Fast and efficient screening of binding conditions for cation-exchange chromatography

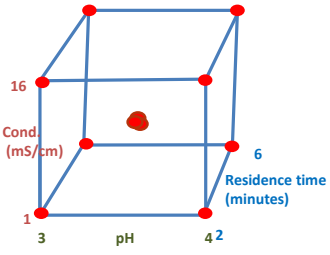
Carina Engstrand and Lars Haneskog; Bio-Works Technologies AB, Uppsala, Sweden

Introduction

Conductivity, pH, residence time and the pI of the protein effects the binding of proteins in ion exchange chromatography. To get the best conditions optimization is necessary. BabyBio™ minicolumns is a new column format, pre-packed with different chromatographic media such as ion exchange media, affinity media and IMAC media, all based on cross-linked agarose. The BabyBio columns are convinient tools for rapid purification of proteins, screening of expression levels or chromatographic conditions. In this study BabyBio S 1ml, packed with the WorkBeads™ S medium, was used for screening of binding conditions for bovine serum albumin (BSA) using Design of Experiments, DoE.

Materials & Methods

Frontal analysis was done using BSA from Sigma (M_r 66 500, pI 4.7) on a BabyBio S 1 ml column. The software Modde v. 10 from Umetrics was used for the DoE setup. The selected factors for the binding conditions were pH, conductivity and residence time. The response metrics was dynamic binding capacity (DBC) at 10% breakthrough. A full factorial design with 3 center points was applied. The design region is shown in Figure 1. The conditions for the 11 experiments are given in the table.



Exp No	Exp Name	Run Order	Incl/Excl	pH	Cond	Res. time	DBC
1	N1	1	Incl	3	1,1	2	20,7
2	N2	9	Incl	4	2,1	2	145,2
3	N3	6	Incl	3	15,5	2	99,3
4	N4	10	Incl	4	15,8	2	132,1
5	N5	3	Incl	3	1,16	6	55,1
6	N6	5	Incl	4	2	6	156,7
7	N7	7	Incl	3	15,4	6	127,8
8	N8	2	Incl	4	16,2	6	149,2
9	N9	8	Incl	3,5	8,9	4	135,4
10	N10	11	Incl	3,5	8,9	4	132,5
11	N11	4	Incl	3,5	8,8	4	138,4

Figure 1: *The design region. The ranges were for pH was 3-4, conductivity 1-16 mS/cm (corresponding to 0-120 mM NaCl), and residence times 2-6 minutes.*

Results & Discussion

The binding capacity of BSA on the BabyBio S 1 ml was high under most conditions in this study. Figure 2 shows the frontal analysis chromatogram for pH 4.0 and low conductivity at 2 minutes residence time. Even at this short residence time the capacity is 145 mg/ml. The model is good as shown in the "Summary of fit" plot (Fig. 3A) and the coeffient plot (Fig. 3B). The contour plot (Fig. 3C) shows the binding capacity at different binding conditions. The highest capacities were found at the highest pH tested, which is opposite to what is generally expected when binding a target substance on a cation exchange column. This non-traditional behavior has been reported earlier and shows the importance of optimization of the binding conditions to establish an efficient purification method. The results may be explained as a pore-exclusion effect, where low conductivity and high net-charge on the protein result in fast and strong binding. This causes the protein to block the pores of the chromatography beads, hindering further binding. The effect is that the binding only occurs on the surface of the beads and the majority of the ligands within the pores are not accessible. The results indicate that the highest pH (4) of the tested range, intermediate conductivity and as long recidence as possible give the highest binding capacity.

Column: *BabyBio S 1 ml*
Medium: *WorkBeads 40 S*
Flow: *0.5 ml/min (2 minutes residence time)*
Sample: *BSA 1 mg/ml in 20 mM Na-citrate, pH 4.0*
Binding buffer: *20 mM Na-citrate, pH 4.0*
Elution buffer: *50 mM Tris, 1 M NaCl, pH 8.0*

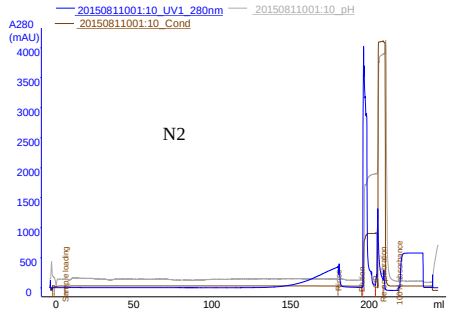
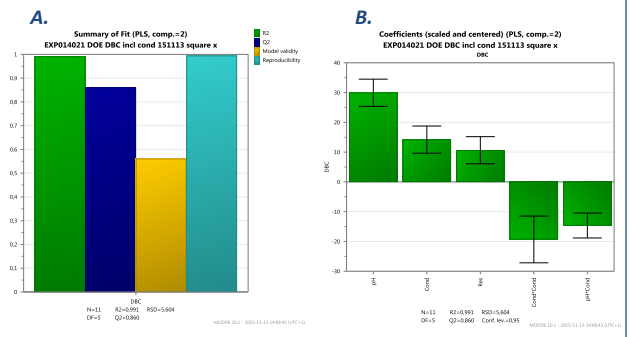


Figure 2: *Example of chromatogram from experiment N2 on BabyBio™ S 1 ml column. The DBC for these conditions was 145 mg/ml.*



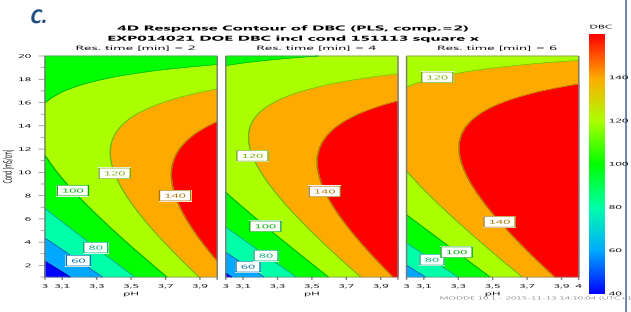


Figure 3: *Evaluation of the study*

Conclusions

BabyBio columns enables fast and efficient screening with small sample consumption

High binding capacity even at short residence times

The highest binding capacities was obtained at pH 4, intermediate conductivity, and long residence time, owing to the pore-exclusion effects at lower pH values.

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