Buffers and Conditions for Use and Maintenance of CHT™ Ceramic Hydroxyapatite

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Abstract

Most previous studies have focused on loss of calcium as the primary mechanism for degradation of CHT. Two lines of evidence suggest that the loss of phosphate may be equally important:

1. Phosphate stabilizes CHT in the presence of buffers that otherwise are independently known to degrade CHT, such as Tris and MES.
2. A conductivity aberration occurs in a phosphate gradient when CHT is previously equilibrated in a buffer from which phosphate is absent.

Comparison of the phosphate gradient abnormality against a gradient of sodium chloride shows that the effect is real and that CHT is absorbing phosphate from the early part of the phosphate gradient. This in turn suggests that non-phosphate buffers dissolve phosphate from the CHT itself. This is apparently not a function of the buffers themselves but simply from the absence of phosphate. This further suggests that inclusion of phosphate in the equilibration buffer should prevent this loss, with the expected result that the phosphate conductivity trace is linear from the beginning. We confirmed this expectation, and as a result strongly recommend that at least 5 mM phosphate be included not only in all CHT buffers, but also in large volume protein samples applied to CHT. It likewise indicates that water should not be used in the cleaning and maintenance of CHT. If a low ionic strength cleaning agent is to be used, then it should contain 5 mM phosphate at a pH not less than 6.5.

Materials and Methods

- **CHT column stability study.** 1 ml column, 600 cm/hr, 0.6 g CHT packed 10 CV buffer A, 24 CV linear gradient to 100% buffer B (0.24 mM PO_4, + buffer A, same pH) 5 CV 1 M NaOH, 1CV 0.5 M PO_4, pH same as buffer A

Cycles

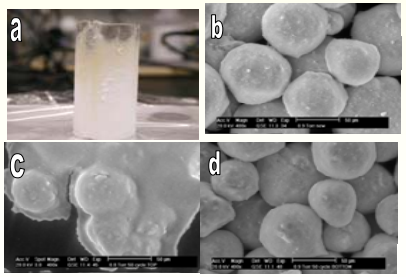
Unpack, wash 3x H_2O, 3x IPA, vacuum dry, 42°C over night

Total weight - tray weight = dry CHT resin weight

- Test buffers and conditions. Buffers: MES, calcium, phosphate and other commonly used CHT buffers). Concentration of phosphate buffer: 0-50 mM. Range of pH: 6.0-8.5. Gradients: sodium chloride, phosphate.

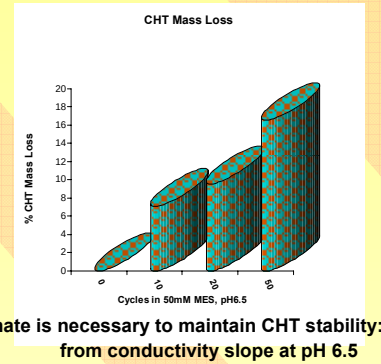
Results

1. CHT column degradation after certain conditions

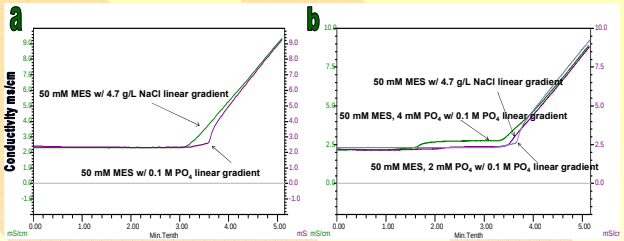


a. Cycled CHT column. Buffer: 50 mM MES, pH 6.5
b. SEM picture for new CHT resins
c. SEM picture for cycled CHT column top.
d. SEM picture for cycled CHT column bottom.

2. Pure buffers degrade CHT: Mass loss vs. cycle numbers

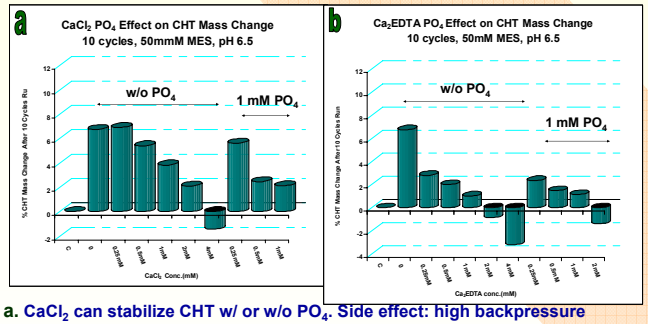


3. Phosphate is necessary to maintain CHT stability: A clue from conductivity slope at pH 6.5



a. Dent formation at conductivity slope in PO_4 gradient for CHT column
b. Dent at conductivity slope in PO_4 gradient for CHT column disappeared after adding 2-4 mM PO_4 in equilibration buffer

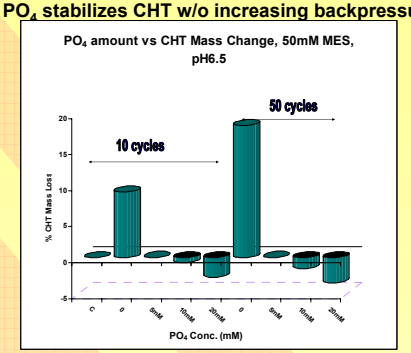
4. Calcium prevents the CHT mass loss, however, with side effect: high backpressure



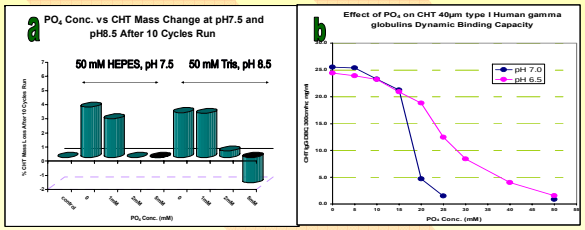
a. $CaCl_2$ can stabilize CHT w/ or w/o PO_4. Side effect: high backpressure

b. Ca_2 EDTA can stabilize CHT w/ or w/o PO_4. Side effect: high backpressure

5. 5 mM PO_4 stabilizes CHT w/o increasing backpressure



6. 5 mM PO_4 or less stabilizes CHT w/o sacrificing IgG binding capacity



a. Less PO_4 needed to stabilize CHT with higher pH buffers
b. 5 mM PO_4 can stabilize CHT without significant reduction of IgG binding capacity on CHT at pH 6.5 (50 mM MES) and pH 7.0 (50 mM HEPES)

7. 5 mM PO_4 prevents CHT mass loss over a wide variety of buffers and pH values

pH	Buffer	10 cycles	50 cycles
5.0	Acetate + 5 mM PO_4	++	
5.5	Acetate + 5 mM PO_4	+	++
6.0	Acetate + 5 mM PO_4	-	+
6.0	Succinate + 5 mM PO_4	+	++
6.5	Succinate + 5 mM PO_4	+/-	-
6.5	Phosphate (5 mM)	-	
6.5	Acetate + 5 mM PO_4	-	-
6.5	MES + 5 mM PO_4	-	-
6.5	Imidazole + 5 mM PO_4	-	+/-
6.5	glycine + 5 mM PO_4	-	-
6.5	arginine + 5 mM PO_4	-	-
6.5	Tris + 5 mM PO_4	-	
7.0	Phosphate (5 mM)	-	-
7.0	MES + 5 mM PO_4	-	

pH	Buffer	10 cycles	50 cycles
7.0	acetate + 5 mM PO_4	-	
7.0	Imidazole + 5 mM PO_4	-	-
7.0	glycine + 5 mM PO_4	-	
7.0	arginine + 5 mM PO_4	-	
7.0	HEPES + 5 mM PO_4	-	
7.0	Tris + 5 mM PO_4	-	
7.5	Phosphate (5 mM)	-	
7.5	MES + 5 mM PO_4	-	
7.5	Imidazole + 5 mM PO_4	-	-
7.5	acetate + 5 mM PO_4	-	
7.5	HEPES + 5 mM PO_4	-	
7.5	Tris + 5 mM PO_4	-	-
8.5	Tris + 5 mM PO_4	-	

Definition of mass loss symbols: "-", no statistically significant mass loss; "+/-", slight (0-1%) loss; "+", small (1-2%) loss; "++", significant (>2%) loss

Conclusions

- It is necessary to add 5 mM PO_4 to CHT buffers and sample solutions to prevent CHT mass loss, especially at pH 6.5 or below
- Higher pH conditions may need less PO_4 to stabilize CHT
- Some chelating agents still harm the CHT column even when 5 mM PO_4 is present (such as acetate or succinate at pH <6.0)
- 5 mM PO_4 does not inhibit IgG (human g-globulin) binding on the column at pH 6.5 or 7
- 10 and 50 cycle studies of CHT mass loss have been done for commonly used CHT buffers w/ 5 mM PO_4

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