

# A 3-mechanism model for adsorption of IgG on CHT™ ceramic hydroxyapatite

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## Introduction

Previous work with IgG discussed interactions of protein molecules by a combination of two mechanisms: phosphorylation and hydrophobic interactions. Phosphorylation involves the interaction of negatively charged amino groups on proteins with the negatively charged phosphate groups on CHT, and calcium metal affinity, which involves the protein carboxyl groups and the positively charged calcium groups on CHT. Although the initial binding of the latter mechanism is also electrostatic, it eventually forms much stronger coordination bonds. Hydrophobic interactions occur between metal ions and the imidazole groups of histidine residues. Metal affinity ions such as calcium, as presented in CHT, can act as electrophiles, seeking the possibility of forming electron pairs with other atoms so that a bond or charge-charge interaction can be formed. Such an interaction has been demonstrated between metal ions and the imidazole groups of histidine side chains in a variety of enzymes. Extension of this observation to IgG which carries six histidine residues on its FC region and other histidine residues on its CDR regions is being investigated.

The present study attempts to determine the relative contributions of the three mechanisms: phosphorylation, exchange, carboxyl chelation and calcium-histidine interaction. Results will be presented demonstrating the effect of pH, phosphate and imidazole on purification performance.

## Experimental

**Chemicals**  
 Protein A eluate, Clarified tissue culture fluid obtained from Avicel Bioscience (Tustin, Ca) was applied to a Millipore column (Bio-Rad) and purified by ion exchange chromatography on a Bio-Rad Mono Q column. All chemicals were purchased from VWR (Beverly Hills, Ca). The phosphate buffers were prepared with sodium phosphate monobasic in nanopure water and were titrated to target pHs with 1M NaOH.

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## Acknowledgement

The authors would like to thank Joshua Kellogg for his assistance with analysis and presentation of DOE data, and Valerie McLaughlin for helpful suggestions with preparation of this poster.

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Figure 1: DBC optimum at pH 6.7 to 6.8

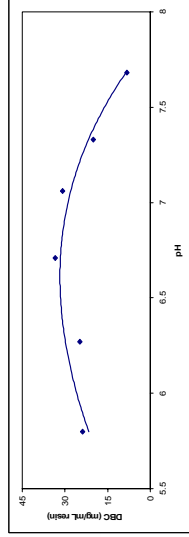


Figure 2: Inverse relation between DBC and PO<sub>4</sub> concentration

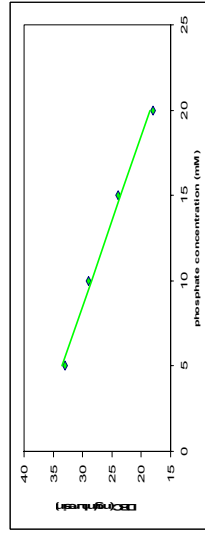


Figure 3: DBC decreases as imidazole concentration increases

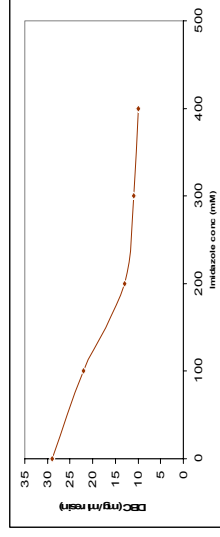


Figure 4: DBC dramatically decreases at 150 mM NaCl

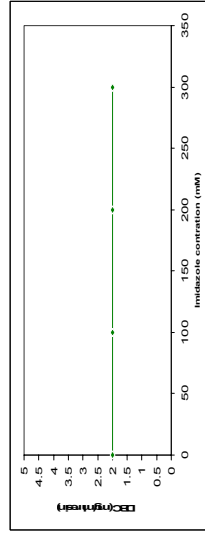
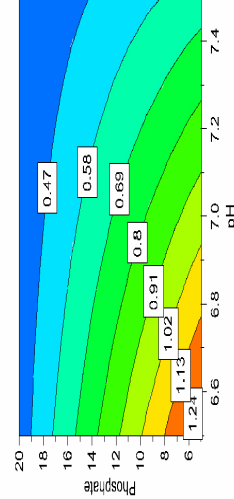


Figure 5: Inverse effect of pH and PO<sub>4</sub> on resolution



## Factorial Design Analysis

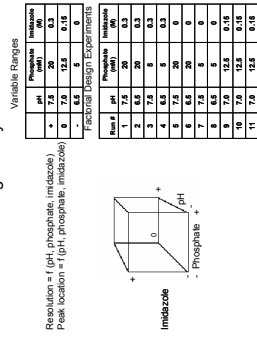


Table 1: Effect of imidazole & NaCl on migration of peak maximum

IgG <sub>1</sub>	% B at peak maximum	Shift index (%)
Control	43	0
0.12M NaCl	34	-21
0.3M imidazole	31	-28
Control	48	0
0.12M NaCl	42	-13
0.3M imidazole	32	-33

Table 2: SFD Results and Conclusions

pH	Resolution	Location of peak maximum
6.5 vs 7.5	Improved at pH 6.5	Higher eluent concentration at pH 6.5
Phosphate 0.005M vs 0.02M	Improved at 5 mM phosphate	Higher eluent concentration at 5 mM phosphate
Imidazole 0M vs 0.3M	No improvement in majority of the cases containing 0.3M imidazole	Higher eluent concentration without imidazole