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Operating Parameters for Glutamic Acid Crystallization in Displacement Ion Exchange Chromatography

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Glutamic acid can be crystallized inside cation exchange column when displacer NaOH concentration is high enough to concentrate displaced glutamic acid beyond its solubility limit. Resulting crystal layer of glutamic acid was moved with liquid phase through the column, and thus could be eluted from the column and recovered in fraction collector. For the purpose of enhancing crystal recovery, effects of operating parameters on the crystal formation were investigated. The increase in the degree of crosslinking of resin favored crystal recovery because of its low degree of swelling. Higher concentration of displacer NaOH was advantageous. If NaOH concentration is too high, however, crystal recovery was lowered due to the solubility-enhancing effects of high pH and ionic strength. The decrease of mobile phase flow rate enhanced crystal recovery because enough time to attain local equilibrium could be provided, but film diffusion would control the overall crystal formation with extremely low flow rate. Lower temperature reduced solubility of glutamic acid and thus favored crystal formation unless the rate of ion exchange was severely reduced. The ion exchange operated by displacement mode coupled with crystallization was advantageous in reducing the burden of further purification steps and in preventing purity-loss resulted from overlapping between adjacent bands.

Key words: crystallization, displacement chromatography, glutamic acid, ion exchange

INTRODUCTION

Ion exchange is commonly used for recovery and separation of amino acids. Many industrial and preparative ion exchange operations are performed in a displacement mode. The advantages of displacement chromatography include capabilities of processing a large feed and of separation and recovery of concentrated products [1-4]. In displacement chromatography, a column is first equilibrated with a weaker carrier solvent. A slug of feed mixture is introduced to the column followed by a solution containing a displacer component which has a greater selectivity to the resin than any of feed components. The front of displacer drives the rearrangement of feed components into adjacent pure zones which move at the same velocity as the displacer front, which is said an isotachic condition is established. The solutes are distributed in the order of decreasing selectivity along the column distance and exit the column as adjacent pure bands. One of the constraints in displacement chromatography is the solubility limit of solute. If the concentration of solute in a pure band exceeds its solubility, the formation of crystal or precipitate takes place in the column. Insoluble inorganic precipitates often cause troubles in normal packed-bed operation and thus the range of displacer should be carefully controlled for displacement chromatography in general [3, 5].

We have reported a crystallized recovery of glutamic acid on a cation exchange column through the displacement mode with NaOH as a displacer [6]. One of the results was that the glutamic acid formed moveable crystal layer in the column and thus pure crystals could be collected from the column without bed clogging and pressure drop. The balanced rates of crystallization and dissolution at boundaries of the crystal layer made the crystal layer move down the column at the same velocity as the solute velocity in the mobile phase. More than 60% of glutamic acid was collected as crystal with 1 M NaOH as a displacer. Fig. 1 shows illustrative equilibrium ion-exchange isotherms for two solutes A and B and displacer D. If the displacer concentration is between C_{dL} and C_{dH}, solutes A and B will form isotachic pure bands. If the displacer concentration is greater than C_{dH} which is determined by the operating line passing through the solubility limit C_S of solute A, solute A will be crystallized in the column.

The objective of this study is to investigate optimal operating parameters for enhancing crystal recovery of glutamic acid. The effects of displacer concentration, degree of resin crosslinking, flow-rate of mobile phase, and temperature are investigated. Also the advantage of crystallization-coupling in ion-exchange for a ternary mixture separation will be discussed.

MATERIALS AND METHODS

Materials and Analyses

Strongly acidic cation exchange resins AG 50W (Bio-rad) as 100-200 mesh beads in H-form were used. AG 50W resins are analytical grade of commercial Dowex 50W resins (Dow Chemical). AG 50Ws are gel type pol-

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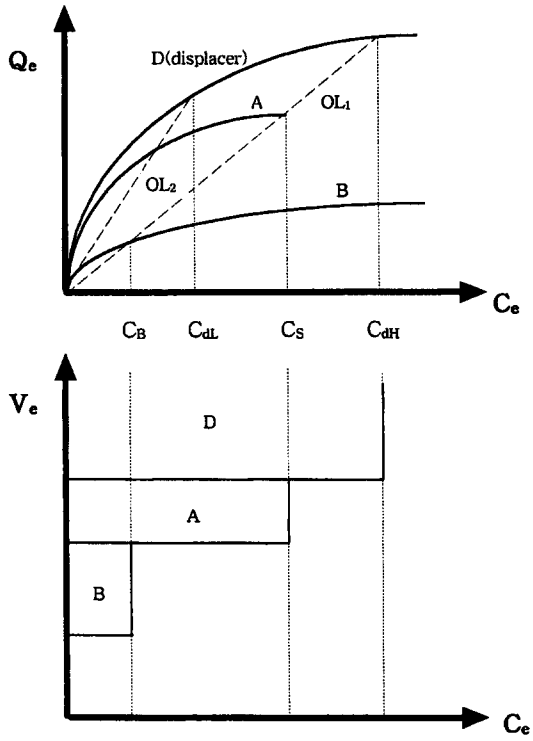

Fig. 1. Hypothetical isotherms with solubility constraint [6]. C_e, equilibrium concentration in liquid phase; Q_e, equilibrium concentration in resin phase; V_e, effluent volume; OL, operating lines; C_{dL}, lower limit of displacer concentration; C_{dH}, upper limit of displacer concentration; C_s, solubility limit of solute A.

ystyrene resins crosslinked with divinylbenzene (DVB) and possess covalently-bound sulfone groups for functionality. Three resins with different degree of crosslinking were used. X4, X8, and X12 represent DVB contents of 4%, 8%, and 12%, respectively. A single lot of resin was vacuum dried in an oven at 105°C and dry resin capacity was found 5.0± 0.2 meq for all of three resins, by batch equilibrium test at 25°C. All amino acids were L-form of tissue culture grade from Fisher Scientific. Concentration of single amino acid was determined by the copper complex method [7]. Gilson 806 HPLC with a Na-form cation exchange column and RI detector was used for the analysis of amino acid mixture. The concentration of sodium ion was determined by AAS(Aurora 1100GF). All experiments were conducted at 25°C and with 1.0 mL/min of flowrate unless specified.

Experimental Methods

To determine equilibrium isotherms, 1 gram of H-form dry resin was gently agitated in 50 mL of predetermined solution for 10 hours in a polypropylene beaker. After equilibrium was reached, the solution was filtered using a Q5 filter paper (Fisher) and the composition in supernatant was analyzed. For column experiments, a polypropylene column of 14 mm ID was slurry packed with 6 gram of H-form resin. The bed capacity was determined as 30± 0.3 meq for Na^+ ion. Regeneration of used resin to H-form was achieved by flowing 1 M HCl through the column. The amount of crystal recovered in fraction collector was determined as the total amount of solute, which was measured after complete dissolution of crystal by repeated vortexing and warming at 40°C, minus the amount in liquid phase.

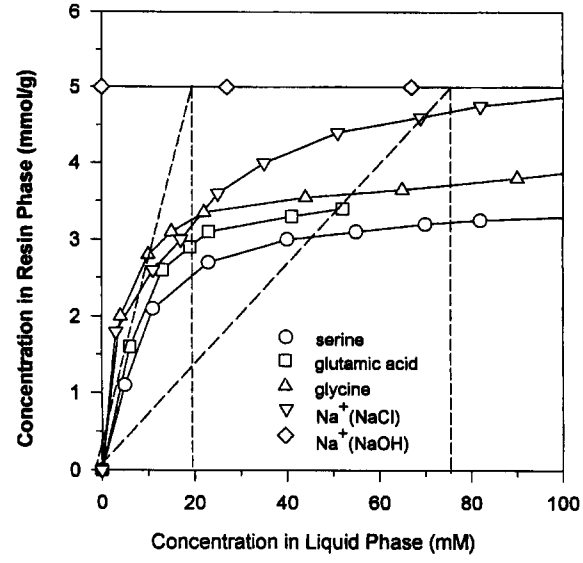

Fig. 2. Ion exchange equilibrium isotherms of amino acids and displacers for AG 50W-X8 resin at 25°C. ---, operating lines.

RESULTS AND DISCUSSION

Equilibrium Ion-Exchange Isotherms

Fig. 2 shows experimentally determined isotherms of amino acids and displacers. In the isotherms of amino acids, the pH varied from point to point in each curve because H^+ ions were released from the resin. The selectivities of amino acid for resin were in the order of alanine > glutamic acid > serine. The solubility limit of glutamic acid was determined to be 52 mM, which is slightly higher than the value reported in literature, 49 mM [8]. Since the resin is strongly acidic, the NaOH isotherm was essentially rectangular [6]. For the separation of a ternary mixture of alanine, glutamic acid, and serine, C_{dL} and C_{dH} are determined as 21 mM and 78 mM, respectively. Fig. 3 shows kinetics of ion exchange for three resins with different degrees of crosslinking. After 1 g of H-form resin was added to 50 mL of 40 mM glutamic acid solution, concentration change in supernatant was analyzed. All three resins showed about 20 mM of final equilibrium concentration after 4 hours, but the approaching rates were different. The decrease of DVB content enhanced the rate of ion-exchange reaction maybe because high degree of crosslinking imposed resistance for the diffusion of glutamic acid to functional groups in resin matrix [9].

Effects of Crosslinking Degree of Resin

Three columns were prepared by packing AG 50W resins with different degrees of crosslinking. Total 3 meq of glutamic acid was equilibriated by feeding 100 mL of 30 mM solution, followed by continuous feeding of 1 M NaOH, which is higher than C_{dH}, 78 mM, in Fig. 2. As NaOH fed, crystallization of glutamic acid took place, forming a discrete white crystal layer. The crystal layer moved down the column and was collected in the effluent stream. Fig. 4 shows the elution profiles of

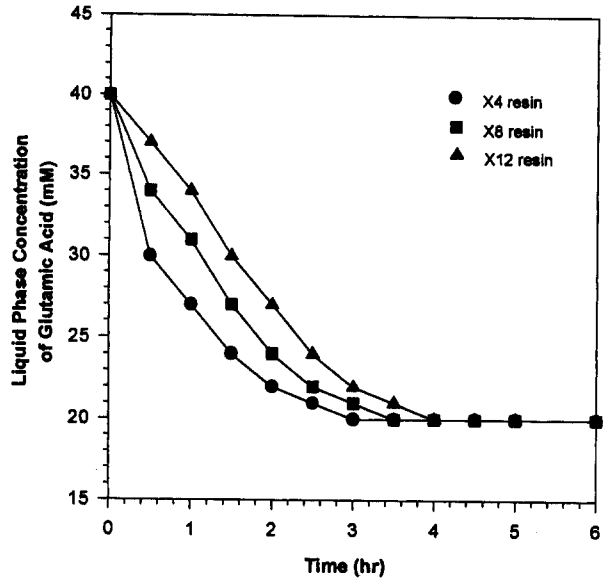

Fig. 3. Effect of the degree of crosslinking on ion exchange kinetics.

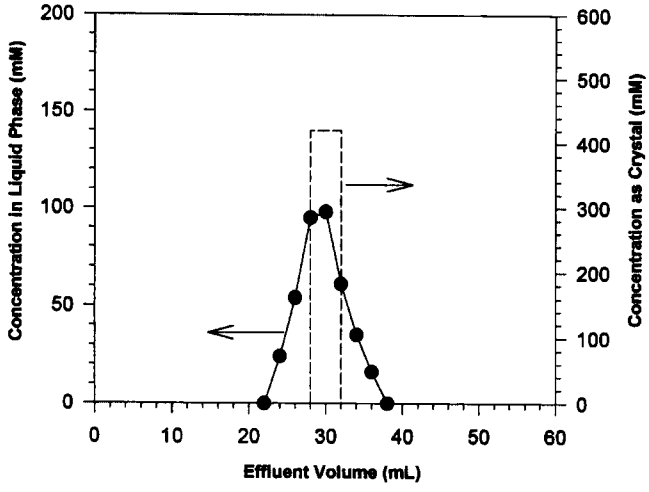

Fig. 4. Crystallized recovery of glutamic acid from AG 50W-X4 column driven by 1.0 M NaOH.

liquid phase and crystal from AG 50W-X4 column. Crystal recovery is defined as the ratio of the amount of crystal collected to the amount of initially loaded. Crystal recovery was about 47% of initially loaded amount, and the position and concentration of peak were at 30 mL of effluent volume and 420 mM, respectively. X8 and X12 resins displayed an identical peak position at about 30 mL, but the differences in peak concentration and crystal recovery are shown in Fig. 5. Increase of crosslinking degree favored crystal recovery and peak concentration. This is maybe because lower degree of crosslinking results in larger hydration volume and swelling [9], which can reduce the volume capacity of the bed. Lower volume capacity dilutes the concentration of released glutamic acid, which is not favorable to crystal formation. The degree of swelling can be envisioned by the change of bed height in Fig. 5 when columns are saturated with H^+.

Effects of Displacer Concentration

The concentration of displacer NaOH is directly related to pH of mobile phase. The effect of displacer

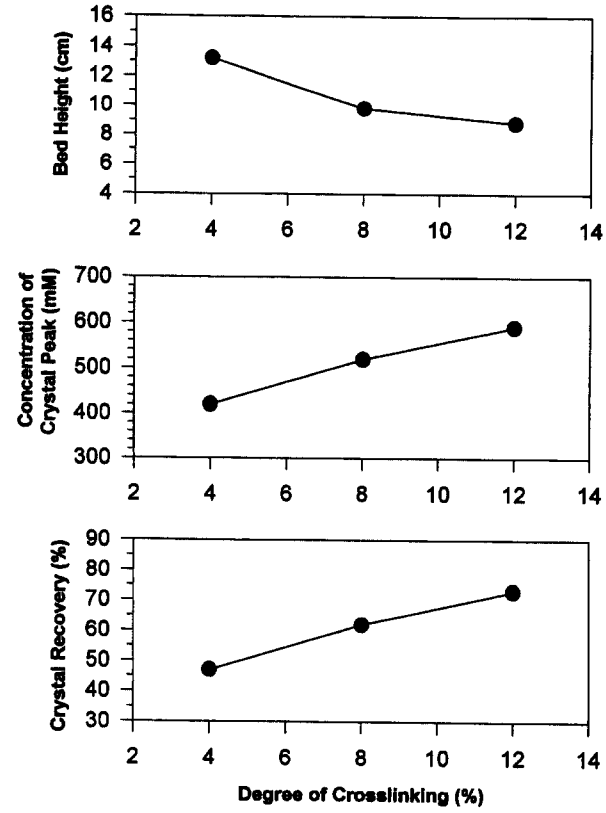

Fig. 5. Effect of the crosslinking degree of ion exchange resin on bed height and crystal recovery.

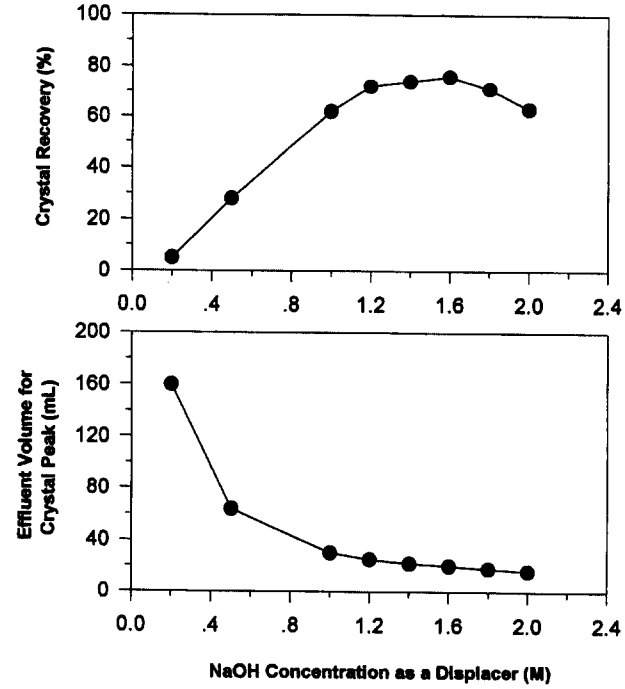

Fig. 6. Effect of displacer concentration on crystal recovery of glutamic acid.

NaOH concentration on the crystal recovery is shown in Fig. 6. The column was packed with 6 gram of AG 50W-X8 resin and loaded with 100 mL of 30 mM glutamic acid. The flow-rate of NaOH was constant at 1.0 mL/min. No crystal was formed when displacer concentration was below 0.1 M. When 0.15 M NaOH was used, crystal was formed temporarily, but dissolved

soon during the propagation and thus not collected out of the column. Crystal recovery had been expected at these displacer levels since 0.1 M or 0.15 M NaOH was higher than C_{dH}, 78 mM in equilibrium isotherms shown as Fig. 2. These observations at this range of NaOH concentration implied that the concentrating effect of NaOH to induce crystal formation was weaker than solubility-enhancing effect of high pH [10-12]. When NaOH level was in the range of 0.2 to 1.6 M, the crystal recovery was increased as NaOH concentration increased, because the concentrating effect of NaOH outran the solubility-enhancing effect. However, with NaOH level higher than 1.6 M, crystal recovery decreased again maybe because the solubility-enhancing effect was dominant due to high pH and ionic strength [13]. The position of effluent volume for elution peak was inversely proportional to the concentration of displacer NaOH.

Effects of Mobile Phase Flow-Rate

Flow-rate of mobile phase can affect band sharpness and resolution. In turn, the degree of concentrating effect and crystal formation are closely related to band sharpness. Fig. 7 shows the effect of mobile phase flow-rate on the crystal recovery. The column was packed with 6 gram of AG 50W-X8 resin and loaded with 100 mL of 30 mM glutamic acid. The NaOH concentration was 1.0 M. The position of effluent volume for elution peak was inversely proportional to flow-rate. Crystal recovery was also enhanced as flow-rate decreased. Lower flow-rate provides enough time for local equilibrium and favors stable ion-exchange reaction, which consequently reduce band spreading and make solute boundary sharper [9]. However, flow-rate below 0.2 mL/min acted unfavorably probably because the flow-rate of this range could not generate sufficient shear for relieving film resistance around resin particles [3, 9], which resulted in less sharp solute boundary and thus reduced crystal formation.

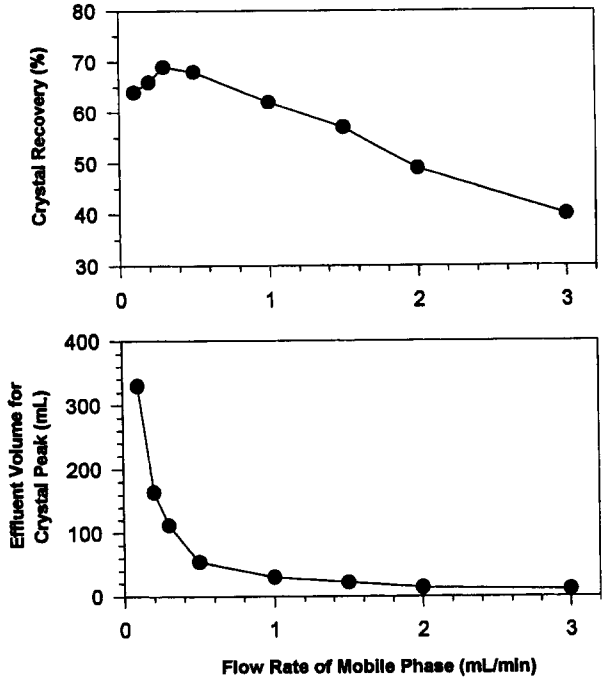

Fig. 7. Effect of mobile phase flow-rate on crystal recovery of glutamic acid.

Effects of Temperature

In general the solubility of amino acids decreases as temperature is lowered [10, 11]. Fig. 8 shows the effect of operating temperature on the crystal recovery. The column was packed with 6 gram of AG 50W-X8 resin and loaded with 100 mL of 30 mM glutamic acid. The flow-rate of NaOH was constant at 3 mL/min and 2.0 M NaOH was used as a displacer. The column used in this study was a hand-made apparatus and it was not appropriate to install water jacket. The temperature of the column was maintained constantly in this study by feeding mobile phase at a specific temperature for an hour prior to experiments and the breakthrough time was shortened by using a high flow-rate and high displacer concentration. Because the bed capacity was 30 meq, it took about 5 min for breakthrough with 2.0 M NaOH at 3 mL/min. After the complete breakthrough, the temperature difference between entrance and exit was within 1.5°C. Crystal recovery was enhanced due to the increased solubility at lower temperature. However, crystal recovery was decreased when the temperature was below 5°C. It seems that temperature below 5°C reduces ion exchange rate, which is not advantageous for the formation of sharp solute boundary [9] and subsequent crystallization.

Separation of a Ternary Mixture

A ternary mixture of 120 mL containing 25 mM each of serine, glutamic acid, and alanine was fed to the AG 50W-X8 column. The selectivities of the three amino acids are in the order of serine < glutamic acid < alanine as shown in Fig. 2. The solubility of glutamic acid is the least among the three. These three amino acids are exited side by side from a cation exchange column when hydrolysate of human plasma is analysed by eluting with sodium citrate [14]. Fig. 9 shows the results of separation driven by 1 M NaCl and NaOH as displacers at 1 mL/min. With NaCl, crystallization of glutamic acid did not occur and three bands were dispersed with significant overlapping. With NaOH, about

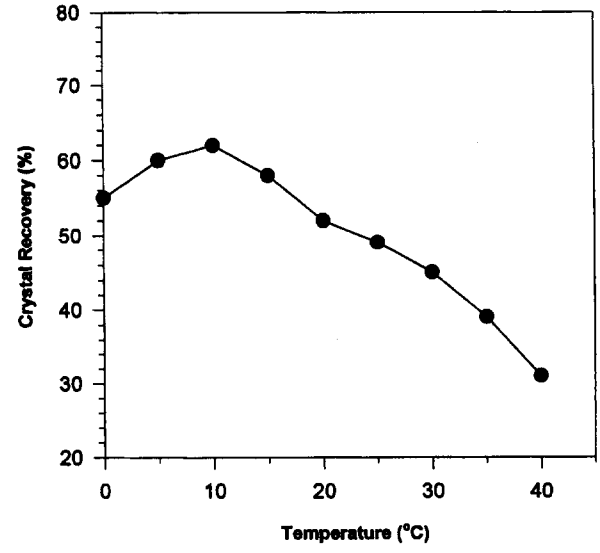

Fig. 8. Effect of temperature on crystal recovery of glutamic acid.

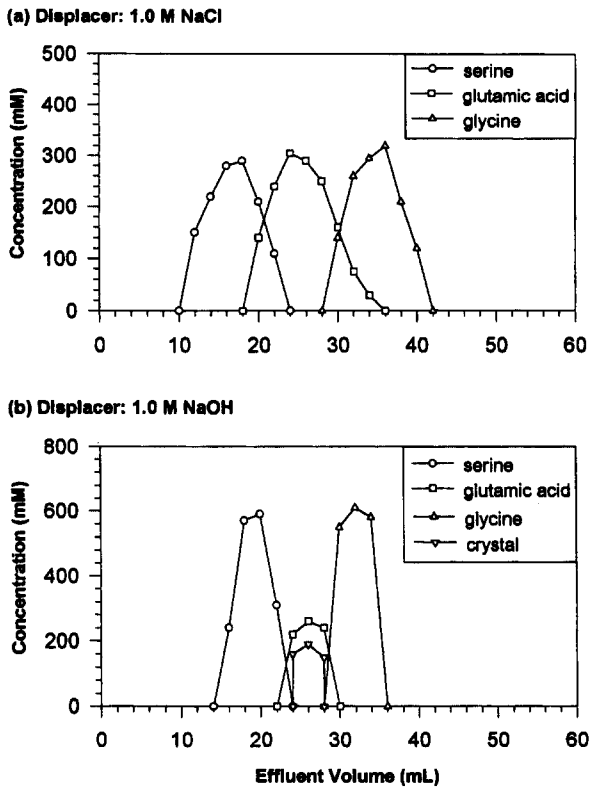

Fig. 9. Displacement ion exchange chromatography of a ternary mixture.

32% of glutamic acid was recovered as crystal which was collected between serine and alanine. The peak concentrations of serine and alanine was about 600 mM which is 24 times higher than the concentration in the feed. Also it can be seen that band overlapping between saturated solutions were greatly reduced by the virtue of crystallization. The collected crystal of glutamic acid could be recovered in pure form by simple filtration or dewatering. Fig. 9 clearly shows that the crystallization-coupled ion exchange operated by displacement mode would be advantageous in reducing the burden of further purification steps and effective in preventing purity-loss which is resulted from overlapping between adjacent bands.

SUMMARY

Glutamic acid can be crystallized inside cation exchange column when displacer NaOH concentration is high enough to concentrate glutamic acid released from the resin beyond its solubility limit. Resulting crystal layer of glutamic acid was moved through the column length and thus it could be eluted from the column and recovered in fraction collector. For the purpose of enhancing crystal recovery, effects of operating parameters on the crystal formation were investigated. The increase in the degree of crosslinking of resin favored crystal recovery because of its low degree of swelling and high volume capacity. Higher concentration of displacer NaOH was advantageous. If NaOH concentration was too high, however, crystal recovery was reduced due to the solubility-enhancing effect of high pH and ionic strength. The decrease of mobile phase flow-rate enhanced crystal recovery because it provided enough time for local equilibrium, but film diffusion would control the overall crystal formation in the case of extremely low flow-rate. Lower temperature reduced solubility of glutamic acid and thus favored crystal formation, unless the rate of ion exchange reaction was severely lowered due to the low temperature. The ion exchange operated by displacement mode coupled with crystallization was advantageous in that it could reduce burden of further purification steps and prevent purity-loss resulted from overlapping between adjacent bands.

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