Fractionation of polyethylene glycol particles by simulated moving bed with size-exclusion chromatography☆

Ming-Tsai Liang*, Ru-Chien Liang

Department of Chemical Engineering, I-Shou University, Kaohsiung City, Taiwan

ARTICLE INFO

ABSTRACT

In this study, a three-section simulated moving bed (SMB) with an open-loop design is used to fractionate polyethylene glycol (PEG) with different molecular weights. The purchased PEGs are mixed and separated by the open-loop SMB. A size-exclusion column with a pore size ranging from 10 to 100 nm, TOSOH GMPW 7.5 mm × 30 cm, is used to separate the mixtures. Based on the Triangle theory, the operating parameters of the SMB are determined and used to separate the three binary mixtures. The results show that the PEG mixtures with molecular weights of 400 and 8000, and those of 1500 and 20,000, are separable, yet those of 1500 and 3500 are difficult to separate by the selected column. The relative elution for molecular weights of 400 and 8000, and 1500 and 2000, is 1.49 and 1.54, respectively, resulting in easy separation. However, the difference in the elution volume for mixtures with molecular weights of 1500 and 3500 is so small that the operation condition is confined to a tiny area on the (m_2, m_3) plane defined by the Triangle theory. This makes robust application of the SMB impossible. Fortunately, it is still possible to obtain pure raffinate with low recovery, but a pure extract is still not possible. It is concluded that the low selectivity of the binary mixture and the fluctuation of the operation result in the difficulty in separating the 1500 and 3000 molecular weight mixtures. This paper presents the operation procedures, including the selection of the column, the discovery of selectivity, the application of the Triangle theory and the experimental results, in order to illustrate how to apply an SMB to the fractionation of PEGs.

1. Introduction

Polyethylene glycol (PEG) is a polyether polymer. It is widely used in industrial applications since its physical properties vary with its molecular weight (MW). In general, the MW of PEG can be determined by the number of ethylene oxide (EO)-units during polymerization. Typical uses of PEG with a low molecular weight include solvents, emulsifiers, and lubricants. In recent decades, PEG with a high molecular weight has been found to have low toxicity, low immunogenicity, and antigenicity, and has thus been used in the pharmaceutical industry, for example, as a carrier for small molecule drugs or as the amphiphilic copolymer for anticancer drugs. To attain PEG with a specific MW, the reaction of PEG is catalyzed by acidic or basic catalysts and terminated by neutralizing the catalyst with acid when the desired molecular weight is reached. It is, therefore, difficult to centre the particle-size distribution in a narrow range. In view of the need for PEG with a narrow particle-size distribution in biomedical applications, in this study our aim was to establish a rapid and effective method by which to fractionate the molecular weight of PEG.

Simulated moving bed (SMB) is a continuous chromatographic technology. Compared to conventional batch chromatography, SMB has the advantages of continuous operation, high product purity, high production yield, high efficiency of absorbents, and low solvent consumption. In addition, SMB can be readily scaled-up for higher volume purification and it can be conducted relatively quickly to meet the demand of tightened schedules in new product development. SMB was first developed by the UOP Company in the 1960s for the production of C8 products in the oil-refining industry [1–3]. In the 1970s, SMB was applied to the separation of fructose and glucose [2]. Despite these large-scale production uses, in 1993, the Separex Co. overcame the dead-volume problem and successfully scaled down the SMB unit (1–1000 kg/y) and applied it to the separation of small molecule chemicals for use in new drug development [2]. After a decade, SMB technology has been increasingly used in the concentration of the crude extract of Chinese herbs [4–6].

Since the fractionation of particles in a dispersion medium can normally be achieved by size-exclusion chromatography (SEC), it is also feasible to apply SMB to particle fractionation. In SEC, a dispersed particle solution is forced to flow through a packed bed with

porous solids, ensuring that particles with a diameter larger than the pore size will not be retained at all, while particles smaller than the pore size will be retained to varying extents according to the size of the particles and the pores in the solids. Mun and Wang showed that insulin particles can be purified by a tandem SMB with SEC [7].

This study employed SMB in an attempt to separate different molecular weights of polyethylene glycol (PEG). Five PEGs with different molecular weights were purchased and three binary mixtures made; SMB was then used to separate them. The Triangle theory was applied to help set up the operation parameters for SMB. It was found that good separation was obtainable for large relative elution. For similar molecular weights of PEG, it was only possible to fractionate large particles from the similar mixture. This work will present how to successfully apply the Triangle theory to the separation of PEGs by SMB with SEC.

2. Size-exclusion chromatography

For size-exclusion chromatography, the velocity of dispersed particles in a porous packed bed can be expressed as [8]:

$$u = \frac{v}{1 + (1 - \varepsilon_P/\varepsilon_e)\varepsilon_P K_D + ((1 - \varepsilon_e)(1 - \varepsilon_P)/\varepsilon_e)\rho_S K_H} \quad (1)$$

where u (cm s^{-1}) is the velocity of the particles in the bed, v (cm s^{-1}) is the interstitial velocity of the suspension medium, ε_e (dimensionless) is the external porosity between the solid packing, ε_p is the internal porosity of the porous packing, ρ_S is the density of the packing material, K_D is the fraction of the void inside the porous packing that allows the particles to penetrate, and K_H (cm^3 g^{-1}) is Henry's constant for adsorption. Inside the microstructure of the packing material, the pores prevent large particles from penetrating into the solid and provide space to retain small particles from effluence. If a particle is very large and the pores prevent penetration, K_D is equal to 0. K_D is set to 1 if a particle is allowed to penetrate all pores inside the packing material.

If there is no interaction between the particles and the packing material, the elution process is an entropy-driven process. The velocity of the particles in the packing bed is:

$$u = \frac{v}{1 + ((1 - \varepsilon_P)/\varepsilon_e)\varepsilon_P K_D} \quad (2)$$

If the size distribution of the pores is a normal distribution with zero mean radius, K_D can be expressed as [9]:

$$K_D = 1 - erf\left(\frac{R}{\sqrt{2}\sigma_a}\right) \quad (3)$$

where R is the Stokes radius of the PEG particle, and σ_a is the standard distribution. Substituting Eq. (3) into Eq. (2) gives:

$$u(R) = \frac{v}{1 + ((1 - \varepsilon_P)/\varepsilon_e)\varepsilon_P[1 - erf(R/\sqrt{2}\sigma_a)]} \quad (4)$$

In conventional chromatography, shock waves and diffusive waves are commonly observed because the velocity is heavily dependent on the adsorption isotherms. For SEC, K_D is dependent on the particle size and the pore-size distribution and independent of the concentration. Therefore, the chromatogram of SEC is normally a symmetrical Gaussian distribution. This is similar to conventional chromatography with linear adsorption. Therefore, the Triangle theory can be employed to set up the operating parameters for SMB.

3. SMB

As shown in Fig. 1, six packed columns were connected in series, with two inputs and two outputs. The columns were numbered
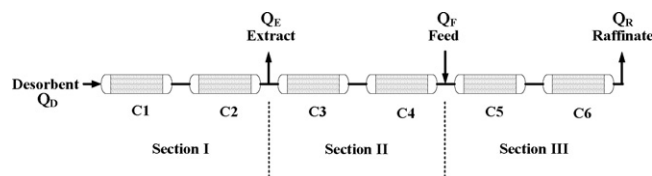

Fig. 1. Flow diagram of SMB used for SEC.

C1 to C6, according to the position in relation to the input of desorbent, which was DI water in this study, and every two columns were grouped as a section. This is called three-section SMB. The dispersed solution of PEG was pumped into the SMB between sections II and III. Small particles were discharged at the extract between sections I and II, and large particles were the effluent at the raffinate. After holding this configuration for a certain time, the position of the inputs and outputs would shift to the next right column. Continuously and simultaneously shifting the inputs and the outputs simulated the flow of a solid inside the column to the left. The countercurrent flow of the solid and the desorbent was fulfilled.

In section I, the porous packing material in the column was washed by fresh DI water before entering section III, and particles entrained by the porous material were eliminated by the fresh DI water to the right and discharged at the extract. Solids in section II carried small particles to the left, and large particles were flushed by DI water to the right and entered section III. Porous material in section III stripped the small particles in the dispersed liquid solution and entrained small particles to section II. Therefore, the porous material in section III acted to reduce the content of small particles and concentrate the large particles in the dispersed liquid. Large particles in the dispersed liquid were concentrated by the porous material in section III and discharged at the raffinate.

In a standard four-section SMB, the fourth section acts to strip solutes from the liquid phase with fresh porous material in order to purify the desorbent for recycling. If the desorbent can be easily purified by other technologies, the fourth section can be eliminated. If the fourth section is removed, the three-section SMB will increase the productivity of the adsorbent; however, the weak retention component is largely diluted. Zang and Wankat showed that the three-section SMB with partial feed or withdrawal operation can be more effective than a standard four-section SMB [10].

The operating parameters for an SMB are the desorbent flow rates in each section and the holding time for the valve switch, called the switching time. If all flow rates and switching times are constant, this is called a standard SMB. The liquid flow rates are usually regulated by liquid pumps. Two pumps are used for the inputs of desorbent and feed, and the third pump is used for regulating the flow rates of the extract discharged from the SMB.

4. Triangle theory

To separate large and small particles, the net flux of the number of particles in each section has to satisfy the following conditions [1,3]:

$$\begin{cases} f_{S,1} > 0; & f_{L,1} > 0 \\ f_{S,2} < 0; & f_{L,2} > 0 \\ f_{S,3} < 0; & f_{L,3} > 0 \end{cases} \quad (5)$$

where the subscripts S and L represent the small and large particles, and 1, 2, and 3 represent the different sections. If the total number of particles remains constant and the dispersed particles do not agglomerate, the net flux in Eq. (5) can be expressed as:

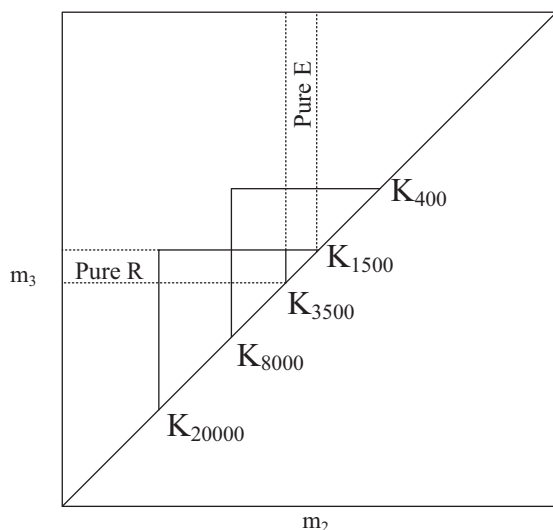$$f_{i,j} = \frac{V(1 - \varepsilon_e)}{At_{sw}}C_{i,j}(m_j - \varepsilon_p(K_D)_i) \quad (6)$$

Fig. 2. Schematic illustration of the Triangle theory.

where V is the volume of the empty column, t_{sw} is the switching time, $C_{i,j}$ is the number concentration of large or small particles in the liquid phase at section j, m_j is the relative volumetric flow rate of liquid to that of solids in section j, and defined as [1,3]:

$$m_j = \frac{Q_j t_{sw} - V\varepsilon_e - V_j^D}{V(1-\varepsilon_e)} \tag{7}$$

where Q is the volume flow rate of DI water, and V^D is the dead volume in each section.

For complete separation, Eq. (5) must be fulfilled. If the products of ε_P and $(K_D)_i$ are defined as the elution constants, K_L and K_S, the relative volumetric flow rate in each section must satisfy the following constraints:

$$\begin{cases} K_L < K_S < m_1 \\ K_L < m_2 < K_S \\ K_L < m_3 < K_S \end{cases} \tag{8}$$

For complete separation, the design of the relative volumetric flow rates in each section has to be located inside the triangle in plane (m_2, m_3), as illustrated in Fig. 2.

In Fig. 2, the subscripted number with the elution constant K represents the molecular weight of PEG. In this figure, three triangles are marked; they represent the three binary systems investigated in this study.

The elution constant was difficult to estimate because of ε_P. However, the elution constant can be treated as resistance to that of a particle flowing through the bed, which is similar to Henry's constant for linear adsorption. By analogy to the method of finding Henry's constant H, the elution constant could be estimated by single column chromatography as in conventional reversed phase chromatography:

$$t_R = t_o \left(1 + \frac{1-\varepsilon_e}{\varepsilon_e}H\right) = t_o \left(1 + \frac{1-\varepsilon_e}{\varepsilon_e}\frac{\varepsilon_P K_D C}{C}\right)$$
$$= t_o \left(1 + \frac{1-\varepsilon_e}{\varepsilon_e}K\right) \tag{9}$$

where t_R and t_o are the retention times of the particles and that of non-retained ones. It is worth noting that the product of $(1-\varepsilon_e)/\varepsilon_e$ and H is the ratio of solute in the solid phase to that in the fluid phase, and by replacing H with K, it can be treated as the ratio of particle numbers entrained by the solid phase to that remaining in the liquid phase. K_D and ε_P are rarely provided by the manufacturer,

yet the elution constant can be easily determined by single column chromatography in the laboratory. It becomes very convenient to use K rather than K_D and ε_P to set up the operating conditions for SMB.

5. Experiments and materials

The PEG standard solutions were purchased from Fluka or Sigma. Five different molecular weights of PEG, MW 400, 1500, 3500, 8000, and 20,000, were used in this study. A GMPW column with pore size <10–100 nm from TOSOH was used, 7.5 mm ID × 30 cm, for the analysis and for the SMB separation. De-ionized water was used as the mobile phase. The porosity of the column was found to be 0.3643, which was determined by calculating the slope of t_R vs V/Q as varying the flow rates of the de-ionized water. The relationship of t_R and V/Q is expressed as:

$$t_R = \varepsilon_e \frac{V}{Q} \tag{10}$$

where t_R is the retention time for the non-retained particles. In order to determine the concentration of unknown samples, calibration curves for different molecular weights of PEG were also prepared. All calibrations had correlation coefficients higher than 0.999.

Six GMPW columns were connected in series to assemble the SMB. The piping design of the three-section SMB is illustrated in Fig. 3. Three Hitachi L-2130 HPLC pumps were installed to control the liquid flow rates in each section. Rotating manifold vales from Valco Co. were controlled by the in-house designed PLC and used to guide the liquid flow. A capillary restrictor was installed in the exit of the raffinate to stabilize the system's pressure and to regulate the flow rate of the raffinate. Extra volume was, therefore, created which could cause contamination when sampling the raffinate effluent after each switching.

6. Results and discussion

6.1. Single column result

Three binary systems were tested by SMB. The elution constant for every purchased standard was measured and found to be 0.5380, 0.4592, 0.4093, 0.3606, and 0.2975 in order of increasing molecular weight from 400 to 20,000. Interestingly, the elution constant could be correlated with the molecular weight of PEG as:

$$K = -0.06098 \ln(M.W.) + 0.9046 \tag{11}$$

The correlation between the elution constant and molecular weight was not unexpected, since the radius of gyration of PEG in water can be correlated to its molecular weight as [11]:

$$R = 0.3675(M.W.)^{0.5152} \tag{12}$$

For two different molecular weights of PEG particles, the ratio of elution constant is called the relative elution, which is similar to the selectivity or relative retention, and defined as:

$$\alpha = \frac{K_s}{K_L} \tag{13}$$

The relative elution for the mixtures with molecular weights of 400 and 8000 was then calculated as 1.49 and 1.54 for 1500 and 20,000. For the mixtures with MWs of 1500 and 3500, the relative elution was 1.12. This made it difficult to set up the separable operating conditions, and a robust operation of the SMB was not feasible. Thus, the results of the separation of the three binary mixtures have been presented.
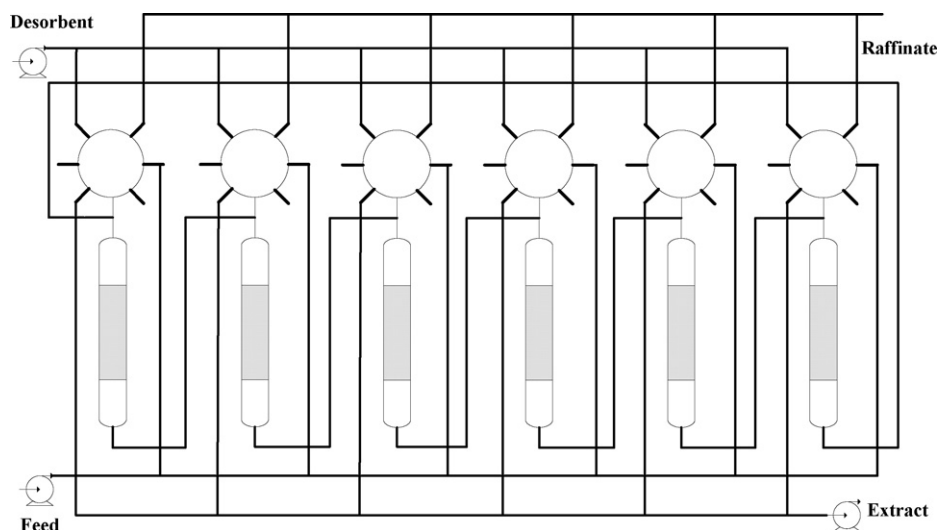
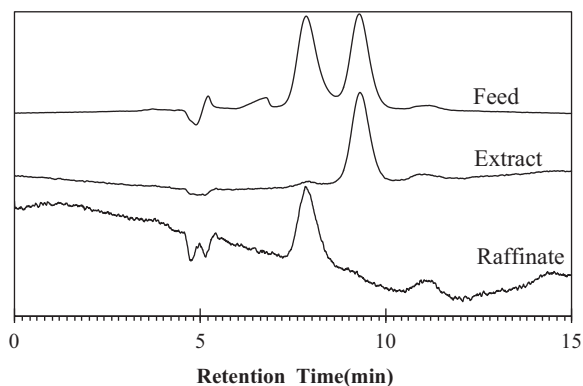**Fig. 3.** Piping design for the three-section SMB.

Fig. 4. Chromatograms of the binary feed and the extract and raffinate after separation.

Fig. 5. Concentration variation in extract at 23.5 min of switching time.

6.2. Separation of MWs of 400 and 8000

Four tests were conducted for the mixtures of molecular weights of 400 and 8000. The results and the operation parameters are summarized in Table 1. The purity is defined as the weight percent of the desired particles in each sample, and the average purity for each experiment is shown in Table 1. The recovery is defined as the weight ratio of the desired particles in the extract and in the raffinate to the total weight of the desired particles. Applying the Triangle theory, the liquid flow rates in each section remained unchanged, and the switching time gradually increased, as shown in Table 1. It was observed that the switching times at 22.5 and 23.5 min were obviously located inside the right triangle on plane (m_2, m_3). Fig. 4 also shows the chromatograms of the binary mixture of the feed, the extract and the raffinate for experiments taken from 23.5 min of switching time.

The concentration variations of the extract and the raffinate with valve switching are recorded and plotted in Figs. 5 and 6. In Figs. 5 and 6, the x-axis represents the number of switching, and the y-axis is the concentration of PEG. The effluents from the extract and the raffinate were only sampled in the daytime; the experiments lasted for two days. Larger fluctuations of the concentration in the raffinate were observed because of sampling contamination inside the capillary restrictor. Normally, an SMB will reach steady state after 6–8 cycles of the rotation of the six columns.

The productivity of an SMB is normally limited by the pressure drop of the packed column. Although a used SMB can stand for 34 MPa, the GMPW column can only operate below 1.0 MPa as recommended by the manufacturer. It was found that the pressure drop of a single GMPW column roughly ranged from 0.3 to

Table 1
Separation of the mixtures of MWs of 400 and 8000.

Run	Switching (min)	Extract (ppm)		Raffinate (ppm)		Purity (%)		Recovery (%)		Remark
		8000	400	8000	400	Extract	Raffinate	Extract	Raffinate	
1	21.5	32.3	65.68	51.5	0	67	>99.9	>99.9	61.3	Pure R
2	22.5	2.8	106.48	69.01	0	97.3	>99.9	>99.9	91.74	Pure R & E
3	23.5	2.05	105.69	71.64	0	98.1	>99.9	>99.9	95.74	Pure R & E
4	24.5	2.93	78.52	58.83	57.71	96.4	56.6	61.8	95.2	Pure E

Feed concentration: MW 8000 = 252.16 ppm; MW 400 = 288.93 ppm. Flow rate (ml/min): Q_F = 0.04; Q_D = 0.5; Q_E = 0.15; Q_R = 0.39.
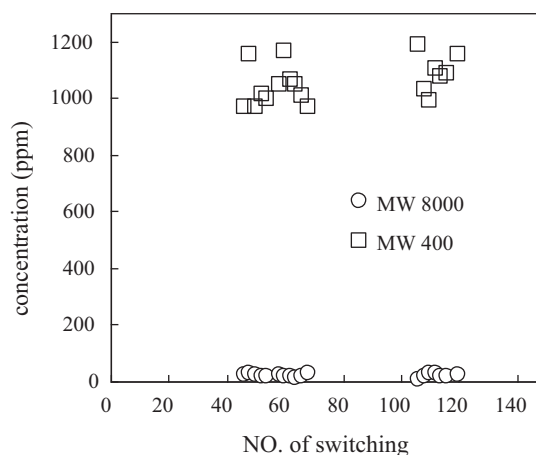
Table 2
Separation of the mixtures of MWs of 1500 and 20,000.

Run	Switching (min)	Extract (ppm)		Raffinate (ppm)		Purity (%)		Recovery (%)		Remark
		20,000	1500	20,000	1500	Extract	Raffinate	Extract	Raffinate	
1	20.5	265.7	489.7	504.4	0	63.6	100	>99.9	65.5	Pure R
2	21.5	0.5	640	401.1	0	99.9	100	>99.9	>99.9	Pure E & R
3	22.5	0	557.5	362	242	100	62.0	70.7	>99.9	Pure E

Feed concentration: MW 20,000 = 1866.5 ppm; MW 1500 = 1674.9 ppm. Flow rate (ml/min): $Q_F = 0.045$; $Q_D = 0.5$; $Q_E = 0.15$; $Q_R = 0.395$.

0.6 MPa with a water flow rate of 1.0 ml/min. Therefore, the liquid flow rates on the SMB had to be reduced to avoid overpressure, and the switching time was correspondingly extended to assure the fulfillment of the Triangle theory.

6.3. Separation of MWs of 1500 and 20,000

Three test runs were conducted for the separation of molecular weights of 1500 and 20,000, and their results are listed in Table 2. Operation conditions represented on the (m_2, m_3) plane are illustrated in Fig. 7. It can be observed that only 21.5 min of switching time was located inside the triangle and it was also close to the apex of the triangle, which represents the optimized operation [1,3]; 20.5 and 22.5 min of switching time gave pure raffinate and pure extract, respectively, as expected by the Triangle theory.

6.4. Separation of MWs of 1500 and 3500

At least twenty tests were conducted to separate the mixtures of molecular weights of 1500 and 3500, yet no feasible separation was found. The difficulty encountered in finding the separable operating conditions was a result of the relative elution being too small to operate. In Fig. 2, it can be observed that the area of the triangle for MWs 1500 and 3500 was very small compared to that of MWs 400 and 8000, and MWs 1500 and 20,000. According to the Triangle theory, it was still possible to obtain pure extract and pure raffinate individually.

When the flow rates of the three pumps were set as those in Table 2 and the switching time was in the range of 24–26.5 min, the PEGs in the feed were totally entrained out from the exit of the extract. If the switching time was increased up to 28 min, pure raffinate was successfully obtained. Furthermore, by increasing the switching time to 29 min, neither the raffinate nor the extract was pure. Extending the switching time up to 35 min by
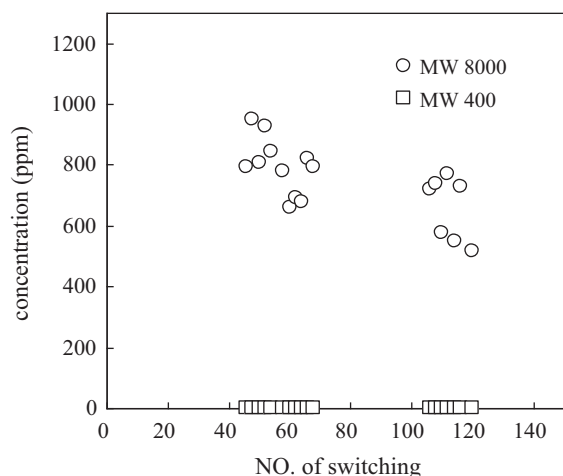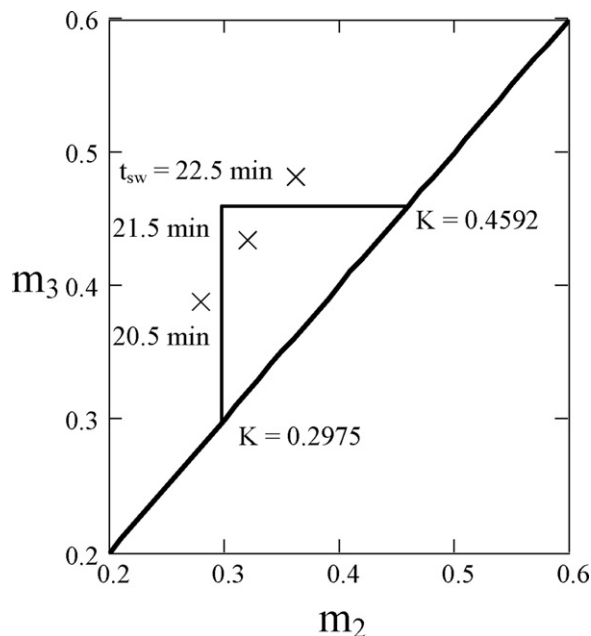

Fig. 7. Illustration of the Triangle theory for the separation of PEGs of MW 1500 and 20,000.

0.5 min increments did not give any pure extract. It was presumed that the capacity of the stationary phase to entrain particles was too small to extract a significant amount of small particles. Replacing packing material with appropriate pore-size distribution should allow separation of the mixture of MWs of 1500 and 3500.

7. Conclusion

In this study, an SMB was successfully applied to separate different molecular weights of PEGs. By analogy to Henry's constant, an elution constant was defined and applied to the Triangle theory to find the separable operation parameters. It was shown that the elution constant could successfully assist in locating the separable triangle on plane (m_2, m_3). For the mixtures of similar molecular weights, separation was difficult and the appropriate pore-size distribution of the porous packing material was crucial.

References

[1] Rajendran, G. Paredes, M. Mazzotti, J. Chromatogr. A 1216 (2009) 709.
[2] G. Subramanian (Ed.), Bioseparation and Bioprocessing: A Handbook, vol. 2, Wiley-VCH, Weinheim, 1998.
[3] G. Subramanian (Ed.), Chiral Separation Techniques: A Practical Approach, 2nd ed., Wiley-VCH, Weinheim, 2001.

Fig. 6. Concentration variation in raffinate at 23.5 min of switching time.

[4] J.X. Cong, B.C. Lin, J. Chromatogr. A 1145 (2007) 190.
[5] F. Wei, Y.X. Zhao, J. Chromatogr. A 1187 (2008) 281.
[6] M.T. Liang, R.C. Liang, L.Y. Wang, H.E. Yen, K.T. Lee, J. Chem. Chem. Eng. 5 (2011) 479.
[7] S. Mun, N.-H.L. Wang, Ind. Eng. Chem. Res. 45 (2006) 1454.
[8] P.C. Wankat, Rate-controlled Separations, Kluwer, Amsterdam, The Netherlands, 1990.
[9] M.L. Maire, A. Ghazu, J.V. Moller, L.P. Aggerbeck, Biochem. J. 243 (1987) 399.
[10] Y. Zang, P.C. Wankat, Ind. Eng. Chem. Res. 41 (2002) 5283.
[11] R. Bhat, S.N. Timasheff, Protein Sci. 1 (1992) 1133.