BIA separations

CIM Convective Interaction Media®

TECHNICAL NOTE

TN002 Chemical stability of CIMac™ Analytical Columns

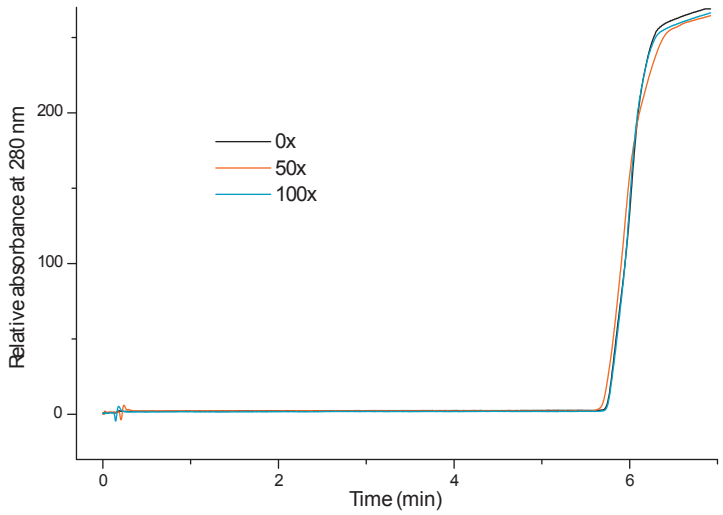
The stability of QA, DEAE and SO3 CIMac™ Analytical Columns was tested according to the CIP (Cleaning In Place) procedures described in the respective Product Specification Sheets (see Table 1). We compared the separation of a mixture of test proteins, the dynamic binding capacity for BSA and the pressure drop after 50 and 100 CIP procedures with the initial characteristics of the columns.

Table 1: CIP procedure for CIMac™ Analytical Columns applied in this study

Reagent	Flow rate (mL/min)	Total number of CVs	Exposure time (min)
1.0 M NaOH	0.5	20	4
Deionized water	1.0	20	2
1.0 M HCl	0.5	20	4
Deionized water	1.0	20	2
Buffer A	1.0	20	2
Buffer B	1.0	20	2
			16

Figure 1 presents breakthrough curves for CIMac™ QA Analytical Column before the first and after 50th and 100th CIP. Similar experiments were also performed for DEAE and SO3 columns and the numerical values of BSA capacities (QA and DEAE) and lysozyme capacities (SO3) are summarized in Table 2.

Figure 1: Breakthrough curves for BSA dynamic binding capacity measurements – CIMac™ QA Analytical Column



Column: CIMac™ QA Analytical Column (5.2 mm I.D. x 5.0 mm);
Product No.: 110.5113
Sample: BSA (0.5 mg/mL) in 20 mM TRIS, pH = 7.4
Flow rate: 1.0 mL/min
Detection: UV at 280 nm
HPLC system: Knauer high pressure gradient HPLC system

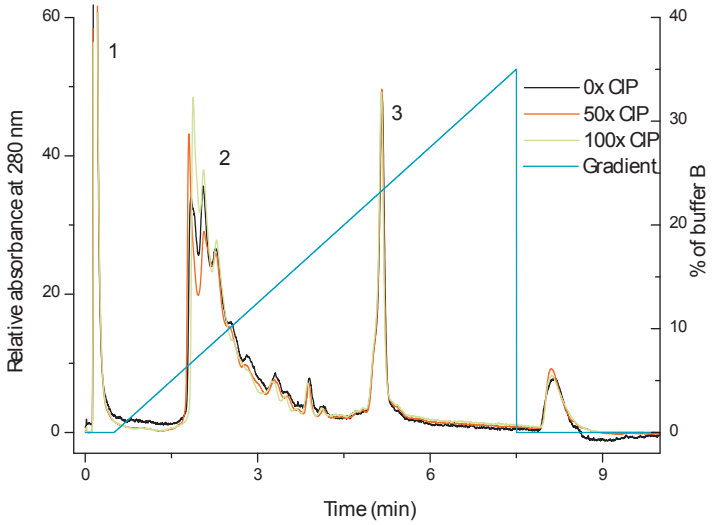
Table 2: Dynamic binding capacities for BSA and lysozyme – QA, DEAE and SO3 CIMac™ Analytical Columns as a function of the number of CIP cycles performed

CIMac™ Analytical Column	Dynamic binding capacity for BSA (QA and DEAE coumn) and for lysozyme (SO3 column) (mg/mL)		
	New column	After 50 CIPs	After 100 CIPs
QA	26.9	27.3	26.5
DEAE	27.3	26.2	26.6
SO3	29.9	28.6	30.3

Columns: CIMac™ QA Analytical Column (5.2 mm I.D. x 5.0 mm); Product No.: 110.5113
CIMac™ DEAE Analytical Column (5.2 mm I.D. x 5.0 mm); Product No.: 110.5114
CIMac™ SO3 Analytical Column (5.2 mm I.D. x 5.0 mm); Product No.: 111.6157
Conditions for QA and DEAE are the same as described in Figure 1.
Conditions for SO3 column are the same as described in Figure 1 except the sample (lysozyme (0.5 mg/mL) in 20 mM phosphate, pH = 7.5)

Separation of proteins on the CIMac™ QA Analytical Column during CIP testing is shown in Figure 2. Similar experiments were also done with DEAE and SO3 columns (chromatograms not shown). Table 3 summarizes the retention times of the last eluted protein from a particular column after 0, 50 and 100 CIP procedures.

Figure 2: Separation of a standard mixture of proteins – CIMac™ QA Analytical Column



Column: CIMac™ QA Analytical Column (5.2 mm I.D. x 5.0 mm); Product No.: 110.5113
Sample: standard mixture of proteins – myoglobin (1), conalbumin (2) and soybean trypsin inhibitor (STI) (3) - in buffer A
Injection volume: 10 µl
Mobile phase A: 20 mM TRIS, pH 7.4
Mobile phase B: 20 mM TRIS + 1.0 M NaCl, pH 7.4
Flow rate: 1.0 mL/min
Gradient: 0 – 35 % buffer B in 3.5 min
Detection: UV at 280 nm
HPLC system: Knauer high pressure gradient HPLC system

Table 3_: Dynamic binding capacities for BSA and lysozyme – QA, DEAE and SO3 CIMac™ Analytical Columns as a function of the number of CIP cycles performed

CIMac™ Analytical Column	Retention time of STI (QA and DEAE column) and of lysozyme (SO3 column) (min)		
	New column	After 50 CIPs	After 100 CIPs
QA	5.16	5.16	5.15
DEAE	3.08	3.08	3.07
SO3	6.06	6.02	6.07

Conditions for QA and DEAE are the same as described in Figure 2, except the gradient for DEAE (0 - 50 % buffer B in 3.5 min)
Conditions for SO3 column:
Sample: standard mixture of proteins – myoglobin, cytochrome C and lysozyme - in buffer A
Injection volume: 10 µl
Mobile phase A: 20 mM phosphate, pH 7.5
Mobile phase B: 20 mM phosphate + 1.0 M NaCl, pH 7.5
Flow rate: 1.0 mL/min
Gradient: 0 – 35 % buffer B in 7 min
Detection: UV at 280 nm
HPLC system: Knauer high pressure gradient HPLC system

The back-pressure was monitored on each tested CIMac™ Analytical Column before and after the CIP testing. The results are shown in Table 4.

Table 4: Monitoring of back-pressure on the QA, DEAE and SO3 CIMac™ Analytical Columns

CIMac™ Analytical Column	Back-pressure (bar)		
	New column	After 50 CIPs	After 100 CIPs
QA	6	7	6
DEAE	7	6	7
SO3	4	4	5

The effects of CIP procedures on the chemical stabilities of the CIMac™ Analytical Columns were evaluated. No relevant deteriorations of protein capacities as well as protein separation after 100 CIP cycles were noticed. There was no significant change in back-pressure on the CIMac™ Analytical Columns after the testing indicating that the columns remain fully intact. Hence, using cleaning and regeneration procedures for CIMac™ Analytical Columns as described in Instruction Manual is fully applicable without any negative consequences on the columns.

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CIM® technology is covered by US patents 4889632, 4923610, 4952349 and 5972218. Other patents pending.

Publication: #TNA0021005