JNC CORPORATION

Super Edge Empty Mini Column

Versatility of empty mini column and performance evaluation of packed column- 2

JNC's empty mini columns can be packed with chromatography media from various manufacturers. In column performance evaluation-1, the quality of packed column was evaluated by theoretical plate number and symmetry factor. In this performance evaluation-2, we will introduce the data on the reliability of the adsorption capacity using the *Super Edge* empty mini column.

1 Compatibility; Comparison of adsorption capacities of commercially available packed columns and columns packed in *Super Edge* empty mini columns

Packed columns are sold by major chromatography resin manufacturers. The resins packed in these commercially available packed columns were purchased in bottles and packed in *Super Edge* empty mini columns, and the adsorption capacities of these were compared. If the comparison data show equivalence, the Super Edge empty mini column is compatible with the commercial packed column. It also shows that the adsorption performance of the resin of each company can be measured correctly.

Packing method
Packing was performed according to the manual using the packing tool enclosed in the starter kit. The manual can be obtained from *Super Edge* website.
https://www.jnc-corp.co.jp/fine/se/index.html

Material
Commercially available packed columns and bottled resins shown below were used.

<<Table1 AEX>>

Manufacturer	Product abbreviation	Bead size µm	Base material
T	DEAE(1)	65	Polymer
	Q(2)	65	Polymer
	Q(3)	75	Polymer
	QAE(4)	100	Polymer
G	DEAE(5)	90	Agarose
	Q(6)	90	Agarose
B	Q(7)	50	Polymer

<<Table2 CEX>>

Manufacturer	Product abbreviation	Bead size μm	Base material
T	CM(8)	75	Polymer
	S(9)	75	Polymer
	SP(10)	75	Polymer
	SP(11)	75	Polymer
G	S(12)	90	Agarose

Chromatography

The dynamic adsorption capacity was measured by performing chromatography under the conditions of Table 3 and 2. A typical chromatogram is shown below. Loading was performed until 10% of the protein concentration was detected, followed by washing and then elution to determine the amount of adsorption and recovery.
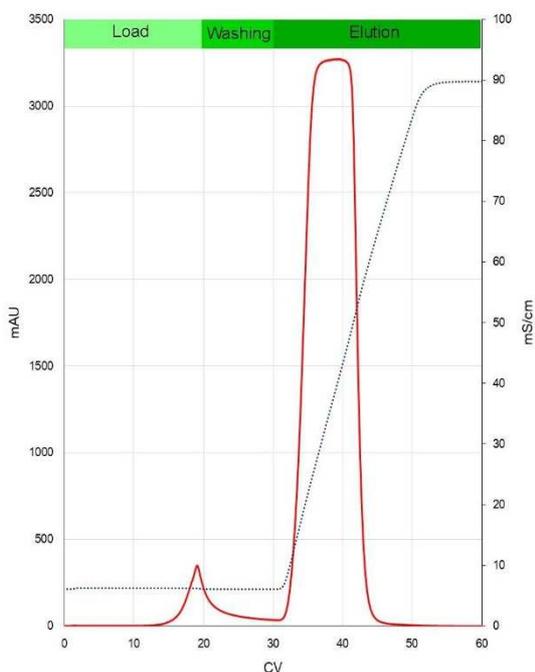
<< Table 3 Chromatography condition >>

Step	CV(column volume) ml
1. Equilibration	5
2. Sample application	Up to 10% break through
3. Column wash	10
4.Elution1（0→100%）	20
5. Elution 2(100%)	10
6. Column wash	10
7. CIP	10 0.1M NaOH
8. Equilibration	20

※Flow velocity: Column residence time 1
1ml column 1ml/ml, 5ml column 5ml/ml

<< Table 4 Protein and buffer condition >>

Mode	Protein	Buffer A	Buffer-B
AEX	BSA 5mg/ml	50mM Tris-HCl, pH8.5	Buffer-A + 1M NaCl
CEX	Lysozyme 5mg/ml	10mM Na-sodium phosphate, pH7.0	Buffer-A + 1M NaCl

Result

Table 5 Comparison of 10% DBC (mg / ml) and recovery rate of *Super Edge* mini column and commercial packed column

	10% DBC			Recovery (%)	
	JNC	Commercial		JNC	Commercial
AEX	BSA				
DEAE(1)	25.2	21.4		105	111
Q(2)	112.7	74.5		98	98
Q(3)	124.8	134.5		100	98
QAE(4)	26.9	19.5		103	118
DEAE(5)	166.2	159.6		101	101
Q(6)	120.2	111.9		97	95
CEX	Lysozyme				
CM(8)	151.9	132.8		101	101
S(9)	155.0	142.9		100	100
SP(10)	78.4	65.6		110	109
※JNC : *Super Edge* mini column,					
※Commercial: Packed column purchased from a resin manufacturer.					

$$y = 0.9671x - 7.4418$$
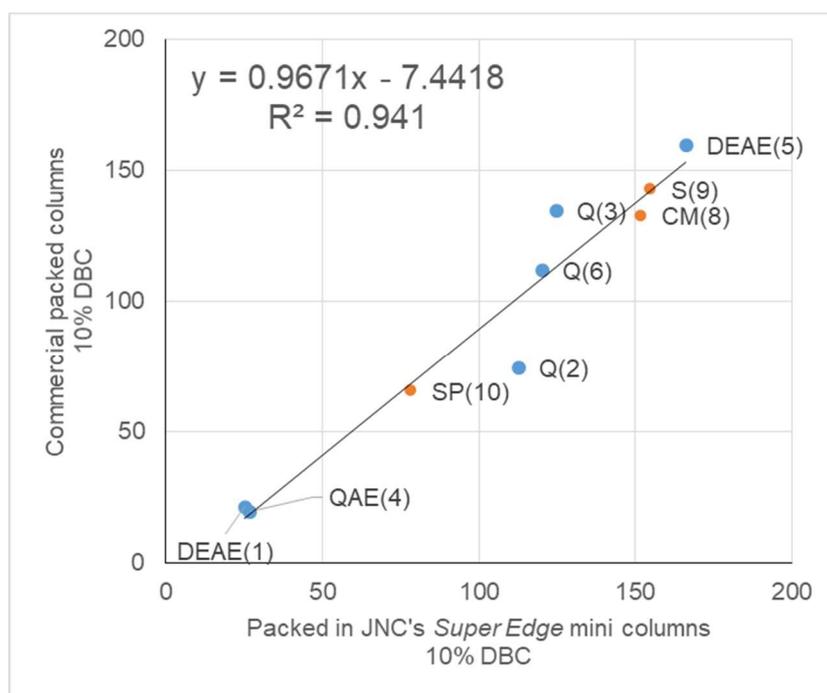$$R^2 = 0.941$$

Fig1. Regression analysis of 10% DBC (mg / ml) of *Super Edge* mini column and commercial column

Comparative data are shown in Table 5 and Fig 1. Q (2) and Q (3) seem to be different in DBC, but the result of regression analysis showed that the intercept was about 1 and the correlation coefficient was close to 1, indicating compatibility.

2   1 ml and 5 ml scalability of *Super Edge* empty mini column

There are two types of JNC's *Super Edge* empty mini columns, 1 ml and 5 ml, and it was investigated whether the adsorption capacity could be measured equally on these scales.

<< Table 6 Column dimension >>

Column volume ml	I.D. mm	Length mm
1	6.7	30
5	14.6	

Material

The resins in Tables 1 and 2 and the following ion exchangers were tested.

<< Table 6 Resin added to comparative experiment >>

Manufacturer	Product abbreviation	Bead size μm	Base material
JNC Cellufine®	A-500	90	Cellulose
	MAX DEAE	90	Cellulose
	MAX Q-h	90	Cellulose
	MAX Q-r	90	Cellulose
	MAX S-h	90	Cellulose
	MAX S-r	90	Cellulose
T	S(13)	100	Polymer
G	S(14)	90	Agarose

Chromatography
The procedure was similar to that described above.

Result

<< Table7 Comparison of DBC with 1ml and 5ml *Super Edge* mini column>>

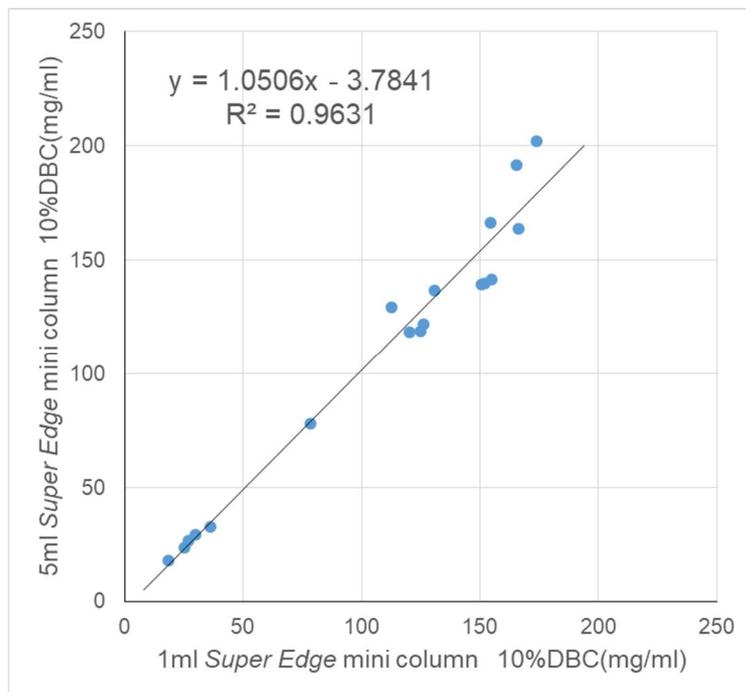|		10%DBC(mg/ml)	
		1ml Column	5ml Column
Cellufine	A-500	18	18
	MAX DEAE	154	166
	MAX Q-h	174	202
	MAX Q-r	131	137
	MAX S-h	165	192
	MAX S-r	126	122
T	DEAE(1)	25	23
	Q(2)	113	129
	Q(3)	125	119
	QAE(4)	27	27
	CM(8)	152	140
	S(9)	155	142
	SP(10)	78	78
	S(13)	30	29
G	DEAE(5)	120	119
	Q(6)	166	164
	S(14)	151	139
B	Q(7)	36	33

Fig2 Regression analysis of DBC on 1ml and 5ml *Super Edge* mini columns

As shown in Table 7 and Figure 2, the *Super Edge* empty mini columns had similar adsorption capacities measured at 1 ml and 5 ml.

3 CIP cycle and stability

We investigated the relationship between the number of times a packed column made with *Super Edge* mini-column was used and its performance. The column was loaded with protein → recovery → CIP → equilibration was repeated, and changes in theoretical plate number, symmetry factor, adsorption amount, and recovery rate were investigated.

Chromatography

Under the conditions of CEX in Table 4, the cycles 1 to 8 of Table 3 were repeated, and the theoretical plate number and symmetry factor As were examined for each cycle.

Column

Cellufine® MAX S-h was packed in 1 ml and 5 ml respectively.

Measurement

Measurements were performed once for each cycle on 3 columns of 1 ml and 5 ml, and the average was calculated from the values of 3 columns.

Result

<< Table 8.　　1ML COLUMN / CIP cycle and column performance >>

Cycle	N/m Average	N/m S.D.	As Average	As S.D.	10%DBC(mg/ml) Average	10%DBC(mg/ml) S.D.	Recovery Average	Recovery S.D.
0	5,738	421	0.93	0.07				
1	5,488	246	0.87	0.01	172.6	4.0	98	0.5
2	5,366	86	0.86	0.06	176.2	4.0	96	0.8
3	5,593	210	0.85	0.05	165.6	3.5	103	1.6
4	5,340	180	0.86	0.02	171.3	2.1	100	0.2
5	5,542	226	0.86	0.02	168.3	3.7	102	0.5
6	5,607	169	0.86	0.01	172.0	4.1	98	0.7
7	5,523	100	0.90	0.01	171.4	4.0	99	0.4
8	5,636	130	0.87	0.02	166.5	4.8	105	0.4
9	5,490	30	0.88	0.03	167.4	3.2	102	0.7
10	5,639	118	0.91	0.05	174.7	3.6	96	0.4

<< Table 9.　　5ML COLUMN / CIP cycle and column performance >>

| Cycle	N/m Average	N/m S.D.	As Average	As S.D.	10%DBC(mg/ml) Average	10%DBC(mg/ml) S.D.	Recovery Average	Recovery S.D.
0	6,849	176	1.22	0.01				
1	6,741	279	1.23	0.04	174.3	1.6	99	1.3
2	6,956	327	1.21	0.02	172.5	2.2	103	2.2
3	7,049	352	1.25	0.02	173.0	1.6	102	3.1
4	6,634	547	1.29	0.04	171.4	1.7	103	1.3
5	7,223	294	1.23	0.02	171.8	1.8	102	1.8
6	7,252	230	1.24	0.02	175.5	1.7	101	1.8
7	7,200	217	1.24	0.03	173.5	1.7	100	0.6
8	7,264	305	1.25	0.04	173.3	1.7	101	1.2
9	7,281	238	1.24	0.01	168.5	1.6	104	0.2
10	7,297	198	1.25	0.03	176.4	1.7	100	1.4
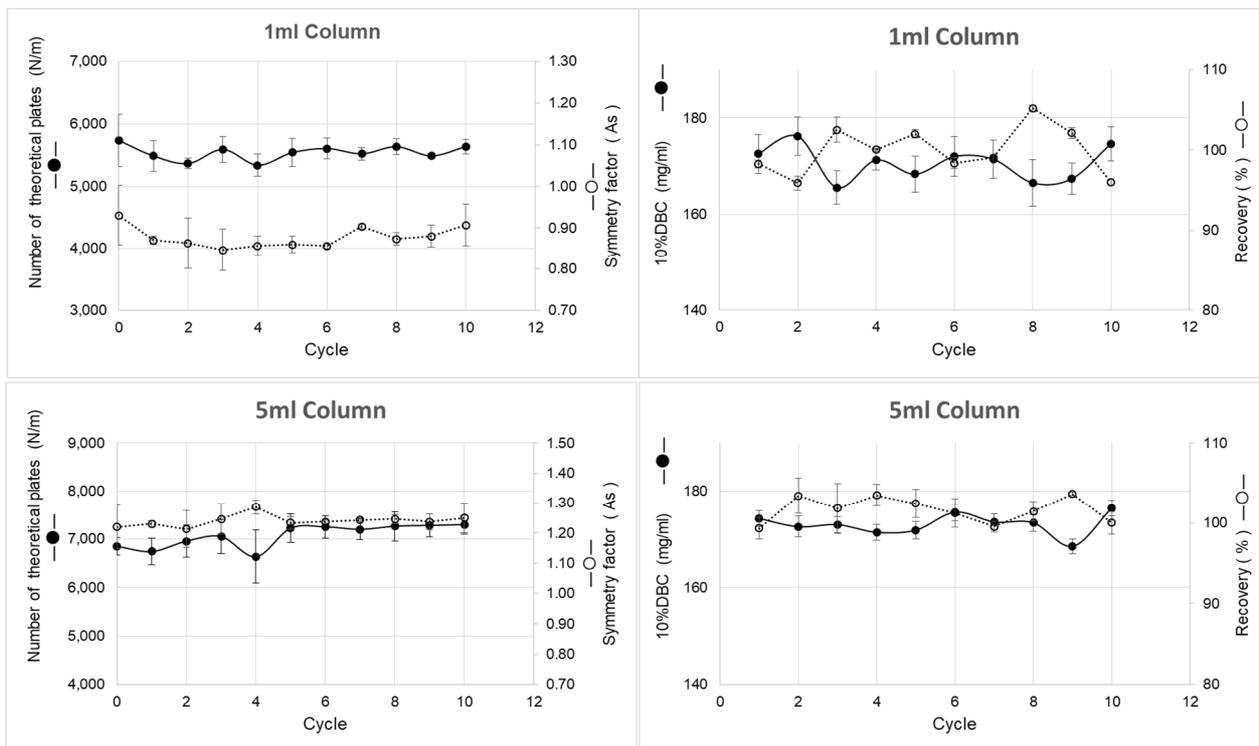
Fig3 CIP cycle and column performance

Tables 8 and 9 show the average value and standard deviation of the results measured for each cycle for 3 columns of 1 ml and 5 ml, respectively. Fig3 is a graph of Tables 8 and 9.

There was no change in the theoretical plate number, symmetry factor, and DBC and recovery rate for each CIP cycle, and no deterioration was observed up to 10 cycles. As far as the tendency of the theoretical plate number and symmetry factor in Fig. 3 is concerned, it seems that there is no sudden deterioration even after 10 cycles. However, care must be taken as DBC is affected by the durability of the packed resin. Please refer to the instruction manual of the resin you actually use and set the CIP conditions before use.

Conclusion

➢ The commercially available packed column and *Super Edge* mini column have the same recovery rate as DBC, and it is possible to purchase a commercially available resin bottle and packed it into the *Super Edge* mini column for performance evaluation.

➢ Since the DBC and recovery rates of 1 ml and 5 ml of *Super Edge* mini-column are the same, it was confirmed that scale-up can be performed easily.

➢ The packed column of the *Super Edge* mini column does not show any deterioration in performance after 10 repeated CIP cycles, and can perform several times chromatography experiments.