Development of an analytical reversed phase column for characterization of intact antibodies using wide pore monolithic silica.

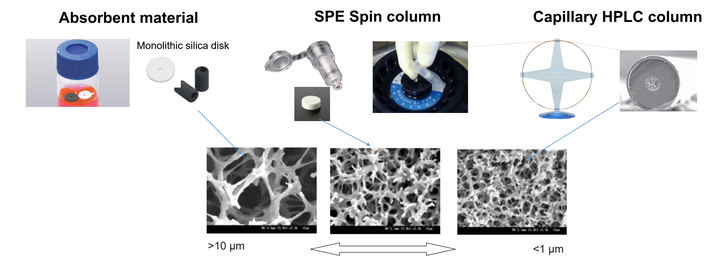
Shigenori Ota, Yuko Yui, Shota Miyazaki, Chiaki Aoyama, Ken Miyashita, Manami Takeda, Shunta Futagami, Tsutomu Sato
GL Sciences Inc.

Introduction

The production and purification of monoclonal antibodies (mAb), comprehensive characterization can be performed at the intact protein on a mass spectrometry. Reversed phase columns with a wide pore (>30nm) particle are used for intact protein chromatography analysis. However, analytical column packed wide pore show low pressure resistance due to the physicochemical durability of support material.

Monolithic silica have been widely studied due to their unique structures that have many advantages as separation media(Figure). Monolith structure and pore size were easily controlled by starting material. In this study, we have developed the monolithic silica column with wide pore size suitable for the intact protein analysis. The performance of the developed column was compared with columns packed with silica particles.

Monolithic silica application fields



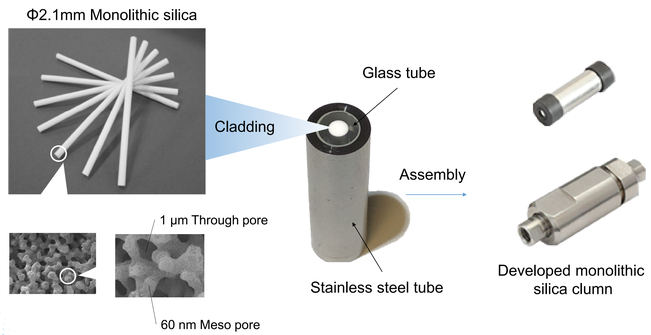
The pores can be sized according to application, resulting in a highly adaptive material for chromatography.

Methods

Monolithic silica gel with an inner diameter of 2.1 mm was prepared via sol-gel processing of tetraethoxysilane. Then, monolithic silica was treated with alkali solution to prepare wide pore size (60nm). The resulting monolithic silica was cladded with a glass tube. After cutting it to an appropriate length, monolithic silica surface was modified with the phenyl group or the C4 group. Reversed phase modified monolithic silica column in this study was fabricated by sealing the cladded silica in a stainless steel tube.

Intact protein (mAb) analysis were performed on a HPLC-UV and TripleTOF 6600 mass spectrometer (Sciex).

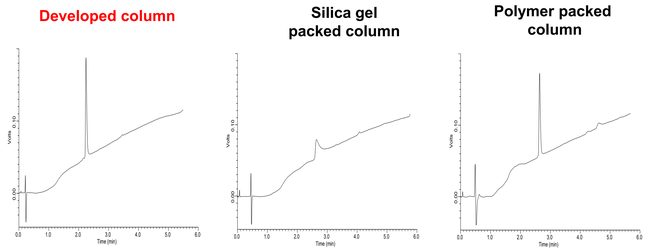
Schematic of the developed monolithic silica column



Results & Discussion

Column performance of the developed column with competition.

The recoveries of intact mAbs was compared on the two particle packed columns (silica gel type and polymer gel type) and developed columns, it appeared that adsorption was less pronounced on the silica gel particle packed, probably due to the size of the small pore size. Developed monolithic column was shown the excellent recoveries to intact mAb. The pressure drop of the developed column is typically low that of a column packed with polymer column.

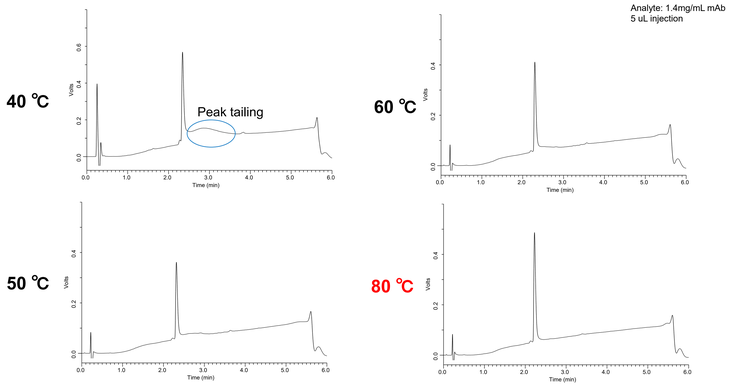


	Developed column	Silica gel packed column	Polymer packed column
Peak height	135451	25136	113250
Pressure drop	1.7MPa	4.5MPa	10.5MPa

Conditions
Eluent: A) 0.075% HCOOH+0.025% TFA - H2O
B) 0.075% HCOOH+0.025% TFA - ACN
A/B=95/5-5min-10/90
Flow Rate: 0.3 mL/min
Detection: UV 210 nm
Oven Temp: 80 ℃
Analyte: 1.4 mg/mL mAb

Temperature effect

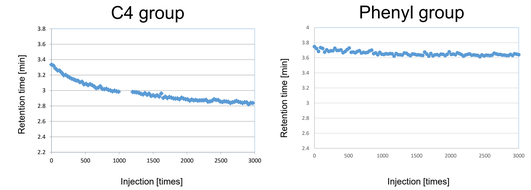
The temperature effect for resolution was compared. The heat resistance of a monolithic column clad with resin was typically up to 50 °C, but clad the monolithic material with glass improved the column's heat resistance to 80℃. Recovery of mAb were significantly improved with elevated temperature, and adsorption was generally acceptable in the temperature range between 60 and 80℃. As temperature increases, peak widths of the protein peaks has decreased and absolute retention of each peak was reduced with increasing temperature. The Stability of the developed column at higher temperature and presence of trifluoroacetic acid has also improved by cladded with a glass tube.



Separation of Intact mAb using a developed monolithic column at different temperatures.

Durability

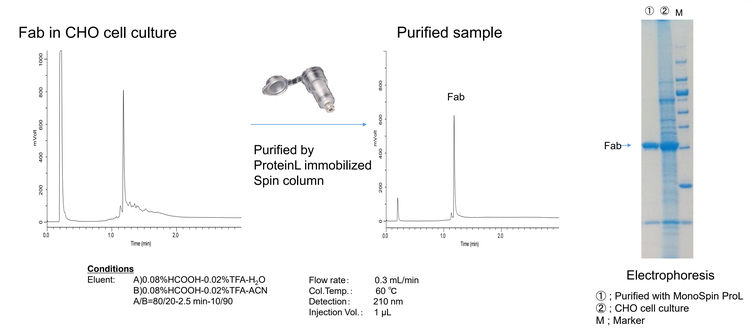
The graph below shows the durability test. In response to the increase of injection times (x-axis), the durability of each column is plotted at y-axis with the retention time of the mAb. The developed column modified phenyl groups shows great durability under low pH and high temperature conditions. The lifetime of phenyl groups modified column is much greater than that of the C4 modified columns. This assures stable analysis over a long period of time.



Conditions
Eluent: A : 0.1 %TFA-H2O
B : 0.1 %TFA-ACN
Gradient: A/B A/B=90/10-2.5min-10/90
Flow Rate: 0.3 mL/min
Oven Temp.: 80 ℃
Injection Vol.: 5 μL

CHO cell culture direct analysis

The developed monolith column has high porosity, it was possible to analyze the culture broth without clogging.

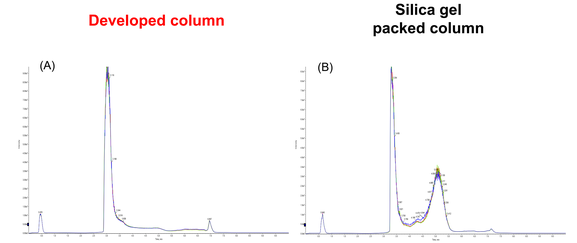


Conditions
Eluent: A)0.08%HCOOH-0.02%TFA-H2O
B)0.08%HCOOH-0.02%TFA-ACN
A/B=80/20-2.5 min-10/90
Flow rate: 0.3 mL/min
Col.Temp.: 60 ℃
Detection: 210 nm
Injection Vol.: 1 μL

① ; Purified with MonoSpin ProL
② ; CHO cell culture
M ; Marker

LC/MS intact mAb analysis

A mAb require buffering salt for stabilization in solution. However, to gain sensitivity in LC-MS analysis of intact mAbs, it is critically important to remove the salt before LC-MS analysis. Figures show the LC/MS data for the intact mAbs with developed column and silica gel columns, respectively. The developed column provided excellent total ion chromatogram peak shapes compared with silica gel column.



Conditions
Eluent: A : 0.1 %HCOOH-H2O
B : 0.1 %HCOOH-ACN
Gradient: A/B=90/10-10min-10/90
Flow Rate: 0.3 mL/min
Detection: TripleTOF 6600
Oven Temp.: 80 ℃
Injection Vol.: 5μL

Intact mAb mass analysis (total ion chromatogram) on (A) an developed column, (B) silica packed column.

Conclusions

- The Novel glass cladded monolithic silica column was developed.
- The developed column was shown to be very suitable for analysis of intact protein (mAb).