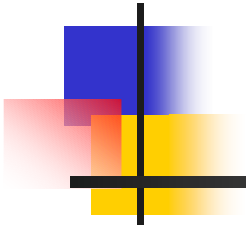


**Optimization on Pore Size of
C8-Bonded Silica Reversed-Phase for
Insulin Purification**





DAISOGEL C8-Modified Phases

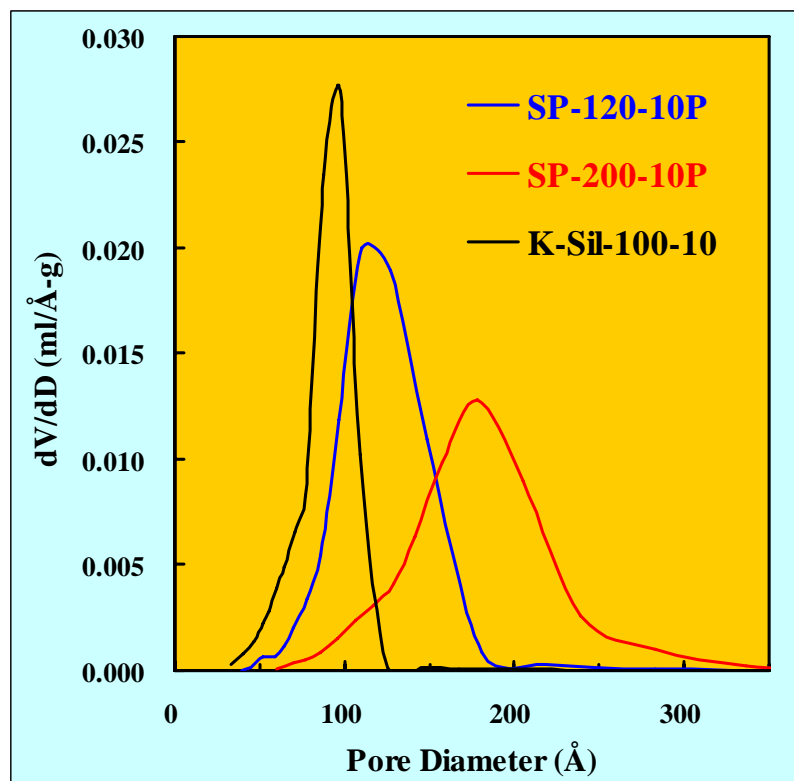
Designed for Insulin Purification

- **200 Å Enlarged Pore Diameter**
Suitable for Medium-Weight Molecules, e.g. Insulin
- **Available in 10-20 μm Spherical Particles**
having Ultra-High Purity
High Resolution and Excellent Peak Shape
Applicable for Preparative Purification
- **High Surface Bonding Density (C8 > 3.5 μmol/m²)**
Combined with New Endcapping Technology (Patented)
Improvement in Phase Stability *against*
Extreme Acidic and Alkalic Environments

Bare Silica Gel Matrixes

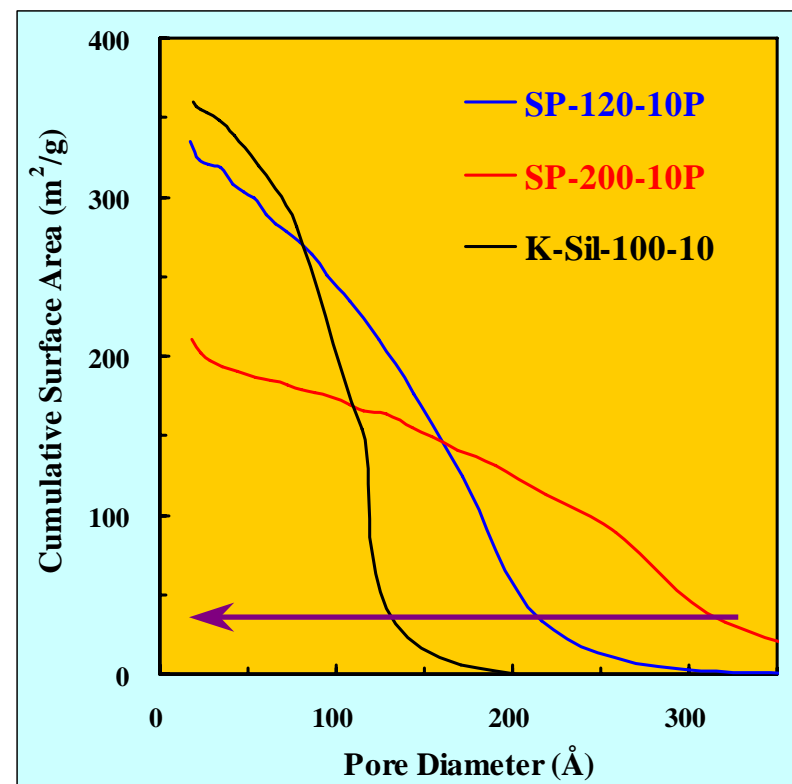
Pore Distribution Region Comparison

Differential Pore Volume



Incremental pore volume was estimated according to B.J.H. method from desorption branch of N₂-physisorption isotherm at 77K.

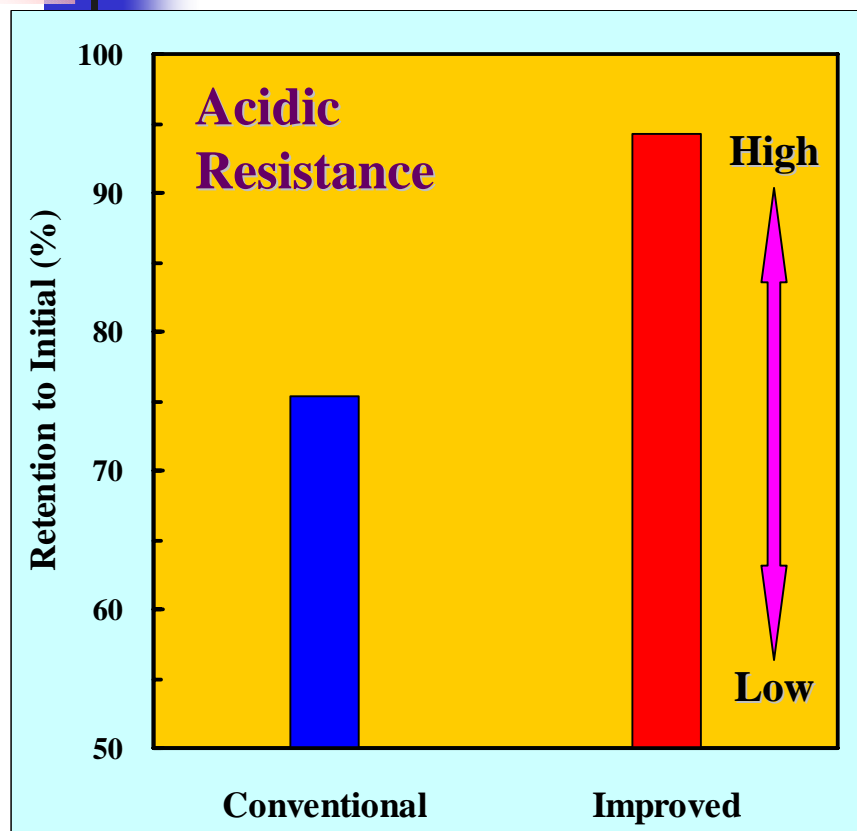
Cumulative Surface Area



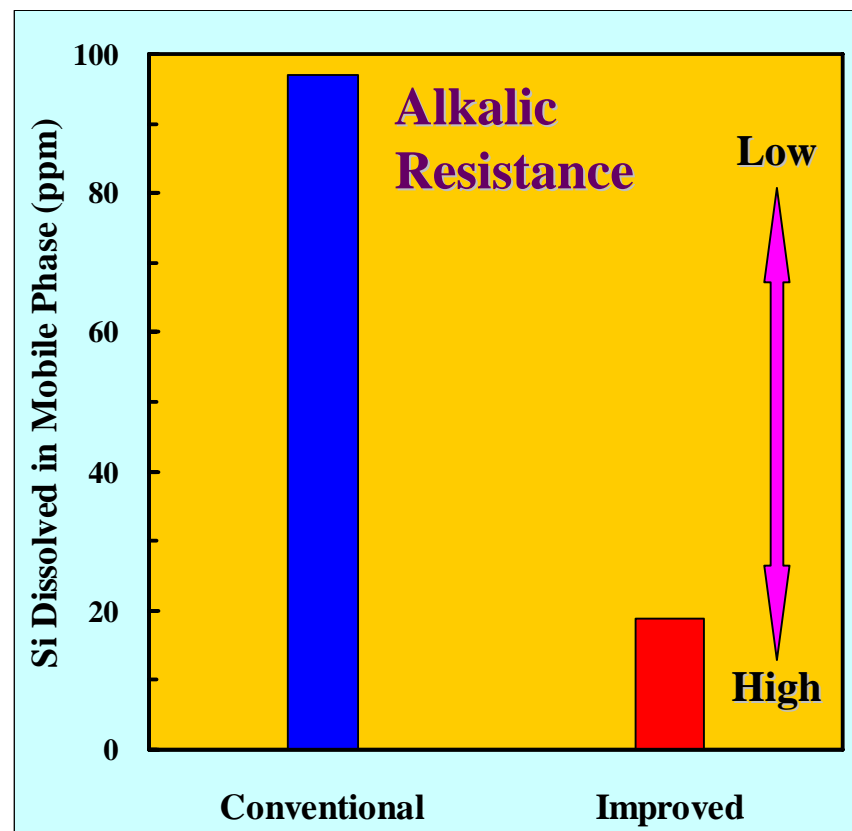
Cumulative surface area was estimated according to B.J.H. method from adsorption branch of N₂-physisorption isotherm at 77K.

DAISOGEL SP-200-10-C8-BIO

Improvement in Phase Stability



Column Dimension: 6 mm I.D. x 250 mm Length;
 Mobile Phase: CH₃CN/1% TFA (pH=1) = 10/90;
 Temperature: 70°C; Flow: 0.9 ml/min; Time for Purge: 20 h.



Column Dimension: 6 mm I.D. x 250 mm Length;
 Mobile Phase: CH₃CN/20mM Na₃PO₄-NaOH (pH=12) = 10/90;
 Temperature: 40°C; Flow: 1.7 ml/min; Time for Purge: 5 h.



Loading Capacity Evaluation

Column Dimension: 4.6 mm I.D. x 250 mm Length

● **Adsorption**

Equilibration: 0.5% TFA *aq.*;

Solute: Human Insulin (Recombinant, Wako, Japan);

Concentration: 10 mg/ml;

Feed: Solute Dissolved in 0.5% TFA *aq.*;

Flow Rate: 0.5 ml/min; Temperature: 30°C; Detector: UV 290 nm.

● **Desorption/Elution**

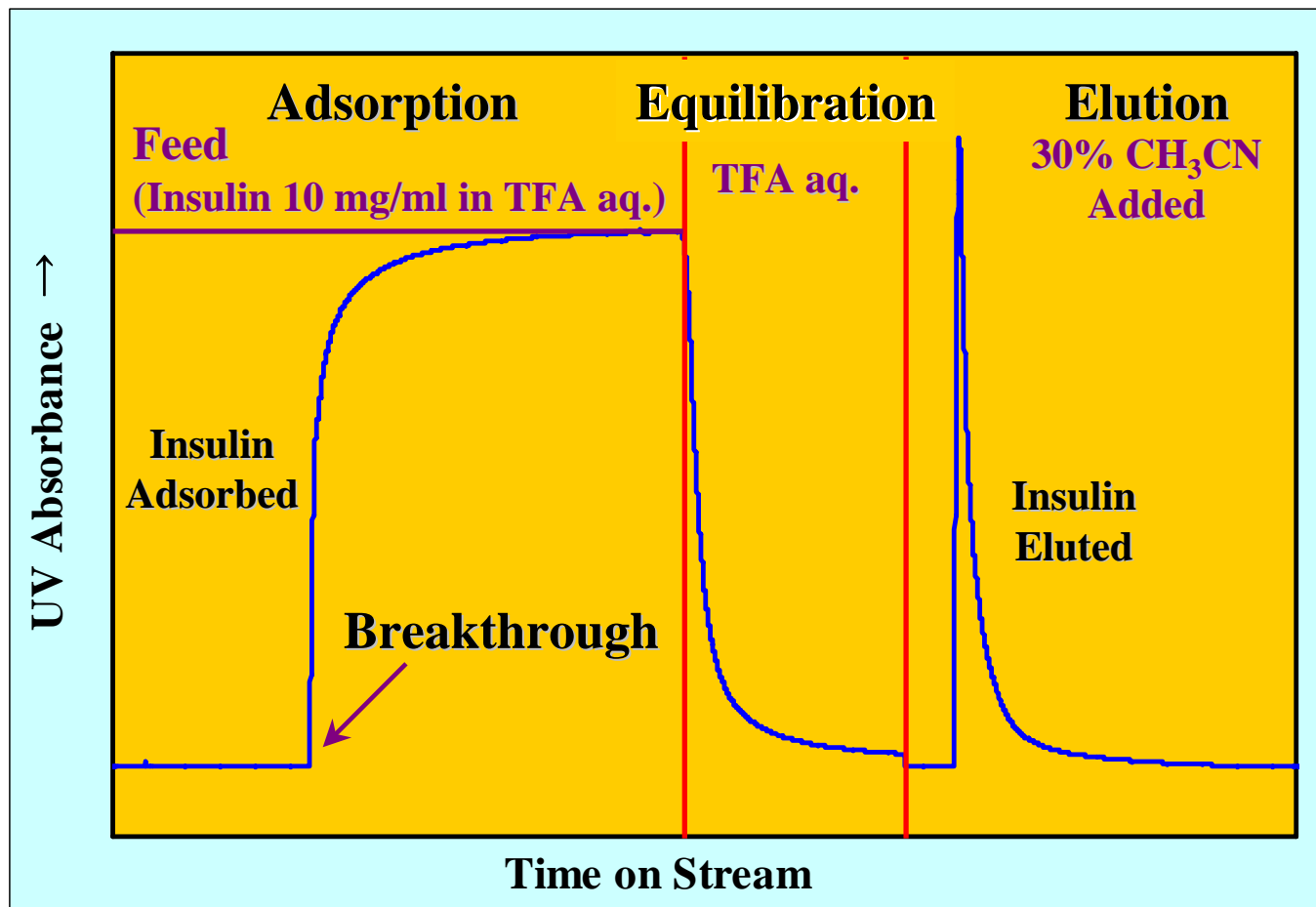
Equilibration: 0.5% TFA *aq.*;

Elution: CH₃CN/H₂O/TFA = 30/70/0.1;

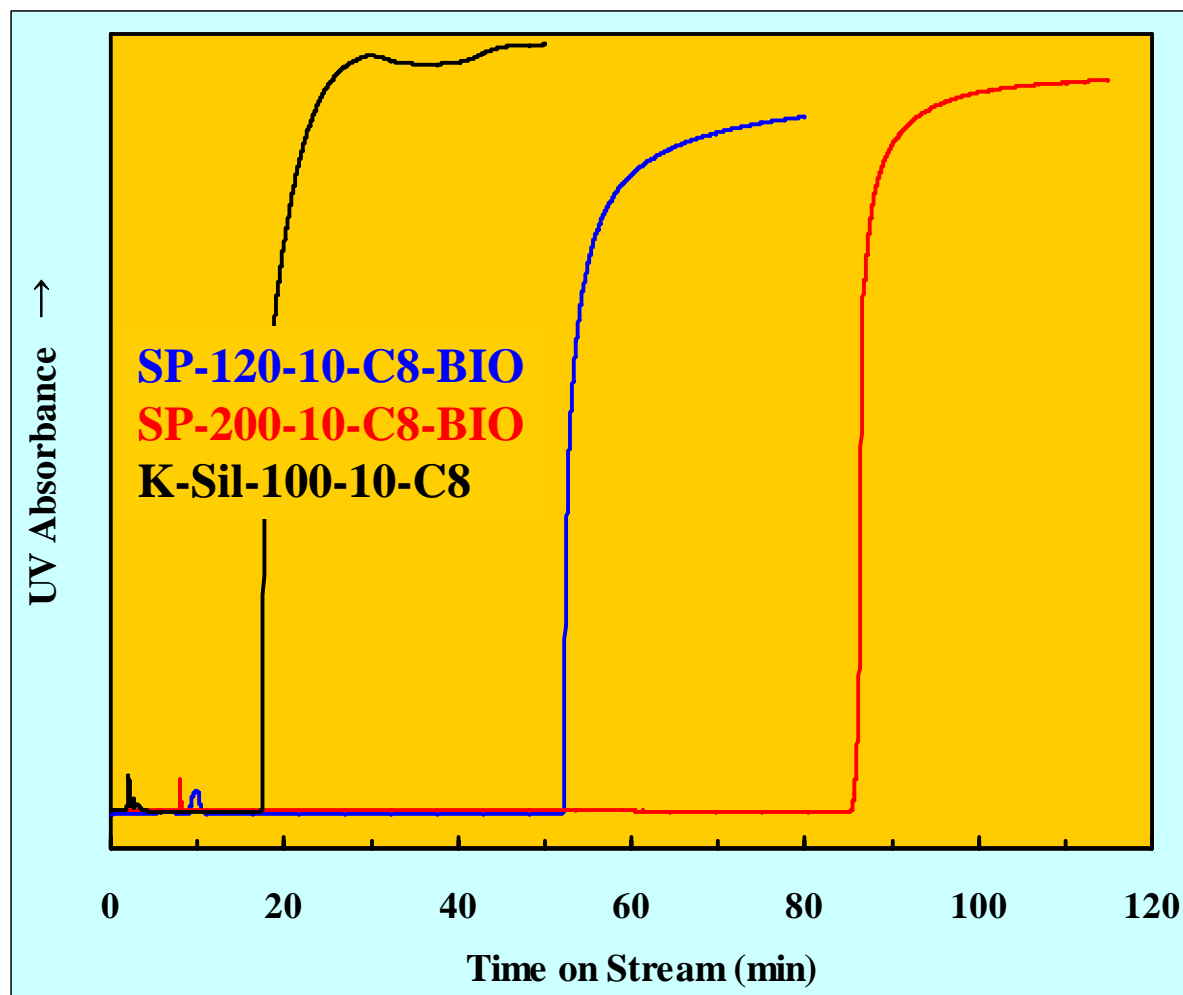
Flow Rate: 0.5 ml/min; Temperature: 30°C; Detector: UV 300 nm.

Loading Capacity Evaluation

Conceptional Illustration of Theoretical Adsorption-Elution Isotherm

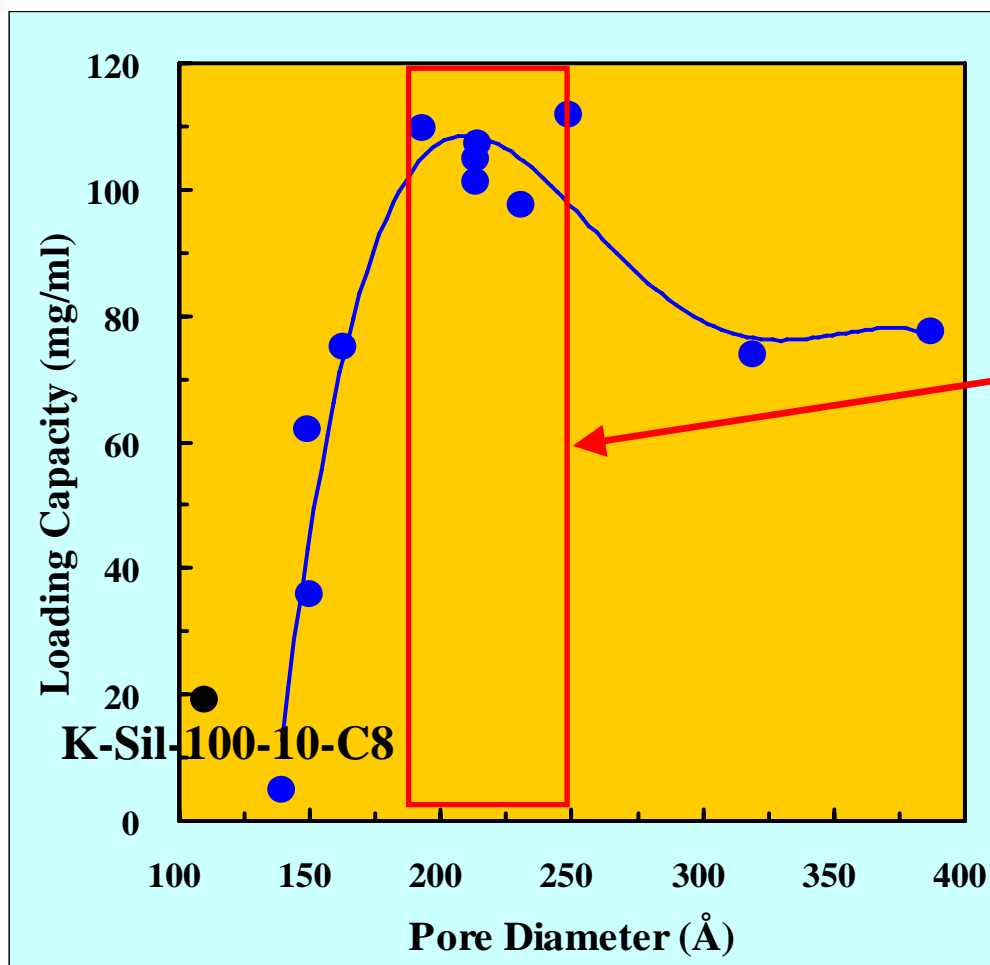


Loading Capacity of Insulin Breakthrough Curve Comparison



Loading Capacity of Insulin

Expanded Pore Diameter Correlation



**Optimum
Pore Range
for
Insulin Molecule
19 - 25 nm**

**Loading Capacity
= $F \times C_0 \times T_b / CV$**

F: Flow Rate;

C_0 : Concentration;

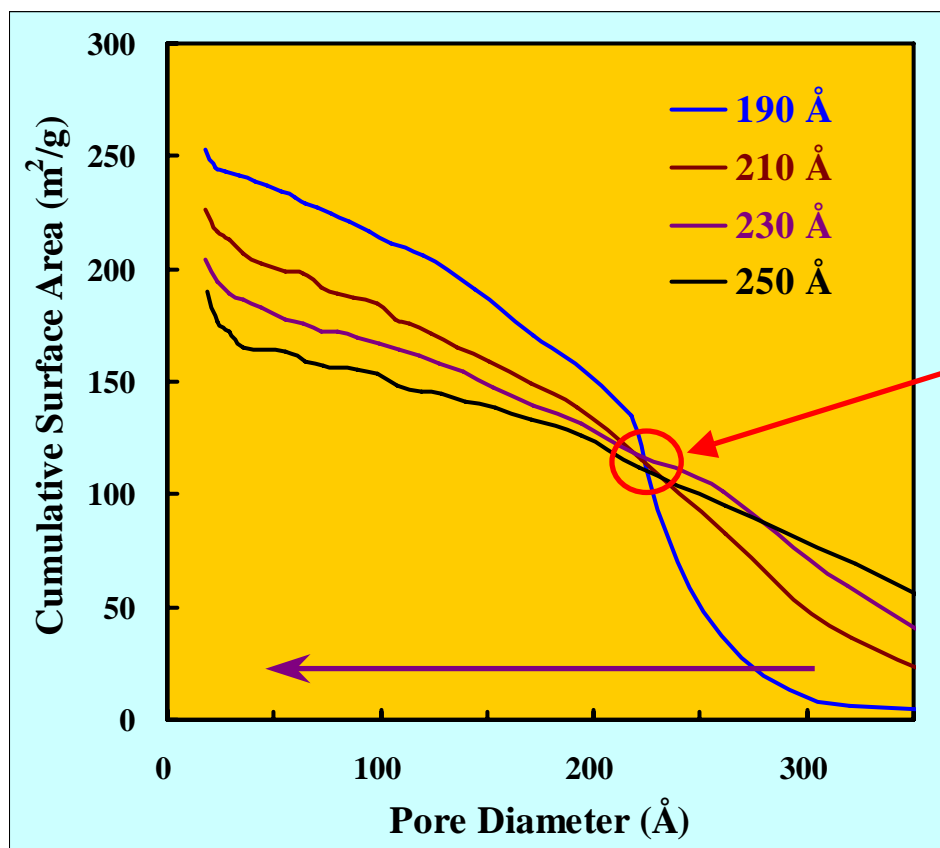
T_b : Breakthrough Time;

CV: Column Volume.

Loading Capacity of Insulin

Detailed Pore Distribution Comparison

Cumulative Surface Area



Effective Pore Diameter for
Insulin Diffusion

22 - 23 nm

Dynamic
Molecular Dimension
of Insulin

Cumulative surface area was estimated according to B.J.H. method from adsorption branch of N₂-physorption isotherm at 77K.



Alkaline Durability Evaluation

Column Dimension: 4.6 mm I.D. x 250 mm Length

- **Alkalic Durability Evaluation**

Mobile Phase: Ethanol/0.1 N NaOH *aq.* (pH=13) = 70/30;

Flow Rate: 2.0 ml/min; Temperature: ambient.

Elution was collected every 50 CV and Si dissolved was analyzed by ICP.

- **Chromatographic Evaluation after Alkali Purged**

Aromatic Standards

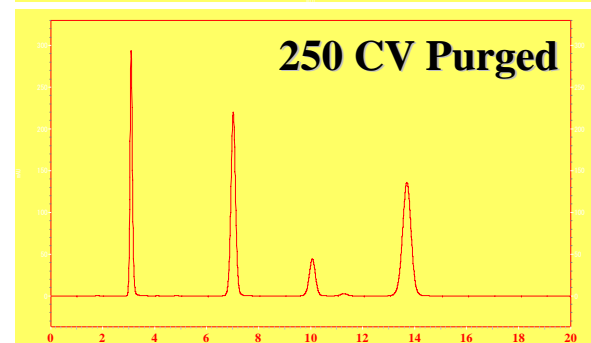
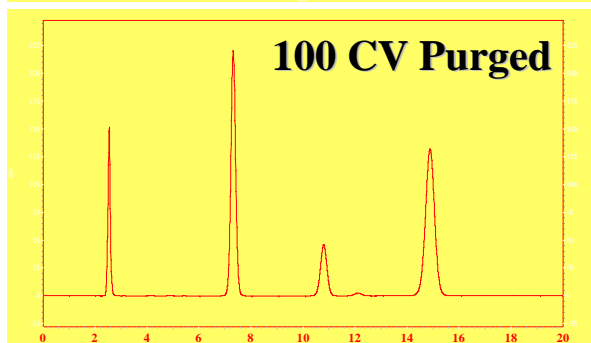
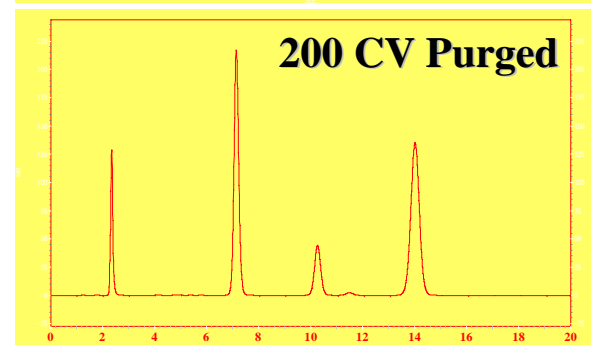
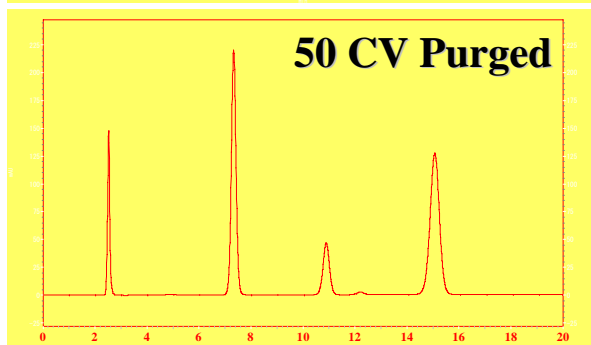
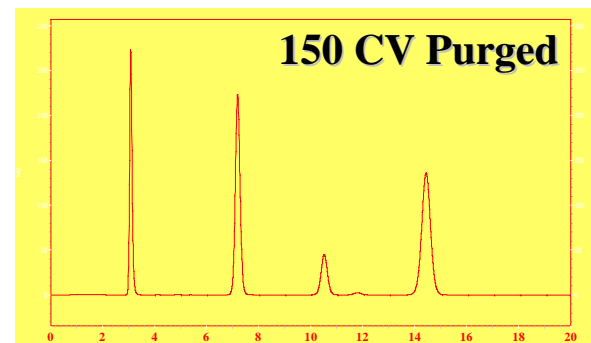
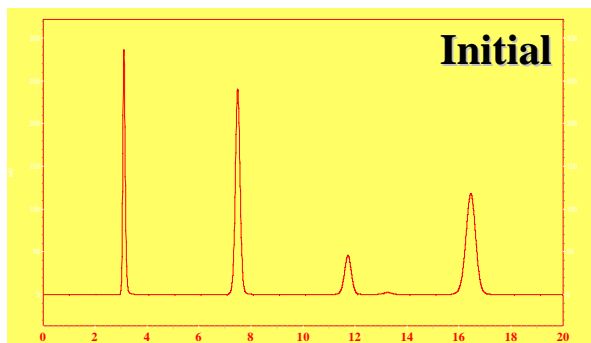
Mobile Phase: CH₃OH/H₂O = 60/40 (100 Å) or 55/45 (200 Å)

Flow Rate: 1.0 ml/min; Temperature: 40°C; Detector: UV 254 nm;

Analyst: 1. Uracil, 2. Methyl Benzoate, 3. Toluene, 4. Naphthalene.

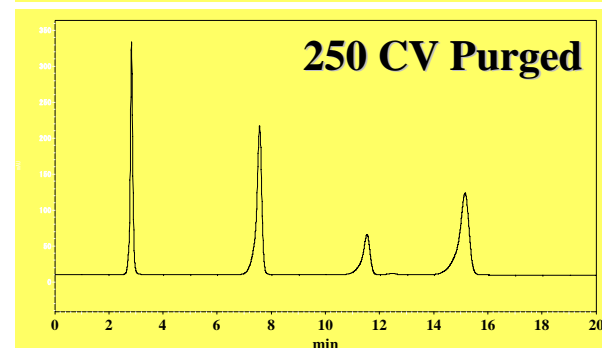
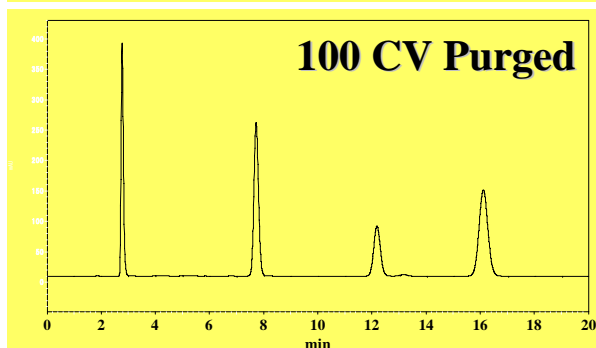
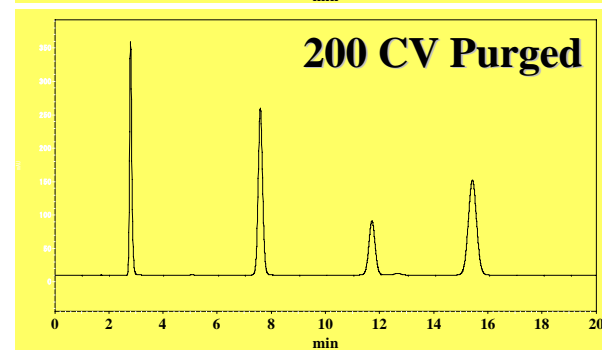
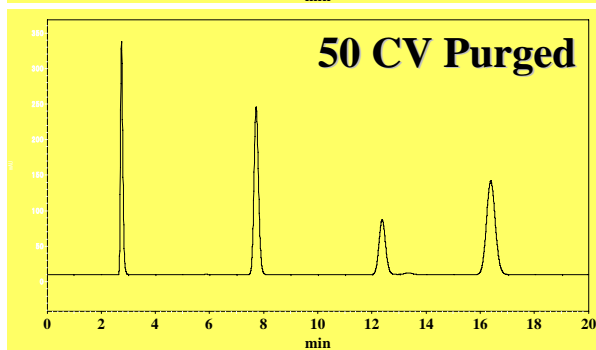
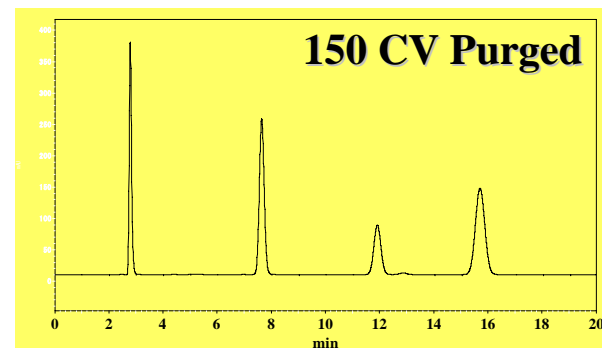
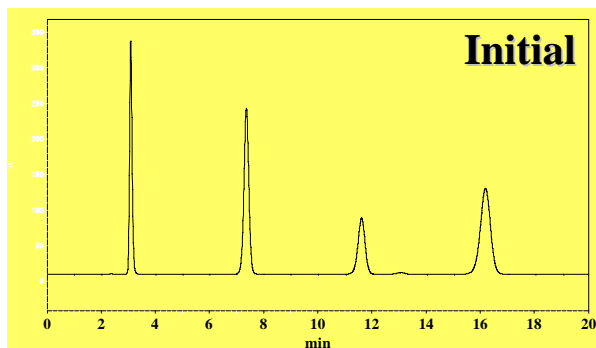
Alkaline Durability (pH=13)

SP-200-10-C8-BIO for Aromatic Standards



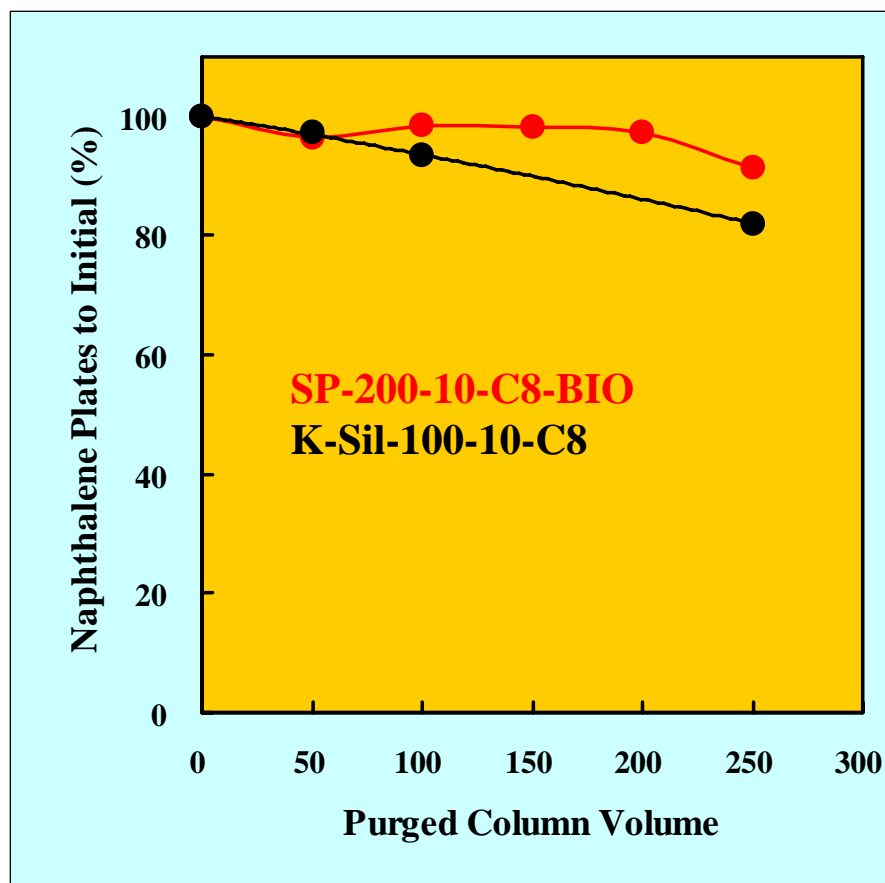
Alkaline Durability (pH=13)

K-Sil-100-10-C8 for Aromatic Standards

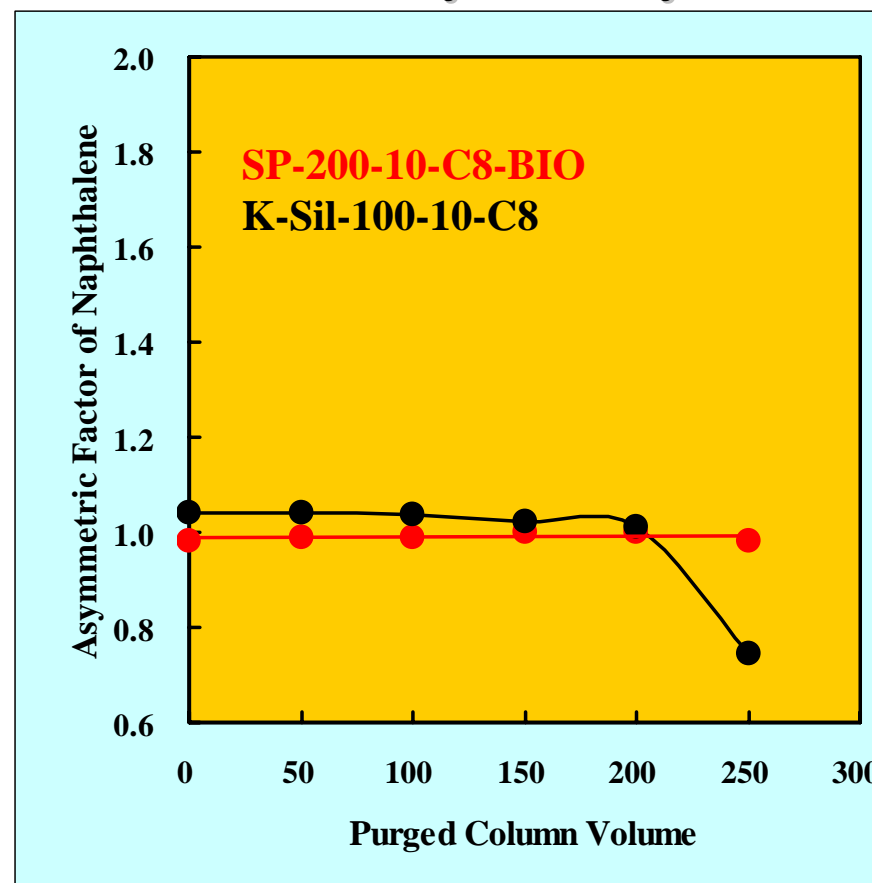


Alkaline Durability (pH=13) Comparison for Aromatic Standards

Theoretical Plates

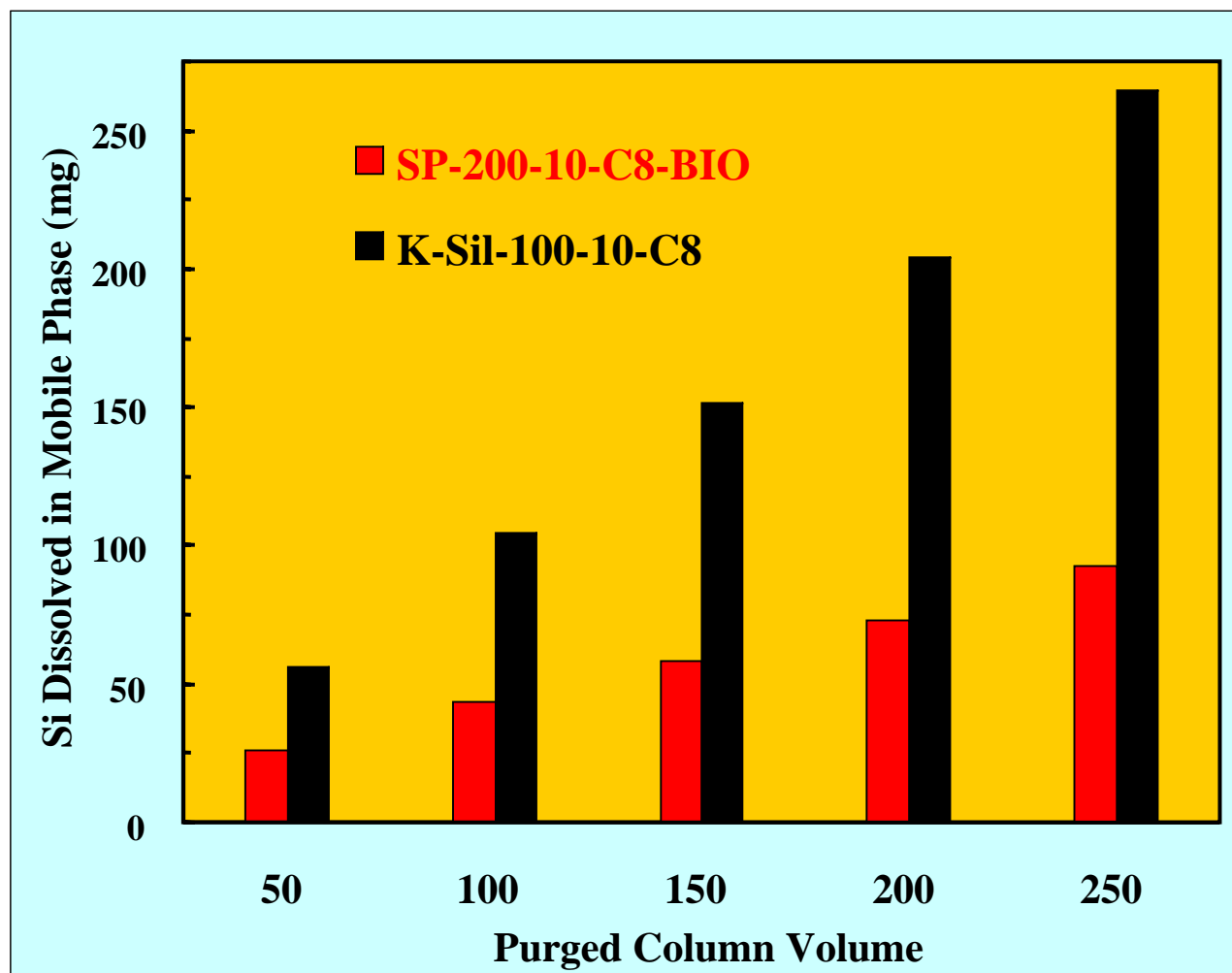


Peak Asymmetry



Alkaline Durability (pH=13)

Comparison of Si Dissolved in Mobile Phase





Summary

- **SP-200-C8-BIO** exhibited the highest loading capacity for insulin estimated adsorption breakthrough point.
- The optimum pore size for insulin molecular diffusion seems to be **19 - 25 nm**.
- **SP-200-C8-BIO** also exhibited better durability in alkaline (pH=13) environment even compared with K-Sil-100-10-C8.
- **SP-200-C8-BIO** seems to be **the best packing material** for insulin purification.