

# Separation and Refining of Amino Acids

(Extracted from the Diaion Manuals pages 280 to 293)

amounts of L- and D-forms. Natural amino acids that are components of proteins are all  $\alpha$ -amino acids in L-form.

Animals cannot bio-synthesize all the necessary amino acids by themselves as plants or bacteria can. Thus, they have to take amino acids as food that can be synthesized enough to grow and to live healthy lives. Such amino acids are called as "essential amino acids" and as for humans there are eight essential amino acids: L-valine, L-leucine, L-isoleucine, L-threonine, L-methionine, L-phenylalanine, L-tryptophane and L-lysine.

Amino acids are commercialized as important enrichment ingredients for foodstuffs and feedstuffs and as seasonings, as conditioning agents and stabilizers for foods, as additives in cosmetics and as pharmaceuticals.

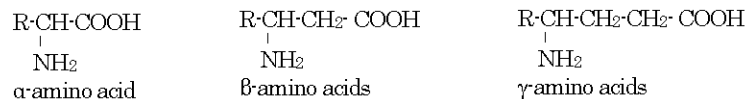
## (1) Manufacturing of amino acids

Amino acids are produced by extraction, organic synthesis, fermentation or enzymatic synthesis. The first industrial manufacturing in 1908 was that of glutamic acid as seasonings separated from the extracts of wheat proteins. In 1955, invented was the fermentation method where bacteria are bred with sugar as carbon source and ammonia as nitrogen source to accumulate amino acid out of their cells in a large amount. L-Glutamic acid is the first industrial application. Most of amino acids can be manufactured by fermentation at present, after selection of wild strains, inducements of mutants and improvements of bacteria fermentation.

Synthetic amino acids should be separated into D- and L-forms by optical resolution, except for glycine that has no DL-forms, methionine and alanine; both D- and L-forms of the last two amino acids can be digested. Optical resolution is industrialized with enzyme aminoacylase.

## 6. Separation and Refining of Amino acids

Amino acid is a general name of the chemical compounds that have both an amino group and a carboxylic group within one molecule, and such amino acids are categorized into  $\alpha$ -,  $\beta$ - and  $\gamma$ -amino acids based on the position of the amino group against the carboxylic acid group, as illustrated below:



Whereas there are more than eighty kinds of amino acids, proteins are made from only twenty six kinds of amino acids, twenty three of which are listed in Table VIII-6-1 and the other three are dibromotyrosine, triiodotyrosine and thyroxine. All  $\alpha$ -carbons except that of glycine are asymmetric carbons that have four different bonds with -H, -COOH, -NH<sub>2</sub> and -C<sub>m</sub>H<sub>n</sub>, and thus amino acids have two different stereo configurations: levorotatory L-form and dextrorotatory D-form. Amino acids synthesized chemically are optically inactive racemic bodies, mixtures of equal

[Table VIII-6-1] Amino acids that constitute proteins

NAME	MW	Chemical structure	pKa	pK <sub>2</sub>	pI
Arginine Arg	174.1		1.82	8.99	10.76
Lysine Lys	146.2		2.20	8.90	9.74
Hydroxyllysine Hyl	162.2		2.13	8.62	8.64
Histidine His	155.2		1.78	5.97 (Im)	7.59
Glycine Gly	75.07		2.35	9.78	5.97
Alanine Ala	89.09		2.34	9.69	6.00
Valine Val	117.1		2.32	9.62	5.96
Leucine Leu	131.2		2.36	9.60	5.98
Isoleucine Ile	131.2		2.36	9.68	6.02
Serine Ser	105.1		2.19	9.21	5.68
Threonine Thr	119.1		2.15	9.12	6.16
Asparagine Asn	132.1		2.02	8.80	5.41

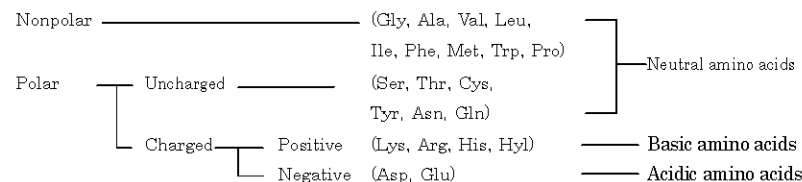
  

NAME	MW	Chemical structure	pKa	pK <sub>2</sub>	pI
Glutamine Gln	146.1		2.17	9.13	5.65
Cysteine (Cys) <sub>2</sub>	240.8		< 1.00	2.1	8.02
Cysteine Cys	121.2		1.82	8.35	10.46
Methionine Met	149.2		2.13	9.28	5.74
Phenylalanine Phe	165.2		2.16	9.18	5.48
Tyrosine Tyr	181.2		2.20	9.11	10.07
Tryptophan Trp	204.2		2.83	9.39	5.89
Proline Pro	115.1		1.95	10.64	6.30
Hydroxyproline Hyp	131.1		1.83	9.66	5.83
Aspartic acid Asp	133.1		1.88	3.85	9.60
Glutamic acid Glu	147.1		2.19	4.25	9.67

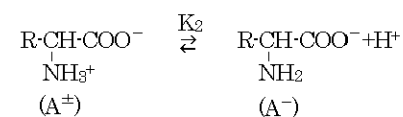
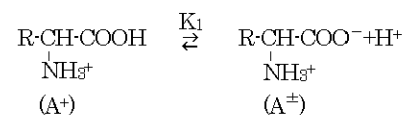
**(2) Dissociation of Amino acids and ion-exchange**

α-Amino acids are defined in a general formula, R-CH(NH<sub>2</sub>)-COOH, and are categorized as follows depending on their properties affected by the side-chain atomic group (R):

(Property of side-chain group R)



All amino acids are amphoteric electrolytes and thus can dissociate as bases in acidic conditions and as acids in alkaline conditions. There is a point where dissociated cations are equal to dissociated anions, and the point is called as "isoelectric point", or pI. When dissociation equations of amino acids are defined



as follows,

dissociation coefficients, K<sub>1</sub> and K<sub>2</sub> are expressed as,

$$K_1 = \frac{[\text{A}^\pm][\text{H}^+]}{[\text{A}^+]}, \quad K_2 = \frac{[\text{A}^-][\text{H}^+]}{[\text{A}^\pm]}$$

Using these equations and applying the pK<sub>1</sub> and pK<sub>2</sub> values in Table VIII-6-1 into them, the dissociation curves are calculated\* and displayed in Fig. VIII-6-1. In other words, neutral amino acids behave in the neutral range between pK<sub>1</sub> and pK<sub>2</sub>, and thus:

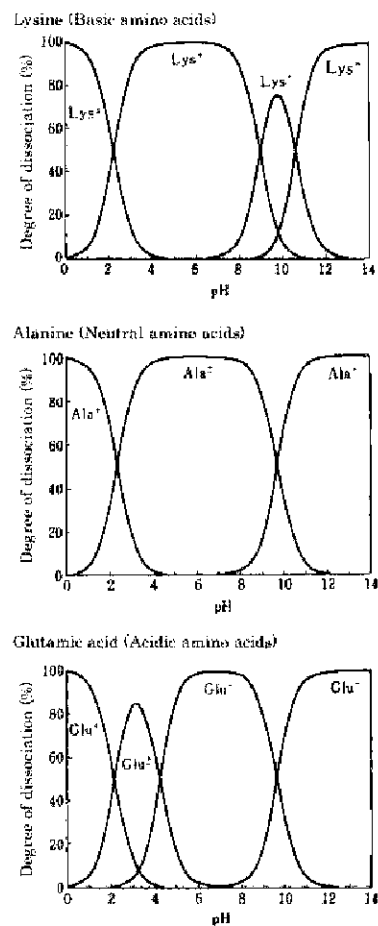
Treated as cations at pH ≤ pK<sub>1</sub>

Treated as anions at pH ≥ pK<sub>2</sub>

Amino acids adsorbed by CERs and AERs can be desorbed and elute with treatment with NaOH or ammonia solutions and HCl or other acid

solutions respectively. In general, CERs are utilized in the treatment of amino acids and ammonia that can be collected in the latter concentration process as eluents. Also, ammonia solution is sometimes used to increase an ionic strength with mixture with ammonium salts.

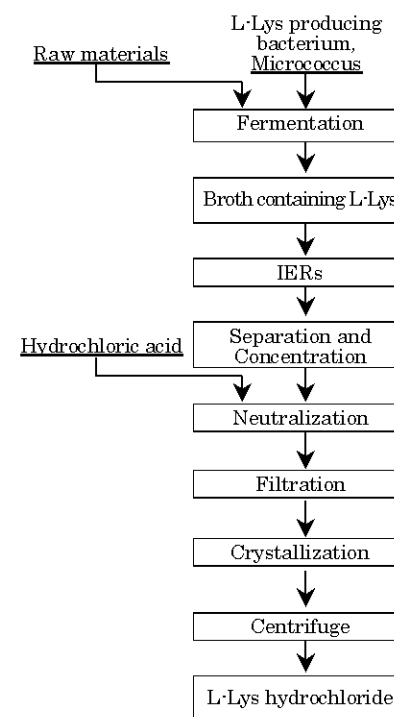
*\*Note: Calculation method of dissociation curves is described in the tables at the end of this book.*



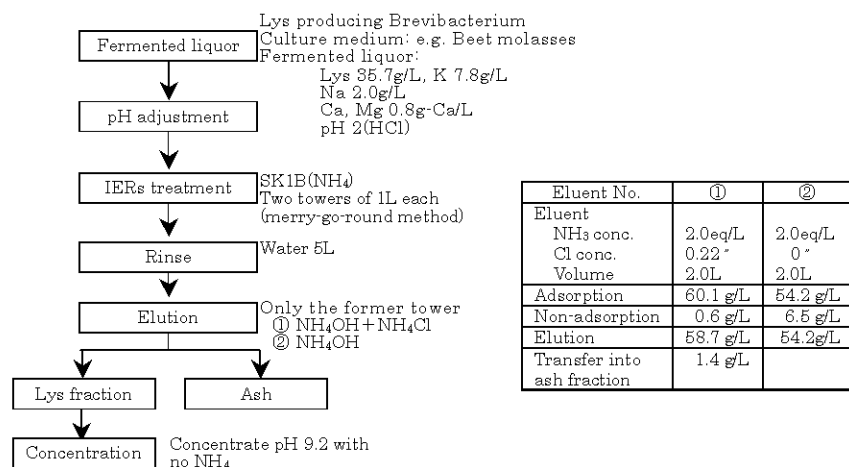
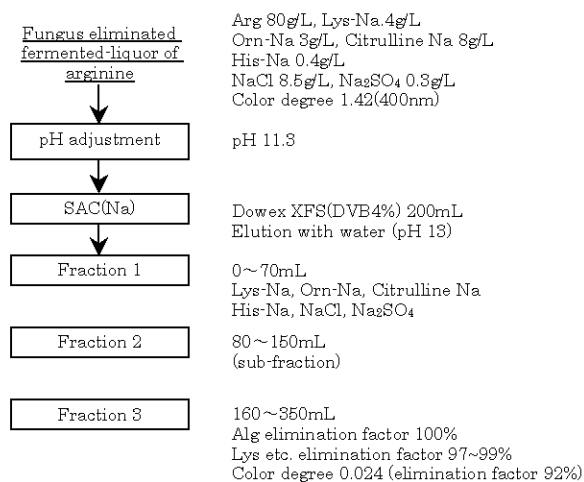
[Fig.VIII-6-1] Dissociation Curves of Amino acids

Manufacturing process of L-lysine hydrochloride is illustrated in Fig.VIII-6-2 and its procedures are as follows: 1) fermented mother liquor is acidified, 2) treated with SACERs that adsorb lysine but pass through most impurities, 3) lysine adsorbed by IERs are desorbed with ammonia solutions or those with ammonium salts.<sup>(81)</sup>

Neutral amino acids are manufactured in similar ways: e.g. histidine<sup>(82)</sup>, phenylalanine<sup>(82)(84)(85)(86)</sup>, leucine<sup>(82)(86)</sup>, isoleucine<sup>(82)(86)</sup>, valine<sup>(82)</sup>, methionine<sup>(82)</sup>, threonine<sup>(83)</sup>, proline<sup>(83)(87)</sup> and tyrosine.<sup>(84)</sup>



[Fig.VIII-6-2] Manufacturing process of L-Lysine by fermentation

[Fig.VIII-6-3] Manufacturing of Basic Amino acids <sup>(81)</sup>[Fig.VIII-6-4] Separation of Arginine <sup>(92)</sup>

Amino acids with low solubility such as phenylalanine sometimes precipitate from IERs that adsorb them during desorption. The constant dissociation with constant alkali addition and with stirring and fluidizing is proposed to avoid such precipitation<sup>(86)</sup> IERs are also applied for other amino acids than lysine and phenylalanine to refine them.

### (3) Separation and Refining by Ion-exclusion method

Because amino acids are uncharged at their Isoelectric points as already explained, neutral amino acids, e.g. glutamine <sup>(88)</sup>, isoleucine <sup>(89)</sup>, valine <sup>(90)</sup> and threonine <sup>(91)</sup>, can be separated from their impurities, e.g. acidic amino acids, by ion-exclusion chromatography with NH<sub>4</sub>-form or Na-form SACERs into acidic amino acids, sulfates, chlorides, colorants and other impurities. Neutral amino acids should be treated in neutral conditions where amino acids are uncharged.

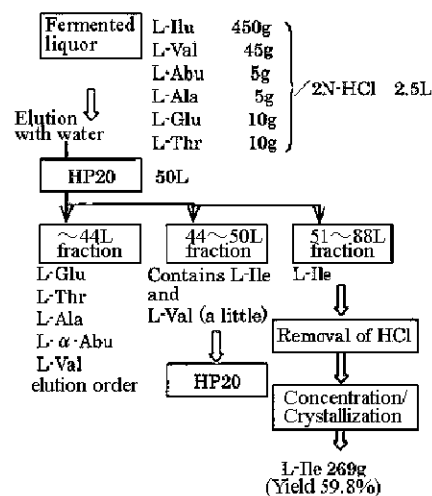
In separating basic amino acids, e.g. separation of arginine from lysine, histidine and other impurities <sup>(92)</sup>, since lysine and histidine exist as anions at the Isoelectric point of arginine, CERs should be in the same ion form as the counter cation of such anions. Arginine flows out after the elution of lysine, histidine and other impurities, but arginine is adsorbed during its elution due to the pH drop when neutral water is used as eluents. Thus, the pH of water should be kept equal or higher than the isoelectric point of arginine, as demonstrated in Fig.VIII-6-4.

### (4) Separation and Refining with Synthetic Adsorbents

Amino acids with nonpolar side chain group can be hydrophobically adsorbed with nonpolar synthetic adsorbents, e.g. DIAION® HP20 and Sepabeads SP207, in the range of isoelectric point. Typical examples are refining of phenylalanine synthesized from cinnamic acid and ammonia with enzyme<sup>(93)(94)</sup>, and refining of isoleucine and tyrosine manufactured by fermentation. <sup>(95)(96)</sup>

Fig.VIII-6-5<sup>(95)</sup> illustrates the refining of isoleucine solution with synthetic resins, e.g. HP20. Isoleucine (pI = 6.02) solutions are made by fermentation of glucose and by treatment with SACERs and hold as impurities byproducts such as valine (pI=5.96, almost as the same as isoleucine's pI) and neutral amino acids, e.g. α-aminolactic acid, alanine and threonine. Water, diluted ammonia water or diluted hydrochloric acid is used as eluents, and glutamic acid, threonine, alanine, α-aminolactic acid, valine and isoleucine elute in this order. The properties listed in Table VIII-6-2 show us that amino acids with larger nonpolar alkyl groups have stronger affinity to synthetic adsorbent HP20.

Synthetic adsorbents decrease their own adsorbing functions after some ten refining cycles, and thus they are regenerated with acetone and NaOH solutions (acetone: 1N-NaOH = 1:1).

[Fig.VIII-6-5] Refining of L-isoleucine with Synthetic Adsorbents <sup>(95)</sup>

[Table VIII6-2] Adsorption of Amino acids by HP20

Order	Amino acid	M.W.	pI	Side chain R	Nonpolar group
1	Glu	147.1	3.22	Polar, negative charged	
2	Thr	119.1	6.16	Polar, uncharged	
3	Ala	89.1	6.00	Nonpolar	CH <sub>3</sub> -
4	Abu	103.1	5.98	Nonpolar	CH <sub>3</sub> -CH <sub>2</sub> -
5	Val	117.2	5.96	Nonpolar	CH <sub>3</sub> -CH(CH <sub>3</sub> )-
6	Ile	131.2	6.02	Nonpolar	CH <sub>3</sub> -CH <sub>2</sub> -CH(CH <sub>3</sub> )-

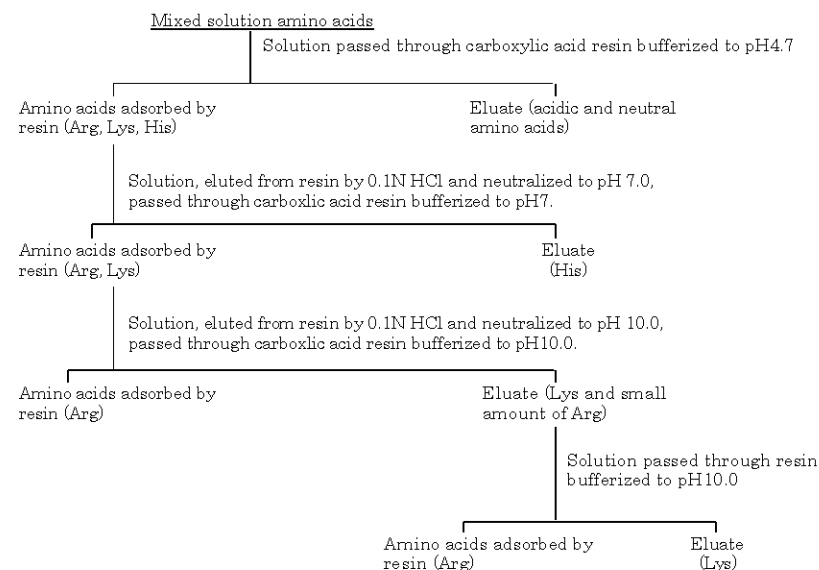
※Abu (α-aminolactic acid): CH<sub>3</sub>-CH<sub>2</sub>-C(NH<sub>2</sub>)-COOH pK<sub>1</sub>=2.29, pK<sub>2</sub>=9.83

### (5) Separation of Amino acids with Weakly electrolytic IERs

Carboxylic acid type WACERs the dissociation coefficients of which are in the weakly acidic region have some buffer power in this region. Thus, basic amino acids that dissociate as cations in the same pH range can be separated with such WACERs. R. Kumin<sup>(97)</sup> reported that neutral amino acids can be separated by the procedures in Fig.VIII-6-6. There is a

disadvantage that WACERs have little adsorbance with amino acids in weakly acidic conditions due to their very strong affinity against H<sup>+</sup> ions.

The dissociation coefficients of WBAERs are, on the contrary, in the weakly alkaline region, and thus WBAERs can adsorb acidic amino acids that dissociate in this range. However, neutral amino acids also dissociate somewhat, and thus the perfect separation cannot be accomplished.

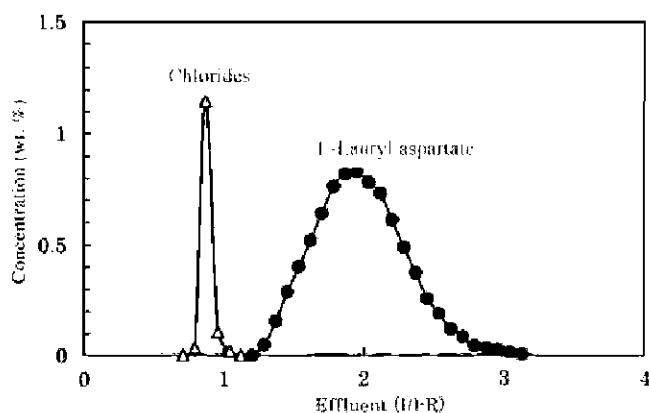
[Fig.VIII-6-6] Separation of Amino acids with Carboxylic acid type WACERs<sup>(97)</sup>

### (6) Separation of Amino acids with amphoteric IERs

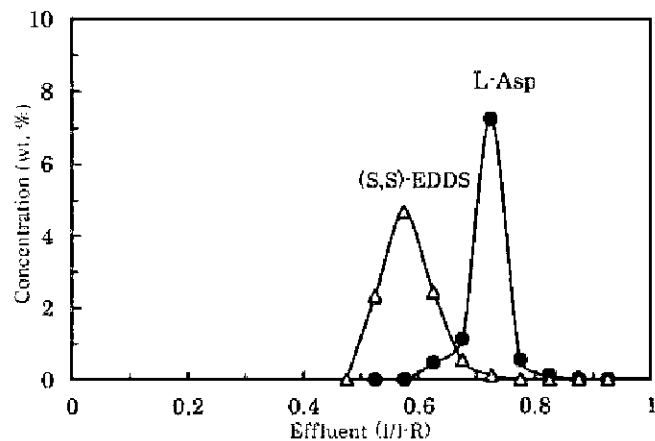
L-Lauryl aspartate reaction mother liquor of the reaction of L-aspartic acid with L-laurylchloride is adjusted at pH 6.5 by the addition of HCl and diluted. This liquor containing 7.32% L-lauryl aspartate and 1.08% chlorides is treated by the DSR01 column at SV 0.5 at 50 °C and then is eluted with water. Fig.VIII-6-7 summarizes this elution curve based on the original literature.<sup>(96)</sup>

Fig.VIII-6-8 also illustrates the separation of S,S'-ethylenediamine-N,N'-disuccinate and L-aspartic acid in the reaction mixtures that are from L-aspartic acid by the DSR01 column at SV 0.36 (1/h) at 50 °C.<sup>(99)</sup>

Amphoteric amino acids show high selectivity toward DSR01 in both examples. Other relevant patents are listed in Table VIII-6-3 through VIII-6-6.



[Fig.VIII-6-7] Separation of Amino acid with amphoteric IER, example-1  
Originated from the table in the patent<sup>(99)</sup>



[Fig.VIII-6-8] Separation of Amino acid with amphoteric IER, example-2  
Originated from the table in the patent<sup>(99)</sup>

[Table VIII-6-3] Patent list of Amino acids purification with strongly electrolytic IERs

Amino acids	IERs/ Eluents and other info.	Patent number
Basic amino acids	SAC(NH <sub>4</sub> )/ eluted with NH <sub>3</sub> aq. or NH <sub>4</sub> salt	JPA1975006778
His, Tyr, Phe, Leu, Ile, Val, Met	SAC(NH <sub>4</sub> )/ eluted with ammonia water	JPA1975126878
Thr, Pro	SAC(NH <sub>4</sub> , H mix)	JPA1978140290
DL-Thr	SAC/ eluted with ammonia water	JPA1984025362
Trp, Phe etc.	MR-type SBA/ Decolor alkaline solutions of amino acids	JPA1985188353
Basic amino acids	SAC(NH <sub>4</sub> )/ Treat at pH 2 the eluent through semipermeable membranes at pH 2-5	JPA1985207593
Cysteinylglycine	SAC of DCB ≥ 8%/ Separate from mixtures of Sys and Gly	JPA1985258199
Basic amino acids	SAC/ Multi-tower, 3 adsorbing and 3 elution	JPA1986024548
Phe	SAC(NH <sub>4</sub> )/ Treat ethylacetate solution after A/C treatments, eluting with conc.NH <sub>3</sub> aq.	JPA1986148147
Trp	SAC/ Adsorbed as well as other amino acids, eluting other coexisting amino acids with acids or inorganic salts solutions, Trp is eluted with NH <sub>3</sub> aq.	JPA1986189267
Phe, Leu, Ile	Porous type SAC(H)/ Eluting from fluidized state	JPA1986200948
Trp, Indole	Porous type SAC/ Trp is eluted with NH <sub>3</sub> aq., Indole adsorbed by porous parts is eluted with 80% IPA	JPA1986234789
Basic amino acids (e.g. Lys)	SAC(NH <sub>4</sub> )/ merry-go-round multi towers, eluted with NH <sub>3</sub> aq. Eluent of Lys conc. < 8g/L is recharged into the next tower	JPA1987061592
Basic amino acids	SAC/ Waste waters from adsorption and elution steps are recycled as rinse waters	JPA1987065690
Gly and Ser	SAC(H)/ Effective particle size 0.15-0.4mm, Gly is adsorbed but Ser is not, eluted with NH <sub>3</sub> aq.	JPA1987111953
Glu	SAC(H, salt)/ Eluted with fermented liquor with Na <sub>2</sub> CO <sub>3</sub>	JPA1987212355
Trp, indole (raw material)	Porous type SAC/ Eluted with NH <sub>3</sub> aq. Then 80% IPA	JPA1987215564
Trp, Ser, Gly and a amino acid of an aromatic ring	SAC(Na)/ Eluted with water	JPA1991227963
Glu etc.	SAC(NH <sub>4</sub> )/ Continuous adsorption and elution by countercurrent multi towers	JPA1992134054
Thr	SAC(H)/ Eluted with NH <sub>3</sub> aq.	JPA1993207886
Amino acids made by hydrolysis of peptides	SAC(H)/ (1) Adsorbed with SAC (2) Hydrolyzed at 100 °C (3) Eluted with water after cooling	JPA1993310657
Lys, Thr etc.	Lys=SAC(NH <sub>4</sub> )/ Eluted with HCl, Thr=SAC(H)/ Eluted with NH <sub>3</sub> aq.	JPA1994133768
Orn	SBA(CH <sub>3</sub> COOH)/ Convert HCl salt of Orn into acetate	JPA1995089914
Hyl	SAC(NH <sub>4</sub> ) → SBA(OH) → Reaction by o-Phthalaldehyde with impurities → HP → SBA(OH)	JPA1995318179
Val, Leu, Ile	SAC(H)/ Eluted with NH <sub>3</sub> aq.	JPA1996009982
Trp	SAC(NH <sub>4</sub> )/ Crude Trp crystallizes	JPA1996103283 JPA1996103284
Pro, Glu, Ala, Val	SAC(H)/ Temp. 40-80 °C, Eluted with NH <sub>3</sub> aq.	JPA1996183768

SAC: Strongly acidic cation exchange resin WAC: Weakly acidic cation exchange resin

SBA: Strongly basic anion exchange resin WBA: Weakly basic anion exchange resin

HP: Synthetic adsorbents AMP: Amphoteric ion exchange resin

Notation in parentheses shows ion-forms

[Table VIII-6-4] Patent list of Amino acids purification with weakly electrolytic IERs

Amino acids	IERs/ Eluents and other info.	Patent number
Fermented liquor from molasses	WBA/ Pretreatment of ion exchange membranes to remove proteins and colorants	JPA1979067093
S-Adenocyl-L-methionine(SAM)	WBA as pH control →WAC(SAM adsorption)/ Eluted with H <sub>2</sub> SO <sub>4</sub> and MaOH	JPA1981145299
DL-Ile	WBA/ Epimerize DL-Alloisoleucine-1,5-naphthalene disulfonate with low molecular fatty acids or aromatic aldehydes, and remove 1,5-naphthalene disulfonic acid	JPA1983008048
S-Adenocyl-L-methionine(SAM)	WAC and HP/ Eluted with H <sub>2</sub> SO <sub>4</sub>	JPA1984029700
Ile	WBA/ Crystallize HCl salt of Ile, and remove HCl	JPA1984062554
Pro	CR(Cu)/ Eluted with water or NH <sub>3</sub> aq.	JPA1988246354
Amino acids	Polyalkylene polyamine type CR(metal ions)	JPA1989022848
Amino acids	Aminocarboxylic acid type CR or aminosulfonic acid type CR(metal ions)	JPA1989258652
Glu	WBA(OH)/ Remove impurities	JPA1990028379
N-Phosponomethyl glycine	WBA(OH)/ Remove more acidic impurities	JPA1991086892
Gly	WAC, SAC/ Adjust pH to 6 after A/C treatment	JPA1992226949
Leu, Ile	CR chelated with metal ions, WAC/ Eluted with NH <sub>3</sub> aq., Separation of Leu and Ile	JPA1993032599
Cys with Met, Phe with Trp	CR chelated with metal ions, WAC/ Eluted with NH <sub>3</sub> aq.	JPA1993058972
Leu, impurities: Val and Ile	CR chelated with metal (Cu) ion, Separation and purification of Leu/ Eluted with NH <sub>3</sub> aq.	JPA1993229995
Gly, monochloroacetic acid and ammonia as raw materials	WBA(f-base) →WAC(H)/ Dissolve crude crystals and charge	JPA1996012632
Gly, with iminodiacetic acid	Exchange Na with WAC(H), then eliminate iminodiacetic acid with WBA(f-base)	JPA2003212829
Amino acids with iminodicarboxylic acids	Eliminate iminodiacetic acid with WBA(f-base)	JPA2005298366
Gly etc.	WAC(H)/ Remove alkali metal salts of amino acids with continuous fluidized-bed IERs, upflow both in adsorption and elution due to low mechanical strength against volume changes	JPA2005343812

SAC: Strongly acidic cation exchange resin    WAC: Weakly acidic cation exchange resin  
 SBA: Strongly basic anion exchange resin    WBA: Weakly basic anion exchange resin  
 HP: Synthetic adsorbents    AMP: Amphoteric ion exchange resin  
 Notation in parentheses shows ion-forms

[Table VIII-6-5] Patent list of Amino acids purification with chromatography e.g. ion-exclusion, ion-retardation

Amino acids	IERs/ Eluents and other info.	Patent number
Gln; impurities= Glu, Pyrolidone carboxylic acids, sulfate ions	SAC(NH <sub>4</sub> )/ ion-exclusion chromatography, Eluted in the order of Glu NH <sub>4</sub> and Gln	JPA1987148459
Ile, acidic amino acids, inorganic salts	SAC(Na)/ Separate Ile with ion-exclusion chromatography	JPA1987255452
Val, acidic amino acids, inorganic salts	SAC(Na)/ Separate Val with ion-exclusion chromatography	JPA1987255453
His; impurities= acidic amino acids, sulfate and chloride anions	SAC(NH <sub>4</sub> )/ ion-exclusion chromatography	JPA1987273961
Arg, Lys, Orn, Citrulline, His	SAC(Na)/ Separate Arg with ion-exclusion chromatography	JPA1987292750
Thr	SAC/ ion-exclusion chromatography	JPA1988000294
Gly, impurities= Iminodiacetic acid	SAC(Na)/ Water expansion chromatography	JPA1990215746
DL-Ser	SAC(Na)/ ion-exclusion chromatography	JPA1990288851
Ser, raw material= Gly	SAC(H)/ ion-exclusion chromatography which used SAC of effective particle size 0.15-0.40mm	JPA1990808796
Trp, Ser, Gly and a amino acid of an aromatic ring	SAC(Na)/ Eluted with water	JPA1991227963
Acylamino acid salts	AMP/ Ion-retardation chromatography	JPA2001247531
Ala, γ-Aminolactic acid, Val, Ser, Leu, Ile from waste waters of beet sugar	SAC(Na), SAC(Ca or Mg)/ Chromatographic separation	JPA2002088036
Basic amino acids	SAC of DCB ≥ 8%/ Simulated moving bed system	JPA2003528602

[Table VIII-6-6] Patent list of Amino acids purification with synthetic adsorbents

Amino acids	IERs/ Eluents and other info.	Patent number
Phe, Cinnamic acid	HP/ Chromatography at pH <9.0	JPA1985104051
Phe, Cinnamic acid	HP/ Chromatography at pH >10	JPA1985104052
Phe, Trp= impurity	HP/ Phe is adsorbed selectively and eluted with ethanol aq.	JPA1985136543
Phe, Cinnamic acid	HP/ Chromatography in the conditions of inorganic salts ≥0.2N	JPA1985181055
Leu, Val	HP with nitro groups	JPA1986028448
Trp	HP/ Treated and decolorated at 80°C	JPA1986126070
Val, Ile and Leu= impurities	HP/ Eluted with water in the order of Val, Ile, Leu	JPA1986178952
Tyr, Phe= impurity	HP/ Phe is adsorbed at pH 11, Eluted with alkalis, acids and alcohols	JPA1986178953
Phe, Cinnamic acid	HP/ Chromatography in the condition of organic acid conc. ≥0.2N	JPA1986194056
Trp	HP/ Eluted with water and ethanol aq. (e.g. colorants)	JPA1986249961
Trp	HP/ Eluted with NH <sub>3</sub> aq.	JPA1988130580
Trp	HP/A/C and HP treatments at pH 1-4 to remove impurities, Trp is not adsorbed	JPA1988177796
Trp	HP/ Treat effluents from UF membranes, eluted with NH <sub>3</sub> aq.	JPA1991200765
Trp, Indole = raw material	HP/ Adsorb indole	JPA1991200766

SAC: Strongly acidic cation exchange resin    WAC: Weakly acidic cation exchange resin  
 SBA: Strongly basic anion exchange resin    WBA: Weakly basic anion exchange resin  
 HP: Synthetic adsorbents    AMP: Amphoteric ion exchange resin  
 Notation in parentheses shows ion-forms