DATA File: TD_EMC_N1_V1_E

Super Edge Empty Mini Column

Versatility of empty mini column and performance evaluation of packed column– 1

JNC's empty mini columns can be packed with chromatography media from various manufacturers. This data file introduces the result of preparing and evaluating packed columns. The data introduced this time is the result of evaluating the packing quality of a packed column using the theoretical number and the symmetry factor (As).

1 Packing quality of packed column of various chromatography media.

Cellufine™ is based on cellulose. In addition, agarose and polymers are used as base materials and provided by various companies. These various types of chromatographic media were packed in empty mini columns and evaluated whether the packing was optimized.

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/english/index.html

Material

The following types of chromatography media are used as representative example.

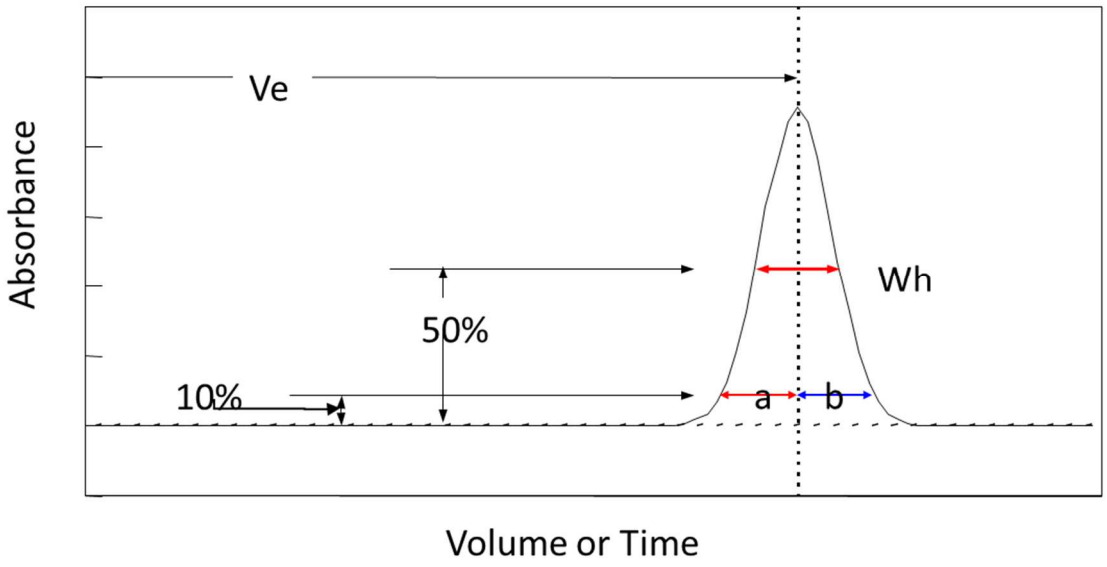
Company	media	bead size	mode	base
JNC	Cellufine Sulfate	dp70 μm	Affinity	Cellulose
JNC	Cellufine MAX Butyl	dp90 μm	Hydrophobic	Cellulose
JNC	Cellufine MAX S-h	dp90 μm	Strong cation exchanger	Cellulose
G	S-CEX(1)	dp30 μm	Strong cation exchanger	Agarose
G	S-CEX(2)	dp50 μm	Strong cation exchanger	Agarose
G	S-CEX(3)	dp75 μm	Strong cation exchanger	Agarose
G	Q-AEX(4)	dp90 μm	Strong anion exchanger	Agarose
T	Sulfate(5)	dp45 μm	Cation exchanger	Polymer
T	S-CEX(5)	dp75 μm	Cation exchanger	Polymer
T	Butyl-HIC(5)	dp100 μm	Hydrophobic	Polymer
B	Q-AEX(6)	dp50 μm	Strong anion exchanger	Polymer

Evaluation method

The apparatus used was a commercially available chromatography system. The flow rate was 30cm/h, and the peak was monitored by UV or a conductivity meter. The peak forming substance was injected at 1% of the column volume.

Method of calculation

The theoretical plates and the symmetry a factor were calculated from the elution peak of marker substance. The calculation method is described below, but these values may be obtained even with the software of the chromatography system, so it is possible to use them as appropriate.



Calculation **HETP = L/N** **N = 5.54× (Ve/Wh)^2** **As=b/a**		
	L	column length （cm or m)
	Ve	elution time or volume
	Wh	half width of peak
	a,b	peak width of 10% of peak height , (a) front, (b) rear
	Note	Ve, Wh and a,b were should same dimension

In this data file, the theoretical plate number per meter (N / m) and the theoretical plate height per particle (RPH) were calculated for comparison of column efficiency.

N/m ＝ N/(m/Column length m)
RPH ＝ HETP/dp
: RPH (Reduction of plate height), dp (Average particle size)

Result

Table1 shows the measurement results of the number of theoretical plates and the symmetry factor of the packed column.

Table1 Performance of packed column

Packed with various resins into *Super Edge* Mini Column

Manufacturer	Product name	Particle diameter dp	HETP measurement: Injection sample 1%CV	Mobile Phase Flow 30cm/h	Column size	Plates /meter	Asymmetry	Flow rate measurement: Flow rate at 0.4MPa (with 0.2MPa F.R*.)
		μm				N/m	As	mL/min
JNC	Cellufine Sulfate	70	2%-aceton	water	1mL	6166	1.0	6.5
					5mL	6929	1.2	11.5
JNC	Cellufine MAX Butyl	90	2%-aceton	water	1mL	6629	0.9	14.0
					5mL	6671	0.9	15.0
JNC	Cellufine MAX S-h	90	2%-aceton	water	1mL	5830	0.9	11.0
					5mL	7076	1.1	14.0
G	S-CEX(1)	30	1M-NaClaq	0.1M-NaClaq	1mL	12056	0.9	5.0
					5mL	11342	1.4	10.0
G	S-CEX(2)	50	2%-aceton	water	1mL	9493	1.0	7.0
					5mL	-	-	-
G	S-CEX(3)	75	2%-aceton	water	1mL	7012	1.1	16.0
					5mL	7971	1.3	18.0
G	Q-AEX(4)	90	2%-aceton	water	1mL	5137	0.9	11.5
					5mL	6338	1.1	13.5
T	Sulfate (5)	45	2%-aceton	water	1mL	8030	1.2	11.0
					5mL	-	-	-
T	S-CEX(5)	75	2%-aceton	water	1mL	7007	1.2	15.0
					5mL	7013	1.2	15.5
T	Butyl-HIC(5)	100	1M-NaClaq	water	1mL	5984	1.2	18.0
					5mL	5100	1.3	16.0
B	Q-AEX(6)	50	2%-aceton	water	1mL	8575	1.0	10.5
					5mL	8621	1.2	18.0

(1) polystyrene/divinyl benzene polymer matrix
(2) high-flow agarose base matrix/ polymer-grafted ligand
(3)Highly cross-linked agarose with dextran surface extender
(4)Highly cross-linked agarose
(5)hydroxylated methacrylic polymer
(6)hydroxylated methacrylic polymer
*F.R. : flow restrictor

The number of theoretical plates can be used as an indicator to determine whether a column has been properly packed. Table1 shows the number of theoretical plates per 1m of column length (N / m). Various values are available in the order of 5,000 to 12,000. It is known that N / m depends on the size of the particle size, and the smaller the particle size, the larger. In this evaluation, there is a correlation between particle size and N / m as shown in Fig. 1.

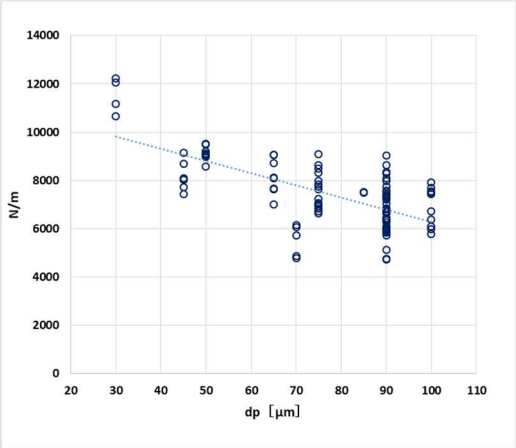


Fig. 1 Relationship between particle diameter (dp) and N / m

According to the guideline of the number of theoretical stages of Q-AEX (4) of Company G, it is sufficient if the number exceeds 3,000 N / m, and it can be said that the measured value of 5,000 this time is in a sufficiently good state.

Reduction theoretical plate number (RPH) is generally 3 or less in terms of the number of theoretical plates per particle. In the case of a large column for a process, if it is 3 to 5, it can be judged that there is good condition. In this data file, PRH was calculated and compared (Fig. 2).

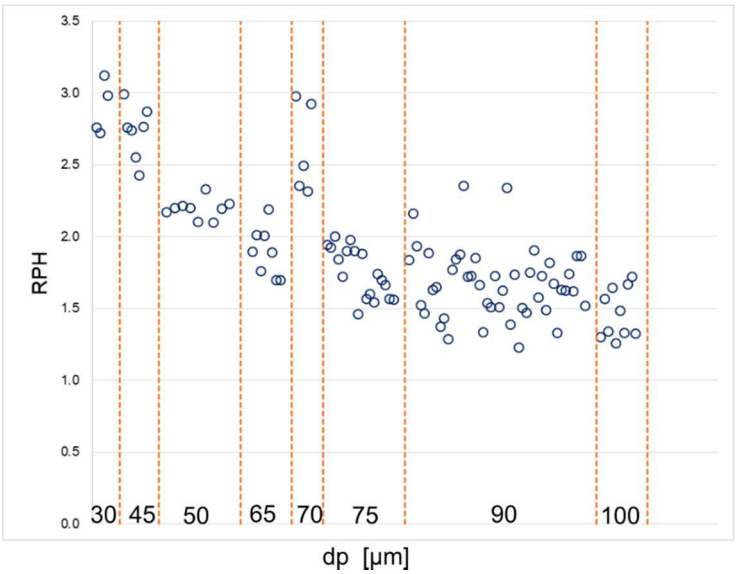
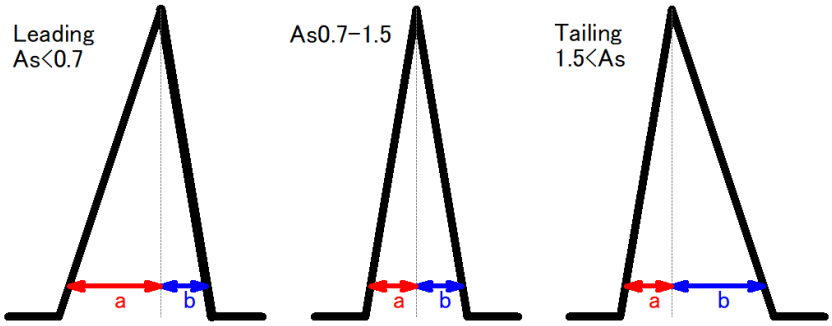


Fig.2 Relationship between reduction plate number (RPH) and particle size (dp)

PRH tended to increase as the particle size decreased. However, it was confirmed that the packing was in good condition with RPH 3 or less.

Symmetry factor (As)

Elution peaks should be sharp and symmetric. The number of theoretical plates indicates the degree of sharpness, whereas “As” indicates the symmetry of the peak. As is ideally 1, but it is said to be good if it is in the range of 0.7 to 1.5.



As = b/a :a>>b Leading Packing pressure is too high

b<<a Tailing Packing pressure is weak

Other causes include, when leading with a combination of mobile phase and peak measurement substance, tailing is observed when the chromatography media interacts with the substance for peak measurement. In such a case, it may be necessary to consider the measurement conditions. Can be measured with acetone/water (or buffer) in most cases, in addition, a method of monitoring the peak with conductivity using salts, alternatively, there is a method of monitoring the peak with UV using a nitrate.

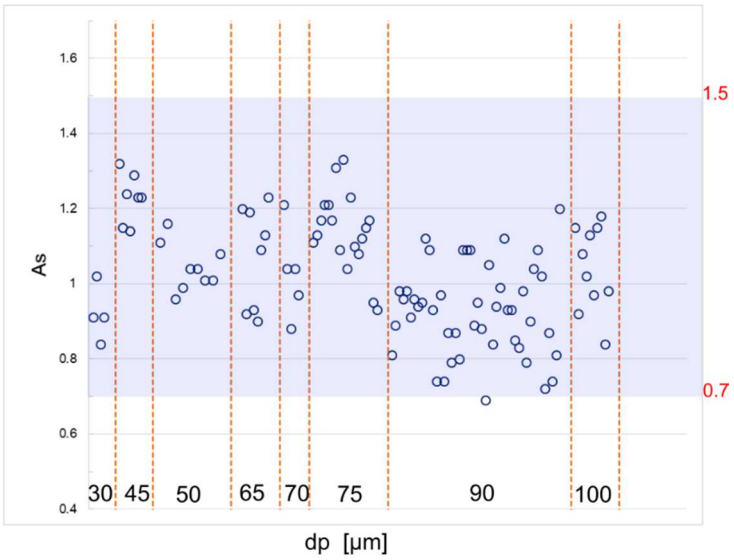


Fig.3 Relationship between symmetry factor (As) and particle size (dp)

Fig.3 shows the relationship between As and dp. Unlike the theoretical plate number, there was no correlation with particle size. The colored area represents 0.7 to 1.5, and the measurement results this time

are within the range, indicating a good packing condition. Fig. 4 shows an example of an actual chromatogram. The graph was repeated 4 times and the baseline was shifted. It is shown that both the peak shape and the reproducibility are good.

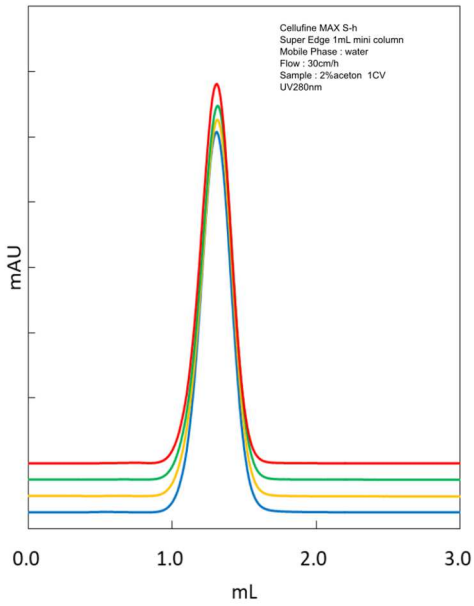


Fig.4 Cellufine MAX S-h/*Super Edge* 1mL column acetone peak shape

Conclusion

Table.2 Statistical summary of packed column variation

		Theoretical plates	Reduction theoretical plate	Symmetry factor
	Column Vol.	N/m ± S.D.	RPH ± S.D.	As ± S.D.
Avarage	1mL	7425 ± 1400	1.89 ± 0.5	1.02 ± 0.15
	5mL	6754 ± 1262	18.2 ± 0.4	1.15 ± 0.16
MAX	1mL	12239	3.12	1.33
	5mL	11342	3.02	1.47
MINI	1mL	4716	1.23	0.69
	5mL	4418	1.33	0.73
N	1mL		107	
	5mL		65	

A high quality packed column could be prepared by packing various chromatographic supports into the *Super Edge* empty mini column of JNC.

- Easy and reproducible packing with the packing tool included in the empty mini column starter kit
- It can be packed with a chromatographic media having a particle size of 30 to 100 μm, various base materials derived from cellulose, agarose, and polymers, and an adsorption system mode of affinity, hydrophobic interaction, and ion exchange.
- The packed column has a reduction theoretical plate number (RPH) of 3 or less and a range of As 0.7 to 1.5, so that high quality packing is possible.