



Monodispersed Hybrid Silica- UniHybrid[®] Eterne

Longer lifetime; higher pH stability

Introduction

Silica reversed phase chromatography (RPC) is a popular purification tool for peptide-based therapeutics such as insulins and GLP-1 compounds. In many cases, high pH conditions are desirable in these chromatography process such as column regeneration by 0.1M NaOH solution, where conventional silica media suffers short lifetime. Hybrid silica particles are well known for high pH stability, but they are less than 10 μm in size and only used in analytical HPLC applications. There are no commercially available hybrid silica particles in 10 μm and larger sizes yet for process scale chromatography in the world, presumably due to low surface area of such hybrid particles, high manufacturing cost, and technical difficulties of their large scale production. Other hybrid coated silica particles have improved lifetime over silica particles at high pH, but still are not as stable as hybrid particles.

NanoMicro Technology is one of the few companies to commercialize monodispersed silica bulk media for separation and purification process. Our innovative Precisely Controlled Silica (PCS) technology allows us to manufacture monodispersed silica particles in large quantity and low cost (several hundred kilograms a batch with almost 100% yield). Based on the PCS technology, we have recently developed monodispersed hybrid silica - UniHybrid® Eterne particles in 10 μm size, which have very high surface area, good mechanical strength, and more than 10 times of chemical stability than silica particles, to meet the high demands of RPC media with high performance and high pH tolerance .

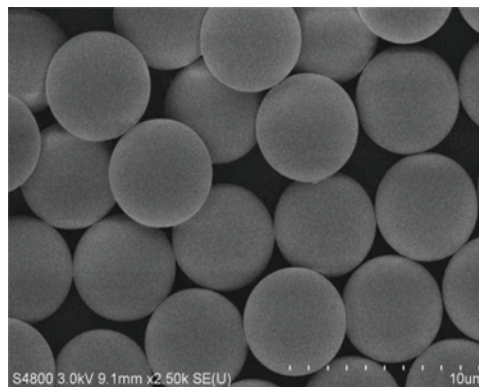


Figure 1. UniHybrid® Eterne SEM

Features of UniHybrid® Eterne



Monodispersed particles



Excellent high pH stability



Long chemical lifetime



Different selectivity at high pH condition

Characteristics

Table 1. UniHybrid® Eterne parameters

| Particle size (μm) | Pore size (\AA) | Surface area (m^2/g) | Pore volume (ml/g) | Stationary phase | Max. pressure (bar) | pH range |
|---------------------------------|----------------------------|--|-------------------------------|------------------|---------------------|----------|
| 10 | 120 | 320 | 1.20 | C18/C8/C4 | 600 | 2-13 |

High pH stability test

UniHybrid® Eterne 10-120 C18 exhibits excellent high pH stability. Naphthalene retention time dropped only ~8% and efficiency unchanged after pH 13 flash for 73 hours (Figure 2). UniHybrid® Eterne C18 maintained good peak shape, efficiency and retention after 73 hours of pH 13 flash (Figure 4) while other competitor's silica based C18 degraded badly after 14 hours of pH 13 flash (Figure 3).

UniHybrid® Eterne 10-120 C18 in 0.1M NaOH flash

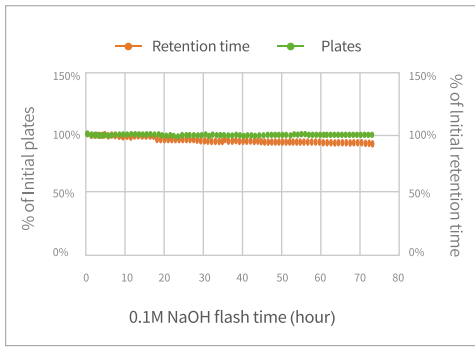


Figure 2. Retention and efficiency changes of UniHybrid® Eterne 10-120 C18 in pH 13 (0.1M NaOH) flash condition

Other competitor's silica based C18

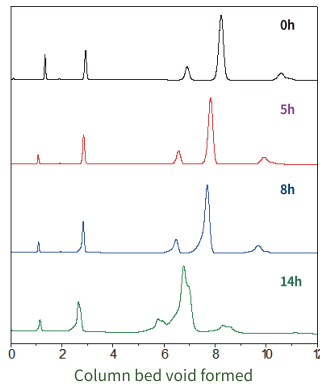


Figure 3. Chromatograms of other competitor's silica based C18 in pH 13 flash condition

UniHybrid® Eterne 10-120 C18

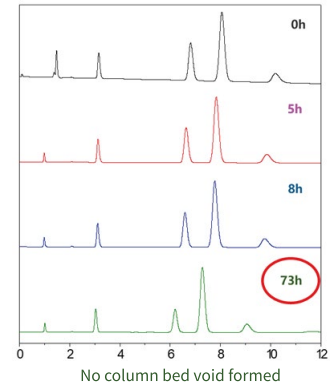


Figure 4. Chromatograms of UniHybrid® Eterne C18 in pH 13 flash condition

Mechanical strength test

UniHybrid® Eterne 10-120 C18 has similar back pressure compared to other silica particles. Figure 5 shows a linear relationship of back pressure of a 2.1 x 150 mm column with flow rate, indicating the particles are strong enough up to 9000 psi (600 bar). UniHybrid® Eterne 10-120 C18 particles were also repeatedly unpacked and reloaded in a 100 x 250 mm DAC column for 20 times without showing the pressure change (Figure 6).

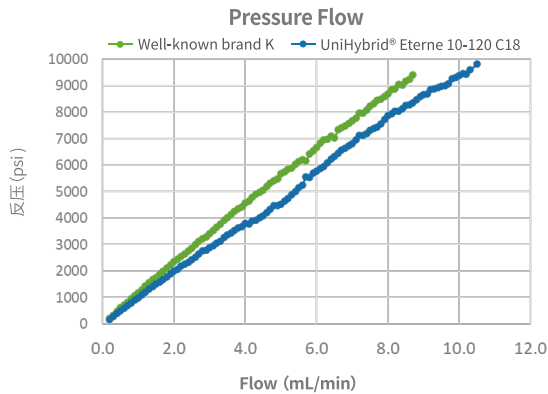


Figure 5. Back pressure vs. flow rate (2.1 mm x 150 mm; in ethanol mobile phase)

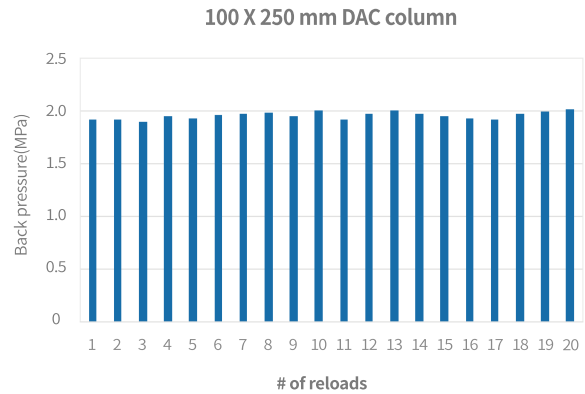


Figure 6. The pressure changes of repeated loading and unpacking of 100 x 250 mm DAC column

Application – Insulin purification

Compared to other competitor's silica particles, UniHybrid® Eterne 10-120 C18/C8 shows similar peak width, but better selectivity and resolution for front and back impurities for insulin purification (Figure 7).

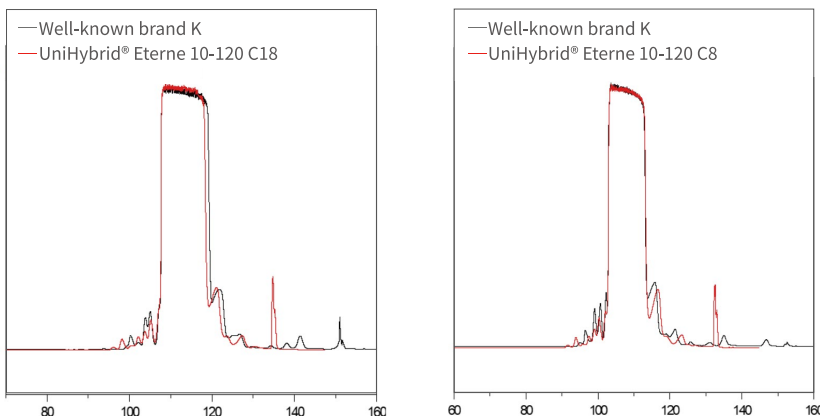


Figure 7. Comparison of UniHybrid® Eterne 10-120 C18/C8 and other competitor's silica C18

Purification condition

Column: 4.6 mm x 250 mm

Mobile phase: A: buffer solution; B: ACN

Flow: 0.42 mL/min

Sample loading: 14 mg/mL-CV

Application – Synthetic semaglutide purification

Semaglutide purification is always challenging among GLP-1 drugs. With UniHybrid® Eterne, NanoMicro is able to provide a better total solution for semaglutide purification.

Strategy: A two-step purification process of semaglutide

- 1st: Purification of semaglutide under basic condition using UniHybrid® Eterne C8;
 - 1st purification sample is usually dirty which requires more 0.1M NaOH washings/regenerations;
 - UniHybrid® Eterne C8 provides a higher yield under basic condition and excellent chemical lifetime for 0.1M NaOH.
- 2nd: Purification under acidic pH 3.5 condition using UniSil® Revo C8.
 - 2nd purification sample is clean which requires less 0.1M NaOH regenerations;
 - UniSil® Revo C8 provides a higher purity.

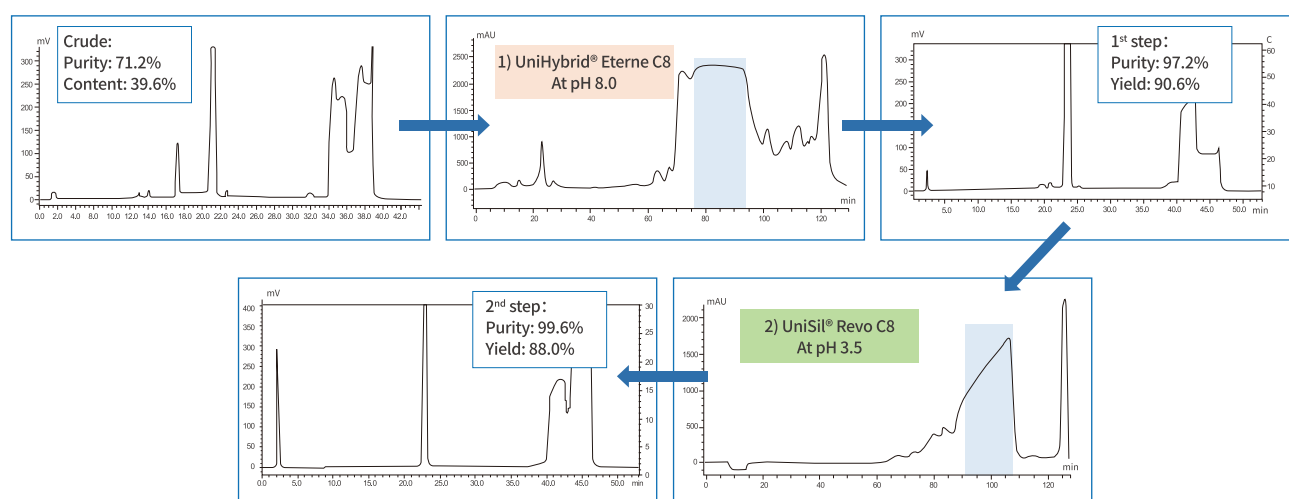


Figure 8. Fully synthetic semaglutide purification flow chart

Ordering information

| Product | Catalog # | Package size |
|------------------------------|--------------|--|
| UniHybrid® Eterne 10-120 C18 | 19801-100012 | 30 g, 50 g, 100 g, 300 g, 500 g, 1 kg, 5 kg, 10 kg, 50 kg, 100 kg |
| UniHybrid® Eterne 10-120 C8 | 19802-100012 | |
| UniHybrid® Eterne 10-120 C4 | 19803-100012 | |

email: info@nanomicrotech.com

Website: en.nanomicrotech.com

United States
NanoMicro Technologies Ins.

Phone: +1-(508) 338-3051
Address: 400 W Cummings Park, Suite
5000, Woburn, MA 01801

India
Suzhou NanoMicro Tech India Private Ltd.

Phone: +91 96548 80558
Address: 1033, Tower A, Ithum Building,
plot-A40 Noida Sector 62, Gsutam Buddha
Nagar Uttar Pradesh 201301, INDIA

China
Suzhou NanoMicro Technology Co. Ltd.

Phone: 400-828-1622
Address: No. 2, Baichuan street, Suzhou
Industrial Park