



## Preparation method of standard molecules for the precise estimation of molecular weight cut-off of membranes by gel permeation chromatography

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### ABSTRACT

In this study, the weighted mass of standard molecular weight compounds (SMWC) was adopted to improve the accuracy of molecular weight distribution measured by the gel permeation chromatography (GPC). To evaluate the impact of SMWC compositions, the mixture prepared by constant, linear-weighted, and polynomial-weighted SMWC (3 g/L total) was used for the calibration of GPC, and results were confirmed with typical membrane-based size-exclusion experiments. The results obtained by GPC analysis revealed that the composition of SMWC in the feed significantly altered the final molecular weight cut-off (MWCO) of same membranes, and the polynomial-weighted SMWC provided the best match with the result of MWCO measured by the single-compound rejection experiment due to the enhanced signal intensity at the higher molecular weight compounds (>20,000 Da). Consequently, the preparation of SMWC in the polynomial manner should be suggested during the calibration and MWCO measurements of membranes by GPC.

*Keywords:* Molecular weight cut-off (MWCO); Gel permeation chromatography (GPC); Ultrafiltration (UF) membrane; Polyethylene glycols (PEGs)

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### 1. Introduction

Recently, the application of membrane separation processes has been growing in a wide range of industries, such as the water treatment, food, and pharmaceutical industries [1]. Generally, membrane pore sizes play a remarkable role in membrane performance, such as permeability and selectivity [2,3]. Membranes can be generally classified on the basis of pore size, such as microfiltration (MF), ultrafiltration (UF), nanofiltration (NF), and reverse osmosis (RO) membranes. The main separation mechanism of MF and UF membranes is size exclusion or sieving by their pores [4,5]. In addition, NF membranes might have pores to separate the molecular

weights of the solutes, typically ranging from 200 to 1,000 Da, which indicates that size exclusion is very important as well as charge effects [2,6]. Hence, the characterization of membrane pore size and pore-size distribution is very important for both manufacturers and membranes users in certain applications [7].

To estimate the membrane pore size and pore-size distribution, several direct and indirect characterization methods have been developed [7,8]. Direct characterization methods include microscopic techniques using scanning electron microscopy (SEM), atomic force microscopy, and transmission electron microscopy. On the other hand,

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indirect characterization methods include solute transport, gas adsorption–desorption, and porosimetric methods, such as thermoporometry, mercury porosimetry, gas–liquid displacement porosimetry, and liquid–liquid displacement porosimetry [7,8]. Unfortunately, the results obtained by these methods may show different characteristics of membrane pore structures due to their different theoretical considerations and capabilities for measurement [9]. Generally, measurement of the mean pore size by direct characterization methods is systematically larger when comparing pore dimensions with a diameter calculated by indirect characterization methods. According to Singh et al. [10], this difference generally can be explained as follows. The membrane pore size measured by microscopic techniques corresponds to the maximum pore size in the case of funnel-shaped pores with two dimensions, while both the minimum and maximum pore sizes are considered in indirect characterization methods [5].

From a practical viewpoint, the molecular weight cut-off (MWCO) of a membrane is generally used to estimate the mean effective pore size and pore size distribution of UF and NF membranes as indirect characterization methods [11,12]. MWCO indicates the lowest molecular weight where 90% of the solute is retained by the membranes. To measure the MWCO, the feed solution is generally prepared with various standard molecular-weight compounds (SMWC), such as polyethylene glycol (PEG), dextrans, and polystyrene sulfonates (PSSs) [13–15]. Among them, PEG is one of the representative macromolecules with dextrans due to a neutral polymer, for which it is possible to exclude the charge effect on the membrane surface during filtration experiments [15,16].

During measurement of the MWCO of the membrane, the solute rejection was calculated by measuring the SMWC concentration of the feed and permeate using several analytical methods such as chromatography, UF filtration, and total organic carbon (TOC) measurement [17]. Among them, the determination of MWCO using TOC measurement is relatively accurate and is independent of the material being characterized. However, the long experiment time during the repeated filtration experiments of SMWC is still a limitation to obtaining the MWCO and pore-size distribution of membranes during TOC measurement [13]. Recently, gel permeation chromatography (GPC) has been widely used due to its simplicity, and a single filtration experiment is sufficient to determine the MWCO and pore-size distribution in an almost continuous manner [13].

However, previous studies have revealed that the signal intensity obtained by GPC analysis could vary significantly due to binary interaction between the solvent, polymer solutes, and gel packing, and this has led to the under-estimation of the MWCO of membranes [13,18,19]. According to Causserand et al. [13], the MWCO of membranes can be under-estimated when retention experiments are conducted with mixed solute in comparison with the single solute. In addition, most MWCO experiments have been conducted using a constant gradient of SMWC by GPC analysis in previous studies [3,20]. In fact, less signal intensity from high molecular weight compounds in the constant gradient of SMWC can generate significant rejection errors during the GPC measurement of MWCO.

Therefore, in this study, the weighted mass of SMWC was adopted to improve the signal intensity of higher molecular weight compounds, and the results were compared with the batch rejection experiment using a single standard molecule.

## 2. Materials and methods

### 2.1. Determination of MWCO

#### 2.1.1. Preparation for the mixture of SMWC

To obtain enhanced signals from GPC, PEG (Sigma-Aldrich, USA) with various average molecular weights of 6,000, 10,000, 20,000, 35,000, and 100,000 Da were prepared and mixed at the various ratios according to the molecular weight of PEG. In detail, the total amount of PEG was set as 3 g/L [21], but the composition of four SMWCs was changed in constant, linear-weighted, and polynomial-weighted manners according to their molecular weights (Table 1). In addition, a single molecular weight of PEG (6,000, 10,000, 20,000, 35,000, 100,000 Da) with 3 g/L of the total amount was also prepared as a feed solution for classical batch rejection experiments to prevent any binary interaction between the various molecular weight compounds [12].

#### 2.1.2. Retention experiments

For the determination of MWCO, a commercial membrane (GE PW-UF, USA) was tested with a mixture of PEG prepared as the feed as shown in Table 1. The test membrane was a flat-sheet type with an effective membrane area of  $2.7 \times 10^{-3} \text{ m}^2$ , placed inside the membrane module. The membrane system was operated in a closed loop so that the pressure on the feed side was stable during the experiment. The prepared feed solution was contained in a stainless steel vessel, which was pressurized by nitrogen gas. The initial flux and cross-flow velocity of the membrane were maintained at  $70 \pm 1 \text{ LMH}$  and  $10 \text{ cm/s}$ , respectively. The feed and the permeate stream were collected to measure the concentration after a stabilization period of 1 h. The same procedure was repeated for filtration experiments using a single molecular weight of PEG. All filtration experiments in room temperature were conducted at least three times to confirm the reproducibility.

#### 2.1.3. Analytical methods

The molecular weight distribution of each feed and permeate solution from the filtration experiments using a mixture of SMWC was measured by GPC (GPC 1260 Infinity, Agilent Technologies, USA). The mobile phase consisted of 0.01 g/L of  $\text{NaN}_3$  and 0.1 g/L of NaCl aqueous solution at a flow rate of 0.8 mL/min and  $25^\circ\text{C} \pm 1^\circ\text{C}$ . A PL aquagel-OH Guard 8  $\mu\text{m}$  column (Agilent Technologies, USA) followed by PL aquagel-OH 60 8  $\mu\text{m}$  and PL aquagel-OH mixed-H 8  $\mu\text{m}$  column in series were used [22]. The columns were calibrated with standard PEG samples with average molecular weights of 6,000, 10,000, 20,000, 35,000, and 100,000 Da according to its retention time. For the comparison and proof of MWCO obtained from GPC, the results were compared with classical batch rejection experiments using a single molecular weight of SMWC. The rejection was determined by the measurement

Table 1  
Composition of PEG in 1 L of feed solution at various weighting functions of SMWC during the GPC measurement

PEG (Da)	Constant (g)	Linear (g)	Polynomial (g)	Single (g)
6,000	0.75	0.30	0.30	–
10,000	0.75	0.40	0.35	–
20,000	–	–	–	3.00
35,000	0.75	0.80	0.40	–
100,000	0.75	1.50	1.95	–
Total	3.00	3.00	3.00	3.00

of dissolved organic carbon concentrations in feed ( $C_f$ ) and permeate ( $C_p$ ) using TOC analyzer.

## 2.2. Determination of pore-size distribution

The pore-size distribution of a membrane is as important as MWCO in evaluating its sieving properties for solutes. The pore-size distributions of the membranes are obtained by the expression of the relationship between the PEG rejection ( $R$ ) and their Stokes hydrodynamic diameter ( $D_p$ ). By ignoring influences such as steric and hydrodynamic interaction between solute and membrane pores, the pore size distribution can be expressed through the following probability density function in Eq. (1) [23]:

$$\frac{dR(d_p)}{dd_p} = \frac{1}{\sqrt{2\pi}d_p \ln \sigma_p} \exp \left[ -\frac{(\ln d_p - \ln \mu_p)^2}{2(\ln d_p)^2} \right] \quad (1)$$

where  $\mu_p$  is the mean effective pore size, which is determined at the solute rejection of 50% ( $R = 50\%$ ), and  $\sigma_p$  denotes the geometric standard deviation, which is defined as the ratio of  $d_p$  at  $R = 84.13\%$  over that at  $R = 50\%$ . Moreover, the molecular weight (MW) of PEG can be converted to the Stokes hydrodynamic diameter ( $D_p$ ) by Eq. (2) [24,25]:

For PEG:

$$D_p = 33.46 \times 10^{-12} \times MW^{0.557} \quad (2)$$

In addition, the average diameter of the membrane pores was analyzed by the digital image processing of the scanning electron microscope images (Magellan 400, FEI Co., USA) with 100 samples of membrane pore [26].

## 3. Results and discussions

### 3.1. Changes in MWCO with variously weighted SMWC

Fig. 1 presents the PEG rejection data obtained at various calibration standard curves by variously weighted SMWC by GPC and TOC analysis. Although the tested membrane was identical, the measured MWCO of the membrane was different to be 14,260, 16,880, and 19,990 Da according to the mixture ratios of PEG molecules in constant, linear,

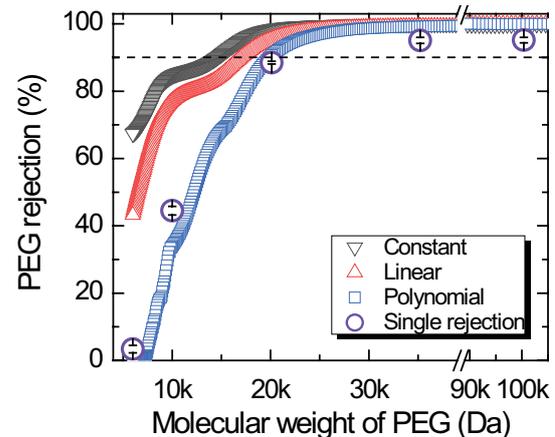


Fig. 1. PEG rejection of the UF membrane according to a various concentration gradient of molecular weights and a single-solute measured by GPC and TOC analysis, respectively.

and polynomial manners during GPC measurement. It implied that the preparation of SMWC could make noticeable differences in MWCO, thus, the mixing of SMWC should be significantly considered during the GPC analysis. Furthermore, polynomial mixing of PEG provided the best match with results from the batch rejection experiment at 6,000, 10,000, 20,000, 35,000, and 100,000 Da of SMWC as shown in Fig. 1. As seen in Fig. 1, the result from constant mixing of SMWC showed under-estimated MWCO and is corresponding with previous studies [13,18].

### 3.2. Pore-size distribution of membranes with variously weighted SMWC

As seen in Fig. 2a, the pore-size distribution and average pore size of the membrane were obtained by a log-normal probability density function (Eq. (1)) of the membrane with the PEG rejection in Fig. 1. The Stokes hydrodynamic diameter ( $D_p$ ) was calculated by Eq. (2). The average pore size of the membrane was obtained as 4.16, 5.25, and 6.30 nm according to the mixture ratios of PEG molecules with constant, linear, and polynomial manner, respectively. In addition, the pore-size distribution becomes broader with an increase in the gradient of higher molecular weight compounds. For the comparison, the pore size distribution was also analyzed using the digital image processing of SEM images (Fig. 2b). From the results of the direct characterization methods, the pore-size distribution was very similar to that of polynomial mixing of SMWC with an average pore size of 6.76 nm. Thus, it is clear that according to Figs. 2a and b, the information of relatively large pore can be better reflected with the increasing gradient of higher molecular weight compounds such as polynomial manner during the estimation of MWCO using GPC analysis.

### 3.3. Signal intensity from GPC

Figs. 3a and b show the signal intensity of the feed and permeate from the rejection experiments according to the mixture ratios of PEG molecules with constant, linear, and polynomial manner, and a single solute of 20,000 Da from

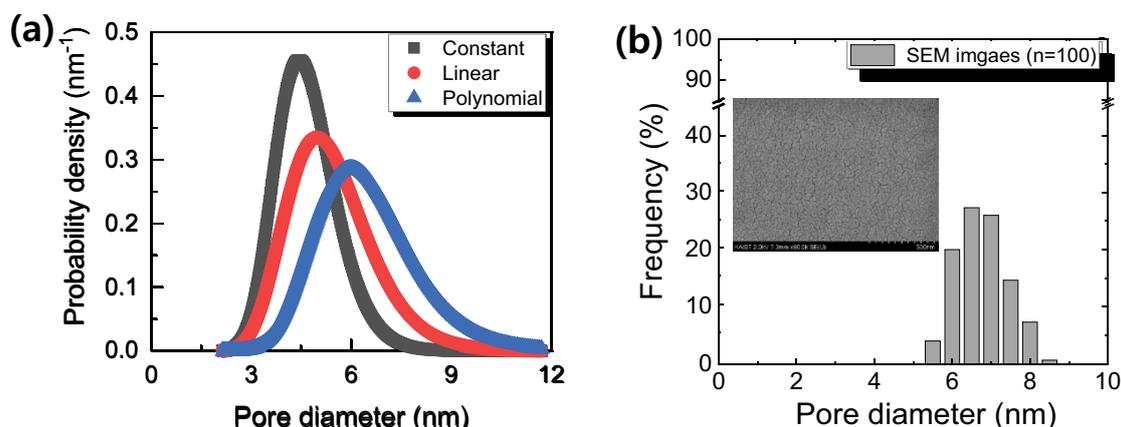


Fig. 2. Pore size distribution of the UF membrane analyzed by (a) a log-normal probability density function of the membrane with the PEG rejection and (b) digital image processing of scanning electron microscopy (SEM) ( $n = 100$ ).

GPC analysis, respectively. The molecular weight of PEG was converted by the retention time from GPC analysis with 6,000, 10,000, 20,000, 35,000, and 100,000 Da of SMWC. The signal intensity is directly related to the molar concentration of feed and permeate [27], and the molar concentration is proportional to the peak area of the refractive index (RI) detector [28]. As seen in Fig. 3a, the various concentration gradients of molecular weights in the feed can significantly influence the signal intensity from GPC analysis. Generally, similar signal intensities were obtained at the higher molecular weight compounds (i.e., higher than 20,000 Da) due to the decreased molar concentration of higher SMWC. Likewise, there were significant differences in the signal intensity at the lower molecular weight region (i.e., smaller than 15,000 Da) due to the increased molar concentration of lower SMWC, especially in constant mixing case. Meanwhile, as seen in Fig. 3b, almost all SMWC higher than 30,000 Da has been removed completely by the membrane, thus the signal intensity of the permeate was not significantly different from the various concentration gradients of SMWC.

To compare the accuracy of data from GPC analysis at various SMWC preparations, the rejection of single molecular weight of PEG 20,000 Da was compared with the data

from batch rejection experiment with membrane. The molecules were rejected  $87.7\% \pm 0.5\%$  by the membrane, and the results from the polynomial SMWC mixture with GPC analysis agreed with that of TOC analysis ( $88.5\% \pm 0.4\%$ ) as shown in Fig. 1. The difference between the rejection results obtained by variously mixed SMWC may be attributed to binary interaction between solvent, polymer solutes, and gel packing during the GPC measurement [13,19] and they have been acknowledged as the major error source of GPC analysis [29]. Thus, the transport of lower molecular weight compounds might be facilitated due to the interactions with higher molecular weight and final results would be biased as the higher rejection of lower molecular weight compounds. The phenomena happened more significantly in the case of constant mixture of SMWC, that the MWCO was shifted to the lower value due to the over-estimation of the rejection of lower molecular weight compounds as shown in Fig. 1. The under-estimation of the MWCO of the membrane was also demonstrated by the evidence from the signal intensity of the feed with various weighting functions of SMWC during GPC measurement. However, as shown in this study, the estimation method for MWCO of membranes can be significantly improved by the preparation of SMWC

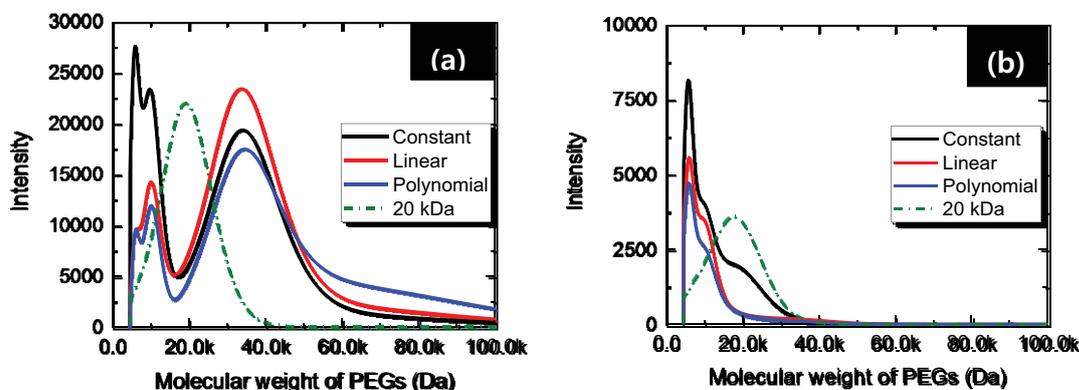


Fig. 3. Signal intensity of (a) feed and (b) permeate in retention experiments according to the mixture ratios of PEG molecules with the constant, linear, polynomial manner and a single solute of 20,000 Da during the GPC measurement.

with polynomial mixture, that the relatively uniform signal intensity of SMWC can be obtained during the GPC measurement.

#### 4. Conclusions

In this study, we proposed an improved preparation method for the composition of SMWCs to measure the MWCO of membranes using GPC analysis. The weighted loading of SMWC can significantly influence the determination of MWCO estimated by GPC analysis. The typically used constant-weighted SMWC mixture resulted in the under-estimation of MWCO value due to the over-estimation of rejection in the range of low-molecular-weight compounds by interactions with other SMWC. Instead, the preparation of SMWC in a polynomial manner can significantly reduce the error from interaction among various molecular weights during the GPC measurement of MWCO. Thus, the preparation of SMWCs with the polynomial mixing for the fast and reliable measurement of MWCO of membranes is recommended.

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#### Symbols

$R$	—	Rejection of PEG
$C_f$	—	Feed concentration
$C_p$	—	Permeate concentration
$D_p^p$	—	Stokes hydrodynamic diameter
$\mu_p$	—	Mean effective pore size ( $R = 50\%$ )
$\sigma_p$	—	Geometric standard deviation ( $R = 84.13\%$ )
$d_p^p$	—	Ratio at $R = 84.13\%$ over that at $R = 50\%$
MW	—	Molecular weight

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