AN060

Preparing small affinity monoliths for antibody purification: Reproducibility of covalent immobilization of recombinant protein A on CIMmic™ CDI-0.1 column

CIMmic™ Monolithic Columns combine the advantages of the CIM® stationary phase with a flexible design and the possibility to operate with syringe. Discs containing the stationary phase can be easily interchanged inside the custom designed housing. Pre-activated chemistries enable immobilisation of numerous ligands and can be used for preparation of affinity chromatographic columns or enzyme reactors. Their small bed volume is particularly suitable for screening purposes and to optimise immobilisation protocols due to economic usage of often expensive ligands.

Carboxyimidazole (CDI) monolithic chromatographic columns are used for covalent immobilisation of proteins, peptides and other amine or thiol containing molecules. The covalent nature of the carbamate bond between the ligand and matrix reduces leaching and improves stability and reusability.
An example described below shows the feasibility of CIMmic™ CDI-0.1 utilisation for covalent immobilisation of recombinant protein A (r-pA). Additionally the example was used for the evaluation of the reproducibility of CIMmic™ CDI columns. The dynamic binding capacity for human polyclonal immunoglobulin (IgG) was used as metric for comparison of the affinity columns.

Immobilisation procedure

The immobilization of r-pA was done by following the manufacturer's protocol (see Further reading). After coupling of affinity ligand (r-pA), the remaining active CDI groups were deactivated via hydrolysis under basic pH conditions.

Recombinant protein A

CIMmic™ CDI activated monolith

Bioaffinity CIMmic™ column with immobilized r-pA (CIMmic™ r-pA column)

Figure 1: Schematic representation of covalent protein A coupling onto CIMmic™ CDI monolith. Carbamate bond between monolith surface and amino group of r-pA establishes strong covalent immobilisation.

Reproducibility of CIMmic™ CDI columns

The reproducibility of CIMmic™ CDI columns was indirectly evaluated by testing CIMmic™ r-pA columns for their polyclonal human IgG dynamic binding capacity (DBC). A representative chromatogram of capacity measurement using CIMmic™ r-pA column is shown on Figure 2 and conditions are provided in the table below. Column saturation was reached after 690 µg of IgG was loaded at 0.5 mL/min (5 column volumes/min) binding flow rate, however using flow rate up to 3 mL/min would result in similar column efficiency. The flat absorbance during loading and steep breakthrough indicate convective mass transport is maintained in the immobilised monolith. Flow through signal corresponds to IgG_3 subclass which does not bind protein A.

Table 1: Chromatographic conditions for IgG DBC testing on CIMmic™ r-pA columns

Column	**CIMmic™ r-pA (r-pA immobilised onto CIMmic™ CDI); bed volume 0.1 mL**
Load	**IgG** (Octagram® 5% (stock conc.: 50 mg/mL), diluted in MPA to final concentration of 0.5 mg/mL. Load until breakthrough.
Flow rate	0.5 mL/min
Mobile phases	MPA: 1.7 mM KH_2PO_4, 5mM Na_2HPO_4, 150 mM NaCl, pH 7.4 MPB: 0.1 M glycine, pH 2.0
Detection	UV at 280 nm
Method	IgG loading (2.5 min); wash with MPA (2 min); elution step to MPB (2 min)
Regeneration/elution	Step gradient to MPB

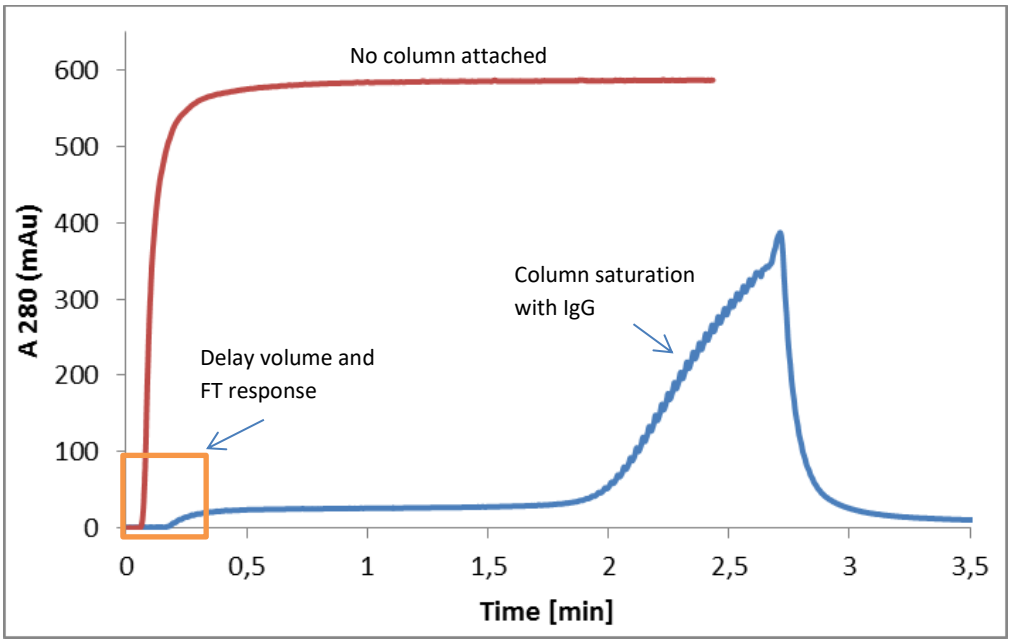

Figure 2: Breakthrough of IgG on CIMmic™ r-pA column. Red - IgG loading without the column attached; blue - dynamic binding capacity of IgG on CIMmic™ r-pA column. Rectangle marks the delay volume when column is attached.

The immobilisation procedure was reproduced 26 times with CIMmic™ CDI columns from three different batches. Relative standard deviation between the DBC values for all columns is less than 6 % (Figure 3). These results demonstrate high inter-batch reproducibility of CIMmic™ CDI columns.

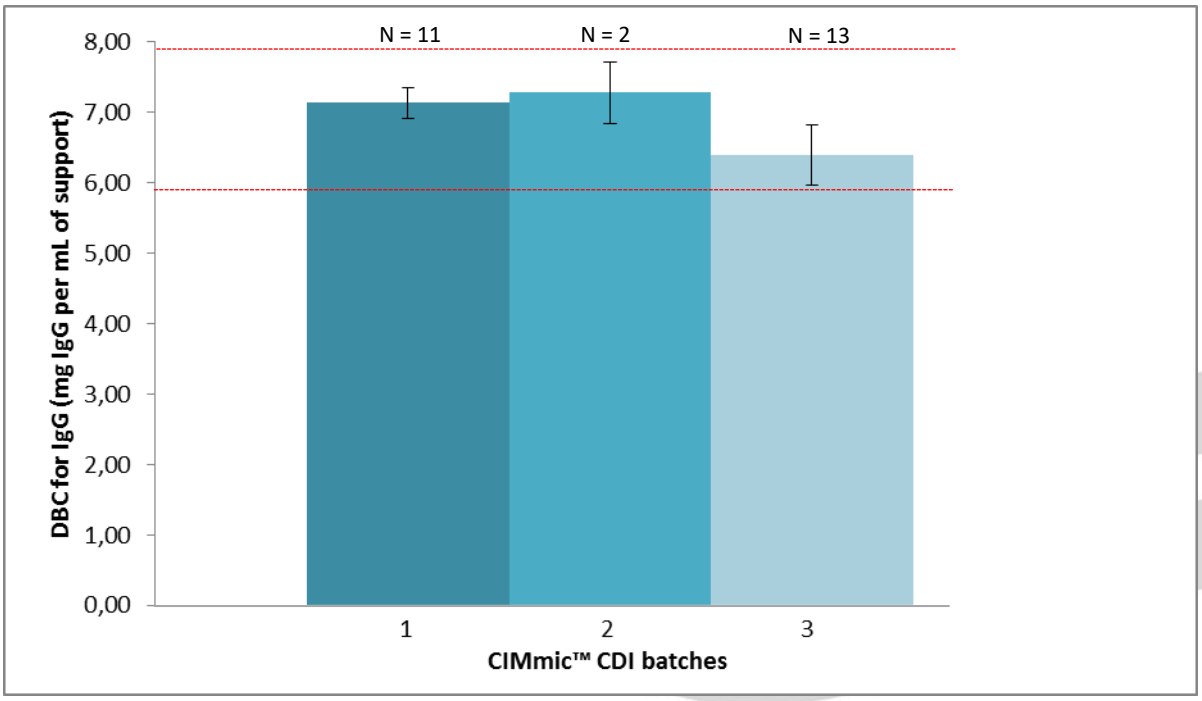

Figure 3: Average values for IgG dynamic binding capacity obtained on three intra-batch CIMmic™ r-pA columns. Number of total CIM™ r-pA columns tested was 26, individual number inter-batch is presented above the column. Error bars represent inter-batch RSD. Red section marks 15 % interval of average DBC value between all three batches.

Conclusions

Carbonyldiimidazole pre-activated CIMmic™ Monolithic Columns (CIMmic™ CDI) are a cost-efficient solution for small-scale screening of immobilisation protocols. Inter-batch reproducibility ensures that immobilisation conditions can be screened and compared over time. It enables coupling of various recombinant proteins, oligonucleotides or organic amines via amino/thiol functional groups and one example is a preparation of recombinant protein A columns. Furthermore, the column can be used for small scale isolation of up to 700 µg IgG from supernatant/plasma samples in a single, very fast chromatographic run.

Further reading

- AN049: Immobilisation of proteins onto CIM® – developing the most efficient affinity chromatographic monolith
- Immobilization procedures for CDI Monolithic Columns
- Jungbauer A., Hahn R., Journal of Chromatography A, 1184 (2008) 62–79
- Černigoj U., Vidic U., Nemec B., Gašperšič J., Vidič J., Lendero Krajnc N., Štrancar A., Podgornik A.,Journal of Chromatography A, 1464 (2016) 72–78

ORDERING INFORMATION

Used product

Catalogue No.	Product description
102.8000-2	CIMmic™ CDI-0.1 (Carbonyldiimidazole) (Pores 2 um)
103.8001-2	CIMmic™ AE-0.1 (Aldehyde) (Pores 2 um)
103.8002-2	CIMmic™ HDZ-0.1 (Hydrazide) (Pores 2 µm)

Services

BIA Separations has a commitment to cater for customer's needs in the field of chromatography and CIM monolithic columns. Beside column production, BIA offers immobilization service. Immobilization of antibodies (Abs) is a challenging task. Let us do the hard work for you. For more information please contact our technical support at help@biaseparations.com.

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