Affinity Chromatography Media

Cellufine® Formyl

Technical Data Sheet

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**
http://www.jnc-corp.co.jp/fine/en/cellufine/

Introduction

Activated Supports for Immobilization of Antibodies, Antigens, Affinity Ligands and Enzymes

The growth of process-scale affinity chromatography has created the need for a new generation of support matrix materials and coupling chemistries suited for the industrial environment. Classical agarose based supports perform poorly at the large-scale for several reasons. They provide poor flow properties in large columns. The widely used cyanogen bromide coupling chemistry has well-documented problems with bond stability and non-specific adsorption. Additionally, even with more modern chemistries, agarose can shed polysaccharide chains, giving rise to significant ligand leakage under mild operating conditions.

Cellufine activated supports provide state-of-the-art laboratory performance at the process-scale without difficulty. The products are based on rigid spherical cellulose beads specially optimized for affinity chromatography to provide very large pore size and high ligand capacity together with high flow rates in large columns. The cellulose backbone offers very low non-specific adsorption without the ligand leakage problems of agarose.

Features

• High flow rates in laboratory and process columns for high throughput

• Low ligand leakage due to exceptionally stable coupling chemistry and support matrix

• Excellent mechanical, chemical and environmental resistance

• High ligand loading capacity

• Compatible with high molecular weight ligands and target proteins due to pore size equivalency with 4 % cross-linked agarose media

• Unreacted formyl groups easily converted during reduction to neutral hydroxyls for low non-specific adsorption

• Built in hydrophilic spacer arms for maximum ligand accessibility and low non-specific adsorption

• No media damage or fines generation with extended mixing to allow use of simple coupling apparatus

• Ligand coupling occurs under mild conditions in short reaction times

• Thermal stability of media allows high temperature reactions

• Long shelf-life of unreacted media

Characteristics	
Substrate	Crosslinked cellulose
MW Exclusion Limit	4,000kD
Standard Particle Size	125 - 210µm
Particle Shape	Spherical
Density	0.7g/ml wet
Shrinkage / Swelling	Will not shrink or swell substantially under changes in pH or ionic strength
Chemical Resistance	Can be used with any salts, non-ionic detergents, organic solvents. Resistant to 0.1M HCl and 0.5M NaOH. (Note: coupled ligand may not be stable under these conditions)
Mechanical Resistance	Will withstand peristaltic pumping and extended mixing
Autoclavable	121 °C for 30 minutes at pH 7
Saturation Capacity	Up to 40mg protein/ml depending upon protein and conditions
Operating Pressure	< 1 bar (15 psi)

Support	Active Group	Spacer Length (atoms)	Density (µmol/ml)
Formyl	Aldehyde	8	15 - 20

Pressure / Flow Characteristics

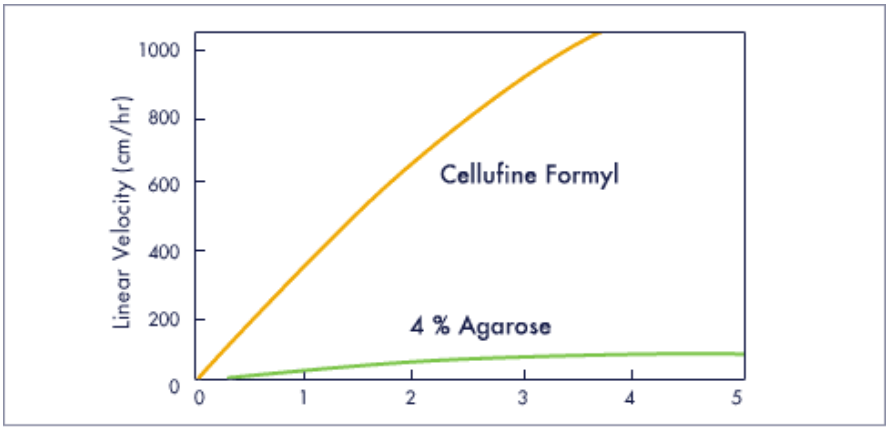

Figure 1
Pressure/flow characteristic of Cellufine Formyl versus 4 % cross-linked agarose gel

Column: 16 x 200mm

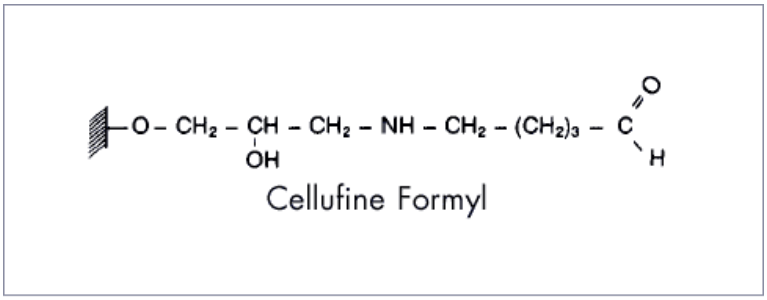

Figure 2
Partial structures of Cellufine activated supports

Applications

Activated Support	Immobilized Molecule	Target Molecule
Cellufine Formyl	Antibodies	Antigens
	Antigens	Antibodies
	Protein A, G	Antibodies
	Lectins	Carbohydrates Glycoproteins
	Cytokines	Receptors
	Enzymes	Substrate/Product
Cellufine Amino	Carboxyl-Containing Proteins and Small Ligands	General Proteins
	Reducing Sugars	Lectins, Receptors
	Heparin	Blood Proteins Growth Factors, Viral Antigens

Table 1
General Applications of Cellufine Activated Supports

Antigen Purification

Figure 3 illustrates the use of Cellufine Formyl coupled with an antibody for largescale antigen purification. In this application the Cellufine affinity column provides a significant concentration and purification of antigen at high yield. The subject column has been used for over 30 months to process over 3,000 liters of starting plasma with no significant degradation in performance.

To produce the gel, 45 liters of horse serum containing anti-HBs Ag antibody were first concentrated and purified by ammonium sulfate precipitation and dialysed into 0.2M phosphate (pH 7) with 0.1 M NaCl. The resulting antibody serum was added to 12 liters of Cellufine Formyl and reacted together with 80 grams of $NaCNBH_3$ at 4 to 8 °C for 24 hours. The antibody gel was then washed with buffer and packed into the column. The process stream consisted of human plasma positive for HBs Ag which had been previously purified by freeze-thawing, centrifugation, ammonium sulfate precipitation and gel filtration chromatography.

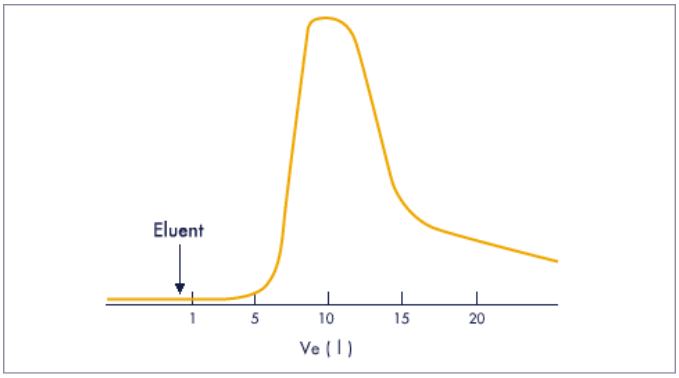

Figure 3
Purification of hepatitis B surface antigen (HBs Ag) with Cellufine Formyl immobilized antibody

Sample:	1200 liters semi-purified HBs Ag-positive human plasma
Column:	140 x 780mm (12 liters) Cellufine Formyl Horse Anti-HBs Ag
Starting/Wash Buffer:	0.1M NaCl, 0.2M phosphate (pH 7) wash volume 200 liters
Eluent:	0.2M glycine/HCl (pH 3)
Flow Rate:	20cm/hr loading/washing 26cm/hr elution
Product Volume:	14 liters (85 x concentration)
Yield:	87 %
Single Step Purification:	149 x

RCA, Purification

Cellufine Formyl can be used to immobilize lectins for glycoprotein purification, as shown in Figure 4. Con A (50mg) was immobilized on 0.5g (wet) of Cellufine Formyl by reacting at 4 °C overnight in 1ml of 0.1M acetate (pH 6.4) containing 1mM $MgCl_2$, 1mM $MnCl_2$ and 1mM $CaCl_2$ under the presence of methyl-alpha-D-mannoside and $NaCNBH_3$. After washing with water, the gel was suspended at 4 °C overnight in 2ml of 1 % glutaraldehyde with $NaCNBH_3$. After a second water wash the gel was suspended for one hour at room temperature in 2ml of 1M Tris/HCl (pH 7.4) and rewashed.

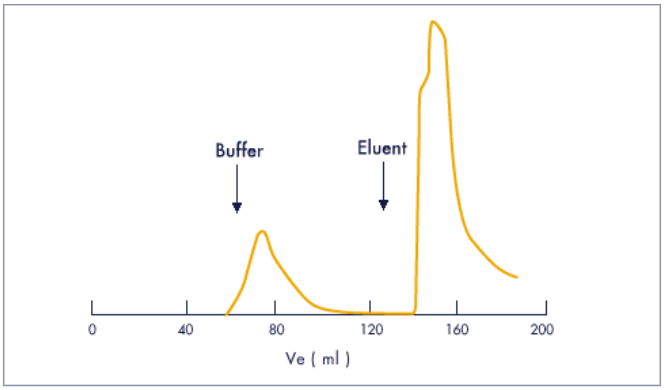

Figure 4
Purification of Ricinus communis agglutinin (RCA_1) on Cellufine Formyl concanavalin A (Con A)

Sample:	66ml RCA_1 (30mg/ml protein)
Column:	0.9 x 9mm (0.6ml) Cellufine Formyl Con A
Starting/Wash Buffer:	0.1M NaCl, 0.2M Phosphate (pH 7.2)
Eluent:	0.2M methyl-alpha-D-mannoside
Flow Rate:	12cm/hr

FUNCTIONAL SELECTION OF ACTIVATED SUPPORTS

There is Cellufine formyl as an activation support of Cellufine. Cellufine formyl is a high-stability, functional packing optimized for an application group. Control of reaction chemistry and ligand density is straightforward.

A GENERAL SUPPORT FOR PROTEINS

Cellufine Formyl

The aldehyde active group on Cellufine Formyl packings reacts with primary amine groups on the ligand to form a Schiff's base complex (see Figure 5). A mild reducing agent is used to convert the Schiff's base to a highly stable linkage. Table 2 illustrates a general ligand coupling protocol.

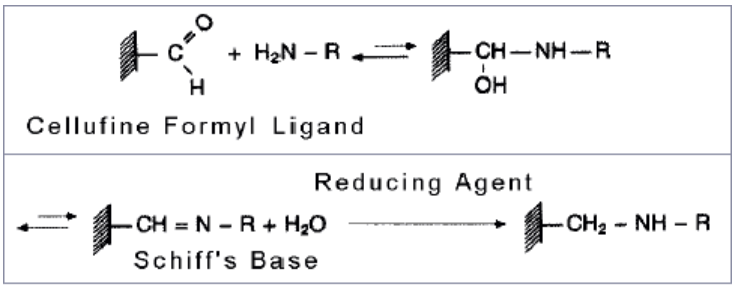

Figure 5
Cellufine Formyl Reaction Mechanism

Reducing Agents

Cellufine Formyl requires a reducing agent for formation of a highly stable linkage. A number of reducing agents are available for good results in virtually any application. The agent should be selected to produce a reasonable reaction rate and yet not be so strong as to damage the protein ligand (such as by reduction of disulfide bonds) or as to reduce the aldehyde groups. Sodium borohydride ($NaBH_4$), sodium cyanoborohydride ($NaCNBH_3$) and a newer, non-toxic reducing agent, trimethylamine borane ($(CH_3)_3NBH_3$) are successful agents, depending upon the particular requirements. For any agent, the quantity required is typically less than 10 milligrams per gram of wet gel.

Coupling with Formyl

The reaction rate of Cellufine Formyl is rapid enough to be practical, yet slow enough to be extremely gentle to most proteins. It also allows for a fine measure of control. The rate may be controlled effectively with temperature to achieve maximum protein stability. The pH of effective couplings ranges between 3 and 10.

The coupling efficiency (the ratio between amount coupled and amount offered) and total ligand density can be varied and optimized quite easily through changes in coupling ligand concentration, pH and temperature. A standard set of conditions will work well for most cases, but optimization over a broad range can be used to improve process economics for specific applications.

1	Wash media with water and filter. Slurry media coupling buffer containing ligand.
2	Stir or shake one-half to two hours.
3	Add reducing agent.
4	Stir or shake 6 to 10 hours.
5	Wash with 0.2M Tris/HCl (pH 7) or 1M ethanolamine in buffer with reducing agent to quench residual aldehydes. Stir or shake 3 to 5 hours.
6	Wash with chromatography elution buffer and then starting buffer.
7	Pack and run column.

Table 2
Typical general protocol used for ligand coupling with Cellufine Formyl

Antibody Purification

Optimization of ligand coupling to activated gels always involves a trade-off between efficiency of uptake (the fraction of ligand offered in the reaction that is actually coupled) and the final ligand loading (mg of ligand coupled per ml of gel). When purified ligand is readily available, a high loading gel can be produced at the cost of low coupling efficiency. In the more common case, however, purified ligand is quite precious, and good coupling efficiency is highly desirable, even at the expense of low loading. Low ligand density may also improve binding specificity in some cases.

The lack of competing hydrolysis reaction in the aldehyde chemistry of Cellufine Formyl makes fine control of the loading and efficiency quite straightforward. In this example, high purity bovine serum albumin is used as an antigen for the purification of rabbit anti-BSA antibody. The coupling reaction was designed to give very high efficiency (98 %) and relatively low ligand density.

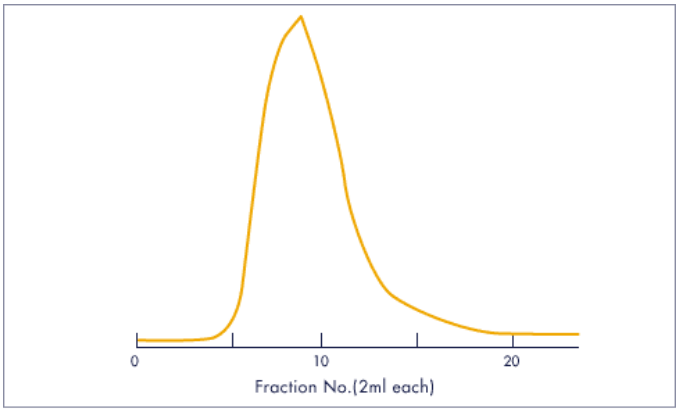

Figure 8
Purification of rabbit anti-bovine serum albumin (BSA) antibody with Cellufine Formyl BSA

Sample:	24ml precipitated rabbit antiserum
Column:	14 x 34mm Cellufine Formyl BSA (5.2ml)
Starting/Wash:	0.05M Phosphate (pH 7.4)
Buffer:	/0.5M NaCl
Eluent:	0.2M Glycine/HCl (pH 2.25)
Flow Rate:	27cm/hr
Yield:	27mg antibody
Single Step	
Purification:	20 x

Cellufine Formyl BSA was prepared by washing 5g (wet) of media with 0.1M phosphate (pH 7.1), adding 5ml of 4mg/ml BSA and stirring for 12 hours at 25 °C. After washing with buffer, the media was suspended in 5ml of buffer containing 0.4M ethanola-mine. After stirring for 4 hours at 25 °C the media was washed with buffer. The BSA coupled was about 3.0 mg/ml media.

Ordering Information

Cellufine Formyl	
Pack Size	Catalogue No.
10 ml	676 944 324
50 ml	19853
500 ml	19854
5 Liters	19855
10 Liters	676 944 335

Contact us

(Technical Support)

(North America & Europe)
JNC America Incorporated
555 Theodore Fremd Avenue, Suite C-206
Rye, NY 10580 USA
TEL: 914-921-5400
FAX: 914-921-8822
E-mail: cellufine@jncamericany.com

(Asia & Others)
JNC Corporation
Life Chemicals Division
2-1, Otemachi 2-Chome, Chiyoda-ku
Tokyo 100-8105 Japan
Tel: +81-3-3243-6150
Fax: +81-3-3243-6219
E-mail: cellufine@jnc-corp.co.jp

(Purchase Orders)

(North America & Europe)
JNC America Incorporated
555 Theodore Fremd Avenue, Suite C-206
Rye, NY 10580 USA
TEL: 914-921-5400
FAX: 914-921-8822
E-mail: cellufine@jncamericany.com

(Asia & Others)
JNC Corporation
Life Chemicals Division
2-1, Otemachi 2-Chome, Chiyoda-ku
Tokyo 100-8105 Japan
Tel: +81-3-3243-6150
Fax: +81-3-3243-6219
E-mail: cellufine@jnc-corp.co.jp