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RAPID COMMUNICATION

A three-zone simulated moving-bed for separation of immunoglobulin Y

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Abstract−Separation of immunoglobulin Y (IgY) from hen eggs is an interesting topic for investigating antibody purification. Understanding of IgY separation by chromatography is necessary to prepare antibody. IgY preparation in SMB was simulated by Aspen chromatography and was experimented by assembling a 3-zone simulated moving bed (SMB). With change of stream flow rates, the SMB performance was studied. Simulation of IgY batch HPLC chromatography was also done and confirmed by HPLC experiments. Based on these, good operating conditions of SMB chromatography were determined. Three-zone SMB equipment was set up by connecting three C_{18}-HPLC columns, four HPLC pumps, and six multiposition valves. Batch chromatography of IgY was conducted to determine the isotherms of IgY and contaminating impurity. The outlet streams of SMB, raffinate and extract were sampled and analyzed by analytical HPLC system. The adsorption isotherms of IgY and impurity were determined as H_{IgY}=0.1 and H_{impure}=1.1. The highest experimental purity of IgY from SMB was obtained as 98% for the operating parameters of Q_{feed}=0.2 mL/min, $Q_{desorbent}$=0.31 mL/min, $Q_{extract}$=0.2 mL/min, $Q_{raffinate}$=0.3 mL/min, and switching time=8.47 min.

Key words: IgY, SMB (Simulated Moving Bed)

INTRODUCTION

Simulated moving bed (SMB) chromatography, whose principle of operation can be described by referring to the equivalent true moving bed configuration, is a continuous chromatographic process developed in the 1960's by UOP (United Oil Products) [1]. With respect to batchwise preparative chromatography, SMB chromatography has high purity, high yield and less mobile phase consumption over batch chromatography in terms of operating costs [2]. It has been used in the petrochemical and sugar [3] industries, and for lipids [4], protein [5] and chiral separations [6]. Nevertheless, SMB chromatography has the disadvantage of high equipment cost and complex operation. Research on reducing the cost of the SMB process has been conducted, such as the three zone SMB process [7].

In complex SMB chromatography, the optimization of the operation conditions relies on the determination of accurate adsorption isotherms. The Langmuir model is the most popular equilibrium isotherm among the various isotherm models. A multi-component Langmuir isotherm explains the competition of two components for available adsorption sites. The competitive Langmuir isotherms in SMB chromatography have been studied by Juza [8], and the linear independent isotherm is sometimes useful in case of SMB separation for proteins [9].

Immunoglobulin Y (abbreviated as IgY) is a type of immunoglobulin which is the major antibody in bird. It is also found in high concentrations in chicken egg yolk [10]. As with the other immunoglobulins, IgY is a class of proteins formed by the immune system in reaction to certain foreign substances. This is because IgY differs both structurally and functionally from mammalian IgG, and does not cross-react with antibodies raised against mammalian IgG. Since chickens can lay eggs almost every day, and the yolk of an immunized hen's egg contains a high concentration of IgY [11], chickens are gradually becoming popular as a cheap source of antibodies for research.

Like IgG, it is composed of two light and two heavy chains. Structurally, these two types of immunoglobulin differ primarily in the heavy chains, which have a molecular mass of 65,100 daltons in IgY, and are thus larger than in IgG. The light chains in IgY, with a molar mass of 18,700 daltons, are somewhat smaller than the light chains in IgG. The molar mass of IgY thus amounts to 167,000 daltons [12].

This study was performed to obtain the optimal separation conditions for IgY in SMB. A small perturbation from one selected operation point was done to obtain an optimum condition. In this work, a linear isotherm from pulse analysis was assumed for SMB separation, and the results of the pulse analysis were used to get isotherm parameters which can be inserted into the simulation model of Aspen chromatography. Isotherm parameters as well as m values of the triangle theory [13] were the basis for simulation, and simulation results were compared with experimental results from the 3 column SMB made in our laboratory.

THEORY

1. SMB Theories

The SMB unit consists of three zones, with each zone having one column connected in series. The feed, desorbent, extract and raffinate ports are placed between these columns (Fig. 1). The port between the columns allows for opening or closing of the inlet (feed, desorbent) and outlet streams (raffinate, extract) at a specified switching time. The counter-current movement between the stationary and mobile phases is simulated by the port movements, as shown in

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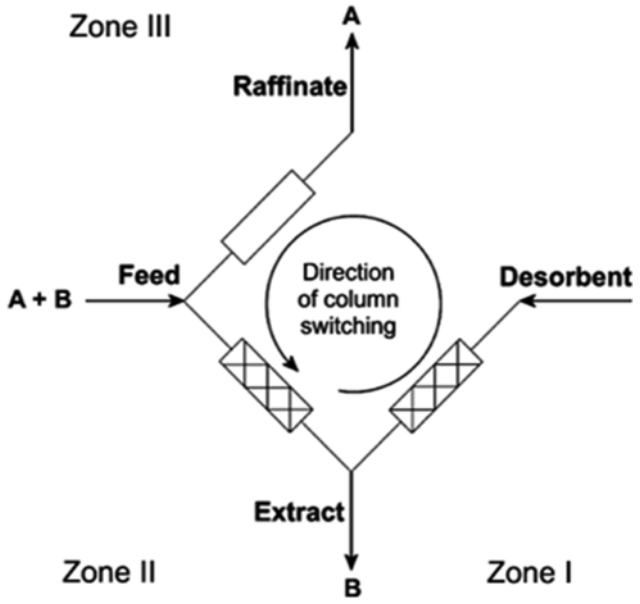

Fig. 1. The schematic diagram of three-zone open-loop SMB chromatography.

Fig. 1. The feed is fed between zones II and III. The less retained IgY and highly retained impure solutes are separated and collected in the raffinate and extract, respectively. The low retained material is IgY and the highly retained one is called as impurity in our case.

The following material balances are used between the stationary and mobile phases [14]:

$$\varepsilon^*\frac{\partial C_i}{\partial t}+(1-\varepsilon^*)\frac{\partial q_i}{\partial t}+v_l\frac{\partial C_i}{\partial Z}=\varepsilon^* E_z\frac{\partial^2 C_i}{\partial Z^2}$$

$$\frac{\partial q_i}{\partial t}=K_f(q_i^*-q_i)$$

$$q_i^*=f_{eq}(C_i)$$

where C_i is the mobile phase concentration of component i, q_i the adsorbed solid phase concentration of component i, * the total porosity, v_l the superficial velocity, and E_Z and K_f the axial dispersion coefficient and the lumped mass transfer coefficient (MTC), respectively. The q_i^* represents the adsorbed solid phase concentration in equilibrium with the mobile phase concentration, and is usually related to the C_i by the Langmuir equation.

The Langmuir isotherm for a single-component is:

$$q=\frac{ac}{1+bc}$$

where q and c are the concentrations of the solute in the stationary and mobile phases at equilibrium, respectively, and a and b are characteristic parameters of the solute in a given system. If the solute concentration is low like proteins, the nonlinear isotherm becomes a linear form of the Henry equation such as $q_i=H_ic_i$. The parameter H_i (Henry's constant) is estimated using pulse analysis of each component with the following equation:

$$t_{Ri}=t_0\,(1+(1-\varepsilon^*)H_i/\varepsilon^*)$$

2. Triangle Theory

For greater understanding of the SMB process, the triangle theory was used by applying the equilibrium theory, which neglected mass transfer resistance and axial dispersion. So the triangle theory proposes separation conditions of SMB under the assumption of linear adsorption isotherms. The triangle theory facilitates the determination of SMB operating conditions.

In zones 2 and 3 of the SMB unit (Fig. 1), IgY and impurity mixture is separated. The more retained component B (impurity extract stream) and less retained component A (IgY raffinate stream) are separated in the standard three-zone SMB unit. In the triangle theory, the key operating parameters are flow rate ratios, m_j, j=1, 2, 3, in the three sections of the SMB unit, according to:

$$m_j=\frac{Q_jt^*-V\varepsilon^*}{V(1-\varepsilon^*)}$$

, where V is the column volume, t^* is the column switching time, i.e., the time between two successive switches of the inlet and outlet ports, $\varepsilon^*=\varepsilon+(1-\varepsilon)\varepsilon_p$ is the overall void fraction of the column, with ε and ε_p, being the bed void fraction and the macroporosity of the stationary phase particles, and Q_j is the volumetric flow rate in the j^{th} section of SMB unit.

These conditions are satisfied for complete separation between two components.

$H_A<m_2<H_B$ (in zone •II)
$H_A<m_3<H_B$ (in zone III)

As illustrated in Fig. 2, the region of complete separation is surrounded by three lines: horizontal, vertical and diagonal. In the regions of pure raffinate or extract, the raffinate or extract stream is pure, respectively. Both raffinate and extract are pure inside the triangle region.

The performance of SMB can be determined by the following criteria: desorbent consumption/feed flow rate (D/F), enrichment of IgY in raffinate (raffinate IgY concentration/feed IgY concentration) which can be calculated by $(m_3-m_2)/m_3$, and productivity of

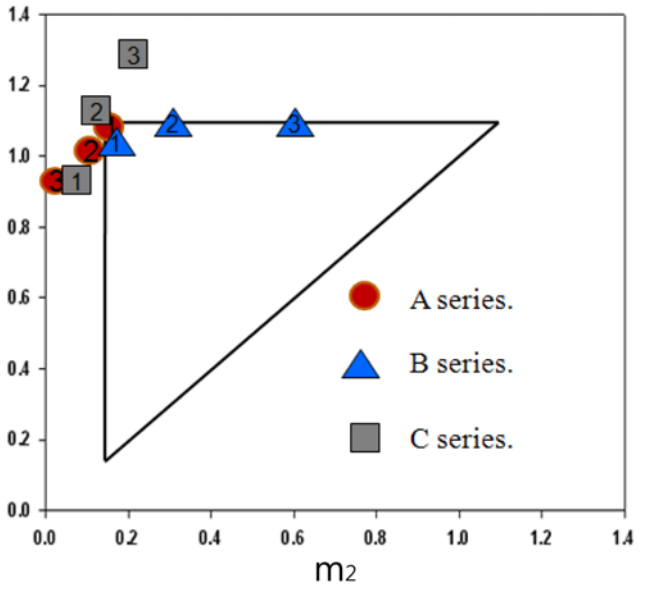

Fig. 2. Triangle diagram showing m_j as the ratio of net fluid flow rate and the adsorbent phase flow rate and starting simulation points.

IgY (IgY amount in feed stream per unit time/column gel amount).

MATERIALS AND METHODS

1. Materials

The IgY and impurity mixture was prepared by the method of the previous report [15], which is shown in Fig. 3. Fresh egg yolk was diluted, centrifuged and treated with delipidification agent Arabic gum powder. The deionized water (DIW) was obtained from Milli-Q system (Waters, USA), and filtered through 0.22 μm filters. The HPLC grade acetonitrile and Arabic gum powder were purchased from Sigma.

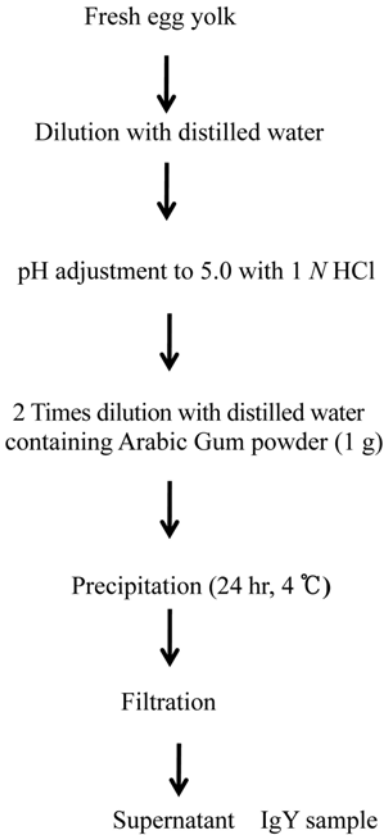

Fig. 3. Schematic diagram of pretreatment steps for IgY purification.

The columns for the batch and SMB experiments were ODS-2 columns (0.46×15 cm, Inertsil, USA). One column was used in the batch experiment, and three columns for the SMB work. Beckman (110B model) HPLC pump was used for the isotherm measurement. Concentrations of IgY and impurity were analyzed by the Beckman pump and an ABI 757 tunable detector. The SMB unit was composed of three columns placed as one column in each zone. Four Younglin pumps (Model 930, 925) controlled the feed, desorbent, extract and raffinate streams. Three rotary valves (Valco 8 position), with one inlet and eight outlet ports, and six port manifolds were used for controlling SMB streams. Each column has one rotary valve connected to the four streams. Each valve changed the flow path at every switching time. The valves were controlled by a software made by a local company (Sunyoung, Korea).

2. Methods

Acetonitrile and distilled water (DIW) were used to design the eluent for IgY. The concentrations of the extract and raffinate samples in the SMB experiment, and peaks from the binary pulse input chromatography, were analyzed by HPLC. A mixture of Acetonitrile/DIW/TFA (85/15/0.1%) was used as the mobile phase at 1.0 mL/min with injection volume of 5 μL, and detected at 254 nm. Identity tests for three ODS-2 columns were performed and the average retention times of IgY and impurity were within 5% value.

A triangle diagram was drawn with the two Henry constants of 0.1 and 1.1 for IgY and impurity, and nine points of m_2 and m_3 around the triangle, whose vertices' coordinates were (0.1, 0.1), (1.1, 1.1), and (0.1, 1.1), were selected to simulate operation performance of those nine points by Aspen chromatography. They are classified as A, B, and C series according to the change of extract, feed, and switching times, respectively. Table 1 shows that the extract and raffinate flow rates vary in the series A runs, the feed flow rates in the series B, and the switching times in the series C. To find optimum operation conditions, SMB chromatography was simulated by Aspen chromatography software. Three series were considered to account for the effects of extract and raffinate flow rate (A), feed flow rate (B), and switching time (C).

RESULTS AND DISCUSSION

The batch chromatography experiments for determining eluent

Table 1. Operation parameters of simulation runs

Run	Flow rates (ml/min)							t^* (min)	m_2 and m_3
	Q_{De}	Q_{Ex}	Q_{Fe}	Q_{Ra}	Q_1	Q_2	Q_3		
A1	0.31	0.2	0.2	0.31	0.31	0.11	0.31	8.47	(0.1, 1.1)
A2	0.31	0.21	0.2	0.30	0.31	0.10	0.30	8,47	(0.03, 1.0)
A3	0.31	0.22	0.2	0.29	0.31	0.09	0.29	8.47	(0.008, 0.9)
B1	0.31	0.2	0.2	0.31	0.31	0.11	0.31	8.47	(0.1, 1.1)
B2	0.36	0.2	0.15	0.31	0.36	0.16	0.31	8.47	(0.3, 1.1)
B3	0.41	0.2	0.10	0.31	0.41	0.21	0.31	8.47	(0.6, 1.1)
C1	0.31	0.2	0.2	0.31	0.31	0.11	0.31	7.47	(0.02, 0.9)
C2	0.31	0.2	0.2	0.31	0.31	0.11	0.31	8.97	(0.1, 1.1)
C3	0.31	0.2	0.2	0.31	0.31	0.11	0.31	9.47	(0.14, 1.3)
D	0.57	0.39	0.2	0.38	0.57	0.18	0.38	4.67	(0.06, 0.5)

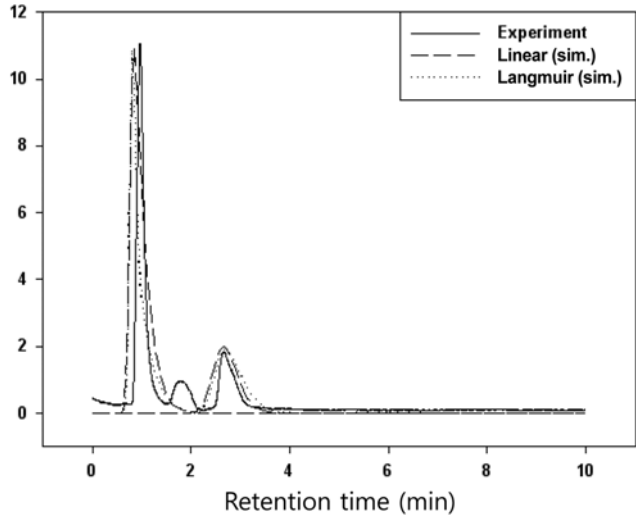

Fig. 4. Comparison of experimental IgY and simulated IgY chromatograms for determining mass transfer coefficient (MTC) for SMB simulations.

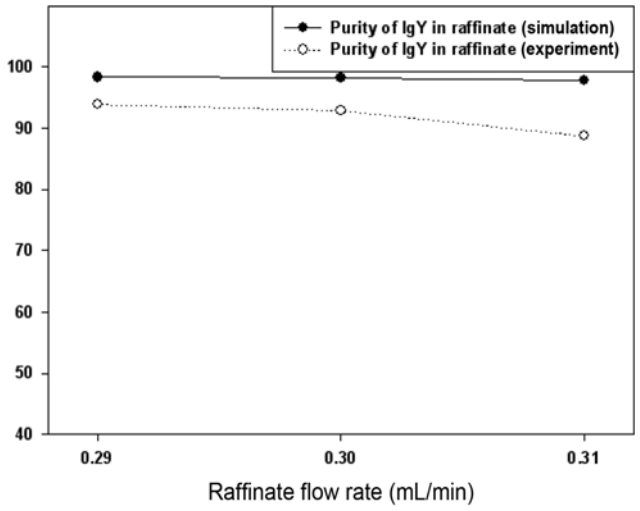

Fig. 5. Variation of simulation and experimental purities of IgY in raffinate when raffinate flow rate=0.29 to 0.31 mL/min.

conditions were successfully performed to separate the IgY and impurity. The peaks of IgY and impurity appear at different retention times by changing acetonitrile mobile phase concentrations [16]. The 85% of acetonitrile was selected to perform SMB experiments. Pulse experiments with three dilution factors of IgY preparation sample from Fig. 3 were performed and isotherms for IgY and impurity were calculated [17]. The isotherm between solution IgY and adsorbed IgY follows a linear line and was measured to be q=0.1 c. The configuration of the SMB was 1-1-1, i.e., one column in each zone. The system parameters for simulation and operating the SMB are listed in Table 1. The MTC of IgY for SMB simulation was obtained as MTC=10 by comparing the simulation with the pulse experiments of IgY, as shown in Fig. 4 chromatograms. The first peak is considered as high molecular weight IgY and following peaks as low molecular weight impurities.

Simulations for IgY purification in SMB have been reported in previous investigations [11,17]. Randomly selected points around and inside the triangle were used to obtain various operation conditions. IgY concentration profiles along columns as well as with time were shown. Highest purity was obtained as 98% at the point (0.1, 1.1). Therefore, A1 was selected as the starting point for the comparison between simulations and experiments. The A series was chosen to understand the effect of raffinate flow rate. The sum of raffinate and extract flow rates should be equal to that of feed and desorbent flow rates in SMB. With the increase of raffinate flow rate, it results in the decrease in the extract flow rate under the condition of fixed feed and desorbent flow rates. The m_2 largely increases from 0.008 to 0.1, whereas m_3 little changes from 0.9 to 1.1. The effect of feed flow rate was studied in the B series. Desorbent flow rate increases with the decrease of feed flow rate by keeping raffinate and extract flow rates constant. The m_2 increases from 0.1 to 0.6, whereas m_3 is unchanged. Switching time was changed in C series to understand its effect on purity.

A1 point suggests that 8.47 min is the starting time and the switching times change from 7.47 (C1) to 9.47 (C3). With the increase of switching time, m_2 and m_3 simultaneously increase from (0.02, 0.9) to (0.14, 1.3). The C points move to the pure extract region. The D point (m_2=0.06 and m_3=0.5) was introduced to show the attainable highest raffinate purity. The target material is the raffinate and so the point of the triangle needs to be in the pure raffiinate zone. However, desorbent flow rate is high in this condition and experiments were difficult to confirm simulations.

In A series runs, the change of extract and raffinate flow rates affects the purities of IgY. As $Q_{raffinate}$ changes from 0.29 to 0.31 mL/min in Fig. 5, simulated purity of IgY slightly decreases from 98 to 97% and experimental purity changes from 94 to 90%. As $Q_{raffinate}$ increases from 0.29 to 0.31 mL/min (A3->A1), $Q_{extract}$ decreases as shown in Table 1. The decrease of outflow $Q_{extract}$ leads to the simultaneous increase of Q_2 and Q_3 by the unchanged Q_{feed}, resulting in more overlapped internal concentration profiles of IgY and impurity across the zones 2 and 3. Therefore, raffinate IgY purity decreases.

The decrease of raffinate purity can be explained in another way. Flow rates of desorbent and flow rates of zone 1 (Q_1) are unchanged in the A series. Flow rates of raffinate are the same as the flow rates of zone 3 (Q_3). However, the extract flow rate is reversely in proportion to the zone 2 flow rate. When extract flow rate increases, Q_2 and Q_3 decrease, that is, both flow rates of zone 2 and zone 3 become lower (Table 1) and m values decrease. Purities of extract and

Table 2. Operation performances of simulation runs

Run	Productivity (g IgY/g gel hr)	D/F	Purity	Enrichment
A1	0.0067	1.55	97.8	0.9
A2	0.0067	1.55	98.2	1.0
A3	0.0067	1.55	98.3	1.1
B1	0.0067	1.55	97.8	0.9
B2	0.0050	2.4	98.7	0.72
B3	0.0034	4.1	98.7	0.45
C1	0.0067	1.55	97.8	0.98
C2	0.0067	1.55	97.8	0.9
C3	0.0067	1.55	97.2	0.89
D	0.0067	2.85	98.3	0.88

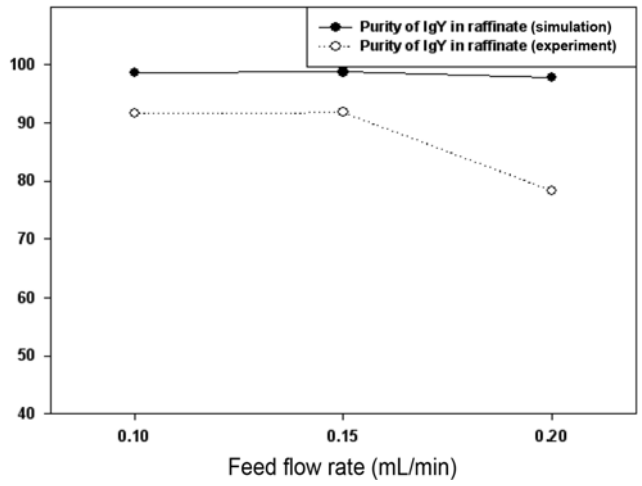

Fig. 6. Variation of simulation and experimental purities of IgY in raffinate when feed flow rate is 0.1 to 0.2 mL/min.

raffinate decrease because m_2 and m_3 move to the left side out of the triangle region where high purity is attainable. From the A series, we understand that raffinate IgY purity decreases by increasing raffinate flow rate.

D/F ratios are calculated as 1.55 in A series and do not change since desorbent and feed flow rates are unchanged (Table 2). Desorbent saving is reported to be low in three zone SMB compared with four zone SMB because the three zone SMB is an open loop process and has no recycle. IgY enrichments can be calculated as $(m_3-m_2)/m_3$ which are near one. They show that IgY concentration does not change during continuous separation. Productivity is about 0.0067 g IgY/(g adsorbent hr) which is one-tenth the value of chiral SMB separation due to the characteristics of high molecular weight IgY as well as low binding capacity of stationary phase [19]. Fig. 5 also shows the comparison of experimental and simulation purities for IgY. The agreements are fairly good except $Q_{extract}$=0.31 mL/min.

Several runs were carried out when feed flow rate, Q_{feed}, was varied from 0.1 to 0.2 mL/min (B3->B1), and purity decrease is shown in Table 2 as well as Fig. 6. The operation conditions are shown in B runs of Table 1. When switching time is kept constant, increasing feed flow rate results in the decrease of m_2 from 0.6 to 0.1 in Table 1 and triangle points move to the left as shown in Fig. 2. The effect of feed flow rate on SMB performance can be explained by referring to m_2 decrease. The m_2 decrease of series B leads to lower IgY purity. The decrease of zone 2 flow rate as well as m_2 explains that contamination of IgY raffinate stream by impurity occurs by the higher loading of sample mixture from feed stream. The B series illustrates that IgY purity decreases with the increase of feed flow rate.

D/F is in the range from 1.55 to 4.1 (Table 2) since desorbent flow rates increases by the decrease of feed flow rates with unchanged raffinate and extract flow rates. Calculated enrichment of IgY becomes lower from 0.9 to 0.45 when feed flow rates decrease from 0.2 to 0.1 due to less difference between m_3 and m_2. This means that IgY concentration in raffinate stream decreases with the increase of desorbent flow rates as well as the decrease of feed flow rate. Fig. 6 illustrates the above-mentioned purity decrease by the phenomena occurring in zone 2. The agreement between simulation

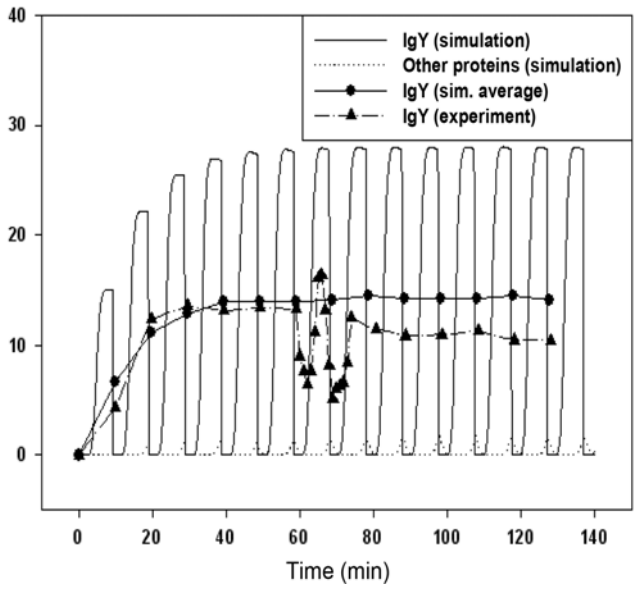

Fig. 7. Simulated transient IgY concentration and assayed concentrations of IgY samples.

and experiment is not good in the case of Q_{feed}=0.2 mL/min.

Fig. 7 shows why experimental purity is under the simulation purity. In the B1 experiment, collected raffinate IgY samples (triangle points) were analyzed and compared with averaged simulation value (circle points) from fluctuated cyclic simulation results. Cyclic concentration profiles of simulation and experiment come from the periodic valve switching. The experimental data between 60-80 mins were obtained by more frequent sampling, and consequently confirm the fluctuating concentration profile. Other experimental data points were obtained by one sampling during one switching time. Simulation average value is higher than that of experiment. Unknown nonlinear phenomena in SMB system occur and the linear assumptions in SMB are not correct. Simulation assumes that equilibrium and linear relation between stationary and mobile phases are attained. The triangle for the nonlinear case is skewed and becomes smaller in area, which results in overestimated purity by simulation. The gap between simulation and experiment becomes larger when

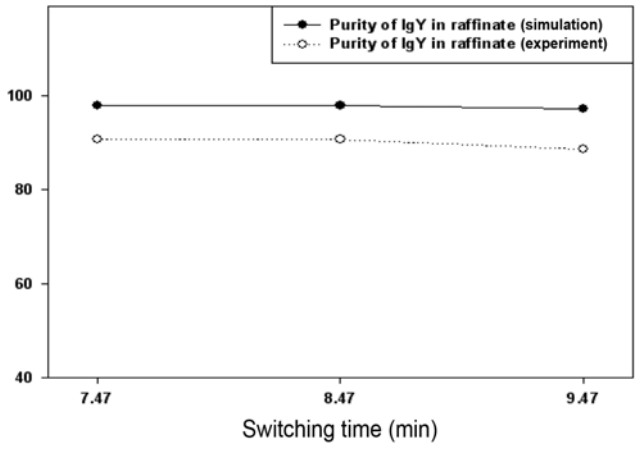

Fig. 8. Variation of simulation and experimental purities of IgY in raffinate when switching time is 7.47 to 9.47 mL/min.

raffinate and feed flow rates increase and m points move far from the diagonal line of the triangle.

C series in Table 1 are intended to understand the effect of switching time on the purity of IgY. Fig. 8 and Table 2 indicate that the increase of switching time slightly lowers the purity of igY. The variation of switching time was under certain limit since the triangle theory limits the values of m_2 and m_3, which should be near the triangle region. The increase of switching time makes m values larger without changes of Q_2 and Q_3, and small decrease of purity is observed. D/F ratio and IgY enrichment 1.55 and 0.89-0.98, respectively, are shown in Table 2.

CONCLUSIONS

IgY separation was studied by Aspen simulation and SMB experiment. The Aspen simulation was conducted under varying conditions of raffinate flow rate (0.29-0.31 mL/min), feed flow rate (0.1-0.2 mL/min), and switching time (7.47-9.47 min) with three ODS-2 HPLC columns (0.46×15 cm) in SMB. Conditions leading to better separation were observed and explained theoretically. Adsorption isotherm was experimentally determined as H_{IgY}=0.1 and $H_{impurity}$=1.1. When raffinate flow rate increases, purity of raffinate IgY decreases. Increasing feed flow rate decreases purity of raffinate IgY. Two observations can be explained by m points movement from the triangle diagram.

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REFERENCES

1. D. B. Broughton, R. W. Neuzil, J. M. Pharis and C. S. Brearley, *Chem. Eng. Pog.*, **66**, 70 (1970).
2. M. B. Park and I. H. Kim, *Korean Chem. Eng. Res.*, **48**, 88 (2010).
3. Y. J. Jeon, M. B. Park and I. H. Kim, *Bioprocess Biosyst. Eng.*, **33**, 97 (2010).
4. Y. B. Lu, Y. W. Yang and P. D. Wu, *J. Zhejiang Univ. Science B*, **7**, 559 (2006).
5. L. C. Keâler, G. Ludmila, R. Ursula and S. M. Andreas, *J. Chromatogr. A*, **1176**, 69 (2007).
6. T. H. Yoon, B. H. Chung and I. H. Kim, *Biotechnol. Bioprocess Eng.*, **9**, 285 (2004).
7. Z. Zhang, M. Mazzotti and M. Morbidelli, *Korean J. Chem. Eng.*, **21**, 454 (2004).
8. M. Zuza, *J. Chromatogr. A*, **865**, 35 (1999).
9. C. H. Lee and Y. M. Koo, *Kor. J. Biotechnol. Bioeng.*, **20**(3), 192 (2005).
10. K. Y. Ko and D. U. Ahn, *Poultry Sci.*, **86**, 400 (2007).
11. S. H. Yoon and I. H. Kim, *Korean Chem. Eng. Res.*, **50**, 866 (2012).
12. B. Meulenaer and A. Huyghebaert, *Food Agricul. Immunol.*, **13**, 275 (2001).
13. M. Mazzoti, G. Storti and M. Morbidelli, *J. Chromatogr. A*, **769**, 3 (1997).
14. M. Zuza, M. Mazzoti and M. Morbidelli, *Trends in Biotechnol.*, **18**, 108 (2000).
15. S. M. Song and I. H. Kim, *Korean Chem. Eng. Res.*, **50**, 659 (2012).
16. S. M. Song, M. B. Park and I. H. Kim, *Korean J. Chem. Eng.*, **29**, 952 (2012).
17. S. M. Song and I. H. Kim, *Korean Chem. Eng. Res.*, **49**, 798 (2011).
18. J. S. Park, W. S. Kim, J. M. Kim and I. H. Kim, *J. Chem. Eng. Japan*, **7**, 624 (2008).
19. E. Lee, J. M. Kim, W. S. Kim and I. H. Kim, *Biotechnol. Bioprocess Eng.*, **15**, 103 (2010).