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Three-zone simulated moving-bed (SMB) for separation of cytosine and guanine

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Abstract−Separation of guanine and cytosine base pairs in nucleotide is an interesting topic for investigation of DNA structure. Therefore, an understanding of nucleotide separation by chromatography is necessary to prepare DNA molecules. Guanine and cytosine separation in SMB was simulated by Aspen chromatography and it was experimented by assembled 3-zone simulated moving bed (SMB) with change of stream flow rates, sample concentration, and desorbent flow rate. The simulation of batch chromatography was also 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 cytosine and guanine was conducted to determine the isotherms of the two nucleotides. The outlet streams of SMB, raffinate and extract were sampled and analyzed by analytical HPLC system. The adsorption isotherms of cytosine and guanine were H_C= 0.5 and H_G=1.05. The highest experimental purity of cytosine and guanine in SMB was obtained as 94.9% and 89.8% with operating parameters of Q_{feed}=0.2 mL/min, $Q_{desorbent}$=0.6 mL/min, $Q_{extract}$=0.2 mL/min, $Q_{raffinate}$=0.6 mL/min, and switching time=7 min.

Key words: Guanine, Cytosine, SMB (Simulated Moving Bed)

INTRODUCTION

The development of modern analytical methods results in the deciphering of bioinformation research in the nucleotide. The nucleotide has many important biological functions in many fields. Guanine in nucleotide is paired with cytosine [1]. Bases change in structure is the main cause of inherited diseases and human cancers [2]. Thus, their separation and analysis have received extensive attention for many years.

High performance liquid chromatography (HPLC), which is commonly used for analysis of purine and pyrimidine bases [3], is known to be a powerful tool for the analysis of nucleotides. HPLC is operated in a batch chromatography mode, and its separation speed is limited due to batch operation [4].

The simulated moving bed (SMB) is a continuous chromatographic countercurrent process that has been widely used on large scale in separation of fine chemicals and biochemicals [5]. Compared to HPLC, column adsorbent usage and the desorbent consumption in SMB are much less than those of HPLC. In addition, high performance of SMB can be achieved even when the adsorbent selectiv-

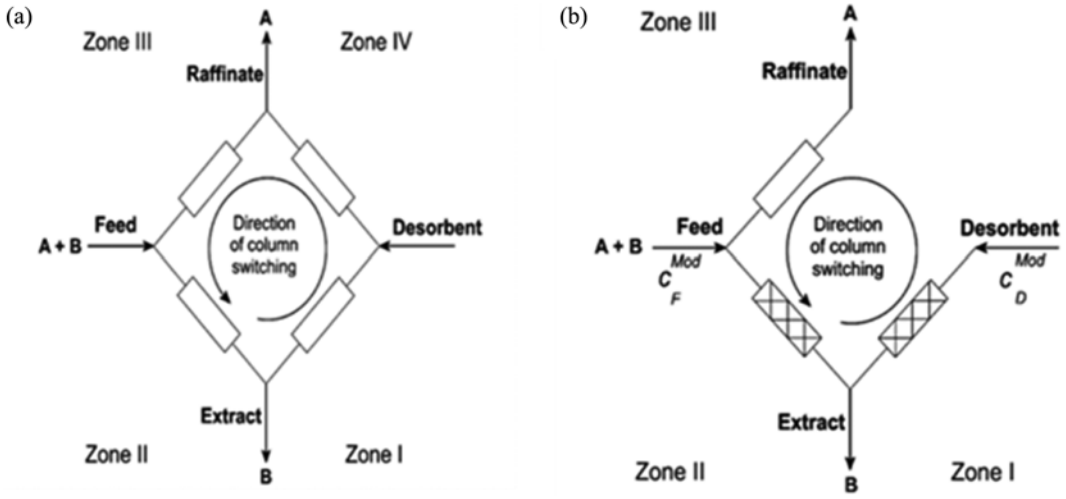

Fig. 1. The schematic diagram of four-zone closed-loop and three-zone open-loop SMB chromatography.

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ity is rather low [6]. Therefore, SMB chromatography is expected to be used in nucleotide separation.

In the SMB process, a continuous multi-column chromatographic process is illustrated in Fig. 1. Conventional fixed bed chromatographic columns can be used, and the inlet and outlet ports to the unit are switched periodically in the direction of the fluid flow so as to simulate the moving bed.

Four-zone SMB is a classical process widely used in industry [7]; however, three-zone SMB in Fig. 1 has many advantages over the four-zone SMB since it does not have a closed recycle line and is free from associated problems such as leakage due to high pressure in the closed line. We reported a novel process development consisting of three columns for the separation of ribose and arabinose by three-zone-SMB [8].

This work aims at evaluating the SMB performance and separation of pure cytosine and guanine in nucleotide. HPLC analysis of them was performed and its experimental results were used to find the effect of SMB operating conditions (desorbent flow rates, feed flow rate and feed concentration in each section of SMB) on purity. It was expected that optimal operation conditions could be obtained from this study and could be useful for preparation of cytosine and guanine by three-zone SMB.

EXPERIMENTAL AND SIMULATION METHODS

1. SMB Unit

SMB consisting of a carousel of three stainless steel ZORBAX 300SB-C18 columns (4.6 mm×250 mm, Agilent, U.S.A) was connected to multiposition valves. These valves were connected to four high pressure liquid chromatographic pumps of feed, desorbent, extract, and raffinate (M925, M925, M930, SP930D, Young-lin, Korea). Water/methanol/acetic acid (90 : 10 : 0.2 v/v) was used as the desorbent (mobile phase).

2. Determination of Purity by HPLC

The composition of the sample from SMB was determined by HPLC with the same ZORBAX 300SB-C18 column used in the SMB. The wavelength of the UV detector (ABI, USA) was set at 270 nm, and the flow rate of the analysis was fixed at 0.5 ml/min. Adsorption isotherms were determined by the pulse input method by varying injection sample concentrations [9]. Pulses of various sample concentration were introduced into columns and the retention times of cytosine and guanine were measured.

3. Column Characterization

The identity of the columns was determined by the retention times of cytosine and guanine in three HPLC columns. The retention times in three columns and their performance should be identical to be used in three-zone SMB.

4. Adsorption Isotherms

Since the concentration of cytosine and guanine in the SMB feed was low, linear adsorption isotherm was applied. Pulses of various sample mixtures were introduced into columns and the retention times of cytosine and guanine were measured. The Henry constant H_i was calculated by the following Eq. (1);

$$H_i = \frac{t_i - t_o}{t_o} * \frac{\varepsilon^*}{1-\varepsilon^*} \quad (1)$$

where t_o indicates the dead volume of the column and ε^* is the total porosity of the column.

5. Triangle Theory

For a 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 operating conditions of SMB for complete separation.

In zones 2 and 3 of an SMB unit (Fig. 1), mixture is separated. So, the more retained component B and less retained component A are separated in a 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:

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

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. And both raffinate and extract are pure inside the triangle region.

6. Aspen Chromatography Simulation

To find optimum operation conditions, SMB chromatography was simulated by Aspen chromatography software. Simulation was performed with parameters given in Table 1. Three series were considered to account for the effects of extract flow rate (A), feed flow rate (B), and feed concentration (C). Q_{De}, Q_{Ex}, Q_{Fe}, Q_{Ra}, Q_1, Q_2, and

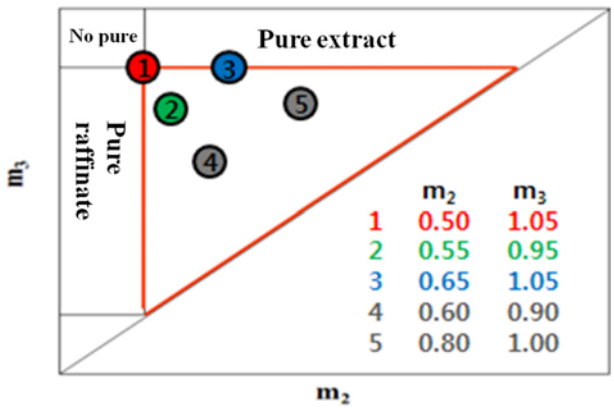

Fig. 2. Triangle diagram showing m_j as of the ratio of net fluid flow rate and the adsorbent phase flow rate and starting simulation points. Experiments in SMB were performed by suitable flow rates from points 1-5.

Table 1. Operation parameters of simulation runs

Run	Flow rates (ml/min)							t^* (min)	Feed con. (mg/ml)	m_2 and m_3
	Q_{De}	Q_{Ex}	Q_{Fe}	Q_{Ra}	Q_1	Q_2	Q_3			
A1	0.6	0.2	0.2	0.6	0.6	0.4	0.6	7.0	0.1	(0.5, 1.05)
A2	0.6	0.3	0.2	0.5	0.6	0.3	0.5	7.0	0.1	(0.2, 0.75)
A3	0.6	0.4	0.2	0.4	0.6	0.2	0.4	7.0	0.1	(0.07, 0.5)
B1	0.5	0.2	0.3	0.6	0.5	0.3	0.6	7.0	0.1	(0.2, 1.05)
B2	0.6	0.2	0.2	0.6	0.6	0.4	0.6	7.0	0.1	(0.5, 1.05)
B3	0.7	0.2	0.1	0.6	0.7	0.5	0.6	7.0	0.1	(0.78, 1.05)
C1	0.6	0.2	0.2	0.6	0.6	0.4	0.6	7.0	0.1	(0.5, 1.05)
C2	0.6	0.2	0.2	0.6	0.6	0.4	0.6	7.0	0.2	(0.5, 1.05)
C3	0.6	0.2	0.2	0.6	0.6	0.4	0.6	7.0	0.3	(0.5, 1.05)

Q_3 stand for desorbent, extract, feed, raffinate, zone 1, zone 2, and zone 3 flow rate, respectively. Point 1 is the important starting m_2 and m_3 pair to be compared other m_2 and m_3 pairs. Switching time t^* is assigned to be 7 min, which is the middle value between retention times of cytosine and guanine in batch HPLC chromatography.

RESULTS AND DISCUSSION

1. Henry Constants

Three HPLC columns used in SMB were tested for identity because identical retention times of cytosine and guanine in the columns are essential for good performance of SMB. Table 2 shows that the three columns are accepted for SMB installation in terms of similar retention times. By measuring the retention times of cytosine and guanine, slope of linear isotherm, i.e., the Henry constant, was obtained.

Table 2. Retention times of cytosine and guanine in C_{18} HPLC columns

Column number	Retention time (min)	
	Cytosine	Guanine
1	6.4	8.6
2	6.3	8.5
3	6.3	8.6

The adsorption isotherms are as follows: the average values of Henry constants are H_C=0.5 and H_G=1.05, where H_C is the Henry constant of cytosine and H_G is for guanine. A triangle diagram was plotted with the Henry constants by drawing lines (m_2=0.5, m_3=1.05) in Fig. 2. Five points were selected for SMB experiments.

2. Simulation Results

After determining the Henry constants, the procedure described by Mazzotti et al. allowed choice of the operational conditions for SMB unit operation [10]. It was assumed that the system operates under conditions dilute enough to be assumed as the linear isotherms.

The conditions chosen for the simulation runs are summarized in Table 1. Three series of simulation runs were performed with various operating conditions. Switching time was determined near the average retention time of cytosine (6.4 min) and guanine (8.6 min).

The effects of the extract flow rate can be seen in Fig. 3. The desorbent flow rate stays at the same value of 0.6 ml/min. The results

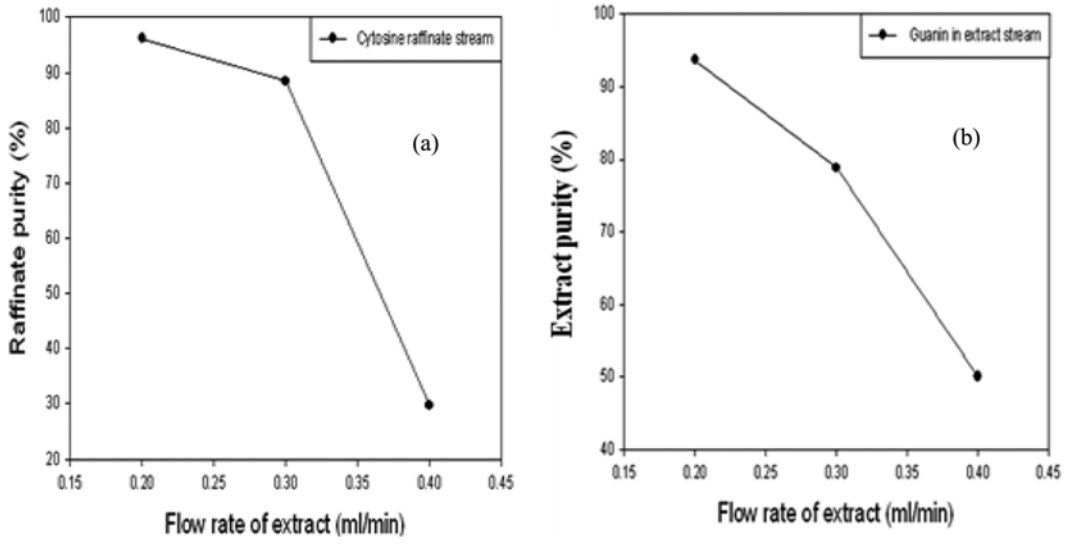

Fig. 3. Variations of simulation purities of cytosine in raffinate (a) and guanine in extract (b) by changing flow rate of extract; $Q_{Desorbent}$= 0.6 mL/min, Q_{Feed}=0.2 mL/min, feed concentration=0.1 mg/mL, and t^*=7.00 min.

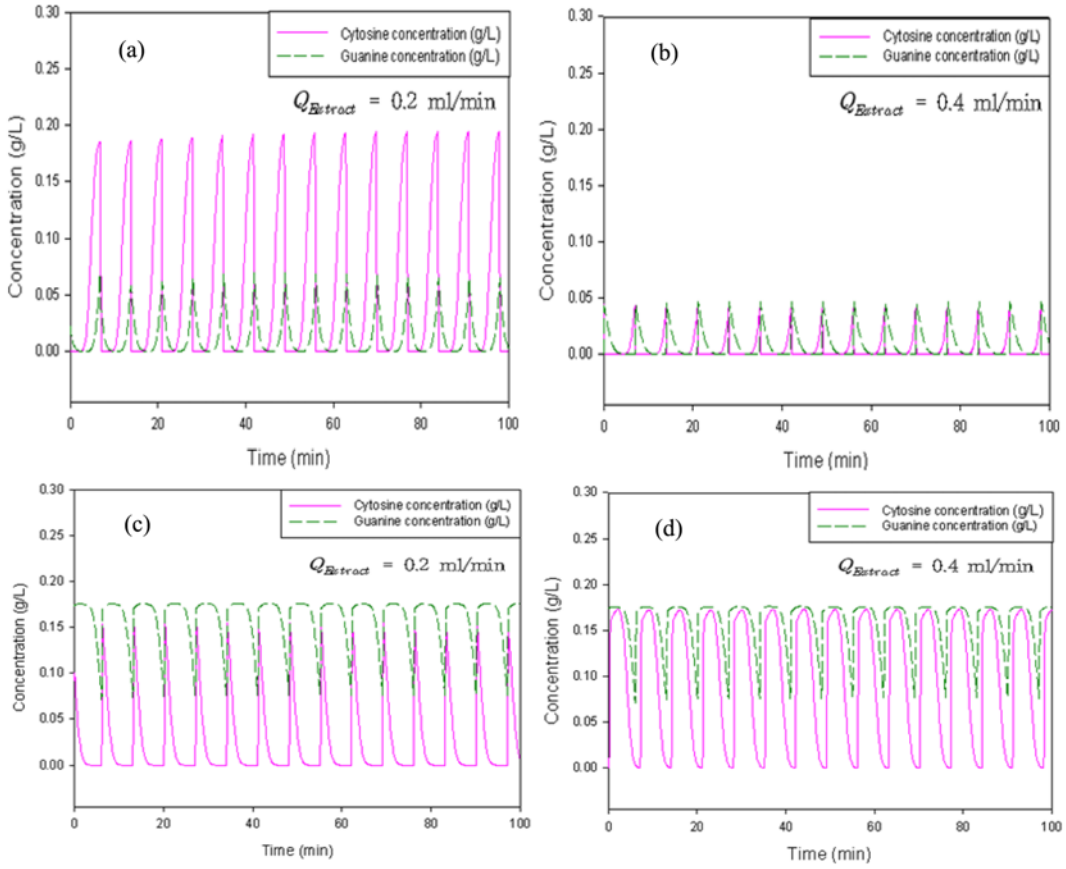

Fig. 4. The concentration profiles of cytosine and guanine in raffinate (a), (b), and in extract (c), (d) by changing extract flow rate from 0.2 to 0.4 mL/min.

of series A show that cytosine and guanine purities decrease when Q_{Ex} increases from 0.2 to 0.4 mL/min. When Q_{Ex} is 0.2 mL/min, higher purity of cytosine and guanine is obtained than Q_{Ex}=0.4 mL/min. Flow rates of desorbent and raffinate are proportional to flow rates of zone 1 and 3 (Q_1 and Q_3). However, extract flow rate is in inverse proportion to zone 2 flow rate. If 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). Hence, purities of extract and raffinate decrease because m_2 goes to the left side out of the triangle region.

Fig. 4(a) and (b) show concentration profiles of cytosine and guanine in raffinate stream with time by increasing extract flow rate from 0.2 to 0.4 mL/min. By increasing extract flow rate, overlapped profiles of cytosine and guanine increase and the peak concentrations of cytosine and guanine become low. Fig. 4(c) and (d) indicate that overlapped profiles of cytosine and guanine in extract stream increase with increasing extract flow rate. Therefore, purities of cytosine in raffinate and guanine in extract decrease with increasing extract flow rate from 0.2 to 0.4 mL/min.

Aspen simulation was performed with changing Q_{Fe} from 0.1 to 0.3 mL/min under the conditions of B series in Table 1. Purities of

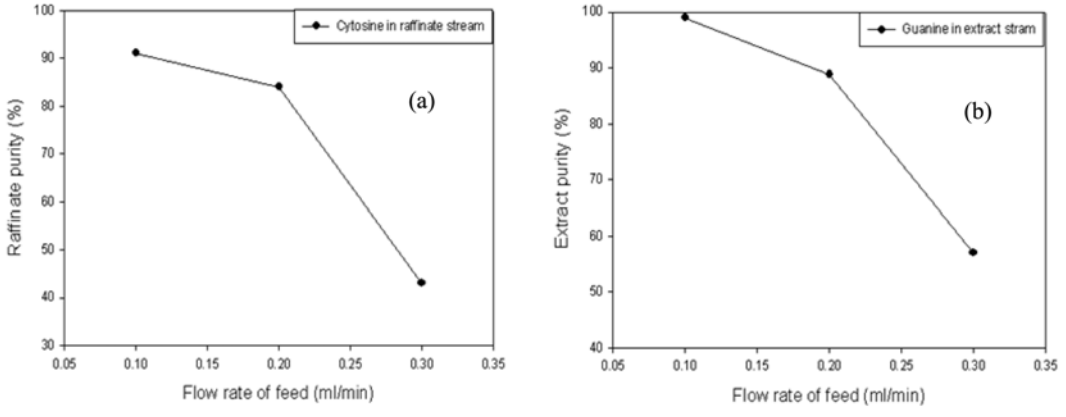

Fig. 5. Variations of simulation purities of cytosine in raffinate (a) and guanine in extract (b) when Q_{Feed}=0.1-0.3 mL/min.

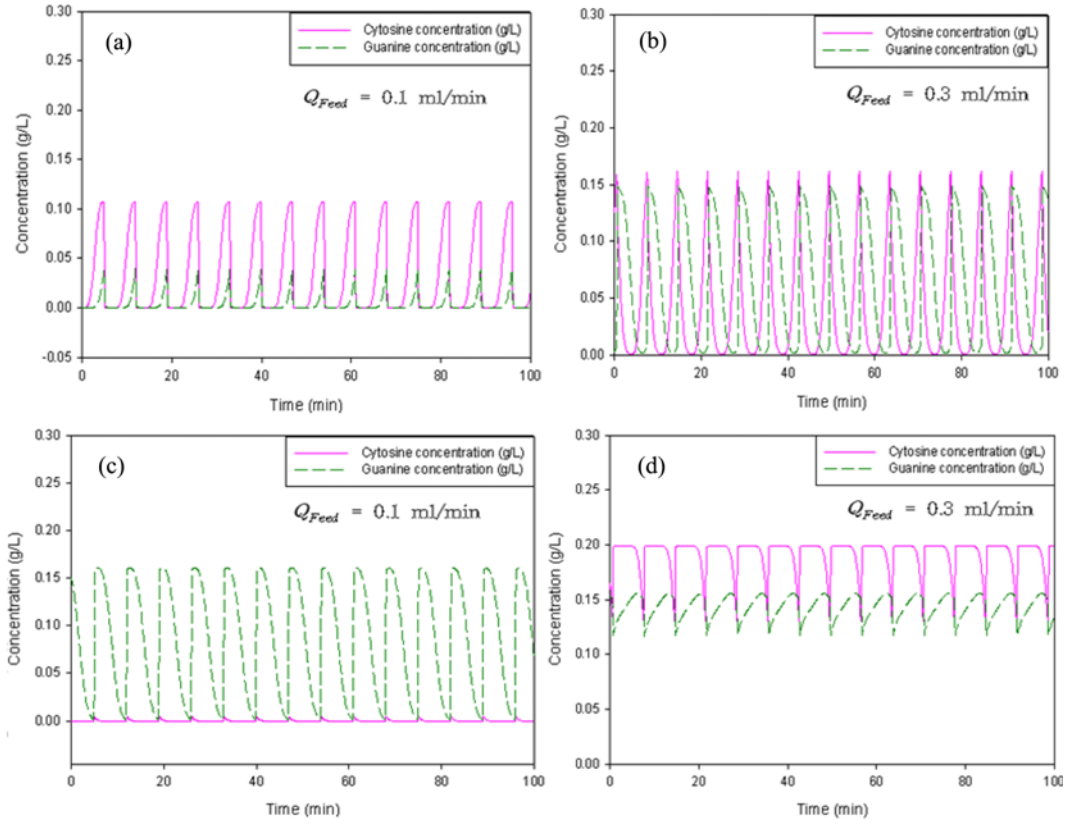

Fig. 6. The concentration profiles of cytosine and guanine in raffinate (a), (b), in extract (c), (d) by changing feed flow rate from 0.1 to 0.3 mL/min.

cytosine and guanine are observed to be lower according to the feed flow increase. The effects of feed flow rate can be explained by referring to m_2 increase in Table 1. The results of series B show that the purities of cytosine and guanine are lower by increasing feed flow rate. Fig. 5(a) shows purities of cytosine in raffinate stream. The purity of cytosine decreases from 91.5% to 44.1% according to the increase of feed flow rate. Fig. 5(b) indicates purities of guanine in extract stream also become lower by increasing feed flow rate. When feed flow rate is increased from 0.1 mL/min to 0.3 mL/min, purity of guanine decreases from 97.8% to 55.4%. Increased overlapped profiles of cytosine and guanine are observed in the concentration profile of cytosine and guanine in Fig. 6.

Fig. 6(a) and (b) show the concentration profile of cytosine and guanine in raffinate stream by increasing feed flow rate. Increased overlapped region of cytosine and guanine is observable at Q_{Fe}= 0.3 mL/min. Same phenomenon in the extract stream is also observed with increased Q_{Fe} in Fig. 6(c) and (d). Purities of cytosine in raffinate and guanine in extract decay since m_2 values are outside the triangle region.

Further Aspen simulation was performed with increasing con-

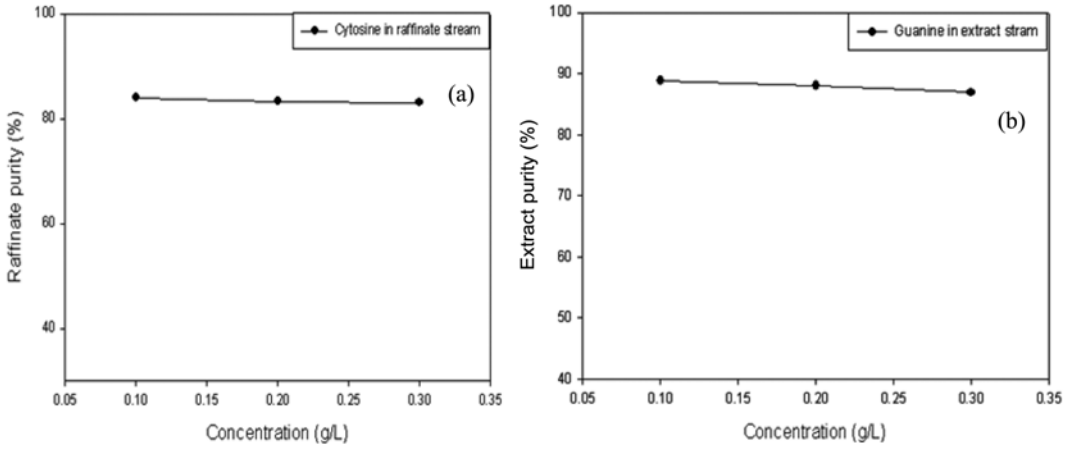

Fig. 7. Simulated purities of cytosine in raffinate (a) and guanine in extract (b) by changing of feed concentration from 0.1 to 0.3 g/L.

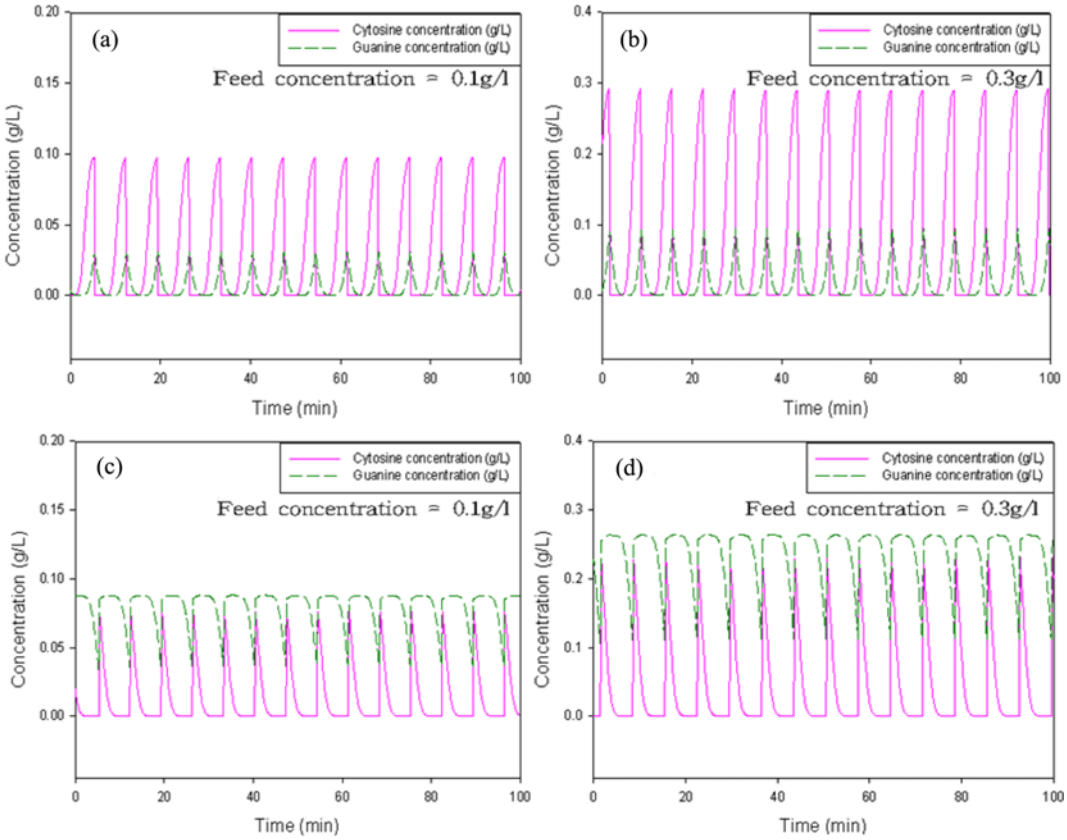

Fig. 8. (a) and (b) are concentration profiles of cytosine and guanine in raffinate, (c) and (d) in extract with changing of feed concentration.

centration of feed from 0.1 to 0.3 g/L with C series in Table 1. The effects of feed concentration can be seen in Fig. 7. Fig. 7(a) and (b) show that purities of cytosine in raffinate and guanine in extract vary by increasing concentration of feed. The purities of raffinate and extract are slightly decreased with increasing concentration of feed. Difference of purity is limited within 1%. The purity of cytosine in raffinate changes from 84.7% to 84.3% as the concentration of feed changes from 0.1 g/L to 0.3 g/L. The purity change of guanine is small (89.1% to 88.7%, Fig. 7b). The reason can be seen in Fig. 8.

Fig. 8(a) and (b) illustrate the concentration profiles of cytosine and guanine in raffinate with the concentration of feed of 0.1 g/L and 0.3 g/L. The increased concentration of feed affects the concentration value of cytosine and guanine, but not the relative ratio of concentration. In Fig. 8(c) and (d), the effect of feed concentration is similar to Fig. 8(a) and (b).

3. SMB Experiments

Three-zone SMB experiments were carried out on the basis of Aspen simulation results. The ideal complete separation points determined by triangle method as No. 1 in Fig. 2 and some experimental runs were made at the points 1-5. Points 1, 2, and 3 in the diagram were experimented. Points 4 and 5 were not possible due to the high pressure of column with higher Q. The experimental purities of point 1, 2 and 3 are shown in Table 3. Among the results, the highest purities were obtained at point 1 (cytosine=94.9% and guanine=89.8%). The simulated purities are 96% for cytosine and 94% for guanine. The agreement between experiment and simulation is fair except guanine purity. The experiments under the conditions of point 2 and 3 were conducted by changing switching times from 7 min to 5.5 min. Lower switching time led to a decrease of cytosine purity since the retention time of cytosine in batch chromatography was 6.5 min and it is higher than 5.5 min, which is unfavorable in cytosine separation in columns.

Table 3. Experimental purities of cytosine and guanine in SMB

Point number	Switching time (min)	Purity of cytosine	Purity of guanine	m_2 and m_3
1	7.00	94.9	89.8	(0.50, 1.05)
2	5.15	89.8	88.1	(0.55, 0.95)
3	5.15	90.0	87.0	(0.65, 1.05)

CONCLUSIONS

Cytosine and guanine separation was studied by Aspen simulations and SMB experiments. The Aspen simulation was conducted with extract flow rate (0.2-0.4 mL/min), feed flow rate (0.1-0.3 mL/min), and feed concentration (0.1-0.3 mg/mL). Conditions leading to better separation were observed and explained theoretically. The adsorption isotherm was experimentally determined as H_C=0.5 and H_G=1.05. When extract flow rate was increased, purities of raffinate and extract were decreased. And increasing feed flow rate decreased purities of raffinate and extract because overlapped profiles of cytosine and guanine increased. However, the increase of con-

centration of feed had little influence on the purities.

SMB experiments were carried out with reference of Aspen simulation. The highest purity of cytosine and guanine was obtained as 94.9% and 89.8% with the values of m_2=0.5 and m_3=1.05.

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