Effect of pH on Gradient Elution of Proteins on Two Types of CHT™ Ceramic Hydroxyapatite

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Introduction

Ceramic hydroxyapatite is a rigid chromatography material that has high durability and protein binding capacity. This makes it a good support for both research and process chromatography. Ceramic hydroxyapatite is available in two types, Type I and Type II. Different particle sizes are available for analytical and preparative work.

The two types of CHT ceramic hydroxyapatite, Type I and Type II, have been studied. The difference in retention times between 16 proteins with isoelectric points between 3.9 to 10.6 at pH from 6.0 to 9.0 has been examined.

The purified proteins were loaded and eluted using a linear gradient of sodium phosphate at pH 6, 7, 8, and 9.

The Type I material showed better binding for acidic proteins; however, both types behaved similarly with retention times increasing with the isoelectric point of the molecule, and decreasing with the pH of the buffer.

Materials and Methods

The hydroxyapatite used was CHT ceramic hydroxyapatite Types I and II (Bio-Rad Laboratories, Hercules, CA).

The different proteins were obtained from Sigma Chemical Company. All chemicals used were reagent grade. All buffers were filtered and degassed prior to use.

The hydroxyapatite was packed into a 4.0 x 100 mm stainless steel analytical column. Proteins were individually injected on the column and eluted using a linear gradient from 10 mM to 400 mM sodium phosphate in 30 min at 0.5 ml/min.

Results and Discussion

The results shown in Figures 1 through 3 are more thorough in scope but similar to those observed previously by others. The molarity of the phosphate buffer required to desorb a protein from the hydroxyapatite is dependent on the isoelectric point of the protein and the pH of the buffer. Certain generalizations can be made based on the findings. Proteins with low isoelectric points elute before proteins with high isoelectric points. Independent of isoelectric point, proteins tend to have longer retention times at pH 6.0 than at pH 9.0, as shown in Figure 3.

Even though CHT ceramic hydroxyapatite Types I and II are chemically equivalent, their chromatographic behavior differs. Independent of buffer pH, proteins have longer retention times on Type I than on Type II, as shown in Table 1 and Figure 1.

Table 1. The proteins used in this study, their isoelectric points and the retention times with CHT ceramic hydroxyapatite Types I and II at different pH. Retention times in min.

		pH 6		pH 7		pH 8		pH 9	
Protein	**pI**	**Type I**	**Type II**	**Type I**	**Type II**	**Type I**	**Type II**	**Type I**	**Type II**
Pepsin	3.9	11.46	9.35	9.73	7.74	10.15	7.34	8.32	1.96
α-Lactalbumin	4.5	9.90	4.29	4.24	2.39	2.68	2.14	1.93	1.98
Transferrin	4.7	13.95	12.10	8.83	7.48	2.73	2.34	1.79	1.83
Bovine serum albumin	4.8	14.84	12.03	9.67	8.06	8.43	7.21	7.13	1.83
Ovalbumin	5.0	10.77	9.43	8.33	3.32	5.34	2.12	2.08	1.88
Carbonic anhydrase	5.3	14.70	13.45	8.89	8.16	7.75	7.28	2.87	2.07
Catalase	5.4	10.60	8.22	7.84	7.31	7.84	2.33	2.60	2.35
Conalbumin	6.8	19.70	16.23	12.93	10.86	11.28	9.57	10.03	1.92
Myoglobin	7.0	17.17	13.66	11.59	9.45	9.70	7.92	5.70	2.15
Ribonuclease	9.7	16.71	14.51	12.16	10.89	10.61	9.33	10.19	7.88
α-Chymotrypsinogen A	9.8	19.61	16.42	14.38	12.50	12.57	11.44	11.37	10.98
Lysozyme	10.5	17.27	15.20	12.70	11.25	11.11	9.83	10.40	8.87
Cytochrome c (reduced)	10.6	27.22	24.26	19.90	17.14	17.51	15.79	16.53	16.26
Cytochrome c (oxidized)	10.6	28.58	24.26	20.86	17.89	18.20	16.43	17.09	16.51

Presented by Dr. Tetsuro Ogawa at PrepTech '95, Industrial Separation Science Conference, February 13–15, 1995, East Rutherford, NJ, USA. Reprinted by permission. CHT ceramic hydroxyapatite is the same as the product formerly known as Macro-Prep® ceramic hydroxyapatite. Information in this poster was current as of the date of writing (1995) and not necessarily the date this version (Rev B, 2010) was published.

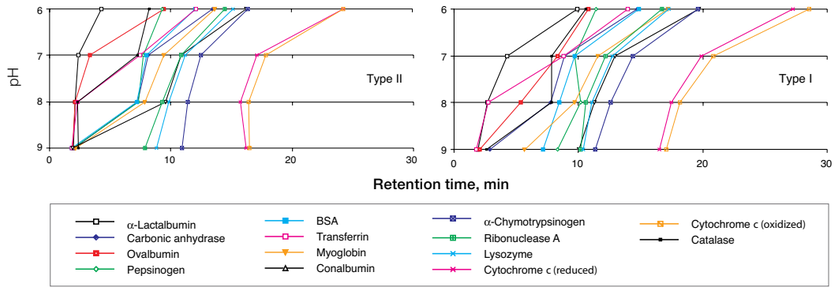

Fig. 1. Changes in retention time with pH of the running buffer. Comparison of CHT ceramic hydroxyapatite Types I and II.

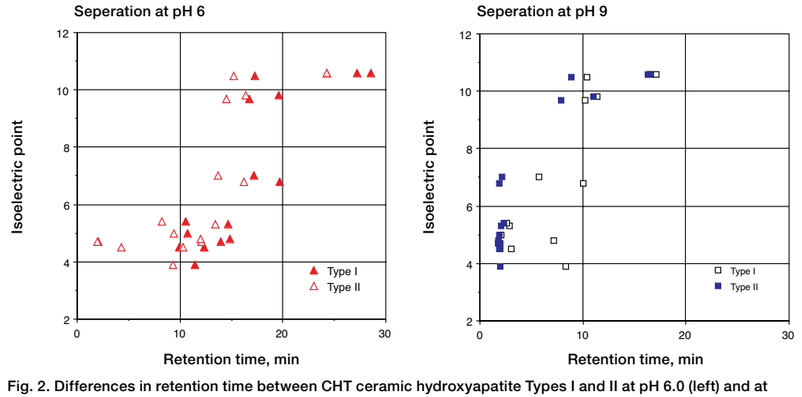

Fig. 2. Differences in retention time between CHT ceramic hydroxyapatite Types I and II at pH 6.0 (left) and at pH 9.0 (right).

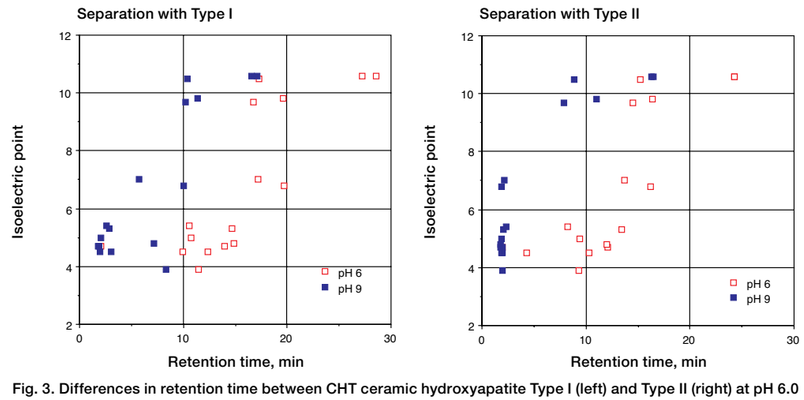

Fig. 3. Differences in retention time between CHT ceramic hydroxyapatite Type I (left) and Type II (right) at pH 6.0 and at pH 9.0.

Summary

This study demonstrates that, through changing the buffer pH and the type of hydroxyapatite used, it is possible to manipulate the binding and retention of different proteins. Specifically, longer retention times, and thus stronger binding, will occur at lower pH using the Type I material.