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Effects of Ionic Speciation of Lysine on Its Adsorption and Desorption Through a Sulfone-type Ion-Exchange Column

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Abstract Lysine produced during microbial fermentation is usually recovered by an ion-exchange process, in which lysine is first converted to the cationic form (by lowering the pH to less than 2.0 with sulfuric acid) and then fed to a cation-exchange column containing an exchanger that has a sulfone group with a weak counterion such as NH_4^+. Ammonia water with a pH above 11 is then supplied to the column to displace the purified lysine from the column and allow its recovery. To enhance the adsorption capacity and for a possible reduction in chemical consumption, monovalent lysine fed at pH 4 was investigated in comparison with conventional divalent lysine fed at pH 1.5. The adsorption capacity increased by more than 70% on a mass basis using pH 4 feeding compared with pH 1.5 feeding. Lysine adsorbed at pH 4 started to elute earlier than that adsorbed at pH 1.5 when ammonia water was used as the eluant solution, and the extent of early elution became more notable at lower concentrations of ammonia. Moreover, the elution of monovalent lysine fed at pH 4 displayed a stiffer front boundary and higher peak concentration. However, when the ammonium concentration was greater than 2.0 N, complete saturation of the bed was delayed during adsorption and the percent recovery yield from elution was lowered, both drawbacks that were considered inevitable features originating from the increased adsorption of monovalent lysine.

Keywords: Lysine, ion exchange, adsorption, desorption

L-Lysine is an essential amino acid, and its global market is steadily increasing as an additive in swine and poultry production [21]. Most commercial lysine is produced *via* microbial fermentation [1, 5, 8, 16, 19] and, upon completion, a typical fermentation broth contains 10%–13% (w/v) lysine, 3.0%–3.5% (w/v) biomass, as well as other components, depending on the ingredients used in the original medium.

The ion-exchange process is the most popular method for the recovery and purification of lysine from fermentation broth [1, 7, 10, 20]. In this process, lysine in the broth, which is usually at pH 6.5–7.0, is first converted to the divalent cationic form by lowering the pH to less than 2.0 with a strong acid such as sulfuric acid; the divalent cation is then fed to a strongly acidic cation-exchange column containing a sulfone group ($-SO_3^-$) with a weak counterion such as NH_4^+. Ammonia water (pH greater than 11) is then used to elute the lysine and to regenerate the depleted bed to the NH_4^+-form. The recovered lysine mixed with ammonium is neutralized by HCl and then lysine is purified through evaporation and crystallization.

Because lysine possesses one α-carboxyl group and two amino groups (one α-amino group and another in a side chain), a lysine molecule can exist as one of four different ionic forms, Lys^{2+}, Lys^{+1}, Lys^0, and Lys^-, depending on the pH. Since the pK_a values for the dissociations of the α-carboxyl group and the α-amino group are 2.18 and 8.95, respectively [2], the dominant ionic forms of lysine in acidic pH are the divalent (Lys^{2+}) and monovalent (Lys^+) forms: Lys^{2+} at pH 2 or less and Lys^+ at pH from 3 to 8. A highly acidic pH (below 2) is used in the ion-exchange process during the lysine adsorption stage to generate divalent Lys^{2+} molecules and also to take advantage of its strong adsorption selectivity for the ion-exchange resin used. However, since the ion-exchange reaction is equivalently stoichiometric [3, 6, 15, 18], divalent lysine Lys^{2+} occupies two sites on the sulfone group in the ion exchanger, utilizing only half of the full theoretical ion-exchange capacity of the resin. Consequently, the adsorbed amount of lysine is always smaller than the theoretical resin capacity. Furthermore, a high-concentration ammonia water with alkaline pH is also required to elute the strongly adsorbed divalent lysine.

Nagai and Carta [12, 13] investigated extensively on the adsorption and desorption characteristics of lysine depending upon ionic composition of lysine under a model situation. The operation strategy of a multicolumn simulated moving

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bed system [14] was also discussed with the feeding of monovalent lysine at a low fixed feed concentration (0.2 M) and desorbent concentration (1.0 M ammonia). It was shown that the adsorption occurs by the stoichiometric exchange, which is governed by the ionic composition of lysine in the feed solution. They also mentioned that the desorption of lysine with ammonia is completely mass transfer controlled, since the negatively charged form of lysine is dominant at pH greater than 11 and excluded from the cation-exchange resin.

In this study, we were especially interested in the desorption behaviors of lysine fed at low pH and high pH under different ammonia concentrations. The adsorption of monovalent Lys^+ and divalent Lys^{2+} was first investigated at a high feed concentration (120 g/l), which is the typical lysine concentration in actual finished fermentation broth. The breakthrough behaviors in the adsorption stage were compared by adjusting the pH of the feed solution to pH 1.5 and 4, through a strongly acidic cation-exchange column. Then, elution profiles of lysine in the desorption stage were compared to investigate the influence of various ammonia concentrations (0.7–2.6 N) and their pH values on their desorption characteristics.

MATERIALS AND METHODS

Ion-Exchange Resin and Column Preparation

Lewatit S1468 monodisperse cation-exchange resin (Lanxess Inc., Germany) was used. This resin contains a styrene-divinylbenzene (St-DVB) backbone and a sulfonyl ion-exchange functional group. According to the supplier's information [9], the exchange capacity and water retention capacity are approximately 2.0 meq/ml and 42%–48%, respectively. The resin particles have a bead size of 0.6± 0.05 mm (minimum 90%) with a density of 1.28 mg/cm^3. The resin was supplied in the Na^+-form; it was slurry-packed in a column with a diameter of 2.5 cm and converted to the NH_4^+-form by running 10% (w/v) NH_4OH through the packed column. The packed bed height, bed volume, and packing density were 18 cm, 83 cm^3, and 0.46 g/cm^3, respectively, in this study when the resin was in the NH_4^+-form. The void fraction of the packed bed in NH_4^+-form was determined as 0.43±0.03 based on pulse injections of 1% (w/v) blue dextran 2M at a 1 SV (specific velocity) flow rate detected by UV absorbance at 280 nm.

Ion-Exchange Experiments

Analytical grade lysine in the form of lysine·HCl was purchased from Sigma (L5626, St. Louis, MO, U.S.A.). The lysine concentration in the feed solution was 120 g/l, and its pH ranged from 5.6 to 5.7. The pH of the lysine feed solution was subsequently adjusted to either pH 1.5 or 4.0 with sulfuric acid. The lysine solution was then fed to the cation-exchange bed using a peristaltic pump with a flow rate of 1 SV, which is defined as the flow rate of 1 BV per hour; in the present experimental system, 1 SV is equivalent to 1.38 mL/min. All operations were carried out at 25°C. In order to estimate its breakthrough characteristics and the adsorption capacity, the lysine concentration in the effluent stream was monitored using a fraction collector and analyzed by the ninhydrin-ferric-derivatization method [4]. After complete breakthrough, the column was rinsed with distilled deionized water until no lysine was detected from the washed-out effluent. The amount of adsorbed lysine (A) was determined as the total amount of lysine fed to the column until breakthrough point minus the summed amount of lysine collected in the effluent stream and rinsed water.

To study the elution characteristics of lysine from the column, ammonium hydroxide (NH_4OH) solution with a concentration ranging from 0.7–2.6 N was used as an eluant. The ammonia solution was fed to the column at the flow rate of 1 SV at 25°C, the eluted lysine concentrations were monitored, and the total amount of recovered lysine (B) was analyzed. The recovery yield of lysine was determined as the ratio of B/A.

RESULTS AND DISCUSSION

Ionic Composition of Lysine at Different pHs

A lysine molecule possesses one α-carboxyl group and two amino groups. The degree of ionization of each group is characterized by the dissociation equilibrium constant of the group with respect to the pH value of the solution. Since the pK values for the dissociations of the α-carboxyl group and the α-amino group are 2.18 and 8.95 [2], respectively, the dominant ionic forms of lysine are Lys^{2+} and Lys^+ in solutions with an acidic pH below 6.

The ionic composition of lysine under other pH conditions can be calculated utilizing the definitions and equilibrium constant values given in Table 1. Rearranging the expressions given in Table 1 for the equilibrium constants, the ionic fractions of Lys^{2+} and Lys^+ can be expressed as Eqs. (1) and (2), respectively [11], and the calculated compositions

Table 1. Dissociation reactions of lysine and the corresponding equilibrium constants.

Dissociation reaction	Equilibrium constant	pK value
$Lys^{2+} \leftrightarrow Lys^+ + H^+$	$K_1 = \frac{[Lys^+][H^+]}{[Lys^{2+}]}$	$pK_1 = 2.18$
$Lys^+ \leftrightarrow Lys^0 + H^+$	$K_2 = \frac{[Lys^0][H^+]}{[Lys^+]}$	$pK_2 = 8.95$
$Lys^0 \leftrightarrow Lys^- + H^+$	$K_3 = \frac{[Lys^-][H^+]}{[Lys^0]}$	$pK_3 = 10.53$

Table 2. Ionic fractions of lysine at different pH values.

pH	Lys^{2+}:Lys^{+}
1.0	0.94:0.06
1.5	0.82:0.18
1.7	0.75:0.25
2.0	0.60:0.40
3.0	0.13:0.87
4.0	0.015:0.985
5.0	0:100

at selected pH values are listed in Table 2. The dominant ionic species are Lys^{2+} at pH 2 or less and Lys^{+} in the range of pH 3 to 8. At pH 1.5, 82% of the lysine exists as divalent Lys^{2+} and 18% as monovalent Lys^{+}. In contrast, at pH 4.0, 98.5% of lysine is monovalent Lys^{+}.

$$\frac{[Lys^{2+}]}{[Lys^{+}]+[Lys^{2+}]}=\frac{1}{1+10^{pH-pK_1}} \quad (1)$$

$$\frac{[Lys^{+}]}{[Lys^{+}]+[Lys^{2+}]}=\frac{10^{pH-pK_1}}{1+10^{pH-pK_1}} \quad (2)$$

Breakthrough of Lysine at Different Feed pHs

Fig. 1 compares the breakthrough curves for lysine fed at pH 1.5 and pH 4. The left-side area of each breakthrough curve below 120 g/l in Fig. 1 represents the corresponding adsorbed mass of lysine, or bed capacity. The solid lines in Fig. 1 are the results of nonlinear regression of which the employed fit is the 5-parameter logistic equation, one of the sigmoidal equations. As these curves show, at pH 4, the adsorbed amount of lysine was much greater than that at pH 1.5. Through the analysis of the collected effluent solutions, the amounts of adsorbed lysine were estimated as 21.93 g and 37.37 g at pH 1.5 and 4, respectively. These amounts corresponded to capacities of 2.95 mmol/g of resin at pH 1.5 and 5.03 mmol/g at pH 4.0 (Table 3). Because divalent Lys^{2+} theoretically occupies two sites on the sulfone groups in the exchanger, whereas monovalent Lys^{+} occupies only one, we expected an increased capacity for the monovalent adsorption at pH 4. As mentioned above, the ionic compositions of lysine are Lys^{2+}:Lys^{+}= 0.82:0.18 at pH 1.5 and Lys^{2+}:Lys^{+}=0.015:0.985 at pH 4. As shown in Fig. 1, the ion-exchange capacity was increased with lysine fed at pH 4, which is composed of 98.5% monovalent lysine, instead of the traditional divalent adsorption at or below pH 2. To maintain 100% Lys^{+}, a more neutral pH, ranging from 5 to 7, should be considered as the feed pH. However, a pH greater than 5 is not recommended for the feed solution since more neutral pH is known to reduce the anticontamination capability during later purification stages.

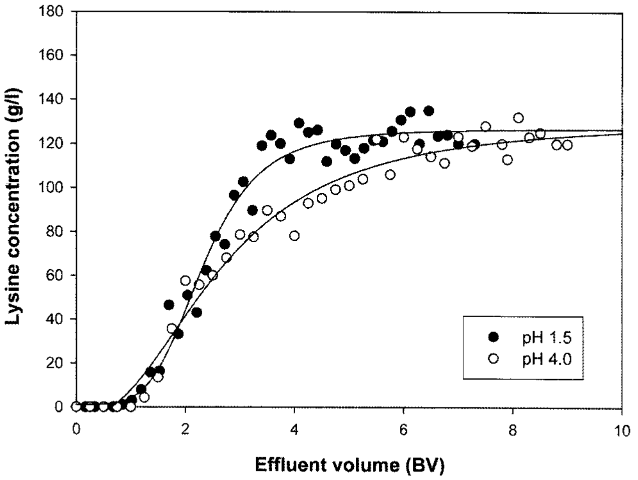

Fig. 1. Effluent profiles of lysine breakthrough in the cation-exchange bed.
●, Feed pH=1.5; ○, Feed pH=4.0.

If we compare the shapes of the breakthrough curves shown in Fig. 1, the breakthrough profile of the pH 1.5 feeding exhibited a sharp increase that amounted to roughly shock-wave propagation; on the other hand, the breakthrough profile of the pH 4.0 feeding revealed a gradual increase of simple wave propagation. This variation in the breakthrough profiles originated from differences in the magnitudes of relative selectivity between NH_4^{+}, Lys^{2+}, and Lys^{+}. Nagai and Carta [12] estimated that $S(Lys^{2+}/NH_4^{+})=3.33$ and $S(Lys^{+}/NH_4^{+})=0.5$ in an ion-exchange system with sulfone-type acidic cation-exchange resin made from ST-DVB copolymerization, where S(i/j) is the selectivity coefficient for the exchange of i and j. As these values also corresponded well with our experiences, we considered these reasonable approximations for our purposes as well. It should be

Table 3. Comparison of adsorption and desorption of lysine depending upon feed pH and eluant concentration.

Feed pH		1.5				4.0			
Lysine concentration in feed		120 g/l							
Adsorbed lysine	bed total	21.93 g				37.37 g			
	per g resin	0.54 g/g (2.95 mmol/g)				0.92 g/g (5.03 mmol/g)			
Eluant concentration (Normality of NH_4OH)		2.6	2.0	1.4	0.7	2.6	2.0	1.4	0.7
Desorbed lysine (g)		22.75	22.34	15.44	14.62	36.56	34.93	30.06	28.43
Recovery yield (%)		100	100	70	66	98	93	80	76

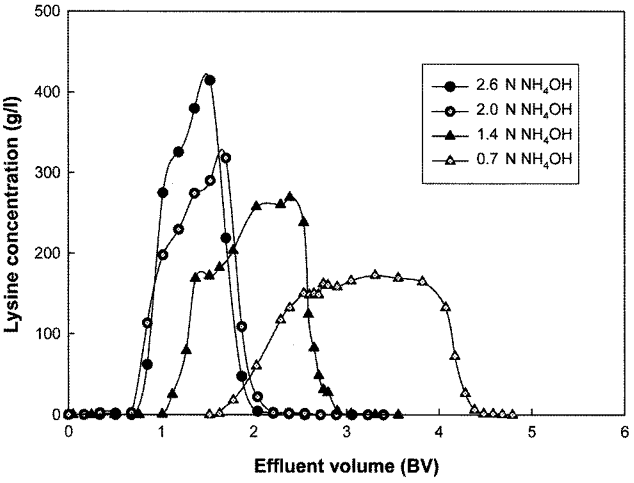

Fig. 2. Influence of different concentrations of ammonia water on desorption of lysine fed at pH 1.5.

noted that the relative selectivity of the resin for Lys$^+$ is less than that for ammonium ion (NH_4^+). Therefore, in a NH_4^+-saturated bed, the self-sharpening behavior of Lys^{2+}, fed at pH 1.5, with its higher selectivity must have resulted in a shock wave as a favorable exchange pattern [3, 17]. At pH 4, the unfavorable exchange of stronger ammonium ions for weaker Lys$^+$ resulted in the simple gradual effluent profile [9]. Furthermore, Lys$^+$ adsorption would have a self-broadening effect at pH 4, resulting in the delay of complete saturation of the bed compared with the Lys^{2+} feeding.

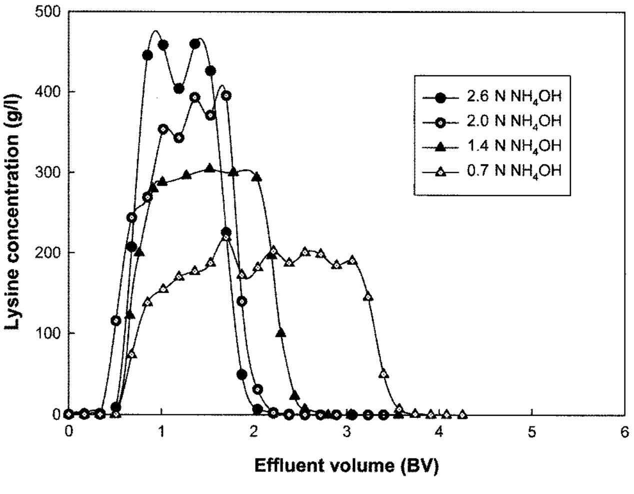

Fig. 3. Influence of different concentrations of ammonia water on desorption of lysine fed at pH 4.0.

Table 4. pH of eluant solution depending upon ammonia concentration.

NH_4OH normality (N)	pH
2.6	11.83
2.3	11.80
2.0	11.77
1.4	11.69
1.0	11.62
0.7	11.54

Elution of Lysine Fed at Different pHs

Adsorption of lysine in different charged states influenced the subsequent desorption behaviors. The elution profiles of lysine fed at pH 1.5 and 4 are shown in Figs. 2 and 3, respectively. In both cases, the effects of the eluant concentration and pH on the lysine recovery were examined by varying the eluant ammonia concentration from 0.7 to 2.6 N; the corresponding pH values are listed in Table 4. More concentrated and narrower desorption lysine bands were obtained with higher ammonium concentrations, according to the mass-action law in the ion-exchange reaction. In the desorption of lysine fed at pH 1.5 with 2.6 N NH_4OH (Fig. 2), lysine eluted from 0.75 to 2.0 BV with a peak concentration of greater than 400 g/l. With 0.7 N NH_4OH, the elution was delayed and the band widened over the range of 1.5–4.3 BV with a greatly reduced peak concentration. For the lysine fed at pH 4 (Fig. 3), the lysine elution period increased from 0.5–2.1 BV to 0.6–3.5 BV as the ammonia concentration was reduced from 2.6 N to 0.7 N.

The desorption peak area and duration for lysine fed at pH 4 (Fig. 3) were always larger and longer than those for pH 1.5 feeding (Fig. 2) under identical elution conditions with the same concentration of ammonia. This is the direct consequence of the amount of adsorbed lysine, which was 70% larger at pH 4 than at pH 1.5, as shown in Table 3. This also explains, in part, why the peak concentration of the lysine effluent was always higher for pH 4 feeding.

The shape of the elution curves also differed between Lys^{2+} and Lys$^+$ elution in Figs. 2 and 3. In particular, the front boundaries were steeper for Lys$^+$ elution (Fig. 3), whereas Lys^{2+} elution exhibited more spreading in the front boundaries (Fig. 2). This spreading tendency became more severe as the ammonium concentration decreased in the eluant solution. The selectivity differences of involved species could also explain this observation. For the desorption of lysine fed at pH 4, most of the adsorbed lysine is monovalent Lys$^+$, which has a lower selectivity than the incoming NH_4^+, $S[NH_4^+/Lys^+]=2$. A shock-wave propagation should occur when an ion of higher selectivity displaces the adsorbed solute of weaker selectivity [3, 17]. At pH 1.5, the major species of adsorbed lysine is divalent Lys^{2+} (Fig. 2), and the selectivity of the incoming ammonium ion is smaller than that of Lys^{2+}, $S(NH_4^+/Lys^{2+})=0.3$. Therefore, the adsorption of NH_4^+ was unfavorable, generating a gradual spread of the lysine band at the ammonium-lysine boundary. This feature also supports, in part, the high peak concentrations that were obtained in Table 3 compared with those in Table 2 using the same ammonia concentration.

The relative differences between the selectivities of the involved species also influenced the onset of lysine desorption. The appearance of desorbed lysine in Fig. 2 (pH 1.5 feeding) was delayed with decreasing ammonium concentrations. The elution band appeared from 0.7 BV of effluent volume with 2.6 N NH_4OH; this was delayed to 1.0 BV and 1.5 BV with 1.4 N and 0.7 N, respectively. The starting points of the elution bands in Fig. 3 (pH 4 feeding) were consistently around 0.5 BV regardless of the ammonium concentration. This implied that the ion-exchange potential of NH_4^+ to replace strongly adsorbed Lys^{2+} was substantially weakened at lower ammonia concentrations (Fig. 2). However, the selectivity of NH_4^+ was so strong compared with monovalent Lys^+ that the initiation of Lys^+ desorption was not influenced much by variation in the NH_4^+ concentration (Fig. 3).

When the ammonium concentration was greater than 2.0 N, the recovery yield of lysine adsorbed at pH 1.5 (Fig. 2) was nearly 100% complete (Table 3). However, it dropped abruptly to 70% and 66% with 1.4 N and 0.7 N ammonium, respectively. On the other hand, the recovery of lysine adsorbed at pH 4 was 98% when eluted with 2.6 N ammonia, and the recovery percent decreased slightly with reductions in the ammonia concentration. It should be noted from Table 3 that the recovery yields of lysine fed at pH 4 were smaller than those of lysine fed at pH 1.5 with ammonia concentrations greater than 2.0 N, the typical ammonium concentration used in manufacturing, even though the majority of lysine adsorbed at pH 4 was monovalent Lys^+ and its selectivity was less than that of Lys^{2+}. The increased amount of lysine adsorbed at pH 4 may be the reason for the relatively incomplete desorption recovery of this lysine compared with that of lysine adsorbed at pH 1.5. The portion of unrecovered lysine was remaining in the exchanger undesorbed, which could be confirmed by eluting the remaining lysine by 2 M NaOH (data not shown) after each desorption experiment. Therefore, it was concluded that the lysine recovery from the cation-exchanger bed is not complete despite that its pH is above 11, unless the ammonia concentration is high enough.

Factors Influencing Lysine Desorption

Desorption of lysine from the cation exchanger can be influenced by two factors: by the displacement of adsorbed lysine with NH_4^+ through the ion-exchange mechanism (ion-exchange factor) and by the loss of positive charge on the adsorbed lysine owing its association with hydroxide ions (pH factor). Both of these factors may contribute jointly to lysine desorption. If the ion-exchange factor is more influential, the lysine desorption will be more sensitive to changes in the ammonium concentration rather than to pH changes. On the other hand, desorption will be more dependent on changes in eluant pH than on changes in the ammonium concentration if the pH factor is more important for lysine desorption.

Since the conversion of adsorbed lysine (either Lys^+ or Lys^{2+}) to its negatively charged form (Lys^-) is directed by the thermodynamically established pK values for the lysine molecule and by the pH of the surroundings, we may expect similar desorption behavior and elution performance, even though adsorption pHs and counterion concentrations differ, if the eluant pH values are high enough to guarantee the dominance of Lys^- (such as above pH 11). This aspect is important for lysine elution because it may allow us to decrease ammonia consumption by reducing the pH of the eluant solution to a pH range that allows similar desorption performance.

The pH values of the eluant used in Figs. 2 and 3 fall in the range where most lysine molecules are the negatively charged Lys^-. Although the ammonium concentration almost quadrupled from 0.7 to 2.6 N, the eluant pH did not change by much, from 11.54 to 11.83 (Table 4). However, according to Figs. 2 and 3, the elution profile changed significantly depending on the ammonium concentration. This result suggests that, under the conditions used in this study, the lysine desorption was strongly influenced by the ion-exchange factor of the ammonium ion rather than by the pH factor. Therefore, it is not appropriate to lower the eluant pH by reducing the ammonium concentration in order to decrease the chemical consumption during bed regeneration, because the ion-exchange factor of the ammonium ion is substantially sacrificed at lower concentrations.

Adsorption of monovalent lysine at pH 4 resulted in several notable differences in its breakthrough and elution characteristics from the conventional strongly acidic feeding (pH 1.5 in this study) that utilizes the high selectivity of divalent Lys^{2+} for sulfone-type St-DVB cation exchanger saturated with ammonium ion. Owing to the equivalently stoichiometric feature of the ion-exchange reaction, the pH 4 feeding increased the adsorption of lysine by more than 70% on a mass basis compared with feeding at pH 1.5. Since the selectivity of Lys^+ for the resin was smaller than that of NH_4^+, the elution of adsorbed lysine fed at pH 4 began earlier than that fed at pH 1.5 when using ammonia water as the eluant solution; the extent of early elution also became more notable with lower concentrations of ammonia. Furthermore, the elution of lysine fed at pH 4 displayed a stiffer front boundary and a slightly higher peak concentration.

These features, especially the enhanced loading capacity, make monovalent adsorption of lysine a potential alternative to the conventional divalent adsorption, if we are willing to accept the following drawbacks that were observed during monovalent feeding at pH 4: First, the time to reach complete saturation of the bed, or breakthrough time, was delayed during the adsorption stage. Second, the percent recovery yield on elution was reduced when the ammonium concentration was greater than 2.0 N compared with the percent recovery from the divalent feeding at pH 1.5. These shortcomings are considered to be inevitable features, which were resulted from the increased adsorption of monovalent lysine. It was possible to substantially reduce the amount of sulfuric acid consumption during adsorption by using pH 4 feeding. However, the consumption of ammonia during the elution stage should not be reduced substantially, because lowering the ammonium concentration sacrificed the recovery yield, even though the elution pH was well above that necessary to guarantee negatively charged lysine.

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