CELLULOSE-BASED LARGE-PORE CHROMATOGRAPHY MEDIA FOR PERFUSION CHROMATOGRAPHY OF LARGE BIOMOLECULES AND BIOPARTICLES

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1 Introduction

As the size of emerging modalities, such as viral vectors used in gene therapy, continues to increase, the performance of conventional porous chromatography media with ~ 100 nm pores is increasingly limited by diffusional hindrance. Alternative media, such as monoliths that utilize convective flow, have been developed to overcome these limitations. However, these media still present challenges, including low hydraulic permeability.

JNC has developed a novel permeable spherical bead, Cellufine MLP, which combines cellulose monolith technology with a conventional porous-bead architecture. Cellufine MLP has a bead diameter of 90 µm, a large porosity, and through-pores larger than 2 µm. This internal monolith-like structure enables fast transport of large biomolecules and bioparticles. In this study, we demonstrate that Cellufine MLP provides fast mass transfer despite its large bead size, indicating a dominance of intraparticle convection. Furthermore, the chromatographic behavior can be described using a perfusion model.

MLP Bead structure

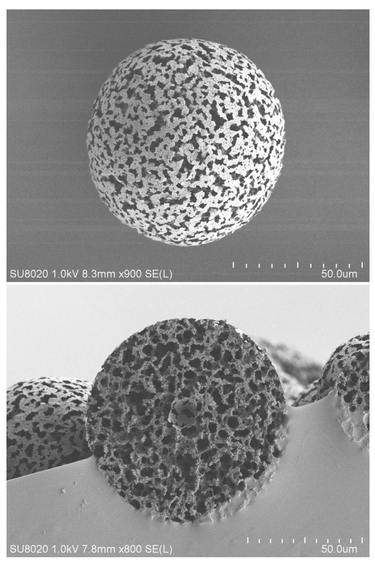
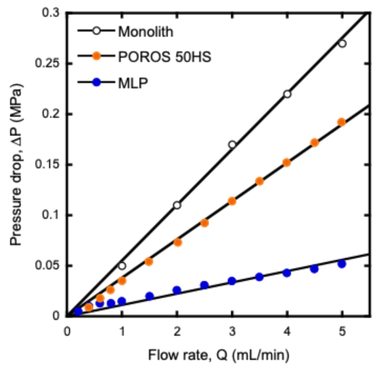


Figure 1. SEM images of MLP bead

Extra-particle porosity; Pressure drop



Intra-particle porosity; iSEC

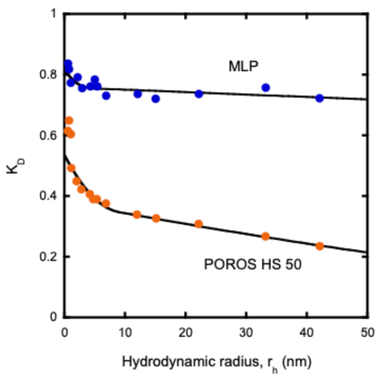


Figure 2. Flow-pressure curves of 0.5 cm x 5 cm packed columns and 0.42 cm height CIM tube monolith (left), Distribution coefficient vs. the hydrodynamic radius of polymer standards from iSEC data (right).

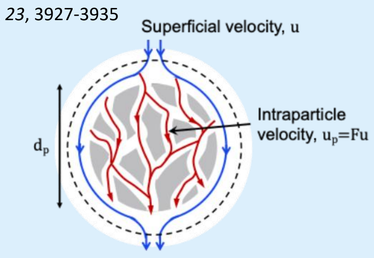
	Monolith	POROS	MLP
d_p (µm)	N/A	43	91
B_0 (mm^2)	0.014	1.0	4.5
ε	0.63	0.33	0.34

*Monolith data from Wu et al. J. Chromatogr A 1147 (2016) 72

	POROS	MLP
ε_M (macro)	0.38	0.76
ε_m (micro)	0.30	0.43
$d_{pore,M}$ (nm)	400	3560
$d_{pore,m}$ (nm)	18	4

Perfusion model

Ref: G. Carta and A. Rodrigues, *Chem. Eng. Sci.*, 48 (1993), *23*, 3927-3935



Convection enhancement factor

$$\frac{\widetilde{D}_e}{D_e} = \frac{(Pe_{intra}/6)}{3}\left[\frac{1}{\tanh Pe_{intra}/6} - \frac{1}{Pe_{intra}/6}\right]^{-1}$$

$$Pe_{intra} = uFd_p/D_e$$

Reduced HETP for non-binding condition

$$h = a + \frac{1}{30}\frac{\varepsilon}{1-\varepsilon}\left(\frac{k'}{1+k'}\right)^2 \frac{\tau_M}{\varepsilon_M K_{D,M}}\frac{D_e}{\widetilde{D}_e}v'$$

$$k' = \frac{1-\varepsilon}{\varepsilon}\left[\varepsilon_M K_{D,M} + (1-\varepsilon_M)\varepsilon_m K_{D,m}\right]$$

For $Pe_{intra} \gg 60$ (Convection dominant)
→ Flow rate-independent HETP

$$\widetilde{D}_e \to \frac{Fud_p}{18} \qquad h \to a + \frac{3}{5}\frac{1}{1-\varepsilon}\left(\frac{k'}{1+k'}\right)^2\frac{1}{F}$$

Rectangular isotherm for DBC analysis

$$\frac{DBC}{EBC} = 1 - \frac{1.03}{\tilde{n}} \quad For\ \tilde{n} > 2.5$$

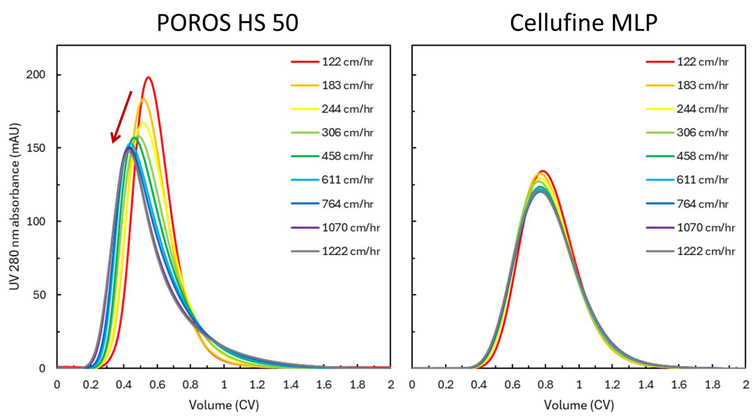
$$\tilde{n} = \frac{60(1-\varepsilon)\widetilde{D}_e}{d_p^2}\frac{L}{u}$$

For $Pe_{intra} \gg 60$ (Convection dominant)
→ Residence time-independent DBC

$$\widetilde{D}_e \to \frac{Fud_p}{18} \qquad \frac{DBC}{EBC} \to 1 - \frac{0.31}{1-\varepsilon}\frac{d_p}{FL}$$

2 HETP Analysis: Non-Binding Condition

IgG (r_h = 5.9 nm)



IgM (r_h = 16.6 nm)

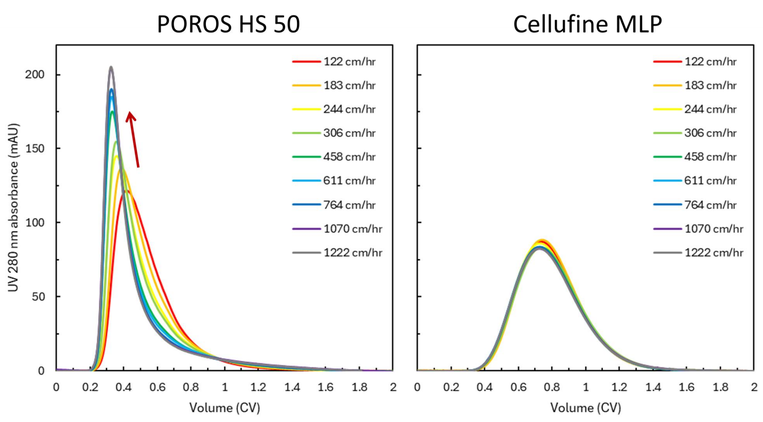


Figure 3. Chromatogram of HETP analysis. Each medium was packed into Tricorn 5/50 chromatography column (0.5 cm ID, 5 cm height). The column was equilibrated with 1 M NaCl, 20 mM sodium phosphate, pH7.0, and 20 µL of protein samples (1.5-2 mg/mL) were injected and eluted with the same buffer at flow rates of 0.2-4 mL/min (60-1220 cm/h).

Comparison with perfusion model

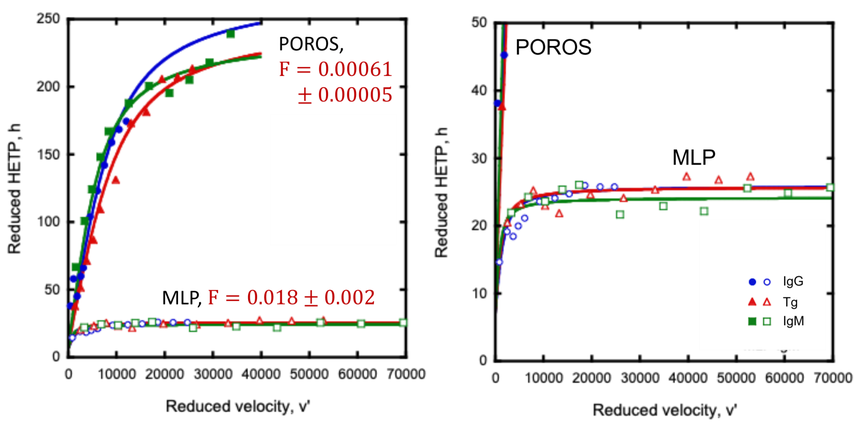


Figure 4. Plots of reduced HETP vs. reduced velocity of each proteins (Symbols: experimental data; Lines: perfusion model). Model parameters are from iSEC measurements except for F and τ_M. Fitted values of F are shown in the graph with τ_M = 2.8±0.4 for POROS and τ_M = 1.2 ±0.2 for MLP.

- A flow-rate-independent peak shape indicates that convective mass transfer dominates, thereby enhancing mass transfer in Cellufine MLP.
- The nearly flat van Deemter curves for Cellufine MLP, with HETP values of approximately 25, suggest a strong contribution from perfusion.

3 Comparison with Perfusion Model: Binding Condition

1. Frontal loading

IgG
$D_e = 1.4 \times 10^{-7}$ cm²/s, F = 0.018

IgM
$D_e = 5.0 \times 10^{-8}$ cm²/s, F = 0.018

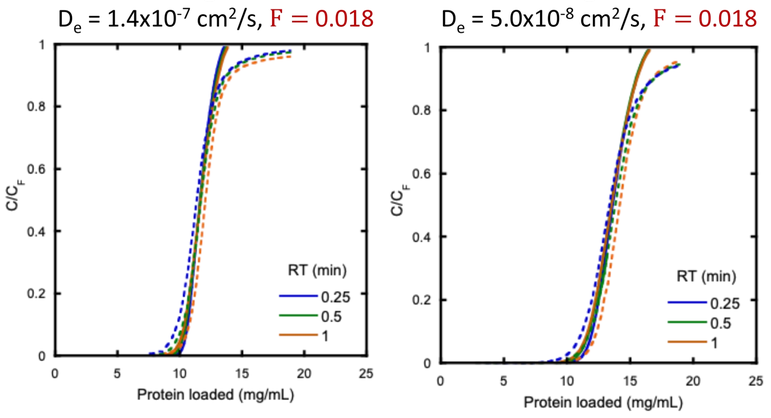


Figure 5. Breakthrough curves of frontal loading (Dashed lines: experimental data for MLP S; Solid lines: perfusion model predictions)

2. Gradient elution (LGE)

A = 3.1×10^{27}, z = 12.3, $D_e = 1.4 \times 10^{-7}$ cm²/s

Varying gradient length
1 min RT, 0-300 mM NaCl

Varying residence time
20 CV, 0-300 mM NaCl

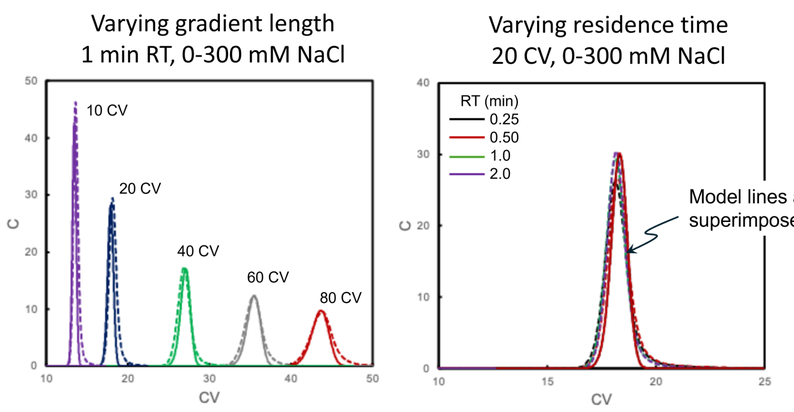


Figure 6. Linear gradient elution of IgG on the MLP S column.(Dashed lines: experimental data for MLP S; Solid lines: perfusion model predictions

Mass transfer modeling

*Carta and Seidel-Morgenstern, *J. Chromatogr. A* 1773 (2026) 466781

Material balance

$$\varepsilon\frac{\partial C}{\partial t} + u\frac{\partial C}{\partial x} + (1-\varepsilon)\frac{\partial \bar{q}}{\partial t} = 0$$

[Discretized by finite differences to simulate axial dispersion]

Rate model

Isocratic and gradient elution*:

$$\frac{\partial \bar{q}}{\partial t} = \frac{15\widetilde{D}_e}{r_p^2}(C - C^*) \qquad C^* = \frac{\bar{q}}{\varepsilon_p K_D + \frac{\varepsilon}{1-\varepsilon}A(C_{Na^+})^{-z}}$$

Frontal loading (R = 0):

$$\frac{\partial \bar{q}}{\partial t} = \frac{3\widetilde{D}_e}{r_p^2}\frac{C}{(1-\bar{q}/q_m)^{-1/3} - 1}$$

- The flow rate-independent breakthrough curves and gradient elution peaks were obtained.
- The perfusion model prediction showed good agreement with experimental results.

4 Conclusion

- ✓ Cellufine MLP having large continuous pore structures was designed for chromatography of large biomolecules and bioparticles
- ✓ Convection dominated transport in MLP beads indicates the potential for high-speed separation of large molecules

C. Mori, G. Carta, *J. Chromatogr. A* 1785 (2026) 467295

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