Adsorption Study of Egg-Derived Influenza Virus with Cellufine™ Sulfate Affinity Chromatography Media

An efficient processing for purifying virus particles is important to develop vaccine. An affinity chromatography medium, Cellufine Sulfate has been used for manufacturing of viral vaccines such as influenza virus, rabies virus and Japanese encephalitis virus. Figure 1 and Table 1 showed a typical chromatogram of purifying egg-derived influenza virus with Cellufine Sulfate. Adsorbed virus particles are eluted from the medium easily with high purity. Here we describe rapid methods to purify egg-derived influenza virus rapidly and easily with Cellufine Sulfate.

Optimal adsorption condition

Influenza virus change and mutate easily and there are well- known to be many different strains. The screening of pH condition is effective for adsorption of inactivated influenza virus to Cellufine Sulfate. Table 2 shows the results of Cellufine Sulfate adsorption study of various egg-derived influenza viruses under optimized pH condition. Allantoic fluid is used as the reference of the study. The study suggested determination of suitable pH condition is important for loading sample with Cellufine Sulfate.

Other Characteristics of Cellufine Sulfate

Cellulose Sulfate is designed as alternative to heparin, which has multivalent activities. Thus, Cellufine Sulfate is suitable for purification of blood coagulate proteins except for virus. As cellulose is a well-known natural product with high chemical and physical stability, Cellufine Sulfate as well as other Cellufine products can be cleaned and regenerated with 0.5 M NaOH solutions. For more information on Cellufine, please consult the JNC Corporation Cellufine website.

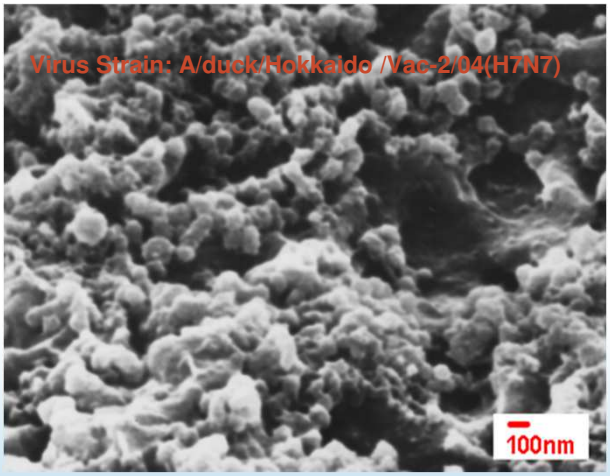


Figure 2. SEM Analysis of the Surface of Cellufine Sulfate after influenza virus loading

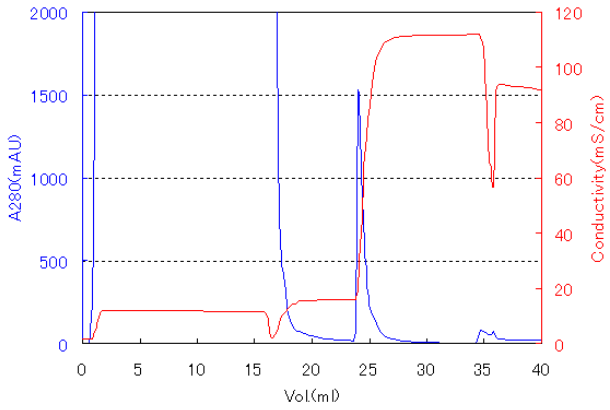


Figure 1. Purification of egg-derived influenza with Cellufine Sulfate
Sample: Allantoic fluid of chiken egg containing inactivated flu virus (H7N7)
Loading volume: 15 ml (total 153,600 HAU)
Column: 6.6 mm ID × 1.5 cm L ,
Equilibration buffer: 10 mM Phosphate buffer (pH7.4)
Washing buffer: Equilibration buffer + 0.15 M NaCl
Elution buffer: 10 mM Phosphate buffer (pH7.4) + 1.5 M NaCl
Flow: 0.5 ml/min (90 cm/h, r.t.=1 min)

Fraction	HA Activity	Protein	DNA
Load	100	100	100
F.T.	1	83	57
Elution	83	10	19

Table 1. Relative recovery of egg-derived influenza with Cellufine Sulfate

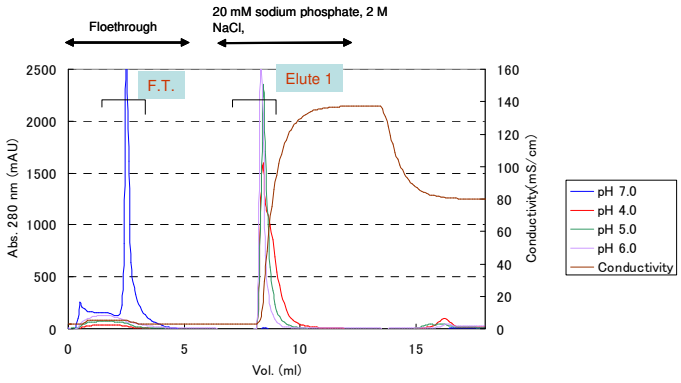
Viral Strains	Loading Condition	Relative adsorption capacity (%)
A strain; Hyogo/YS/2011 (H1N1)pdm	HEPES (pH 7.0)	100
	Allantoic fluid	93
A strain;duck/Hokkaido /Vac-1/2004(H5N1)	Acetate (pH 5.0)	100
	Allantoic fluid	30
A strain; duck/Hokkaido/ Vac-2/2007 (H7N7)	Bicine (pH 8.0)	100
	Allantoic fluid	100
A strain; duck/Mongolia/ 119/2008(H7N9)	MES (pH 5.5)	100
	Allantoic fluid	No adsorption
B strain;Hokkaido /30/1990	Tris (pH 9.0)	100
	Allantoic fluid	85

Table 2. Adsorption study of egg-derived influenza virus to Cellufine Sulfate under optimized pH condition

Purification of Virus Like Particles with Cellufine™ media

	Cellufine Sulfate	Cellufine MAX Butyl (LS)	Cellufine MAX AminoButyl
Type	Affinity	Hydrophobic	Mix mode
Matrix	Cellulose particles	Highly cross linked cellulose particles	
Ligand	Sulfate ester	Butyl (Low substance)	Butyl +Primary Amine
Elution buffer	NaCl	Lower conductivity buffer	Detergent

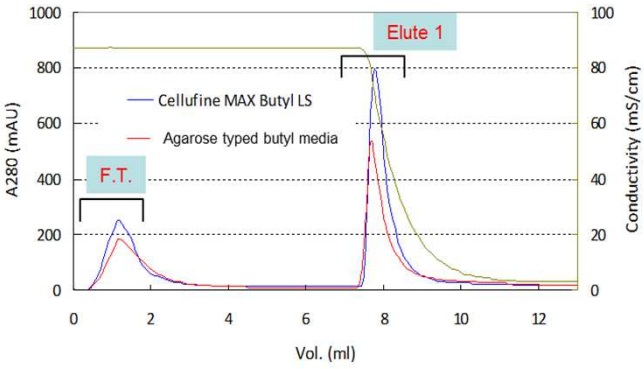
Purification of r-HBsAg with Cellufine™ Sulfate



Run condition
Load, Equilibration and wash: 0.02 M sodium phosphate, pH 6.0-7.0 or 0.05M sodium citrate, pH 4.0-5.0
Elution : 0.02 M PB, 2 M NaCl, pH 7.0
Flow rate: 220 cm/hr

pH	Load	F.T.	Elute 1
4	100	9	68
6	100	26	62
7	100	100	0

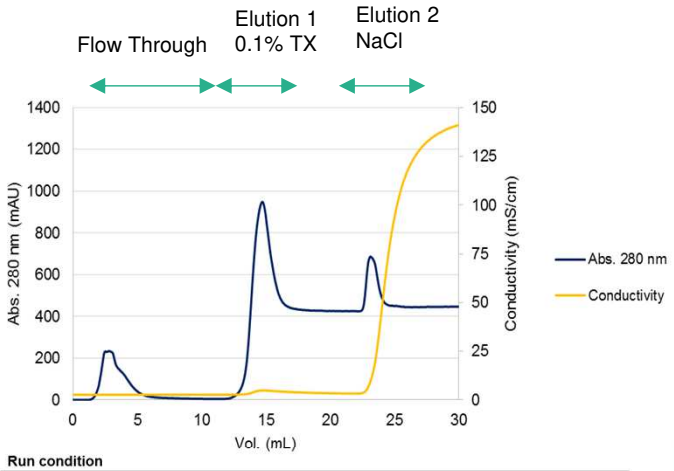
Purification of r-HBsAg with Cellufine™ MAX Butyl LS



Run condition
Load, Equilibration and wash: 0.02 M PB, 0.6M (NH4)2SO4, pH 7.0
Elution 1: 0.02 M PB, pH 7.0
Flow rate: 220 cm/hr

	Load	F.T.	Elute 1
Cellufine MAX Butyl LS	100	29	48
Agarose Typed media	100	26	39

Purification of r-HBsAg with Cellufine™ MAX AminoButyl



Run condition

System	AKTA explorer 10S	Program	
Media	Amino + Butyl	Equilibration	20 mM sodium phosphate, pH 7.0
Column	Φ16 x 500 mm (10 ml)	Wash	20 mM sodium phosphate, pH 7.0 (8CV)
Sample	2 ml of partially purified HBsAg	Elute 1	20 mM sodium phosphate, 0.1%TX-100, pH 7.0 (8CV)
Flow Rate	0.5 ml/min(90 cm R.T. 2 min)	Elute 2	20 mM sodium phosphate, 0.1%TX-100, 2M NaCl, pH 7.0 (8CV)

	VLP		Protein		Nucleic acids	
	nU	%	ug	%	ug	%
Load	4260	100	2320	100	1398	100
Flow through	480	11	350	13	207	15
Elution 1	2060	48	770	30	279	20
Elution 2 (NaCl)	172	4	1190	46	649	46

Cellufine

Cellufine is the liquid chromatography media for the purification of proteins, enzymes and other bio-active substances. Since it is made from spherical cellulose particles having high chemical stability, high mechanical strength and bio-compatibility, it is suitable for the production in pharmaceutical and food industry. Leaking from this matrix is much less than that from the synthetic polymer media.
The production of Cellufine is guaranteed by ISO 9001 and 14000.

ADSORPTION

ION EXCHANGE

DEAE Weak Anion
- Cellufine A-200 90 µm (Ave)
- Cellufine A-500 90 µm (Ave)
- Cellufine A-800 90 µm (Ave)
- Cellufine MAX DEAE 90 µm (Ave)

QA Strong Anion
- Cellufine Q-500 90 µm (Ave)
- Cellufine MAX Q-r 90 µm (Ave)
- Cellufine MAX Q-h 90 µm (Ave)

CM Weak Cation
- Cellufine C-500 90 µm (Ave)
- Cellufine MAX CM 90 µm (Ave)

S Strong Cation
- Cellufine S-500 90 µm (Ave)
- Cellufine MAX S-r 90 µm (Ave)
- Cellufine MAX S-h 90 µm (Ave)

mAb Aggregate removal
- Cellufine MAX GS (Graft S) 90 µm (Ave)

ProA

mAb Capture
- Cellufine SPA-HC 70 µm (Ave)

AFFINITY

Virus & Heparin Binding Proteins
- Cellufine Sulfate 80 µm (Ave)
- Cellufine MAX DexS-HbP 90 µm (Ave)
- Cellufine MAX DexS-VirS 90 µm (Ave)

Endotoxin Removal
- Cellufine ET cleanL 80 µm (Ave)
- Cellufine ET cleanS 90 µm (Ave)

Nucleic Acid Related Molecules
- Cellufine Phosphate 90 µm (Ave)

Activated Supports
- Cellufine Formyl 150 µm (Ave)

HYDROPHOBIC INTERACTION

- Cellufine MAX Phenyl 90 µm (Ave)
- Cellufine MAX Phenyl LS 90 µm (Ave)
- Cellufine MAX Butyl 90 µm (Ave)

MIXED MODE

VLPs
- Cellufine MAX AminoButyl 90 µm (Ave)

mAb Polishing
- Cellufine MAX IB 90 µm (Ave)

PARTITION

GEL FILTRATION

Purification of bio-molecules and proteins by molecular size

MW 50 - 3,000 kDa
- Cellufine GCL-2000HF 90 µm (Ave)

Salt and solvent removal and buffer exchange
- Cellufine GH-25 80 µm (Ave)

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