to develop an optimized method. The process is very similar for hydrophobic interaction chromatography (HIC) except that the salt gradient decreases.

Thermo Scientific™ CX-1 pH gradient buffers can be used in conjunction with a quaternary LC system such as the Thermo Scientific™ Vanquish™ Flex UHPLC system to greatly simplify the task. The CX-1 pH buffer system is unusually resistant to unwanted shifts in pH during the salt gradient. It is easy to control the pH in the range of 5.6 to 10.2 by adjusting the ratio of buffer A to buffer B. While the CX-1 pH buffer system was originally designed for pH gradient separations, it can be creatively applied to other method development situations where convenient and robust pH control is desired. Once optimum pH and salt gradient conditions are known, a simplified binary gradient can be designed for routine applications.



Important notes

- The pH range of the CX-1 pH buffer system is 5.6–10.2. It is possible to exceed the safe operating range of silica-based columns such as the Thermo Scientific™ MAbPac™ HIC-10 or Thermo Scientific™ MAbPac™ HIC-20 HPLC columns.
- The optional pH and conductivity detector will assist in optimizing the elution conditions. The actual pH and conductivity will vary from the nominal values during the gradient and can be affected by the column chemistry. This information is useful for making robust methods.
- If you choose perchlorate salts for Thermo Scientific™ DNAPac™ PA200, be aware that the pH sensor is not compatible with perchlorate salts which can precipitate in the sensor.

Materials required

- Mobile phase:
  - A: Deionized water
  - B: Salt solution. The type and concentration will depend on the type of column used. See Table 1.
  - C: CX-1 pH buffer A, undiluted (pH 5.6)
  - D: CX-1 pH buffer B, undiluted (pH 10.2)
- Vanquish Flex Quaternary UHPLC system (other quaternary LC systems can be used)
- Thermo Scientific™ UltiMate™ 3000 VWD-3000 with pH-conductivity detection module PCM-3000 (optional but recommended)
- Ion exchange or HIC column, any of the following:
  - Thermo Scientific™ ProPac™ Elite WCX column, 4 x 150 mm, 5 µm (P/N 302972)
  - Thermo Scientific™ MAbPac SCX-10 column, 4 x 150 mm, 5 µm (P/N 085198)
  - Thermo Scientific™ ProPac SAX-10 column, 4 x 250 mm, 10 µm (P/N 054997)
  - MAbPac HIC-10 column, 4.6 x 100 mm, 5 µm (P/N 088480)
  - MAbPac HIC-20 column, 4.6 x 100 mm, 5 µm (P/N 088553)
  - MAbPac HIC-Butyl column, 4.6 x 100 mm, 5 µm (P/N 088558)
  - DNAPac PA200 column, 4 x 250 mm, 8 µm (P/N 063000)
- Protein or oligonucleotide sample to be analyzed
- Thermo Scientific™ Chromeleon™ Chromatography Data System, version 7.0 or equivalent

Table 1.

Column	Mobile phase B	Initial %B	Final %B	Gradient time
ProPac Elite WCX, 4 x 150 mm, 5 µm	1.0M NaCl	0	20	15
MAbPac SCX-10, 4 x 150 mm, 5 µm	1.0M NaCl	0	20	15
ProPac SAX-10, 4 x 250 mm, 10 µm	1.0M NaCl	2	50	25
MAbPac HIC-Butyl, 4.6 x 100 mm, 5 µm	3.0M NaCl	90	0	20
DNAPac PA200, 4 x 250 mm, 8 µm	1.0M $NaClO_4$	2	50	25
DNAPac PA200, 4 x 250 mm, 8 µm	3.0M NaCl	2	50	25
MAbPac HIC-10, 4.6 x 100 mm, 5 µm	2.0M Na_2SO_4	75	0	20
MAbPac HIC-20, 4.6x100 mm, 5 µm	2.0M Na_2SO_4	75	0	20

Table 2.

Program no.	% Eluent C	% Eluent D	Nominal pH
I	10	0	5.60
II	9	1	6.06
III	8	2	6.52
IV	7	3	6.98
V	6	4	7.44
VI	5	5	7.90
VII	4	6	8.36
VIII	3	7	8.82
IX	2	8	9.28
X	1	9	9.74
XI	0	10	10.20

Protocol

1. Select the column appropriate to the analysis.
2. Select the salt for mobile phase B from Table 1 (Note: There are two options for DNAPac PA200). Prepare the mobile phase and filter it.
3. Select the pH range for the survey from Table 2. Take into account the pH limits for the column.
4. Write a series of instrument method programs, one for each pH step.
    1. Mobile phases A and B form the salt gradient. Use the initial %B, final %B, and gradient time from Table 1. This gradient will be the same for the entire series of programs. %A and %B always add up to 90%.
    2. Mobile phases C and D control the pH. Use %C and %D from Table 2. These remain constant for the entire program. Each program has a different %C and %D, but they always add up to 10%.
5. Write the sequence of injections. For each pH step, make two blanks followed by two samples. The first blank is to equilibrate the column and system after the pH shift; the second blank is for diagnostics and optionally for baseline subtraction.
6. Install the mobile phases on the LC system. Make sure that any incompatible solvents have been flushed out of the system with water before installing the high-salt mobile phase B. Install the CX-1 pH buffers without any dilution; buffer A is mobile phase C, and buffer B is mobile phase D.
7. Run the sequence.

Related products

Description	Part number
CX-1 pH Gradient Buffer Kit; 125 mL Buffer A + 125 mL Buffer B	083274

Current versions of product instructions are available at **thermofisher.com/chromexpert**

Find out more at **thermofisher.com/phgradientbuffer**

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