Hydrophobic Interaction Chromatography Media

Cellufine® Butyl
Cellufine® Phenyl

Technical Data Sheet

JNC CORPORATION
Life Chemicals Launch Office

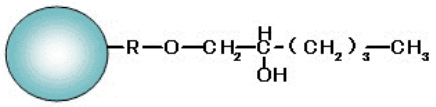
2-1, Otemachi 2-Chome
Chiyoda-ku, Tokyo 100-8105 JAPAN
Phone +81-3-3243-6150
Fax +81-3-3243-6219
e-mail: **cellufine@jnc-corp.co.jp**
Cellufine web: **http://www.jnc-corp.co.jp/fine/en/cellufine/**

Introduction

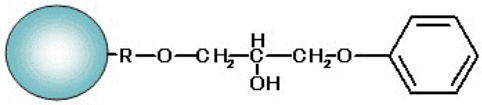
For purification of proteins and macromolecules

Hydrophobic Interaction Chromatography (HIC) is a method which separates proteins on the basis of their differential interactions with a mildly hydrophobic surface.
HIC media are porous chromatography particles, manufactured from crosslinked cellulose to which either a butyl or phenyl functionality has been covalently bonded via a short spacer. Factors which affect hydrophobic interactions include: salt concentrations, temperature, pH, surfactant and organic solvents. Usually the higher the ionic strength (salt concentration) the stronger the hydrophobic bond. Consequently the interaction is enhanced by conditions inverse to that of ion exchange chromatography. HIC is, therefore, an effective complementary tool for separating and purifying substances which are difficult or cannot be separated by ion exchange.

Partial Structure



Butyl



Phenyl

Features

- Spherical particles exhibiting high mechanical strength
- Butyl and Phenyl functionality
- Pre-swollen
- Virtually no shrinkage or swelling
- Stable in organic solvents and surfactants
- Stable coupling chemistry
- Resistant to 0.2 M NaOH
- Autoclavable (121 °C, 20 min)

Benefits

- High flow rates allowing rapid chromatography and direct scale-up
- Enables optimum selectivity to be obtained
- Easy packing
- Easy large scale operation. No shrinkage at high salt concentrations
- Enables range of solvent systems to be utilized
- Resistant to cleaning and elution conditions
- Sterilizable
- Regulatory support

Characteristics	
Support Matrix	Crosslinked Cellulose
Particle Size	ca. 40 – 130 µm
Particle Shape	Spherical beads
Exclusion Limit	4,000 kD
Functional Group	Butyl, Phenyl
Shrinkage/Swelling	Negligible
pH Stability	pH 1 – 13
Environmental Resistance	Resistant to 0.2M NaOH
Operating Pressure	Up to 1 bar (15 psi)
Solvent Resistance	Resistant to detergents, organic solvents, salts
Supplied	Suspension in 20 % Ethanol
Density	1.3 ml/g wet gel
Autoclavable	121 °C, 20 min

Hydrophobicity of Matrix

The degree of hydrophobicity increases in the order of Butyl < Phenyl . In general hydrophobic proteins will be more strongly adsorbed to Cellufine Phenyl than Cellufine Butyl. However, if the protein is too strongly adsorbed, difficulty may be experienced in elution. The aromatic nature of the Cellufine Phenyl may, in certain cases, give improved selectivity compared to either the Butyl matrices. Consequently it is difficult to generalize and each application needs to be evaluated separately to select the optimal media functionality.

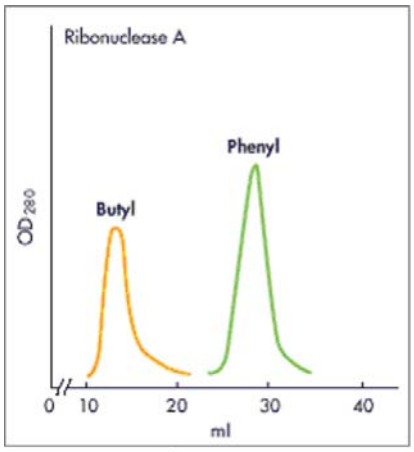


Figure 1
Variation of the Hydrophobic Degree

Column :	8.2 x 150 mm
Column Vol. :	8 ml
Buffer :	2.0 – 0.0M Ammonium Sulfate in 0.01M phosphate, pH 7.0
Flow Rate :	1.32 ml/min
Sample :	5 mg/3 ml– 100 µl

Figure 1. The retention increases with an increase in the carbon chain and aromatic structure of the functional group as a result of stronger hydrophobic interaction.

Flow Properties

The semi-rigid structure of Cellufine HIC, combined with the spherical bead shape, gives excellent flow rates with higher operating pressures. Flow rates in excess of 100 cm/hr are achieved at pressure drops of 1 bar, even in large diameter process columns.

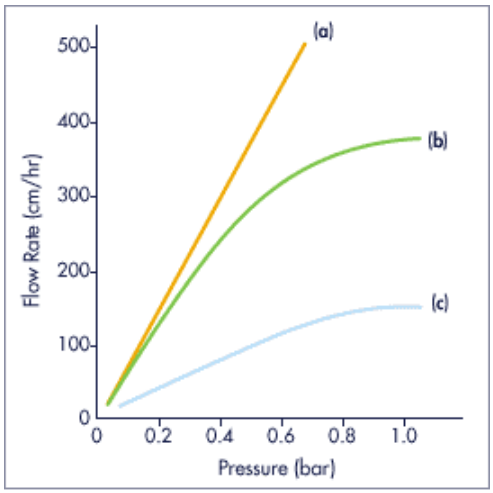


Figure 2
Flow Rate vs. Pressure Drop for Different Column Diameters with HIC Cellufine

Buffer :	0.01M Phosphate buffer (pH 7.0)
Temperature :	23 °C
Columns :	(a)22 x 300 mm (V_c = 0.11 liters) (b)90 x 200 mm (V_c = 1.27 liters) (c)250 x 250 mm (V_c = 12.26 liters)

Adsorption Capacity and Recovery Ratio

	Cellufine Butyl		Cellufine Phenyl	
	Adsorption mg/ml	Recovery %	Adsorption mg/mL	Recovery %
BSA	25	87	30	92
Catalase	42	62	35	71
Myoglobin	19	62	11	63
Glucose Oxidose	38	97	37	99
Ovalbumin	31	87	31	89

Sample concentration : 0.1 %
Adsorption buffer : 0.1M phosphate buffer, pH 7.0 + 2M $(NH_4)_2SO_4$
Recovery buffer : 0.01M phosphate buffer, pH 7.0

Ordering Information

Cellufine Butyl		Cellufine Phenyl	
Pack Size	Catalogue No.	Pack Size	Catalogue No.
Mini-Column 5 x 1ml	19905-51	Mini-Column 5 x 1ml	19900-51
Mini-Column 5 x 5ml	19905-55	Mini-Column 5 x 5ml	19900-55
100 ml	19905	100 ml	19900
500 ml	19906	500 ml	19901
5 Liters	19879	5 Liters	19881
10 Liters	676 973 335	10 Liters	676 975 335

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 Launch Office
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 Launch Office
2-1, Otemachi 2-Chome, Chiyoda-ku
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