Separation and Purification of Pharmaceuticals and Antibiotics

1. Antibiotics

(1) Outline

Antibiotics are "chemical substances produced by microorganisms which, in minute quantities, inhibit or suppress the proliferation of other microorganisms". This is defined in 1942 by Selman Abraham Waksman in the USA. Antibiotics were developed in Britain following the discovery of Penicillin in 1929 by Alexander Fleming: He found that Blue mold, Penicillium, dissolves Staphylococcus staphylococci and inhibits its growth and he named the extract from Penicillium as Penicillin. Many new antibiotics have been found since 1940's, and there are more than 1,500 antibiotics. Principal ones are listed in Table IX-1-1.

Most of these antibiotics are manufactured by fermentation or by semi-synthesis that combines fermentation and organic synthesis, though chloramphenicol is by synthesis. Fermentation is carried out by Actinomycetes, mold (hyphomycetes) or bacteria in culture media with glucose, sucrose, lactose, starch or dextrin as carbon source, with nitrate, ammonium salt, corn steep liquor, peptone, meat extract or yeast extract as nitrogen source and with a little amount of inorganic salts.

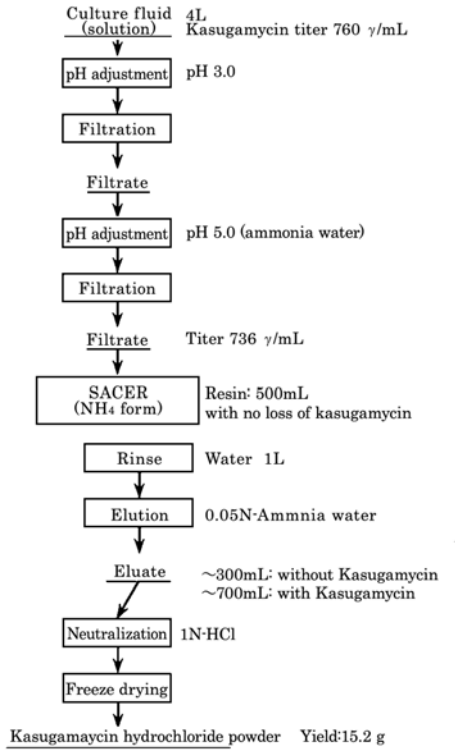
Penicillin-type antibiotics are manufactured by semi-synthesis, i.e. they are synthesized by the reaction of organic acid chlorides or organic acid anhydrides with 6-APA, 6-Aminopenicillanic acid, that is the product of enzymatic decomposition from Penicillin G, benzylpenicillin, or Penicillin V, phenylmethylpenicillin. Cephem-type antibiotics are also manufactured from fermented cephalosporin C through 7-ACA, 7-aminocephalosporanic acid, as an intermediate. Tetracycline derivatives are synthesized by semi-syntheses.

Since the concentration of antibiotics in the culture fluids is generally low in the manufacturing by fermentation, concentration and purification should be carried out at the same time. Thus, extraction by solvents, precipitation and adsorption with IERs or synthetic adsorbents are applied for antibiotics manufacturing.

[Table IX-1-1] Principal Antibiotics

ß-Lactams	Penicillins	Penicillin-G (benzylpenicillin), Oxacillin, Chloxacillin, Dichloxacillin, Nafcillin, Methicillin, Amoxicillin, Ampicillin, Ticarcillin, Piperacillin, aspoxicillin, Ciclacillin, Sulbenicillin, Talampicillin, Bacampicillin, Pivmecillinam, lenampicillin, Phenethicillin, Carbenicillin etc,
	Cephalosporins	Cephalosporin C Cefazolin, Cefatrizine, Cefadroxil, Cefalexin, Cefaloglycin, Cefalothin, Cefaloridine, Cefoxitin, Cefotaxime, Cefoperazone, Ceftizoxime, Cefmetazole, Cefradine, Cefroxadine etc.
	Other ß-lactams	
Aminoglycosides	Kanamycins	Amikacin, Kanamycin, Dibekacin
	Gentamycins, Sisomycins	Gentamicin, Micronomicin
	Streptomycins	Streptomycin
	Spectinomycins	Spectinomycin
	Fradiomycins	Fradiomycin,
	Astromicins	Astromicin
Macrolides		Erythromycin, Oleandomycin, Carbomycin, Kitasamycin (Leucomycin), Josamycin, Spiramycin, Midecamycin
Tetracyclines		Oxytetracycline, Tetracycline, Demethylchlortetracycline, Doxycycline, Minocycline, Methacycline
Peptides		
Streptogramins		
Chloramphenicols		Chloroamphenicol
Others		Enramycin, Gramicidin, Colistin (Polymixin E), Cycloserine, Novobiocin, Bacitracin, Fusidic acid, Polymixin B, Rifampicin, Lincomycin etc.
Quinolones		
Antituberculous		
Anti-tumor antibiotics		Actinomycin D, Chromomycin A3 (Toyomycin), Daunorubicin Sarkomycin, Bleomycin, Mitomycin C etc.
Antibiotics acting on mold		Amphotericin B, Griseofulvin, Trichomycin, Nystatin, Pyrrolnitrin etc.

(2)Purification with Strongly Electrolytic IERs



[Fig.IX-1-1] Purification of Kasugamycin with SACERs [109]

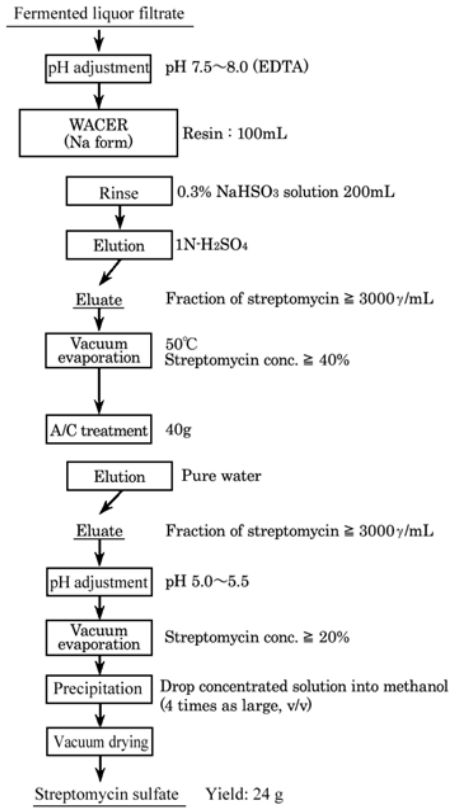
Antibiotics are categorized into four groups based on their dissociations: Basic ones with e.g. amino groups, acidic ones with e.g. carboxylic acid groups, amphoteric ones with both basic and acidic groups and the ones with no such groups. Though basic and acidic antibiotics can be adsorbed by SACERs and SBAERs respectively, fermented antibiotics are generally stable only in neutral or weakly acidic conditions and they may decompose or deteriorate in basic or acidic conditions. Thus, regenerated-form IERs could not used for such antibiotics.

Basic antibiotics, e.g. kasugamycine[109] illustrated in Fig.IX-1-1, ozemycin [110] and hortimycin A (fortimicin A) [111], can be separated from impurities in fermented mother liquor by filtration, neutralization of the filtrate, adsorption with ammonium-form SACERs and elution with ≈0.5N

aqueous ammonia. Acidic antibiotics, e.g. 7-aminocephalosporanic acid [112], on the other side, can be similarly purified adsorption with acetic acid-from SBAERs and elution with aqueous ammonium chloride.

Molecular weights of antibiotics spread from ≈100 of cycloserine to nearly 2,000 of gramicidin, a peptide. Both IERs and non-ionic synthetic adsorbents should have micro-pores within which objective materials can diffuse sufficiently, for them to be applied for antibiotics purification. Thus, such properties related with the micro-pores and the crosslinkage degrees are important factors to select proper resins.

Strongly electrolytic IERs can be also used in salt-conversions, e.g. formation of free bases from inorganic salts of kanamycin [113] and conversion into sodium salt from potassium salt of penicillin.



[Fig.IX-1-2] Purification of Streptomycin with WACERs [115]

(3) Purification with Weakly Electrolytic IERs

The pK values of WACERs are in weakly acidic region and thus they can ion-exchange in the range from weakly acidic to basic conditions, as already explained at the clauses concerning the refining of amino acids. The pK values of WBAERs are, on the contrary, in weakly basic region and thus they can ion-exchange in the range from acidic to weakly basic conditions. Based on the above-mentioned property, streptomycin [114) 115)] can be purified by adsorption with WACERs of regenerated-, NH_4- or Na-form and elution with diluted mineral acid solution following pH adjustment of the filtrate of fermented mother liquor, as illustrated in Fig.IX-1-2.

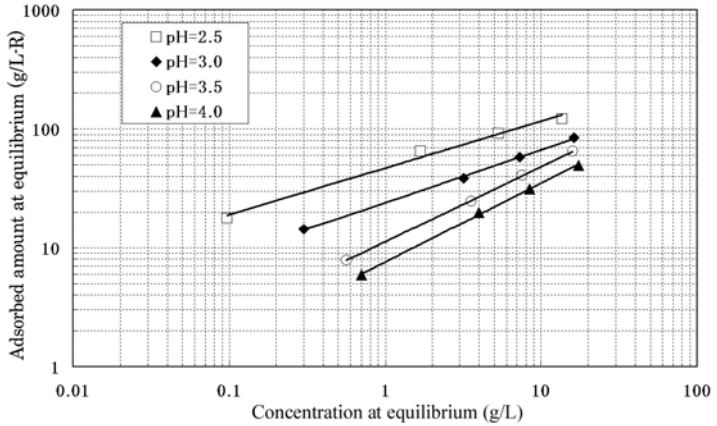
Other basic antibiotics than streptomycin, e.g. josamycin and isepamycin, can be purified in similar ways: pH adjustment of the filtrate of fermented mother liquor to weakly acidic or neutral, adsorption with IERs and elution with diluted mineral or organic acid solutions or aqueous ammonia.

Acidic antibiotics, e.g. cephamycin C [118], on the other side, can be purified by adsorption with WBAERs of free basic- or hydrochloride-form and elution with alkali solutions or weakly alkaline salt solutions. Porous WBAERs can be sometimes applied for decoloring in the purification process of antibiotics.

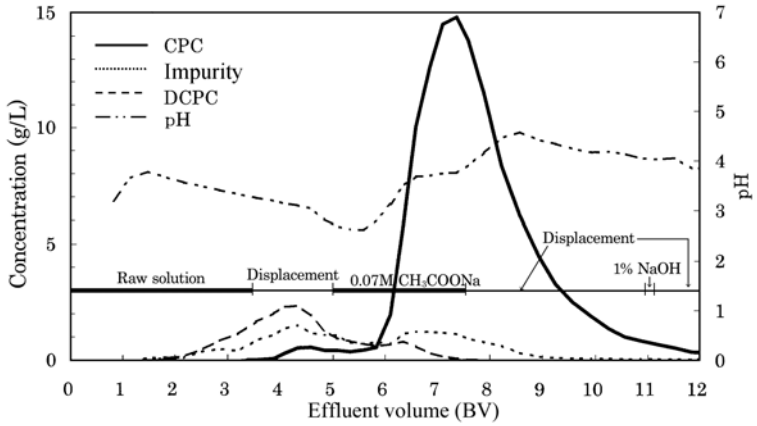
(4) Purification with Synthetic Adsorbents

Synthetic adsorbents are mostly used in the purification process of antibiotics. They are applied not only for the antibiotics with no electrolytic groups but also for those with carboxylic acid groups since the dissociative groups rarely dissociate in weakly acidic conditions where such antibiotics are stable. Synthetic adsorbents can adsorb hydrophobically such un-dissociated antibiotics in weakly acidic conditions particularly. Hydrophilic organic solvent, e.g. lower alcohols and acetone, its aqueous solution or alkaline solution is used as eluents.

Figures IX-1-3 and IX-1-4 illustrate the adsorption isotherm of cephalosporin C to SP825 and its purification with SP825L, and they show that cephalosporin C is adsorbed much at low pH.



[Fig.IX-1-3] Adsorption isotherm of cephalosporin C to SP825 at 5°C

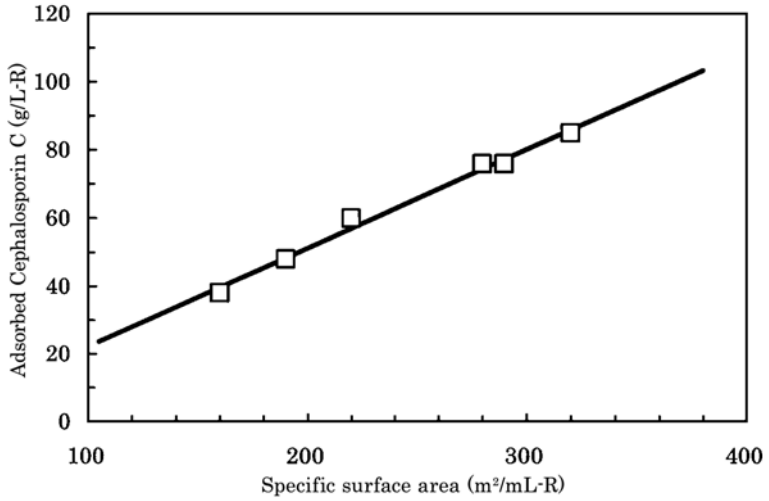


[Fig.IX-1-4] Purification of cephalosporin C with SP825L

Raw liquor:		Conditions:	
CPC	9.968g/L	CPC Feed	3.51L/L-R
pH	2.8	Displacement	1.5L/L-R
Load	35g/L-R (3.51BV)	Elution-1	0.07M CH_3COONa 2.5L/L-R
Flow rate	SV=1.0	Displacement	Demineralized water 3.5L/L-R
Temp.	5°C	Elution-2	1% NaOH 0.1L/L-R
		Displacement	Demineralized water 0.9L/L-R
Results:	CPC Purity	90%	
	Recovery	93%	

CPC: cephalosporin C DCPC: Deacetyl cephalosporin C

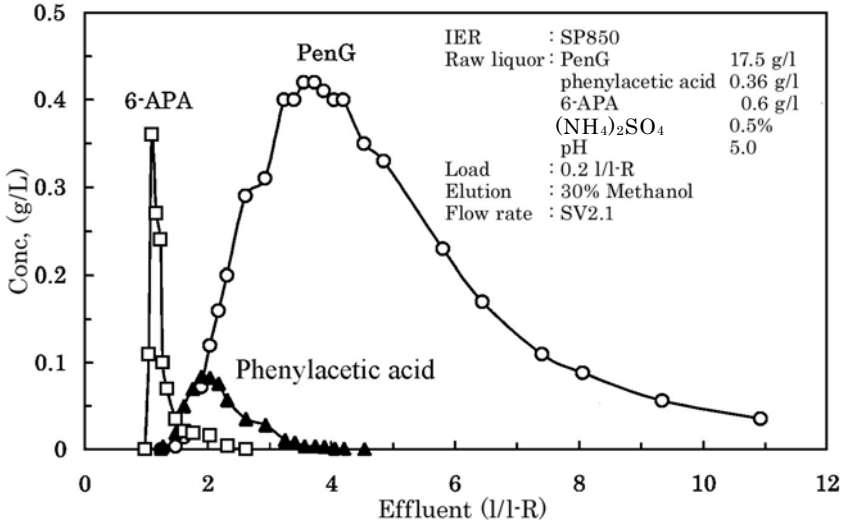
Synthetic adsorbents should be selected depending on the molecular sizes of the objective compounds, since the properties such as pore distributions, pore volumes and specific surface areas differ from grades of synthetic adsorbents. The ones with pores of over 60Å-diameter and with large specific surface area are suitable for cephalosporin C, for example. DIAION™ HP series resins show the positive correlation of their specific surface areas with the adsorbed quantity of cephalosporin C, as demonstrated in Fig.IX-1-5.



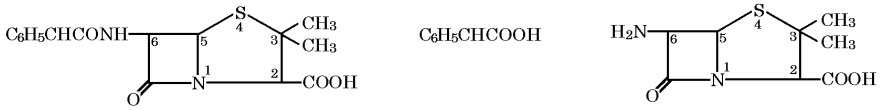
[Fig.IX-1-5] Adsorbed Cephalosporin C vs. Specific surface areas of Synthetic adsorbents

Since penicillin G is produced in 90 ~98% purity when phenylacetic acid is used as a precursor, it is not necessary to be separated from other relevant penicillins. However, it should be separated from 6-aminopenicillanic acid, 6-APA, generated by the cleavage of 6-position and unused phenylacetic acid. Fig.IX-1-6[119] illustrates such separation with Sepabeads SP850 and it shows us that 6-APA is separated almost perfectly and phenylacetic acid remains only in the first fraction of penicillin G. Applying of simulated moving bed chromatography may be suggested.

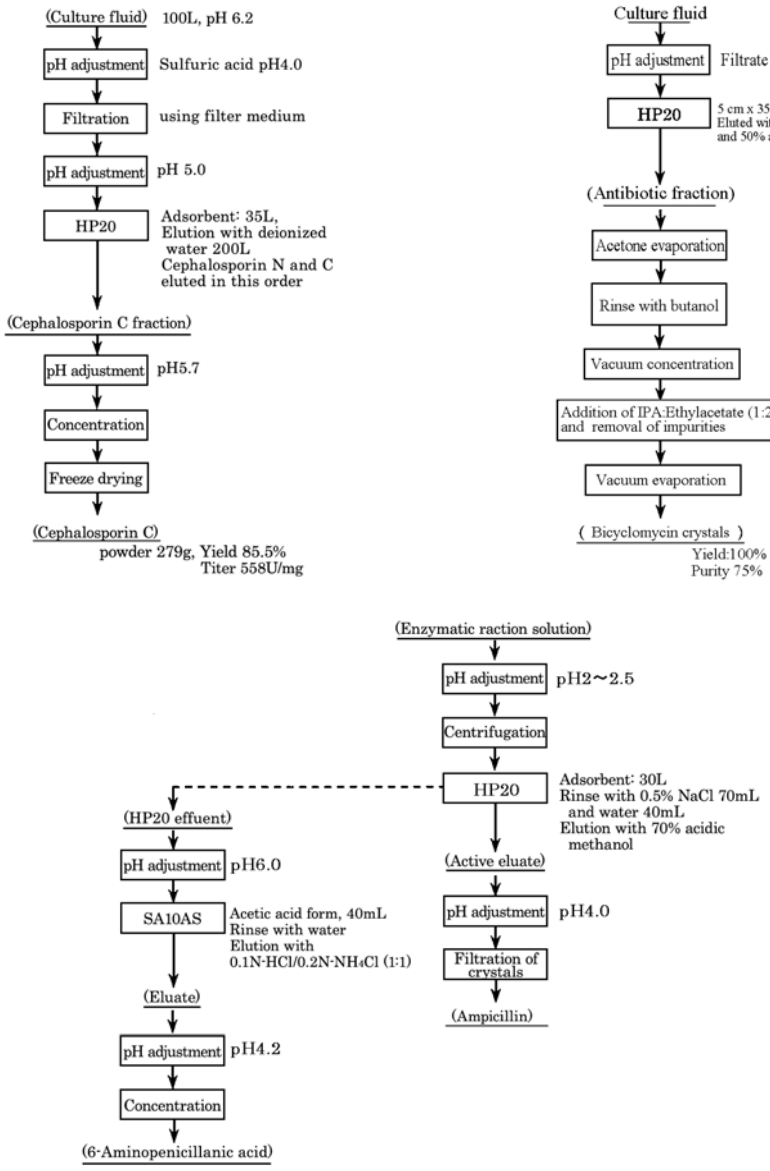
The relevant patents regarding application of synthetic adsorbents for antibiotics purification are listed in Fig.IX-1-7 and Table IX-1-2.



[Fig.IX-1-6] Purification of Penicillin G with SP850 [119]



Penicillin G phenylacetic acid 6-APA



[Fig.IX-1-7] Patented example to apply Synthetic adsorbents in antibiotics purification [120][121][122]

[Table IX-1-2] Patented example to apply Synthetic adsorbents in antibiotics purification

Antibiotics	Resin	Summary	Patent Application No.
Macrolides	HP21 SK104S	1) Filtration of fermented liquor 2) Adsorption by HP21, 3) Rinse with water 4) Elution with 25% acetone 5) Effluent treated with SK104S, 6) Rinse with water 7) Elution with 0.4% $NaHSO_3$	JP Appl. 1992-235996
Reveromycin B,C,D: polyketides	HP20	1) Filtration of fermented liquor 2) Adsorption by HP20 3) Elution with 30% MeOH to remove impurities 4) Elution with 100% MeOH	JP Appl. 1993-194525
Desalaninebenanomicin A derivative: antifungal agent	HP20	1) Dissolve pyridine salt with water 2) Adsorption by HP20 3) Elution with acetonitrile	JP Appl. 1993-202087
7-(5-carboxy-5-hydroxypentanamide)-3-cetoxymethyl-3-cephem-4-carboxylic acid: Cephems	HP20SS	1) Filtration of reaction mother liquor 2) Adsorption by HP20SS 3) Elution with water	JP Appl. 1995-82274
Mitomycin: antitumor	HP20	1) Filtration of fermented liquor 2) Adsorption by HP20, 3) Rinse with 20% methanol 4) Elution with 80% methanol	JP Appl. 1996-245626
Cepacidine: antifungal	HP20	1) Extraction with IPA, 2) Precipitation/ Filtration 3) Dissolve with 50% IPA, 4) Dilution to 1/10 conc. 5) Adsorption with HP20 ,6) Elution with 50% IPA	JP Appl. 1998-5000098
Tubelactomicin: antifungal	HP20	1) Filtration of fermented liquor 2) pH adjustment to 2, 3) Adsorption by HP20 4) Elution with 50% aq. acetone	JP Appl. 1999-217382
Nothramicin: antifungal, antitumor	HP20	1) Filtration of fermented liquor 2) Adsorption by HP20 3) Rinse with water and 30% methanol 4) Elution with 50% aq. acetone	JP Appl. 1998-251289
Lankacidin derivative	HP20	1) Preparation, 2) Adsorption by HP20 3) Rinse with water and 20% methanol 4) Elution with 50% methanol	JP Appl. 1994-321950
Daunomycin: anthracyclines	HP20	1) Dissolve crude powder with acidic water (pH2.5, HCl), 2) Adsorption by HP20, 3) Rinse with acidic water, 4) Elution with methanol in acidic water (pH2.5, 70:30)	WO99/11650
Antitumor compound, PF1201	HP20 SP20SS	1) Filtration of fermented liquor 2) Adsorption by HP20 3) Rinse with water and with 25% methanol 4) Elution with acetone: 0.2N HCl (1:1) 5) Cromatographic separation with SP20SS with eluent of 0.1% Trifluoroacetic acid/ 80% methanol	JP Appl. 2000-72798
Anthracyclines	HP20	1) pH adjustment to 2.0 (phosphoric acid) 2) Sedimentation/ decantation 3) Rinse sediments with acetone of pH 2.9 (phosphoric acid), 4) Treat supernatants with HP20 5) Elution with 90% acetone (pH 2.4)	JP Appl. 2002-53589
Caprazamycin: antifungal	HP20	1) Filtration of fermented liquor 2) Add extracts from bacteria with methanol 3) Adsorption with HP20 4) Elution with deionized water, 50% methanol, 80% methanol, 80% acetone, and acetone 5) objective compound is in 80% methanol fraction	JP Appl. 2003-12687
Captomycin: lipopeptides	FP-DA13 HP20 HP20SS	1) Supernatant of fermented liquor 2) Adsorption by FP-DA13 3) Rinse with 30mM NaCl (pH6.5) 4) Elution with 300mM NaCl (pH6.0-6.5) 5) pH adjustment to 3.5 (phosphoric acid) 6) UF membrane, 7) Purification by hydrophobic	JP 2003-520807

		chromatography with HP20, HP20SS	

2.Purification of other Pharmaceuticals

Other pharmaceuticals can be purified in the same ways as antibiotics. That is to say, basic and acidic ones can be ion-exchanged with CERs and AERs respectively and amphoteric ones can be ion-exchanged with CERs or AERs in accordance with the pH of their mother liquor. Those with no dissociation groups can be adsorbed with synthetic adsorbents. Furthermore, IERs can be sometimes applied to convert the counter ions of pharmaceuticals into other salts.

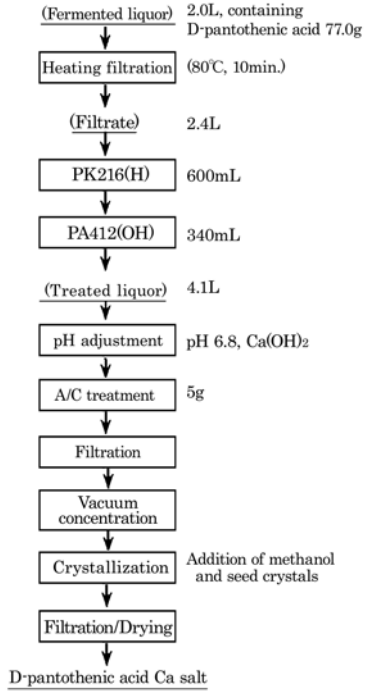
Fig.IX-2-1 illustrates separation of impurities by IER treatment. D-pantothenic acid, a component of Coenzyme A, is important in metabolism of carbohydrates and fatty acids. It is purified, in Fig.IX-2-1, by heating and filtration of the fermented liquor made from glucose, corn steep liquor, ß-alanine and inorganic salts such as ammonium sulfate to remove bacteria and insoluble matters, and then by treatment with H-form PK216 and OH-form PA412 to remove the remaining impurities.(123) PK216 and PA412 are applied in this application to eliminate cations and inorganic anions with organic compounds more acidic than pantothenic acid, respectively.

Fig.IX-2-2 illustrates purification of pharmaceutical ingredients by IER treatment. In this illustration, the activator of angiotensin-converting enzyme inhibitor, that is important as a hypotensive agent or as a preventive or curative medicine, is purified as follows from the extracts of spent barley roots from beer manufacturing: a) treatment with activated carbons, b) filtration with kieselgur, c) UF treatment, d) Adsorption by PA318, e) elution with 0.5N-HCl, f) Adsorption by Dowex 50W x2 (SACER of 2% cross-linkage) and g) elution with 0.5% aqueous ammonia (124). Since the active ingredient is amphoteric, it can be treated both with CERs and AERs. Activated carbons and synthetic adsorbents can be used to remove impurities because the active ingredient is difficult to be absorbed by them due to its high polarity. UF membranes are applied to remove proteins.

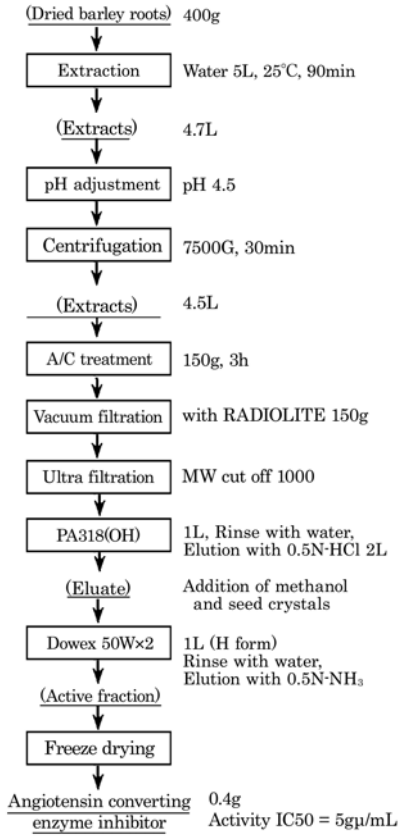
As an example to convert the counter ions of pharmaceuticals, Fig.IX-2-3 illustrates the formation of ascorbic acid from sodium ascorbate, sodium salt of Vitamin C, with CERs.

Synthetic adsorbents are widely used in pharmaceutical purification. Figures IX-2-4 and IX-2-5 illustrate examples of a pharmaceutical ingredient and a synthetic pharmaceutical, respectively. The former Fig.IX-2-4 demonstrates the manufacturing process of triterpenoidesaponin, a therapeutic medicine for dysfunction of cerebral memory, e.g. amnesia caused by aging, stress and alcohol drinking or Alzheimer's-like dementia: The extract from dried puerariae flos by 20% ethanol is adsorbed by HP20 already filled with alcohol and water, rinsed with water and eluted with ethanol (126). The latter Fig.IX-2-5 also illustrates the manufacturing process of an aziridinecarboxylic acid derivative, preventive and therapeutic medicine for bone diseases, e.g. osteoporosis, malignant hypercalcemia and Paget disease.

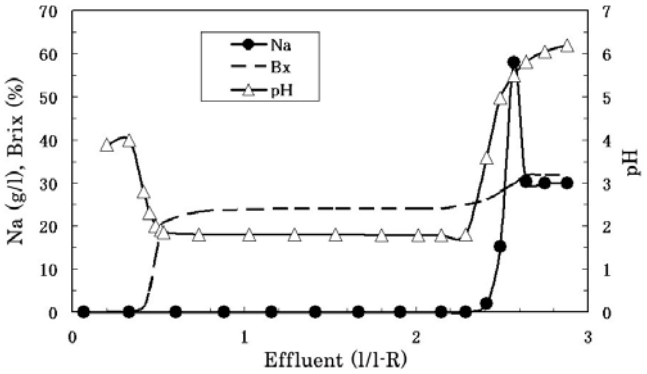
Synthetic adsorbent HP20 is applied in the process to produce Compound-2 from Compound-1, the reaction product of N-(Boc-(2S)-phenylalanyl)-1-amino-3-methoxypropane made from 3-methoxypropylamine and Boc-L-phenylallanine with (2R,3R)-ethylhydrogen-aziridine-2,3-dicarboxylate, diphenylphosphorylazide and triethylamine (127).



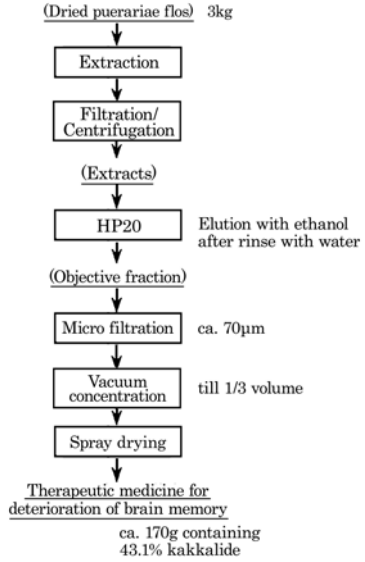
[Fig.IX-2-1]
Purification of D-pantothenic acid (123)



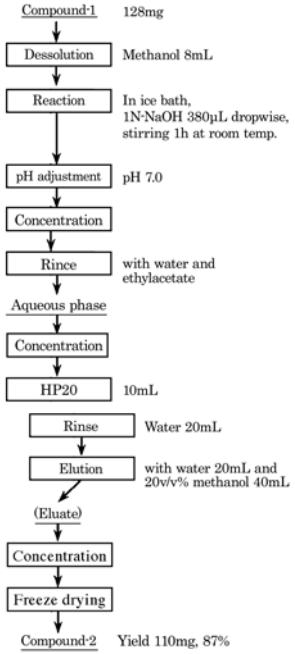
[Fig.IX-2-2]
Purification of activator of angiotensin converting enzyme inhibitor (124)



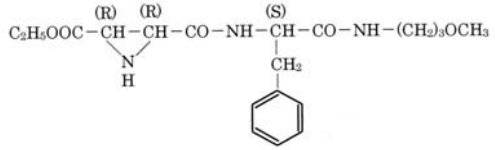
[Fig.IX-2-3] Formation of Ascorbic acid from Sodium ascorbate(125)
Resin: SK116, 750mL
Raw liquor: Sodium ascorbate 25%, Na 30.4g/L, Ca 750mg/L, Brix 31.7%



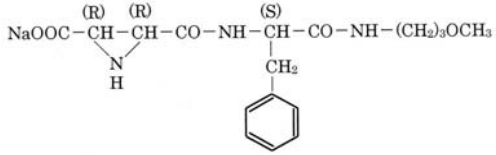
[Fig.IX-2-4]
Manufacturing process of Triterpenoidesaponin (126)



[Fig.IX-2-5]
Manufacturing process of Aziridinecarboxylic acid derivative(127)



(Compound-1):
N-(N-((2R,3R)-3-ehtoxycarbonylaziridine-2-carbonyl)-(2S)-phenylalanyl)-1-amino-3-methoxypropane



(Compound-2):
N-(N-((2R,3R)-3-ehtoxycarbonylaziridine-2-carbonyl)-(2S)-phenylalanyl)-1-amino-3-methoxypropane monosodium salt

References:

(109) Japan Science and Technology Agency, Purification method of kasugamycin, JPB1968008003

(110) Mercian Corp., Manufacturing method of ozemycin, JPB 1976033194

(111) Kyowahakko Kogyo Co., Ltd., Purification method of Hortimycin A, JPB 1984007318 (JPA 1978006487)

(112) Riken, Japan and Ajinomoto Co.,Inc., Pesticides for agriculture and gardening, JPB 1977038092

(113) Meiji Seika Kaisha, Ltd., Purification method of kanamycin salts, JPB 1961007047

(114) Kagaku-Kenkyusho, Purification method of streptomycin with cation exchange resin in carboxylic form, JPB 1954005248

(115) Takeda Pharmaceutical Co., Ltd., Purification method of streptomycin mineral acid salts, JPB1967026626

(116) Astellas Pharma Inc., Collecting method of a new antibiotic (Josamycin S), JPB 1970005032

(117) "Balkanpharma-Razgrad" Actzionerno Drugestvo, Manufacturing method of isebamycin, JPA 2004043481

(118) Merck & Co., Ltd., Collecting method of antibiotics, JPB 1983030040 (JPA 1976106790)

(119) Nippon Rensui Co.Ltd., In-house Technical Sheets

(120) Meiji Seika Kaisha, Ltd., Collecting and purification methods of cephalosporin C, JPB 1979017833

(121) Astellas Pharma Inc., Purification method of bicyclomycin, JPB 1982009799 (JPA 1975 101586)

(122) Banyu Pharmaceutical Co., Ltd., Manufacturing method of antibiotics by fermentation, JPB 1983008838 (JPA 1975117990)

(123) Takeda Pharmaceutical Co., Ltd., D-pantoic acid, D-pantothenic acid and their manufacturing methods, JPA 1994261772

(124) Kikkoman Corp., Angiotensin converting enzyme inhibitors and their manufacturing method, JPA 2004051519

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(126) Ohta's Isan Co., Ltd., Remedy for hypofunction of brain memories and its preparation method, JPA 2006206556

(127) Takeda Pharmaceutical Co., Ltd., Aziridinecarboxylic acid derivatives and their manufacturing method and uses, JPA 1997221470