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Article

Development of high-resolution HPLC columns packed with monodisperse polymer particles

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1. Introduction

Polymer particles with sizes in the range 3 to 20 μm are extensively used for analytical purposes such as environmental analysis including air samplers, solid-phase extraction materials, and HPLC columns. For air samplers, in addition to applications to environmental chemicals such as formaldehyde, NOx, and VOC, precise pore size adjustment technology and background reduction techniques are essential in order to measure individual exposures and exposures in working environments. For solid-phase extraction, we have developed materials that exploit polymer particles to make hydrophobic interaction, ion exchange, chelates, and other functionalities which are widely use in water quality, medical and pharmaceutical analysis. Development of HPLC columns has began around 1980 and resulted in the development of a series of polymer-based columns which have turned into commercial products, including analysis SEC columns, preparative SEC columns, sugar and organic acid analysis columns, aqueous SEC columns, reverse-phase partition columns, and ion chromatography columns.

For these products, polymer particles were normally synthesized using suspension polymerization method which requires time consuming classification techniques of sieves or air jets in order to extract targeted polymer particles size range. However, recent increase of demand for smaller particles and sharper particle size distributions to improve the properties of products has led to attention to produce monodisperse particles[1)]. The seed polymerization method is utilized for monodisperse particles and time consuming classification can be avoided. Here we present on our investigations of column applications for monodisperse particles produced by seed polymerization (hereinafter referred to as “monodisperse particles”) which gives great improvements on column performance.

2. Monodisperse particles

A schematic overview of our method for synthesizing monodisperse particles using seed polymerization is shown in Figure 1. Seed particles ranging in size from 0.4 to 1 μm are produced via soap-free emulsion polymerization[2)]; these particles then absorb monomers and porogen to swell by a factor of 10 to 500, after which they are polymerized via thermal polymerization in configurations that preserve particle shape[3)].

Comparison of particle size distributions between monodisperse particles and suspension polymerization particles with size classification is shown in figure 2. Figure 3 shows an SEM image of monodisperse particles.

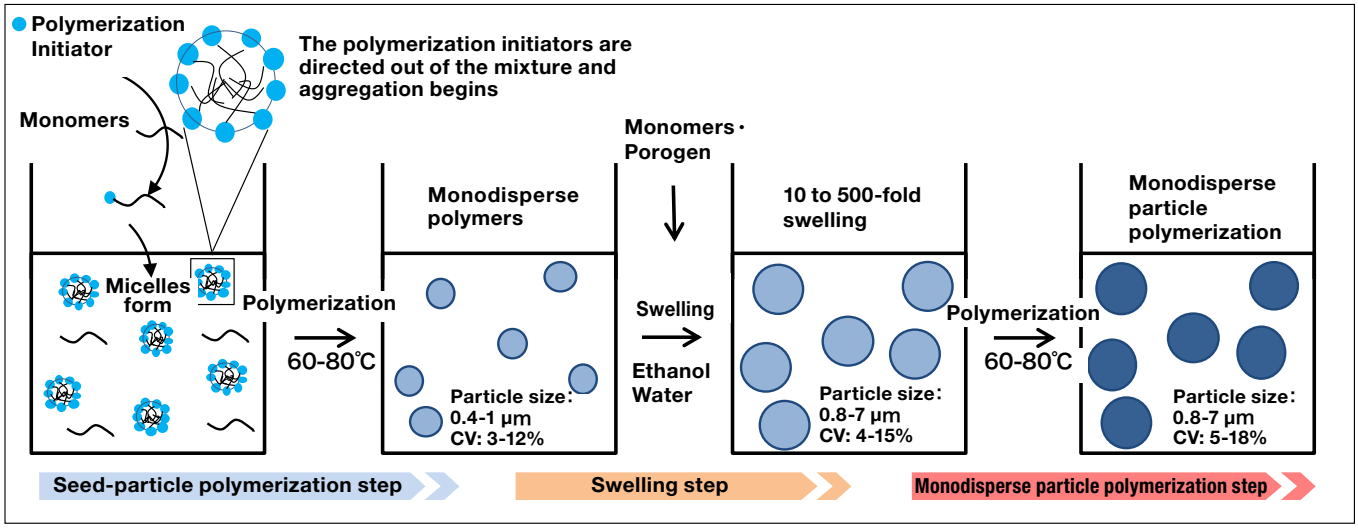


Fig.1 Seed polymerization method for synthesizing monodisperse particles.

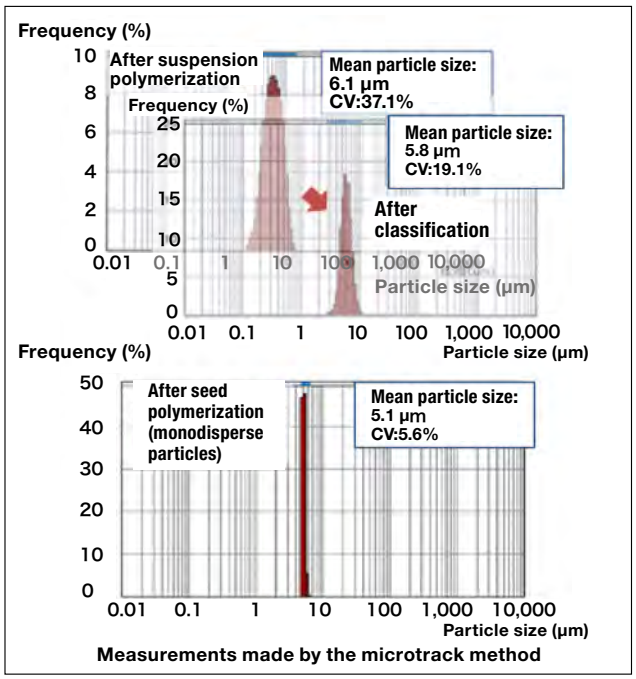
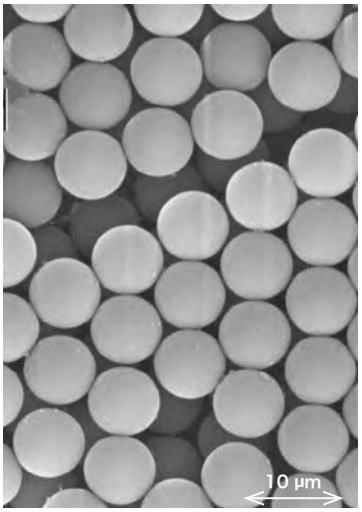


Fig.2 Comparison of particle-size distributions for monodisperse particles and particles produced by suspension polymerization.



Instrument: SU-70 (Hitachi High-Technologies).
Accelerating voltage: 2 kV.

Fig.3 SEM image of monodisperse particles

From Figures 2 and 3, suggest that the CV value (standard deviation / mean particle size × 100), which quantifies the sharpness of the particle size distribution for monodisperse particles, is smaller than the values seen for particles formed by suspension polymerization and the corresponding particle classification.

Having thus confirmed that the seed polymerization method yields monodisperse particles with sharper particle size distribution than suspension polymerization, we next synthesized both acrylic base hydrophilic and styrene based hydrophobic monodisperse polymer particles and applied these polymers to SEC columns for proteins and other organic compounds analysis.

3. Analytical SEC columns

3-1 Particle characteristics

For SEC analysis of proteins, hydrophilic acrylate monomer was utilized to create monodisperse particles using seed polymerization method. In order to improve the theoretical plate number, a mean particle size of 3.5 µm was selected for the monodisperse particles. Moreover, the ratio of cross-linkage of monomer was increased by 1.7 folds to reduce column pressure. Table 1 compares the properties of monodisperse particles (W540-SM) to conventional suspension polymerization particles (8-12 µm) Gelpack W540S SEC column, and Figure 4 shows the particle size distributions. The seed polymerization yields a CV of 5.8%, which is smaller than suspension polymerization particles; in addition, the swelling ratio was reduced due to the increase of crosslinking monomers ratio.

Table 1 Properties of acrylic-base polymer particles.

Particle	Ratio of cross-linking monomer ***	Mean particle size **** (µm)	CV (%)	Swelling ratio*****
W540-SM*	1.7	3.5	5.8	1.2
W540S**	1.0	9.5	23.7	1.3

* Monodisperse particles synthesized by seed polymerization

** Particles synthesized by suspension polymerization

*** Value obtained with the ratio of cross-linking monomers set to 1.0 for W540S

**** Measurements obtained using the microtrack method

***** Swelling ratios were measured by acetone solution and the calculation of the swelling ratio was performed as follows; Swelling ratio = swelling volume/dried volume

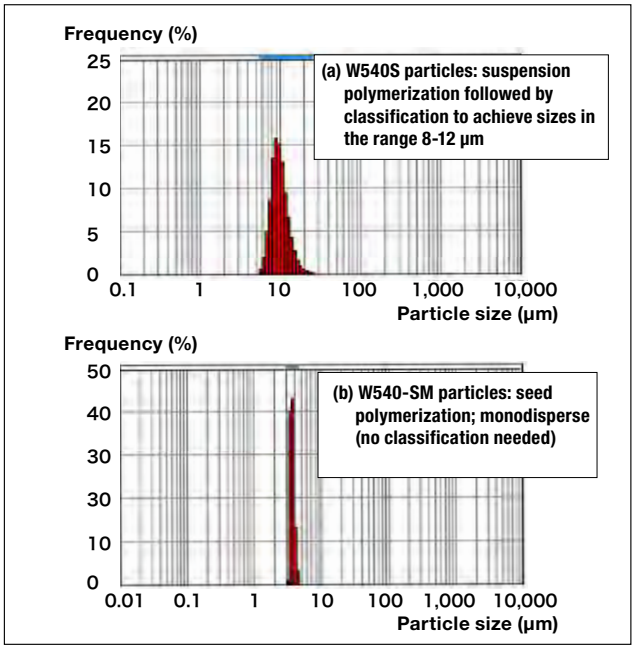


Fig.4 Particle size distributions for suspension polymerization particles and for monodisperse particles.

3-2 Column properties

A stainless-steel column (4.6 mm I.D. × 150 mm L) was packed with the monodisperse particles and its synthesize process is described above. The column (W540-SM) was then assessed using device 1 (HPLC) and device 2 (UHPLC). Table 2 shows the theoretical plate number and the column pressure, and Figure 5 shows chromatograms.

The specifications of newly developed W540-SM column (4.6 mm I.D. × 150 mm L) and conventional Gelpack W540S column (7.5 mm I.D. × 300 mm L) are shown in Table 2. For the conventional Gelpack W540S column's height equivalent of a theoretical plate (HETP)[4] was consistent at 23 µm (theoretical plate number 13,000) for both devices 1 and 2. In contrast, newly developed W540-SM column's analytical results by device 1 shows HETP was 21 µm (theoretical plate number 7,100), which is not significantly different from that of the conventional product; however, for device 2 the HETP was 9 µm (theoretical plate number 16,000), which indicates a significant improvement and close to the theoretical value. Since the cell size and flow pathway are smaller for device 2 than for device 1, there is less diffusion within the device 2 than device 1 [5], and thus the small monodisperse particle column W540-SM exhibited performance similar to that of conventional products. Therefore for future compatibility with HPLC systems, we believe that it is important to develop analytical columns using monodisperse particles with sizes on the order of 3.5 µm.

Table 2 Properties of SEC columns for analytical purposes

Column	Column dimensions (mm)	Flow rate (mL/min) / Linear velocity (mm/sec)	Measured values for device 1**			Measured values for device 2**			Theoretical value[4)]	
			Theoretical plate number N***	HETP**** (μm)	Column pressure P (MPa)	Theoretical plate number N	HETP H (μm)	Column pressure P (MPa)	Theoretical plate number N	HETP H (μm)
W540-SM	ϕ4.6×150	0.35 (0.35)	7,100	21	2.8	16,000	9	3.5	21,000	7
W540S	ϕ7.8×300	1.00 (0.35)	13,000	23	2.2	13,000	23	2.4	17,000	18

* Device 1: HPLC LaChrom Elite (Hitachi High-Tech Science), UV detector (Cell volume: 13 μL), tube size: 0.3 mm

** Device 2: UHPLC ChromasterUltra Rs (Hitachi High-Tech Science), DAD (diode-array) detector (cell volume: 2.2 μL), tube size: 0.1 mm

*** N=5.54 × (retention time / half-width)

**** HETP = height equivalent of a theoretical plate
HETP = column height (μm) /N

Measurement conditions: eluent: 1/15 N trisodium phosphate (pH7) + 0.2 MNaCl solution, sample: 1% alanine aqueous solution, injection volume: 5 μL

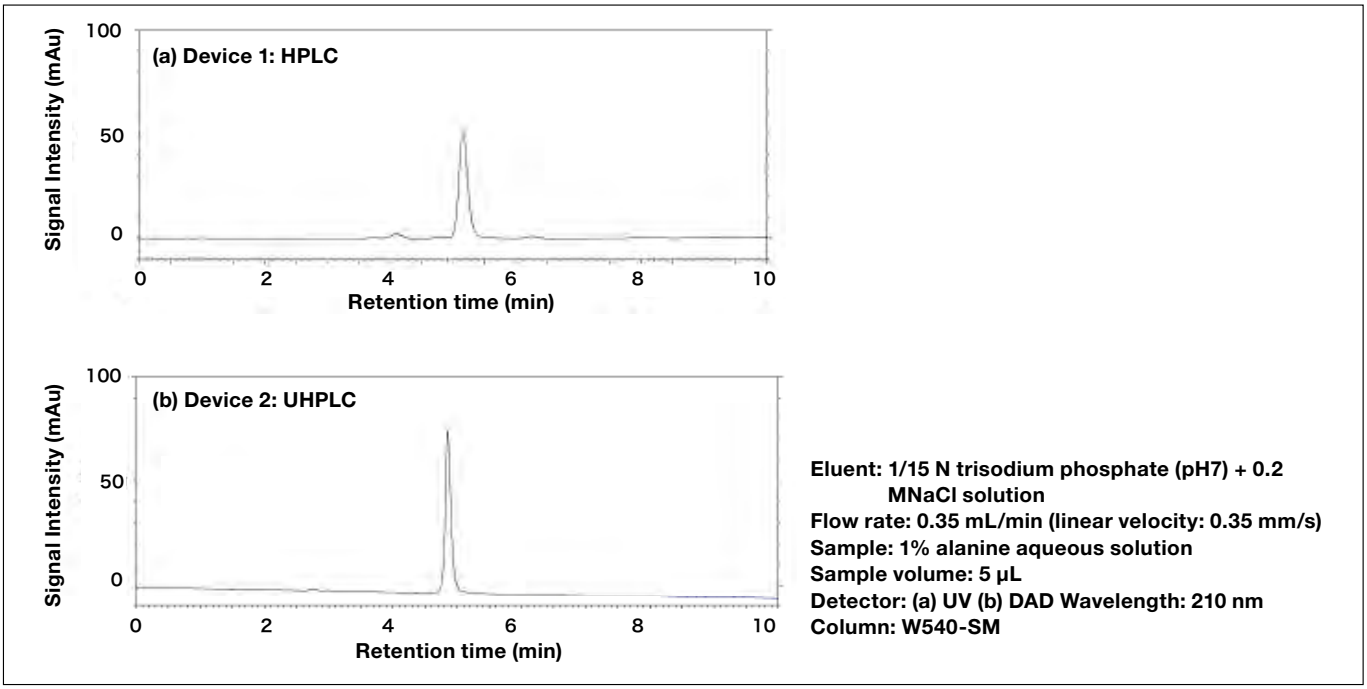


Fig.5 Comparison of chromatograms for the two devices.

4. Preparative SEC columns

4-1 Particle properties

For preparative SEC columns, two types of monodisperse particle with different exclusion limit (P212MD, P213MD) were synthesized using styrene-based monomers via seed polymerization method. To improve the theoretical plate number, the mean size of the monodisperse particles is 8 µm, which is smaller than the size of suspension polymerization particles (14 µm). In addition, the ratio of cross-linking monomer is set to a high value in view of the expected reduction in column pressure. Table 3 shows the properties of monodisperse particles and suspension polymerization particles. The suspension polymerization particles were classified in the size range 10 to 20 µm for use with conventional SEC columns (Gelpack P212, P213 for sampling). The particle size distributions of P212L and P212MD were shown in Figure 6. Table 3 indicates that the CV of monodisperse particles (CV=7.2 to 7.6) is smaller than suspension polymerization particles after sieve classification (CV = 18.6 to 25.0). The reduction of swelling ratio in monodisperse particles due to the increase in cross-linking monomer ratio was shown in Table 3.

Table 3 Properties of styrene-based particles.

Particle	Ratio of cross-linking monomer***	Mean particle size****(µm)	CV(%)	Swelling ratio*****
P212MD*	3.8	8.0	7.2	1.3
P213MD*	3.8	8.2	7.6	1.2
P212L**	1.0	14.6	18.6	2.1
P213L**	1.3	13.6	25.0	2.1

* Monodisperse particles synthesized by seed polymerization
** Particles synthesized by suspension polymerization
*** Value obtained with the ratio of cross-linking monomers set to 1.0 for P212L
**** Measurements obtained using Coulter-counter method
***** Swelling ratios were measured by acetone solution and the calculation of the swelling ratio was performed as follows; Swelling ratio = swelling volume/dried volume

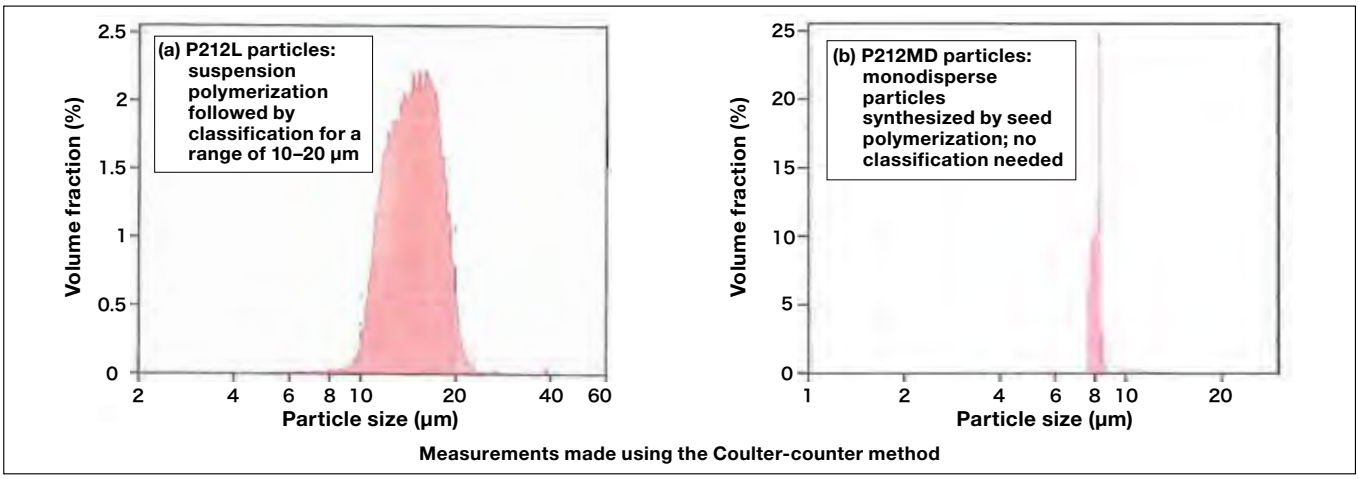


Fig.6 Comparison of particle size distributions for monodisperse particles and suspension polymerization particles.

4-2 Column properties

We packed monodisperse particles and suspension polymerization particles into stainless-steel columns (20 mm I.D.× 600 mm L) to obtain columns P212MD, P213MD, P212L, and P213L. The characterizations of these columns were shown in Table 4.

Table 4 Properties of SEC columns.

Column	Measured value*			Theoretical value**
	Theoretical plate number N	Peak symmetry	Column pressure P (MPa)	Theoretical plate number N
P212MD	40,000	1.1	2.1	38,000
P213MD	41,000	1.1	2.1	37,000
P212L	17,000	1.0	2.4	21,000
P213L	24,000	1.0	1.7	22,000

* Sample: 0.1% benzene 500 µL, eluent: chloroform
Flow rate: 7.5 mL/min
Device: LC-9101 (Japan Analytical Industry Co., Ltd.)

** N = Column length /(particle size × 2)

The theoretical plate number of monodisperse particles packed P212MD and P213MD columns were 40,000 and 41,000 respectively. These values are 2.3 times of conventional columns (P212L and P213L), since the particle size distribution is sharper and this is consistent with theoretical value based on the particle size. Moreover, even though the monodisperse particle size was reduced to 8µm, the column pressure remained the same as suspension polymerization particles packed column since ratio of cross-linking monomer was increased. The calibration curve shown in Figure 7 indicates that the exclusion limit molecular weight is 5,000 for P212MD and 20,000 for P213MD .

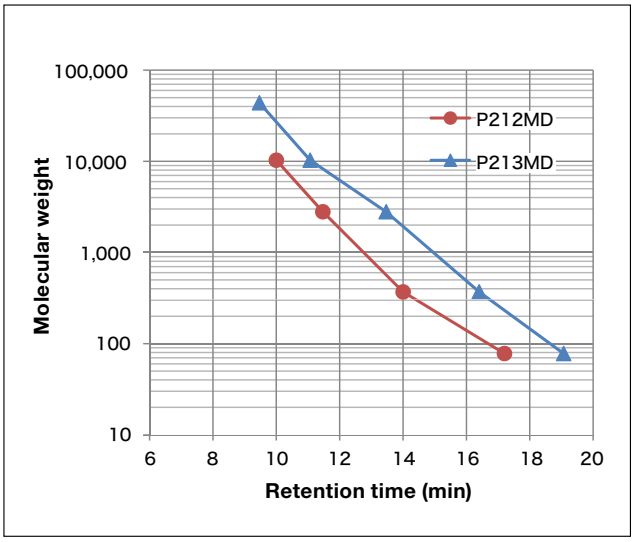


Fig.7 Calibration curves

The relationship between flow rate and theoretical plate number for monodisperse particle columns and chromatograms are shown in Figures 8 and 9. Both P212MD and P213MD column showed theoretical plate number exceeding 30,000 even at flow rate of 15 mL/ min, which is twice as much as conventional columns. The enhancement of flow rate in monodisperse particles columns were achieved by increased ratio of cross-linking monomer despite the face that the particles size in monodisperse particles packed column were smaller than conventional columns. Moreover, high resolution results are acquired since monodisperse particles enable to separate sample in consistent rate.

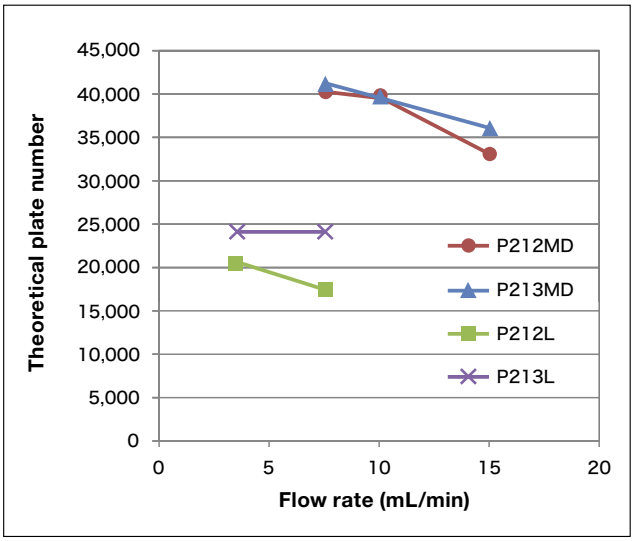


Fig.8 Relationship between flow rate and theoretical plate number

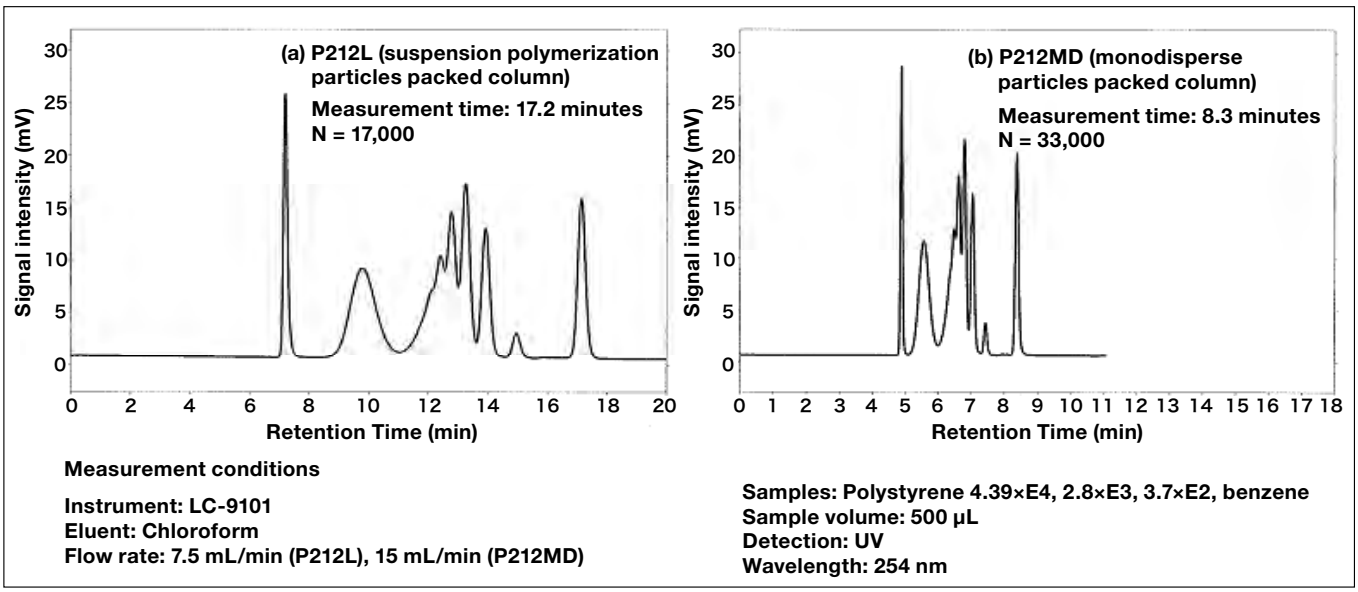


Fig.9 Comparison of chromatograms obtained from columns of suspension-polymerization particles and monodisperse particles

5. Conclusions

We investigated the applications of monodisperse polymer particles to HPLC columns.

- Analytical results on 4.6 mm I.D. × 150 mm L column packed with hydrophilic acrylic monodisperse particles for protein analytical SEC column using UHPLC system showed theoretical plate number of 9 μm (compared to 23 μm for conventional columns).

- Analytical results on 20 mm I.D. × 600 mm L column packed with styrene monodisperse particles for preparative SEC showed theoretical plate number of 40,000 (compared to 20,000 for conventional columns). Even when the flow rate was increased to 15 mL/min which flow rate is twice as much as conventional column, the theoretical plate number remained above 30,000.

Based on these findings, we conclude that the application of monodisperse particles to HPLC columns offers the promise of significant improvements in the column performance, including in areas such as separating ability and throughput.

6. Future work

For protein analysis SEC columns, our goal is to gain more compatibility on separation with various HPLC and UPLC equipment. For preparative SEC columns, we will introduce column which is capable of analyzing samples in which exclusion limit molecular weight of 100,000. Our goal is to bring these products to the market near future.

References

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