Desalting using Cellufine® GH-25

JNC CORPORATION

Life Chemicals Division

2-1, Otemachi 2-Chome
Chiyoda-ku, Tokyo 100-8105 JAPAN
Phone +81-3-3243-6150
Fax +81-3-3243-6219
e-mail: **cellufine@jnc-corp.co.jp**
Cellufine web: **http://www.jnc-corp.co.jp/fine/en/cellufine/**

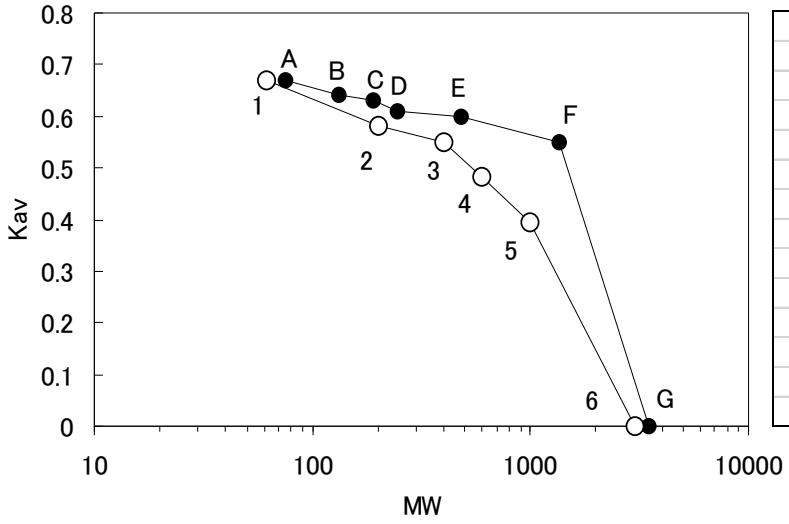
Table of contents

Cellufine® GH-25 is a spherical porous particle made only of natural cellulose. In the Cellufine® series, this Cellufine® GH-25 type is manufactured for desalting. Since cellulose has many hydrogen bonds in intermolecular, it has higher mechanical strength than dextran and agarose, which are the materials of conventional gel filtration media. Cellufine® GH-25 has a pressure resistance that is incomparably greater than conventional soft gels, which is the first feature. Therefore, the elution can be performed at a high flow rate, which is particularly advantageous when performing industrial desalting.

The second feature of Cellufine® GH-25 is its unique separation pattern. As can be seen from the relationship between the molecular weight and Kav in Fig. 1, the difference between Kav for molecular weight of 1,000 and 3,000 for molecular weight is large. This indicates that it is very advantageous for separating low-molecular compounds having a molecular weight of 1,000 or less from high-molecular compounds such as proteins.

The third feature of Cellufine® GH-25 is that there is almost no non-specific adsorption to proteins. This is particularly important when desalting is performed industrially, and it is economically important that 100% of the target protein is recovered.

This document describes the characteristics of Cellufine® GH-25 by describing many experimental examples, especially for desalting.



	M.W. marker	MW
1	Ethylen glycol	62
2	PEG	200
3	PEG	400
4	PEG	600
5	PEG	1000
6	PEG	3000
A	Gly	75
B	Gly2	132
C	Gly3	189
D	Gly4	246
F	Calucium pantothenate	477
E	Vitamin B12	1355
G	Insulin B chain	3495

Fig.1 Relationship between molecular weight and Kav

1. Properties

Particle size	:	45 — 105 μm
Exclusion limit		
polyethylene glycol	:	2,500
protein(peptide)	:	3,500
Water absorption		2.0±0.2 g/g
Swelling degree		3.0～4.0 ml/g

2. Flow rate

Cellufine® GH-25 has a very high flow rate and shows good separation ability. Fig. 2 shows the results obtained by packing Cellufine® GH-25 by the gravity sedimentation method and adjusting the applied water pressure by the height of the water surface to measure the flow velocity. FIG. 3 shows the flow rate of a column packed with a pump.

FIG. 2 and 3 shows that Cellufine® GH25 has better flow performance than the conventional gel. Further, FIG. 3 shows that the pressure and the flow rate have a linear relationship even when the pressure is increased to 0.3 MPa, indicating that the pressure resistance of Cellufine® GH-25 is excellent.

3. Physical and chemical properties

Acid and alkali resistance

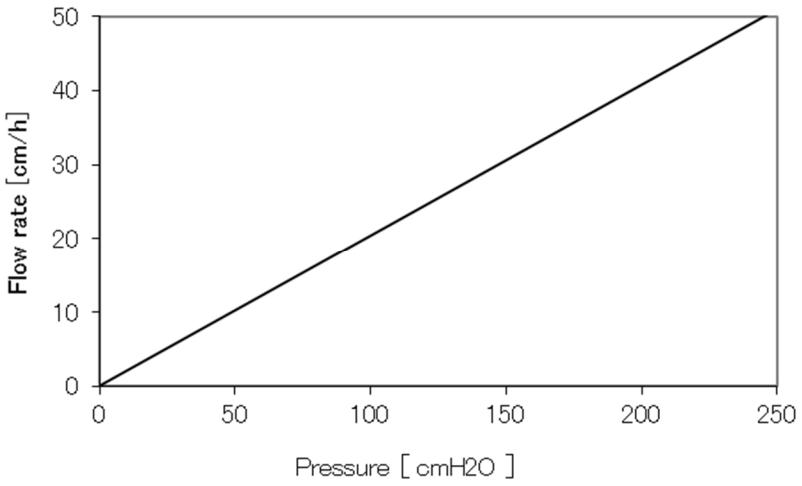
Cellufine® GH-25 is stable against acids and alkalis. Cellufine® GH-25 did not change shape and performance when 0.1N hydrochloric acid or sodium hydroxide for 30 days at room temperature or 1N hydrochloric acid or sodium hydroxide for short-time washing at room temperature.

Heat resistant

Cellufine® GH-25 can be autoclaved (120 degrees, 60 minutes).

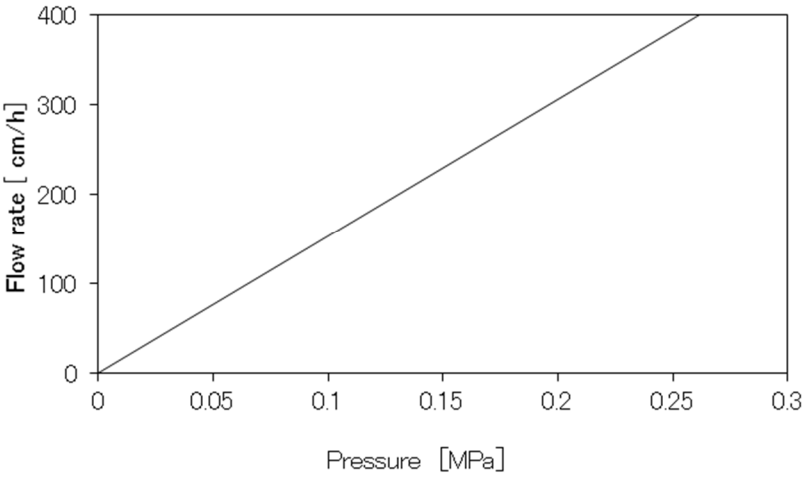
Stability in special solvents

Even when stored in 6M urea and guanidine hydrochloride at room temperature for 7 days, there was no change in shape or change in desalting ability.



Column	: 2.2 x 90 cm
Eluate	: 0.05M Ammonium formate
Temperature	: 25℃

Fig.2 Relationship between pressure and flow velocity（water head pressure）



Conditions are the same as Fig.2

Fig. 3 Relationship between pressure and flow velocity

Nonspecific adsorption of proteins

As a result of examining the adsorption of various proteins shown in the table below, almost no protein was adsorbed, and the recovery was close to 100%. Lysozyme, which is a basic protein, is generally adsorbed on a gel and tends to have a low recovery rate or a delayed elution position. Cellufine® GH-25 does not have this tendency, and a recovery rate of nearly 100% can be obtained. When eluted with water or a very low concentration salt solution, a slight tailing can be seen. Table 1 shows the recovery data of various proteins.

Table 1 Recovery test of various proteins from Cellufine® GH-25

Sample	recovery（%）	Sample	recovery（%）
albumin	９８	Ferritin	９２
Lysozyme	９６	Fibrinogen	１００
γ-globulin	９２	Apoferritin	１００
Cytochrome c	９８	Chymotrypsinogen	９０
Myoglobin	９６	DNA	１００
β-lactoglobulin	１００	Adenosine	１００
Catalase	１００	Tryptophan	１００

（conditions）

Column	: 1.3×12cm（16ml）
Sample	: 10mg／5ml
Eluate	: 0.05M Tris-HCl, pH7.5 +0.1M KCl
Volumn	: 50ml

4　Durability

A desalting test of albumin was carried out for 1,000 cycles for 250 days under the following conditions, but no change in the desalting ability was observed.

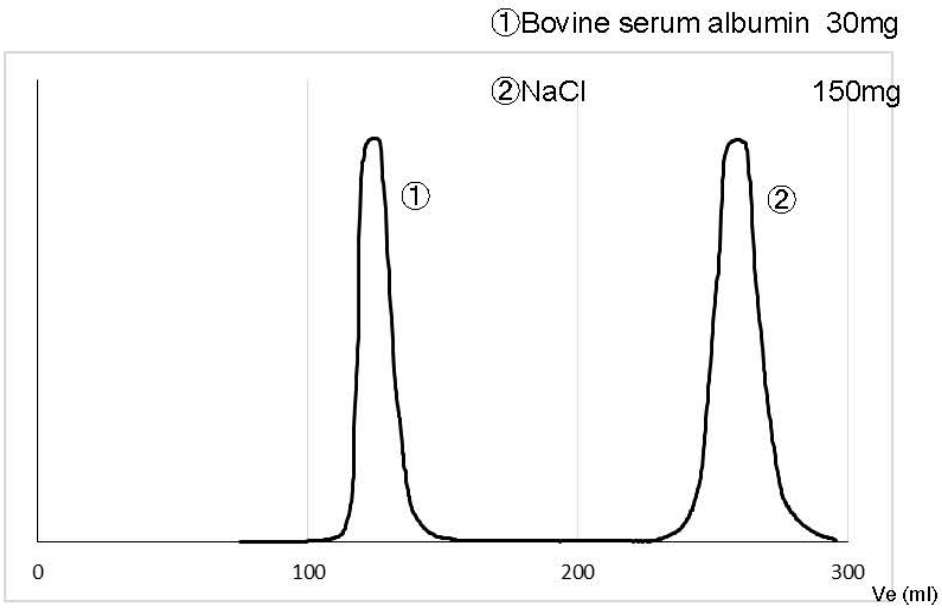
（conditions）

column	: 2.6×55cm（292ml）
sample	: 8ml　（BSA　3.75%，NaCl　18.75%）
Eluate	: 0.05M　Ammonium formate
Flow rate	: 130ml/h　（24.5cm/h）

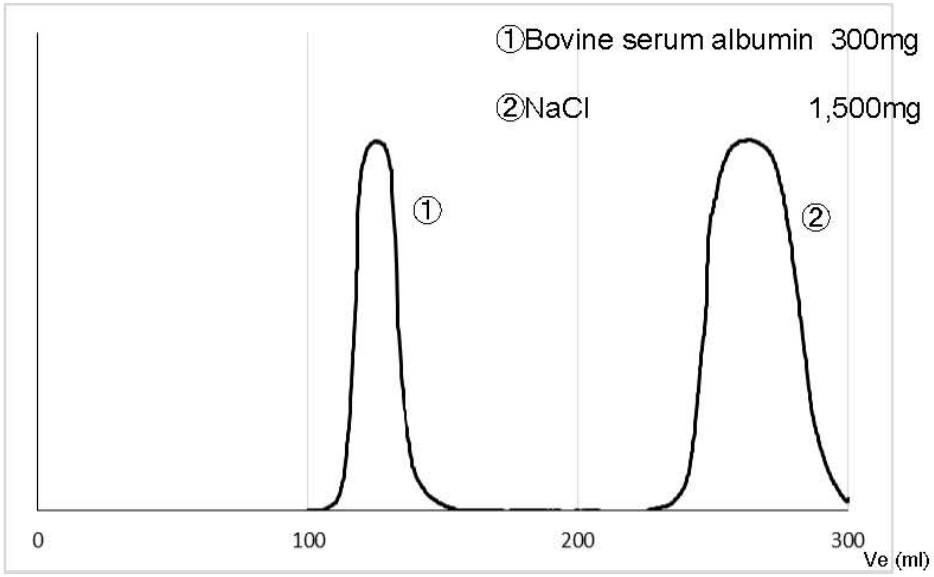
Application of Cellufine® GH-25

1. Desalting from Albumin

(I) sample 2ml

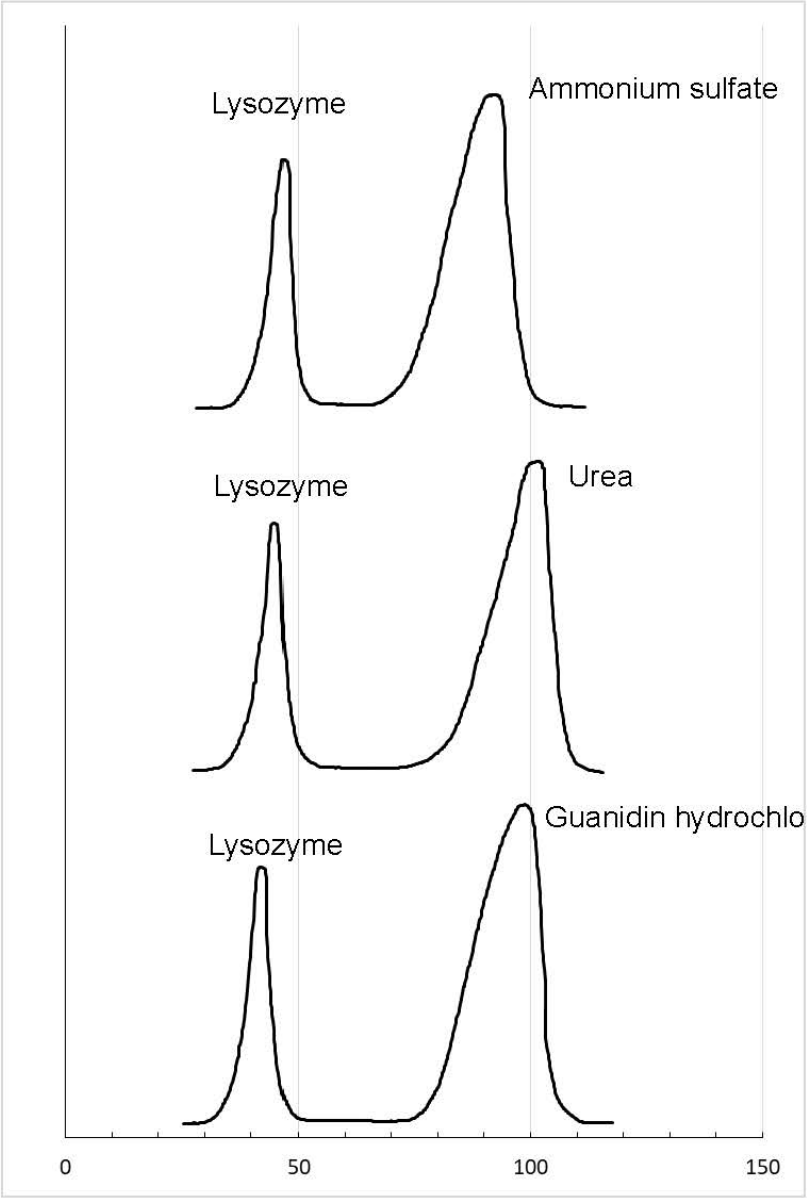


(II) sample 8ml



Column : 2.6x 60cm (Vt=318ml)
Gel : Cellufine® GH－25
Eluate : 0.05M ammonium formate
Flow rate : 36ml/hr (6.8cm/hr)
Detection : RI

2. Desalting from Lysozyme

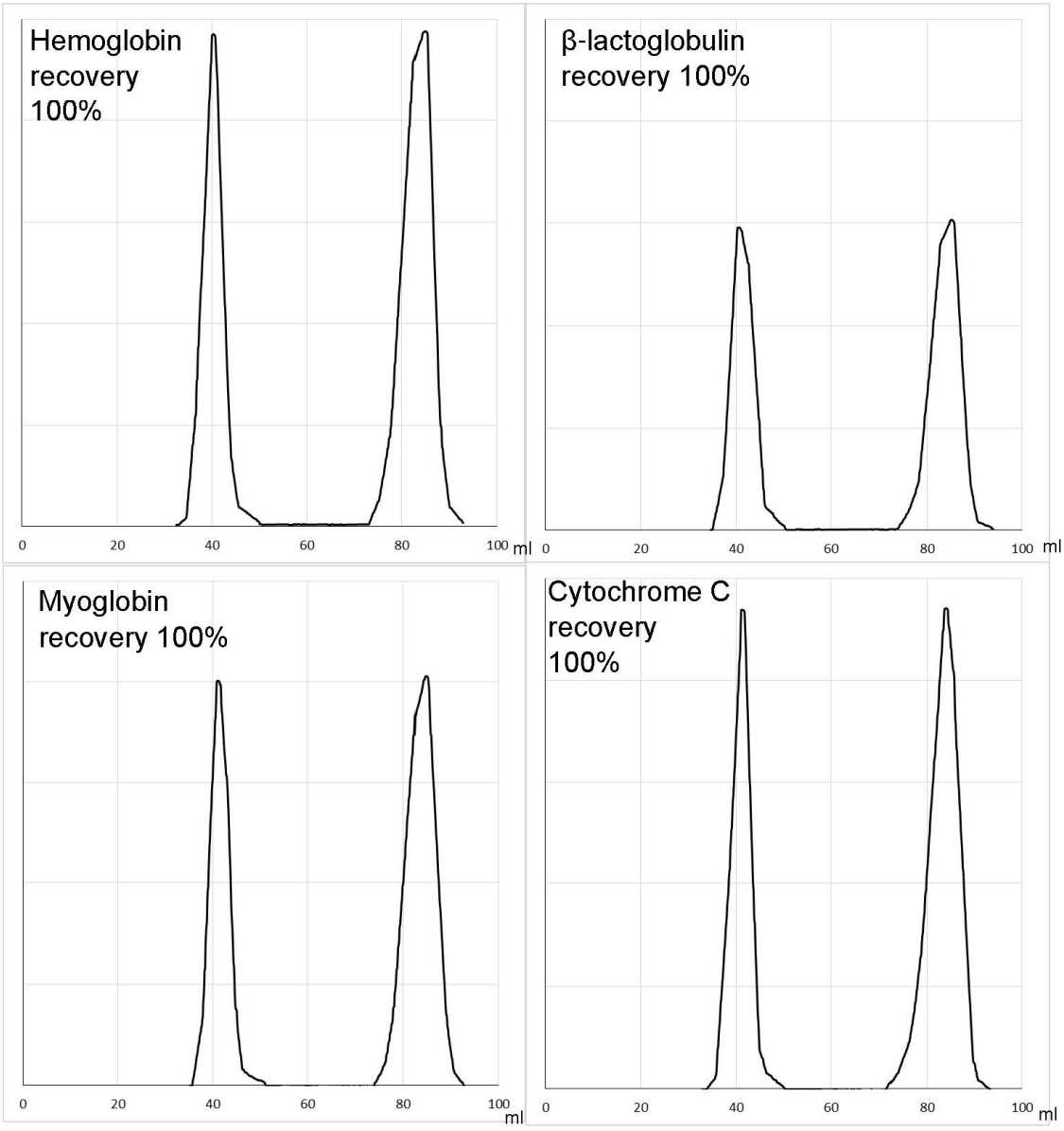


Column : 1.6 x 52cm (Vt = 104ml)
Gel : Cellufine® G H － 2 5
Sample : 2 m l Lysozyme 30m g /Salt 150㎎
Eluate : 0.05M ammonium formate
Flow rate : 42ml/hr (21cm/hr)
Detection : R I

3. Desalting from other proteins

An example of separation of hemoglobin, myoglobin, β-lactoglobulin, cytochrome C and sodium chloride was shown.

The separation of NaCl from these proteins is very sharp and nearly 100% recovery.



Column : 1.5 x 4 9 c m （V t ＝ 8 6 m l ）
Gel : Cellufine® G H ― 2 5
Sample : 2 m l ①Protein 50m g 、②N a C l 250m g
Eluate : 0.05M Ammonium formate
Flow rate : 30m l / h r （１７㎝/ h ）
Detection : A280nm(protein） 、silver nitrate titration method （N a C l ）

4. Removal of alcohol from human serum albumin production process

The step of removing alcohol from the alcohol-fractionated albumin is particularly important when the product is used for pharmaceuticals. Table 2 shows the results obtained by examining various conditions using Cellufine® GH-25.

In particular, in the experiment of No. 3, 4 and 6 the content of ethanol was 0.001% or less, and the recovery of albumin was almost 100%. Better results were obtained than conventional gel filtration media throughout all experiments.

Table 2. Removal of ethanol from human serum albumin

Conditions (room temp,)							
Exp.No.	1	2	3	4	5	6	7
Column ID (cm)	5	5	2.6	2.6	5	5	2.6
Column L (cm)	68	67	82×3	82×3	76.5	76.5	26.6
Column Volume[Vt](ml)	1335	1316	1306	1306	1502	1502	141
Flow Rate (ml/min)	570	2010	560	440	2010	590	1014
Linear velocity (cm/h)	29	102	105	83	102	30	191
1 cycle time (h)	2.3	0.7	2.3	3.0	0.7	2.5	0.1
Sample							
Exp.No.	1	2	3	4	5	6	7
sampl Vol. [Vs] (ml)	310	310	310	400	360	360	34
Vs/Vt (%)	23	24	24	31	24	24	24
Albumin conc.(%)	12	12	12	12	12	12	12
Ethanol conc.(%)	4.8	4.8	4.8	4.8	4.8	4.8	4.8
Result							
Exp.No.	1	2	3	4	5	6	7
Recoved volumn [Vs'] (ml)	546	525	403	477	550	550	46
Vs'/Vs	1.76	1.69	1.30	1.19	1.53	1.53	1.35
Albumin recoved conc, (%)	6.6	6.9	9.0	9.9	7.4	8.0	8.5
Ethanol residual conc. (%)	0.002	0.010	<0.001	0.001	0.023	0.001	0.002
Albumin recovery (%)	97	974	98	98	94	102	96

Next, the results of experiments performed at 9 ° C. and 0 ° C. are shown in Table 3. No. 12, 13 and 14 are comparison data using other products under the same conditions. It can be seen that Cellufine® GH-25 is superior in residual ethanol concentration.

Table 3. Removal of ethanol from human serum albumin (at low temperature)

Conditions (9 degrees C)							
Exp.No.	8	9	10	11*	12	13	14
Gel	GH25	GH25	GH25	GH25	Other	Other	Other
Column ID (cm)	3.2	3.2	3.2	3.2	3.2	3.2	3.2
Column L (cm)	53.3	53.2	53.1	52.8	53.3	52.2	51.2
Column Volume[Vt](ml)	429	428	427	425	429	420	412
Flow Rate (ml/min)	70	255	544	291	70	255	544
Linear velocity (cm/h)	9	32	68	36	9	32	68
1 cycle time (h)	6.1	1.7	3.6	6.8	6.1	1.6	0.8
Sample							
Exp.No.	8	9	10	11*	12	13	14
sampl Vol. [Vs] (ml)	86	86	86	86	86	86	86
Vs/Vt (%)	20	20	20	20	20	20	21
Albumin conc.(%)	12	12	12	12	12	12	12
Ethanol conc.(%)	10	10	10	10	10	10	10
Viscosity (cp at 9 deg. C)	6	6	6	10	6	6	6
Result							
Exp.No.	8	9	10	11*	12	13	14
Recoved volumn [Vs'] (ml)	139	146	149	144	141	148	147
Vs'/Vs	1.62	1.70	1.73	1.67	1.64	1.72	1.71
Albumin recoved conc, (%)	7.4	7.0	6.8	7.5	7.2	6.8	6.7
Ethanol residual conc. (%)	0.007	0.006	0.012	0.002	0.014	0.032	0.097
Albumin recovery (%)	100	99	98	105	98	98	95

＊ Sample temperature -7 ℃ gel filtration temperature 0 ℃ (10cp)

5. Large scale desalination with Cellufine® GH-25

An experimental example of the removal of ammonium sulfate by Cellufine® GH-25 is shown in Table 4. The sample loading was 32%, albumin recovery was 98%, and ammonium sulfate was almost completely removed. Moreover, the dilution ratio of the sample was within 1.14 times. These results indicate that Cellufine® GH-25 is superior to conventional gel filtration media even in large-scale processing.

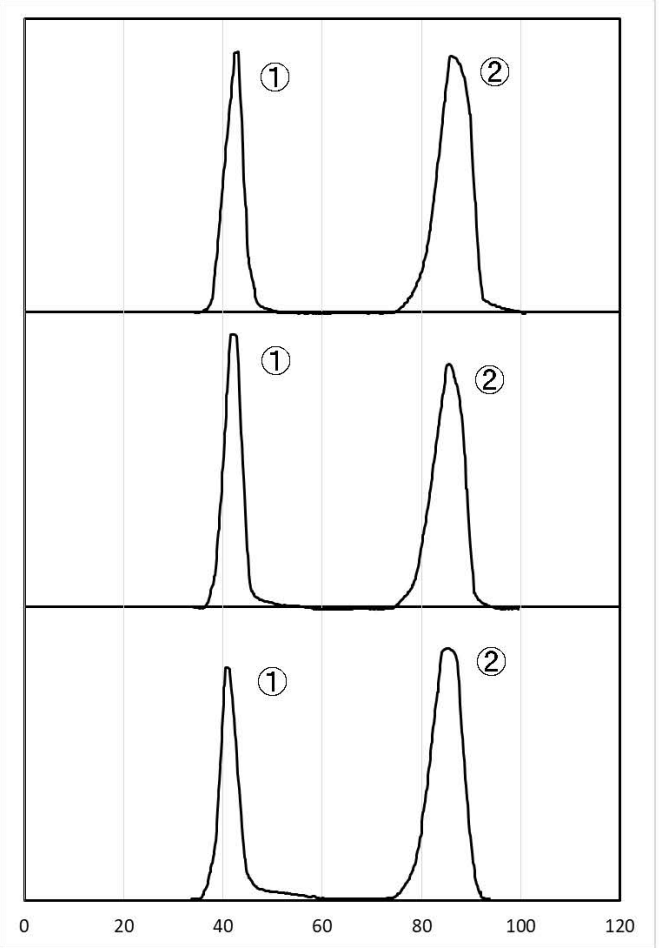
Table 4. Desalting of ammonium sulfate from albumin by Cellufine® GH-25

Condition				
Exp.No.	1	2	3	4
Column ID (cm)	14	14	14	14
Column L (cm)	87	87	87	87
Column Volume[Vt] (L)	13	13	13	13
Flow Rate (L/h)	6.7	13.5	6.7	13
Linear velocity (cm/h)	44	88	44	84
1 cycle time (h)	2.0	1.0	5.7	2.9
Operating pressure (MPa)	0.04-0.05	0.09-0.1	0.03-0.04	0.06-0.08
Sample				
Exp.No.	1	2	3	4
sampl Vol. [Vs] (L)	4.3	4.3	4.3	4.3
Load Vs/Vt (%)	32	32	32	32
Processing speed (L/h)	2.2	4.3	0.8	1.5
Albumin conc.(%)	5.6	5.6	5.6	5.6
Ammonium sulfate conc. (%)	24	24	24	24
Result				
Exp.No.	1	2	3	4
Recoved volumn [Vs'] (L)	4.7	4.8	4.9	4.9
Dilution rate Vs'/Vs	1.09	1.12	1.14	1.14
Albumin recoved conc, (%)	5.0	4.9	4.8	4.8
Ammonium sulfate residual conc (%)	0.007	0.011	0.021	0.038
Albumin recovery (%)	98	98	98	98

Effect of various conditions on desalting

1． Effect of salt concentration

The salt concentration of the eluate does not prevent desalting. Lysozyme, which has relatively high adsorptivity, can be desalted without being adsorbed on the gel even when distilled water is used.



Eluate：
0.05M
ammonium formate

recovery 100%

Eluate：
0.005M
ammonium formate

recovery100%

Eluate：
distilled water

recovery100%

Column：1.5x50cm (Vt=88ml)
Gel：Cellufine® GH-25
Sample：2ml ①Lysozyme 25mg ②NaCl 125mg
Flow rate：33ml/hr (18.6 cm/hr)
Detection：A280nm(Lysozyme） Silver nitrate titration method法（NaCl)

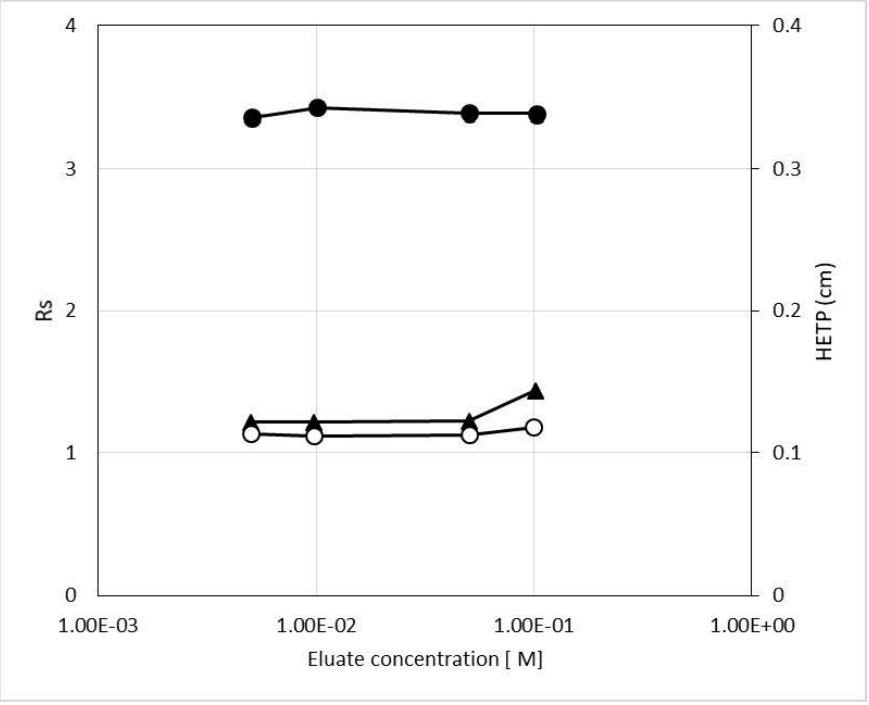
Further, the relationship between the salt concentration of the eluate and the “height equivalent to a theoretical plate” (HETP) and the “Resolution” (Rs) was obtained from experimental values and shown in a graph. Even if the concentration of ammonium formate was changed, both parameters hardly changed.

HETP=Lx16x(W/4Ve)

L：Column length ，Ve：Elution volume，W：Peak width

Rs=2(V2-V1)/(W1+W2)

V1，V2： Elution volume ，W1，W2：Peak width

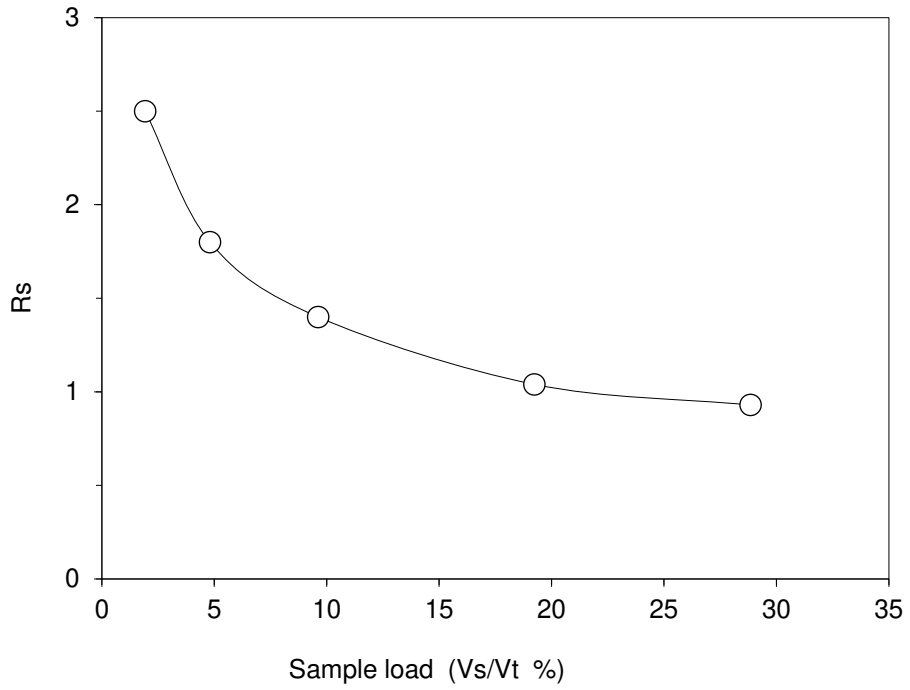


（Condition）

Column size	: 1.6 x 52 cm (104ml)
Gel	: GH-25
Sample	: 2ml(Lysozyme30mg/Ammonium sulfate 150mg)
Flow	: 40ml/h (20cm/h)
Detection	: RI

2. Sample volume

In industrial desalination processes, the sample volume can be 10% or more of the column volume. The relationship between the sample volume and the resolution (Rs) is shown in the following graph. It is shown that sufficient separation can be achieved even when a sample of 30% of the column volume is added.

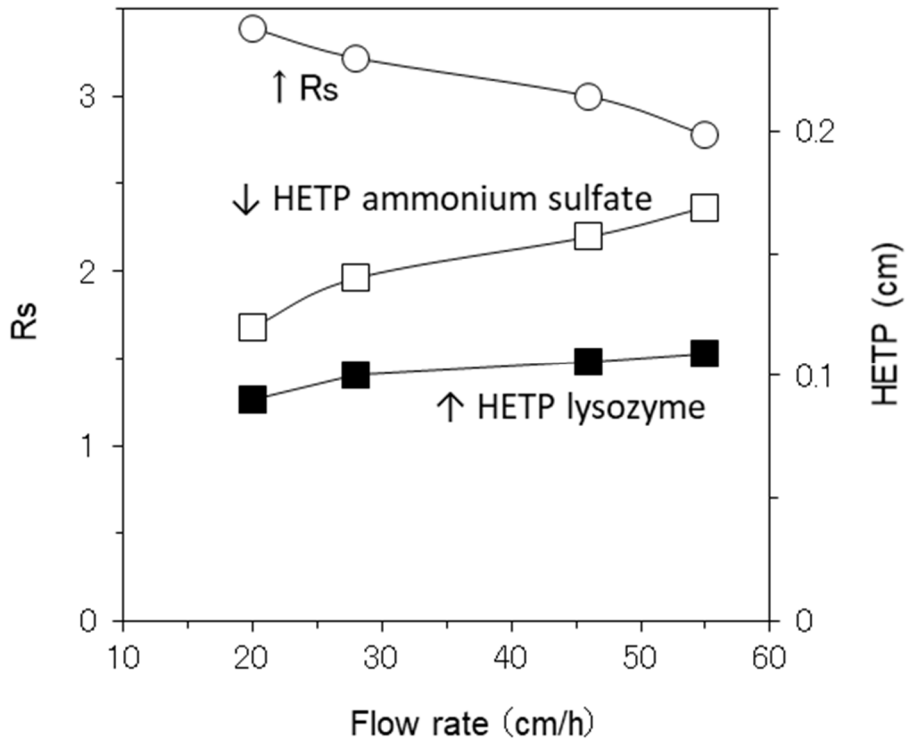


（Condition）

Column	: 1.6 x 52 cm (104ml)
Gel	: GH-25
Sample	:（Lysozyme 1.5% / Ammonium sulfate 7.5%）
Eluate	: 0.05M Ammonium formate
Flow	: 40ml/h (20cm/h)
Detection	: RI

3. Flow Rate

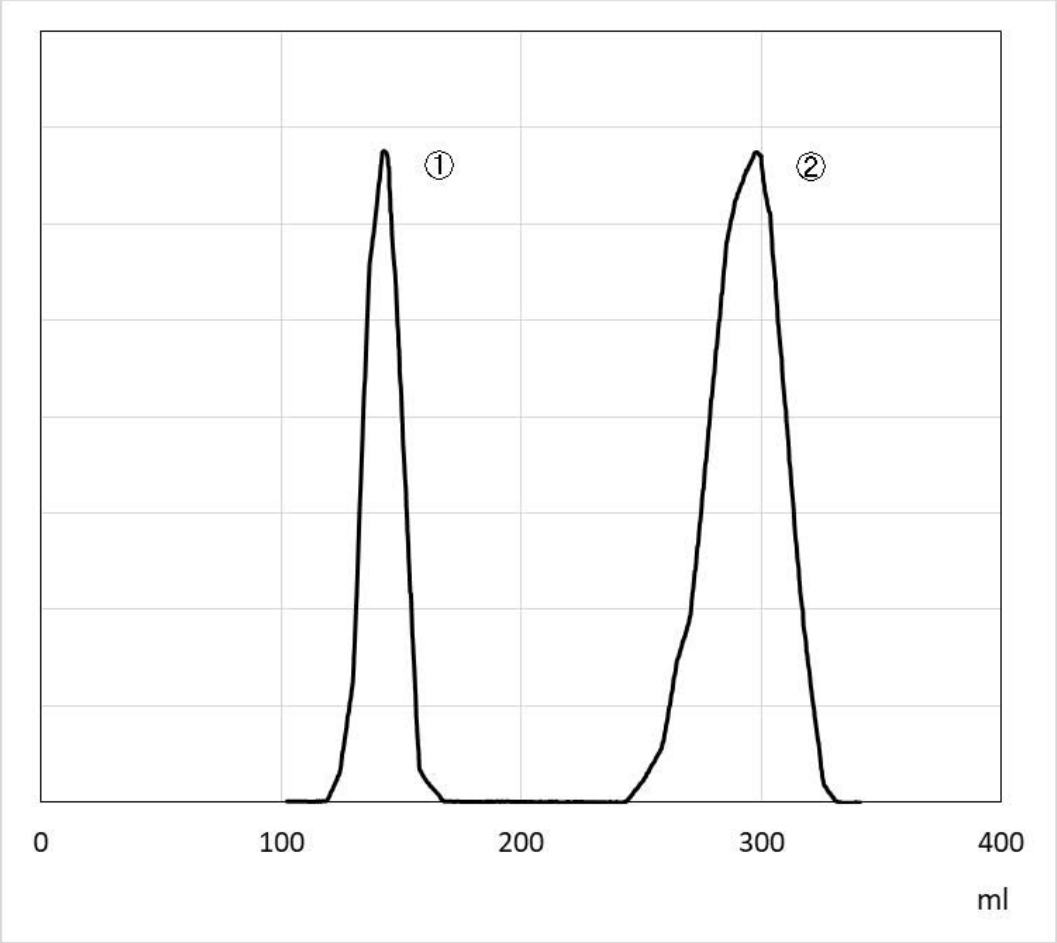
A major feature of Cellufine® GH-25 is that a very high flow rate can be obtained compared to conventional media. Gel filtration is usually performed at a flow rate of about 10 to 60 cm / hr, and separation becomes worse at a higher flow rate. The relationship between flow rate, resolution and HETP is shown below. Cellufine® GH-25 has no significant change in resolution and HETP at a flow rate of 60 cm / hr or less, and can be sufficiently desalted even at a high flow rate.



（Condition）

Column size	: 1.6 x 52 cm (104ml)
Gel	: GH-25
Sample	: 2ml(Lysozyme30mg/Ammonium sulfate 150mg)
溶出液	: 0.05M Ammonium formate
Detection	: RI

Next, the results of desalting of albumin at high flow rate condition are shown in the figure below. The required time is about 25 minutes, and both bovine serum albumin and sodium chloride are completely separated with almost 100% recovery.



（Condition）

Column	: 2.2 x 90cm (342ml)
Gel	: GH-25
Sample	: 10ml（①BSA375mg / ②NaCl 1,875mg）
Eluate	: 0.05M Ammonium formate
Flow	: 850 ml/h （224cm/h）
Detection	: BSA A280nm / NaCl Silver nitrate titration method