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Separation of Bioactive Natural Products using Synthetic Adsorbents

Separation Materials Laboratory, Mitsubishi Chemical Corporation

Separation Materials Division, Mitsubishi Chemical America

MITSUBISHI CHEMICAL CORPORATION

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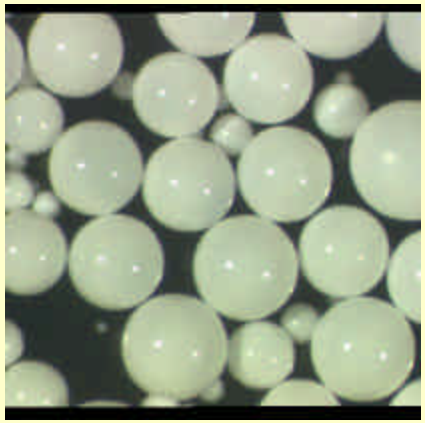

DIAION ® HP20

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Introduction of Mitsubishi Chemical Corporation

- Established on October 1st ,1994
  By the merger of
  - Mitsubishi Kasei (Established in 1930) and
  - Mitsubishi Petrochemical (Established in 1956)
- Capital; 1,369 million US$ (March 2000)
- Sales; 15,754 million US$ (Consolidated, 2000 F. Year)
- Employees; 33,465 (Consolidated, March 2000)
- R&D Expense; 628 million US$ (March 2000)

- The Largest Chemical Company in Japan

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Introduction of Separation Materials Division

- History of DIAION ® Ion Exchange Resins
  - 1935 – Ion exchange phenomenon of polycondensation resin was found in Great Britain
  - 1946 – Production of DIAION ® A, K (polycondensation resins)
  - 1950 – Development of Polystyrenic ion exchange resins started
  - 1955 – Production of DIAION ® polystyrenic cation exchange resins
  - 1956 – Production of DIAION ® polystyrenic anion exchange resins
- Offers Products of a Wide Variety over 400
  - ❖ **Leading Company of Synthetic Adsorbents in the World**
  - DIAION ® ion exchange resins and synthetic adsorbents
  - SEPABEADS ® synthetic adsorbents and ion exchange resins for protein
  - MCI ® GEL HPLC packing materials
- 50% Share in Japanese Market

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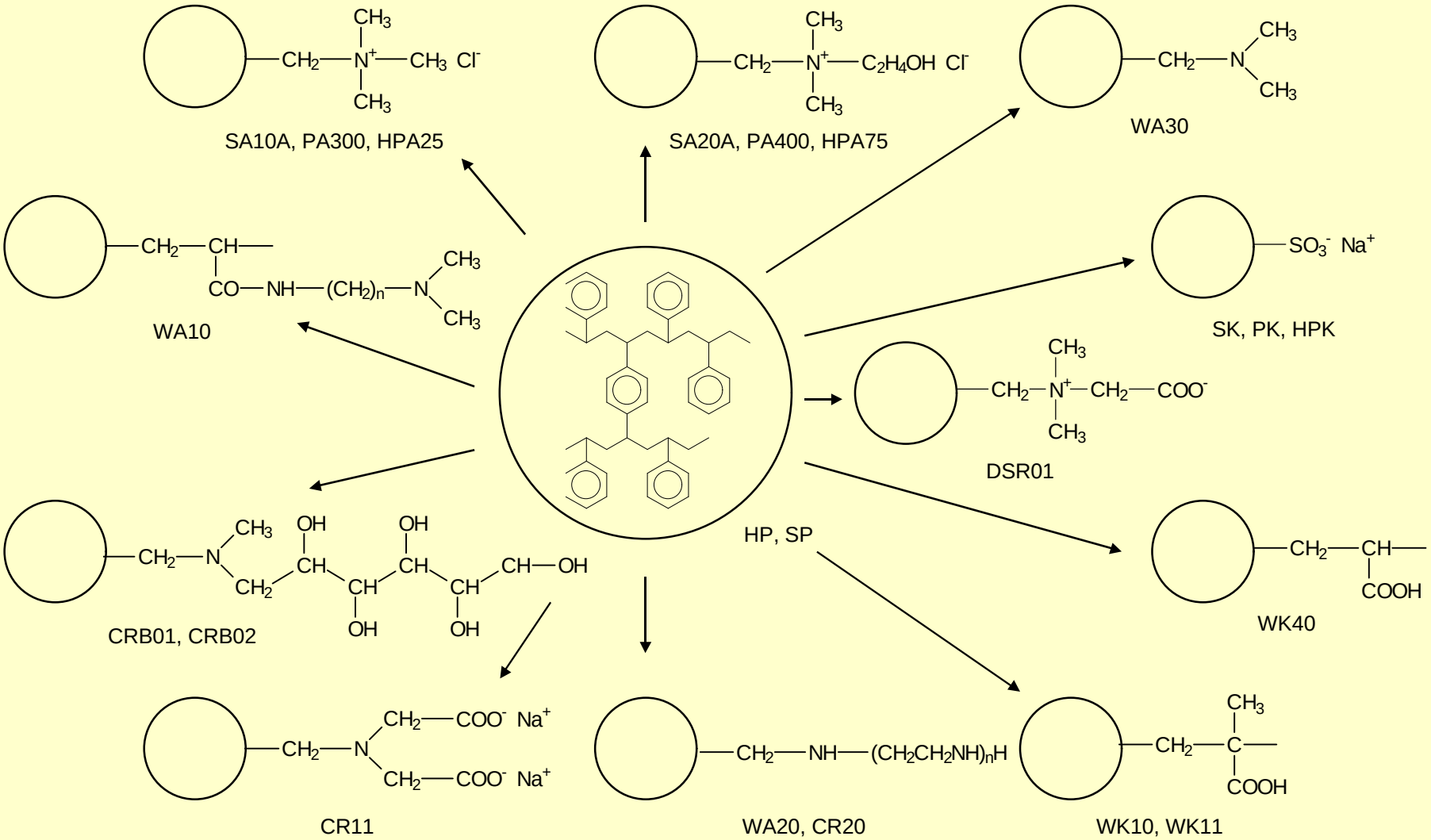
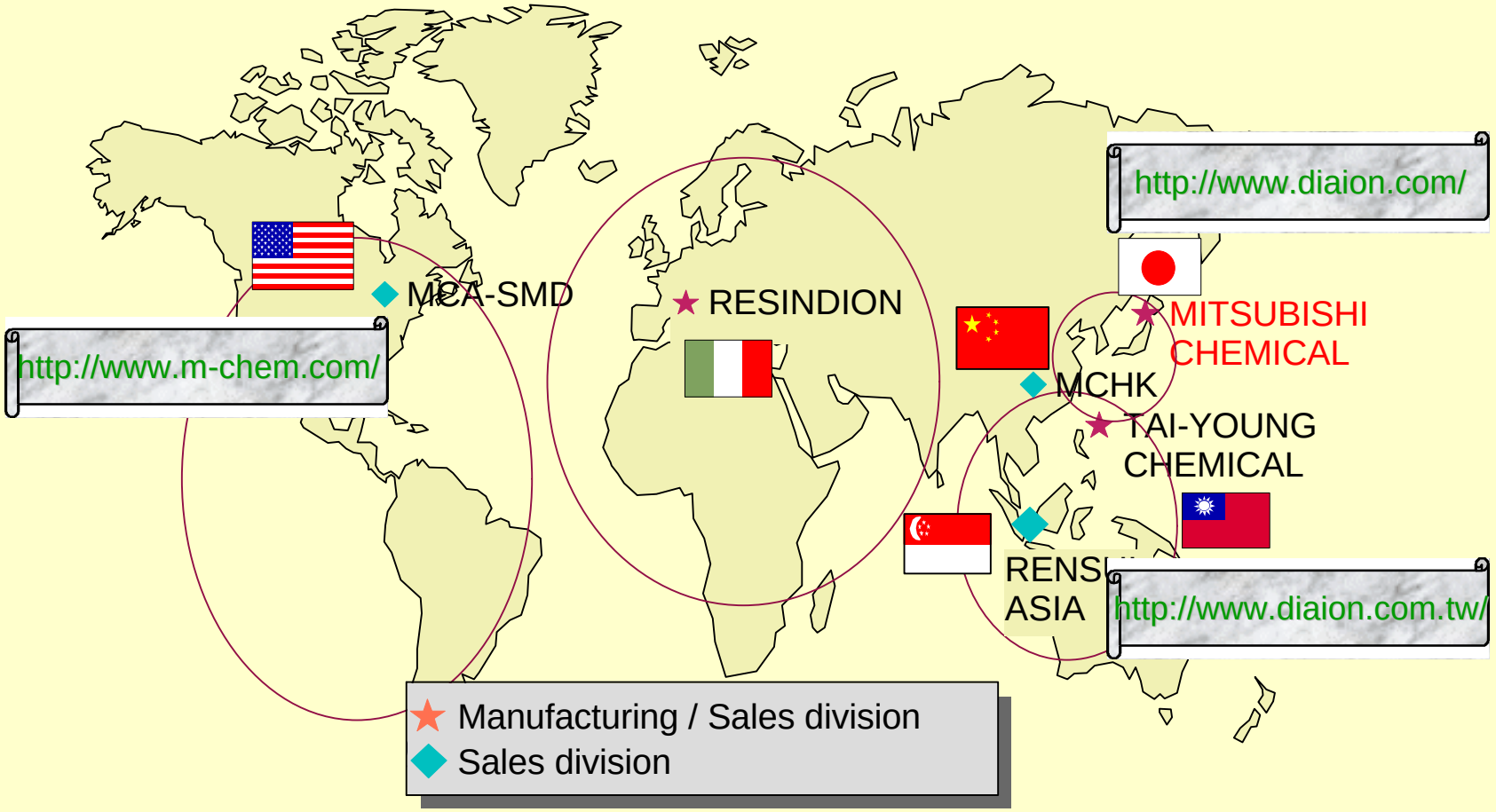
Synthetic Adsorbents and Ion Exchange Resins



Worldwide Network of DIAION ® and SEPABEADS ® Business



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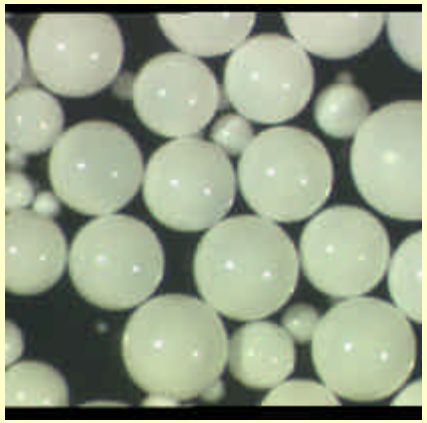
What are Synthetic Adsorbents?

- Spherical crosslinked polymer particle with porous structure
- No ion-exchange groups or functional groups
- Adsorb organic compounds on the surface due to hydrophobic interaction
- Highly porous structure
  - Pore diameter :
    several tens – several hundreds angstrom
  - High adsorption capacity
  - Selective adsorption utilizing size exclusion effect



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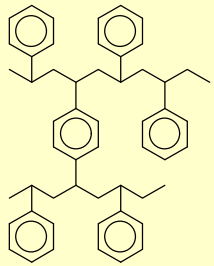
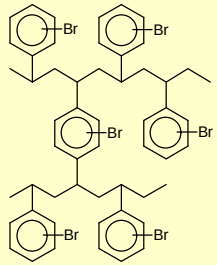
Chemical Structure of Synthetic Adsorbents

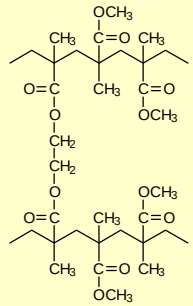
- Polystyrenic Adsorbents – Standard type
  - DIAION ® HP20, HP20SS, HP21
  - SEPABEADS ® SP70, SP700, SP825, SP850
  - MCI ® GEL CHP20A, Y, P, CHP55A, Y



- Chemically Modified Polystyrenic Adsorbent
  - SEPABEADS ® SP207
    Higher adsorption capacity
    High gravity (1.2)
    – Applicable to Expanded Bed Adsorption or Countercurrent operation



- Polymethacrylic Adsorbent
  - DIAION ® HP2MG
  - MCI ® GEL CHP2MG, CHP2MGY
    More polar than polystyrenic resins
    Applicable to normal-phase adsorption



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What Kind of Compounds can be Separated?

- Compounds with Moderate Solubility in Water
  - Highly Soluble Compounds: Ion Exchange Resin, Gel Filtration
  - Insoluble Compounds: Solvent Extraction, Normal Phase Adsorption
- Compounds with Aromatic Groups
  - Suitable for Polystyrenic Adsorbents
  - Benzene Rings
  - Conjugated Double Bonds
- Compounds with Hydrogen Bonding Effects
  - Suitable for Polymethacrylic Adsorbents
  - Carboxylic Groups, Ester Groups
  - Amino Groups, Amide Groups

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What Kind of Compounds can be Separated?

- Products from Plants
  - Herbal Drugs
  - Natural Pigments
- Antibiotics from Fermentation Broth
  - Penicillin, Cephalosporin C
  - Spiramycin
- Peptides and Proteins
  - Insulin
  - Peptide Antibiotics
- Functional Food Additives
  - Vitamins

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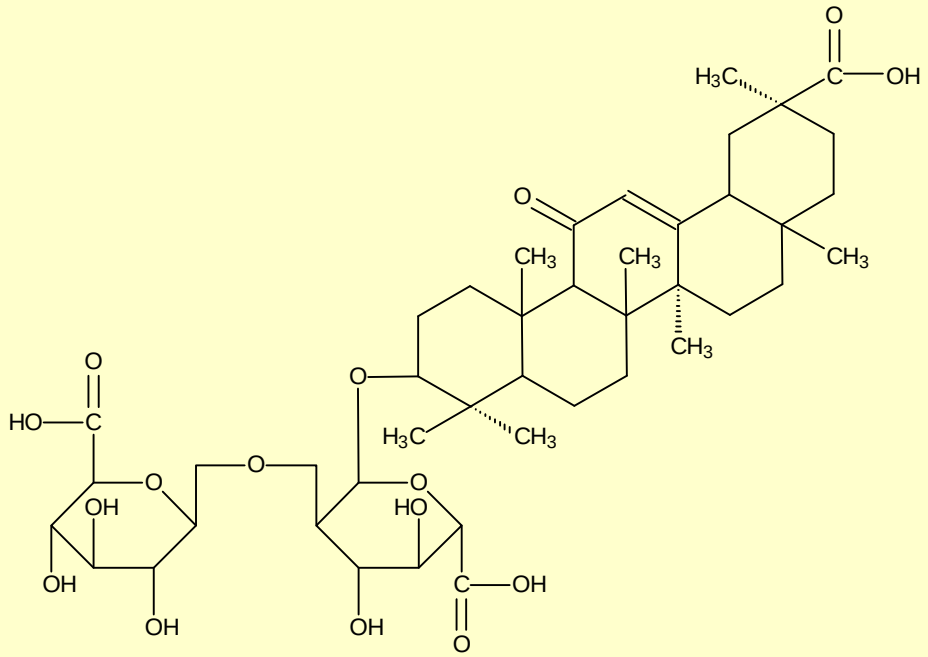
Separation of a Herbal Drug: Glycyrrhizic Acid

- Structure of Glycyrrhizic Acid (Mw: 823)



Analytical separation of glycyrrhizic acid on polystyrenic and polymethacrylic adsorbents

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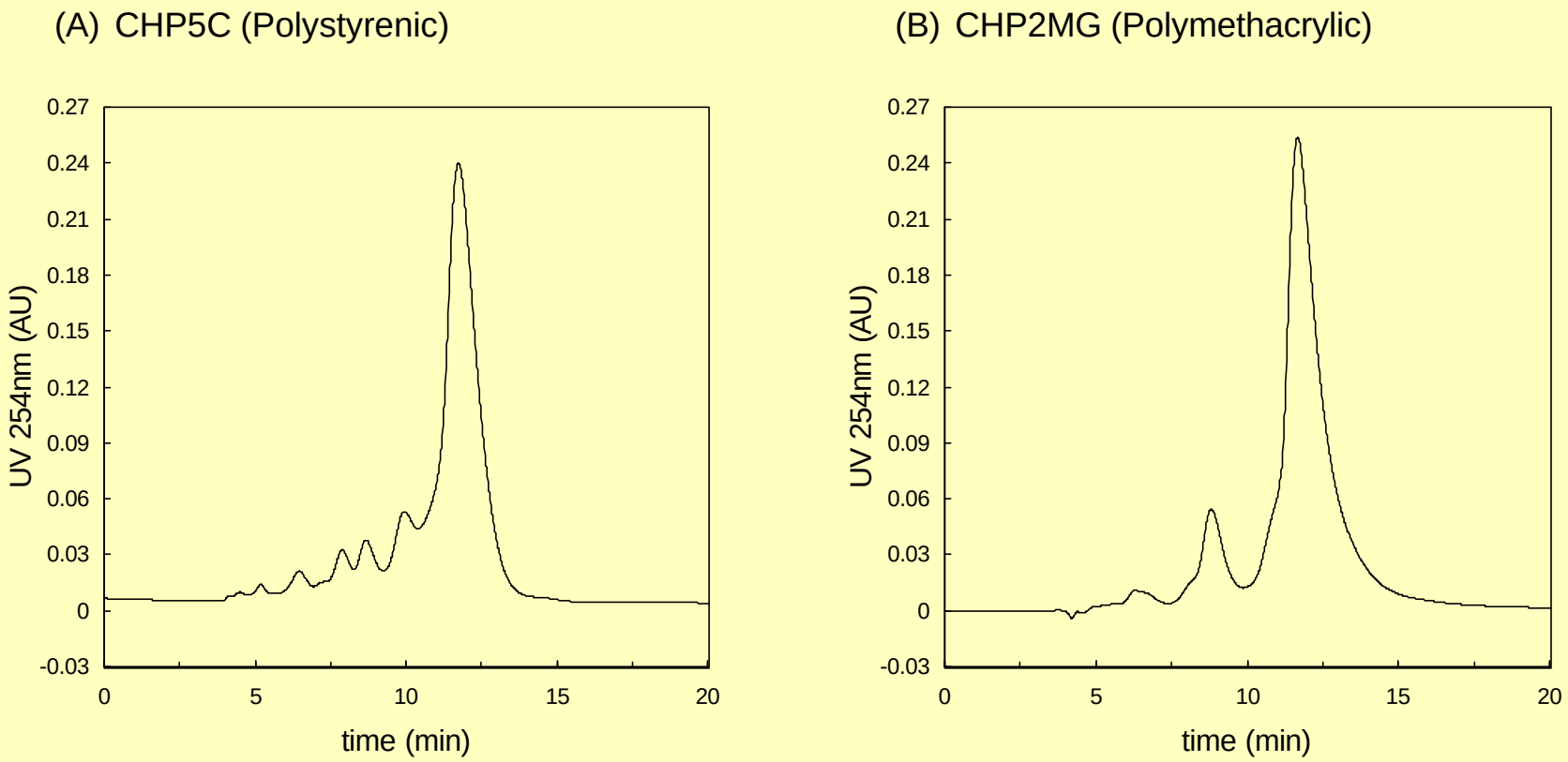

Figure. Separation of glycyrrhizic acid on polystyrenic and polymethacrylic adsorbents.
Conditions:

(A) Adsorbent, CHP5C (10µm); Column size, 150mm x 4.6mm I.D.;
Eluent, MeOH/20mM Citrate (pH4.0)=65/35; Flow rate, 0.5ml/min.

(B) Adsorbent, CHP2MG (10µm); Column size, 150mm x 4.6mm I.D.;
Eluent, MeOH/20mM Citrate (pH4.0)=55/45; Flow rate, 0.5ml/min.

Sample: Glycyrrhizic Acid (Sigma G-2137, 2mg/ml). Injection: 10µl.

Analytical Separation of Taxol on a Polystyrenic Adsorbent

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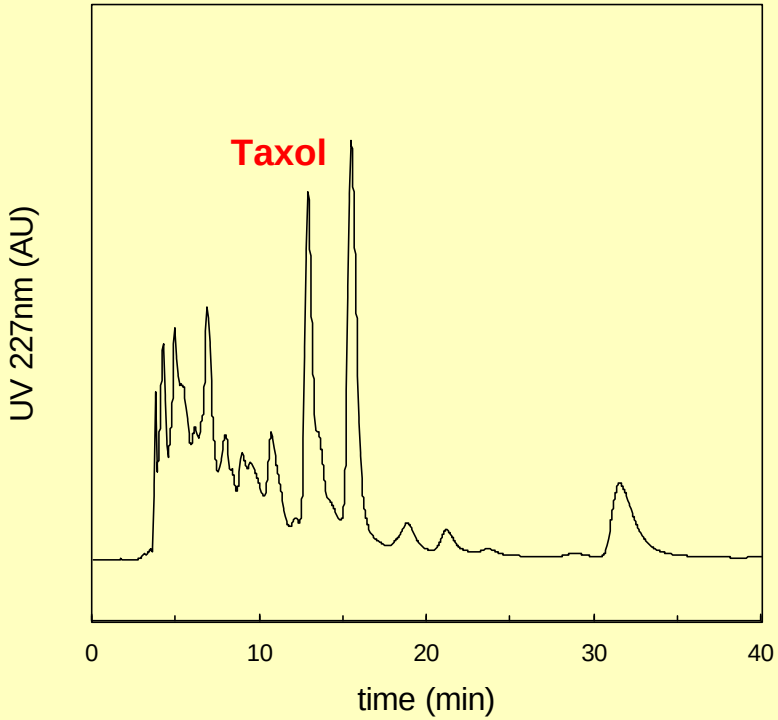

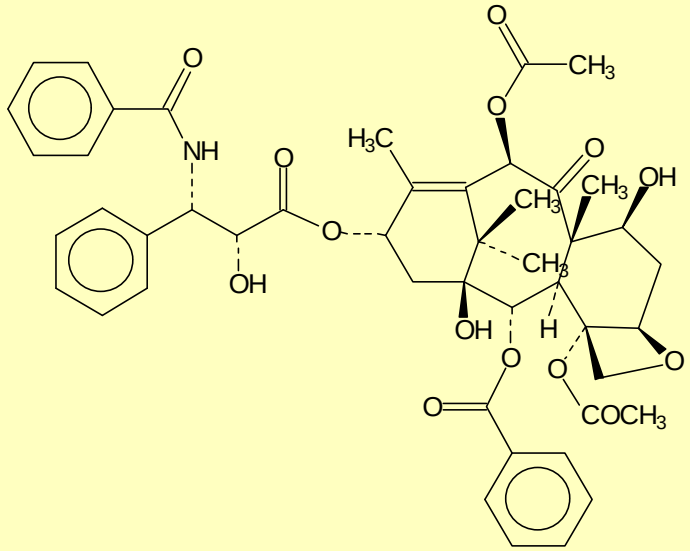

Figure. Separation of taxol on a polystyrenic adsorbent.
Conditions:
Adsorbent, CHP10M (4μm); Column size, 150mm x 4.6mm I.D.;
Eluent, AcCN/H2O=60/40; Flow rate, 0.5ml/min.

Separation of curcumins on polystyrenic and polymethacrylic adsorbents

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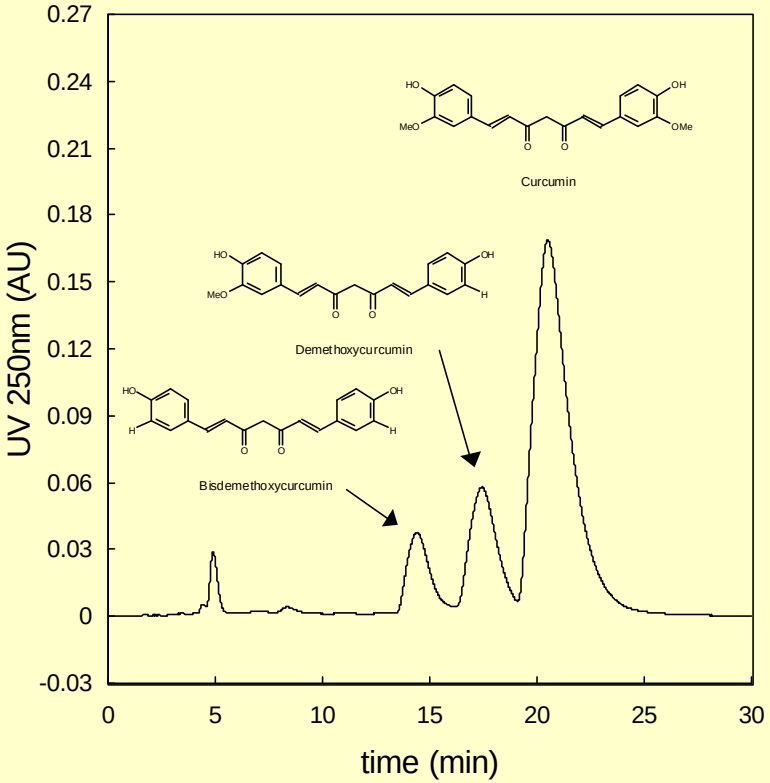
(A) CHP5C (Polystyrenic)



(B) CHP2MG (Polymethacrylic)

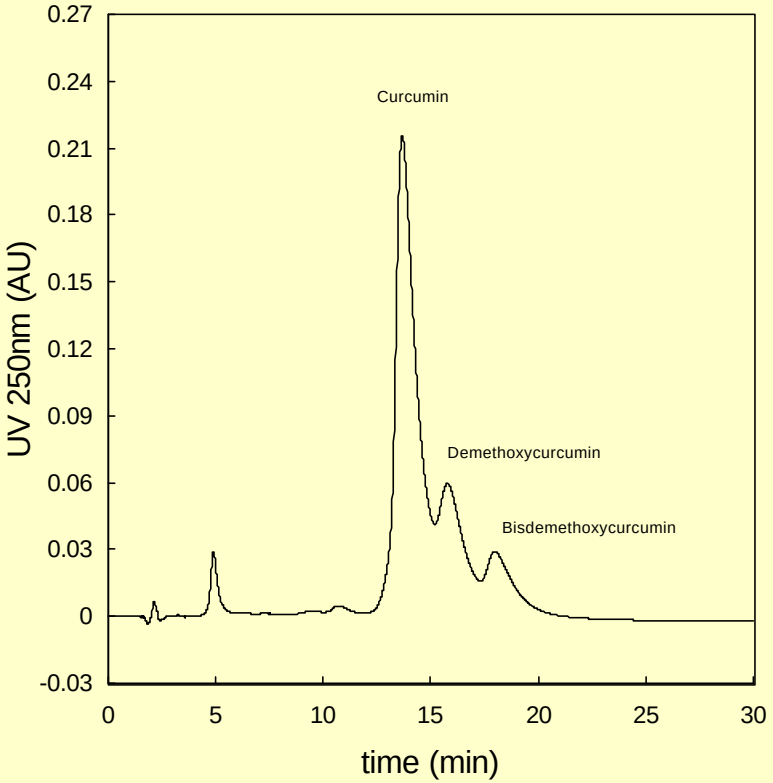


Figure. Separation of curcumins on polystyrenic and polymethacrylic adsorbents.
Conditions:

(A) Adsorbent, CHP5C (10μm); Column size, 150mm x 4.6mm I.D.;
(B) Adsorbent, CHP2MG (10μm); Column size, 150mm x 4.6mm I.D.;
Eluent, AcCN/0.04M phosphoric acid=50/50; Flow rate, 0.46ml/min.

Separation of Penicillin Antibiotics – Effect of Resin Size on Separation

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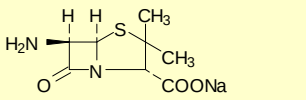
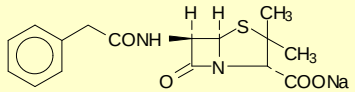
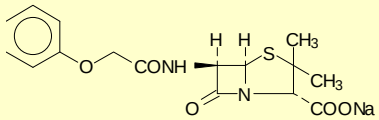


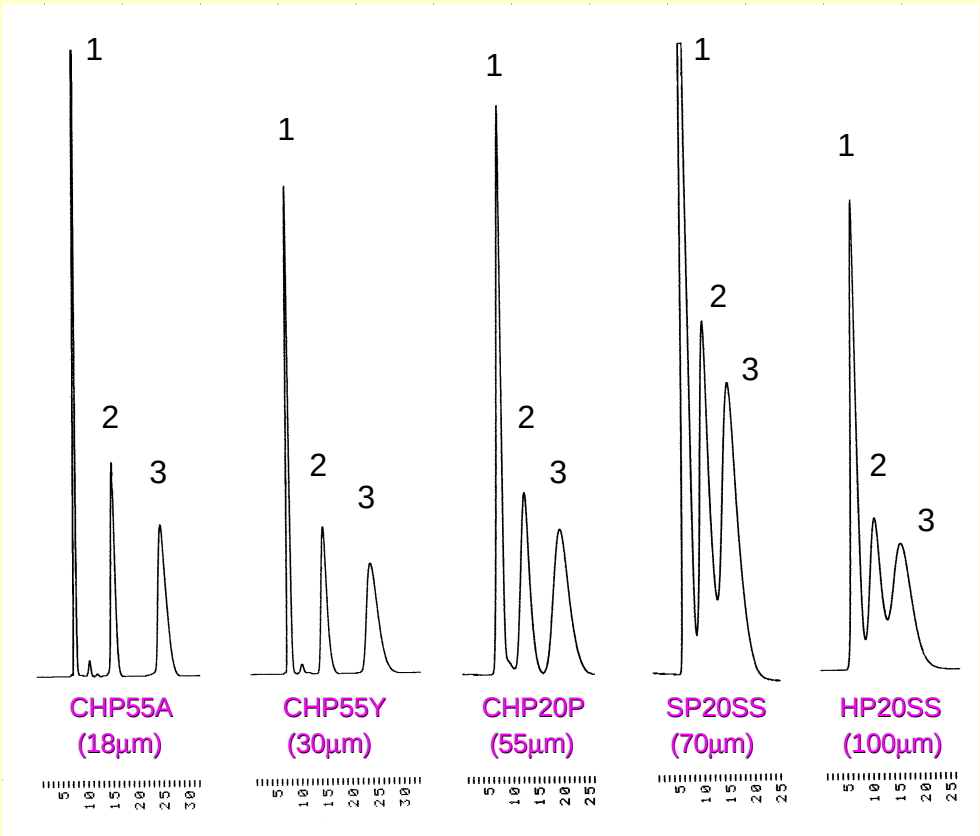
6-Aminopenicillanic acid



Penicillin G



Penicillin V



Column size, 250 x 10mm I.D.; Eluent, MeOH/50mM phosphate (pH8.0) = 60/40;
Flow rate, 2.18ml/min; Detection, UV 254nm.
Samples: 1= 6-aminopenicillanic acid (1g/L), 2= penicillin G (1g/L), 3= penicillin V (1g/L).
Injection: 100μl

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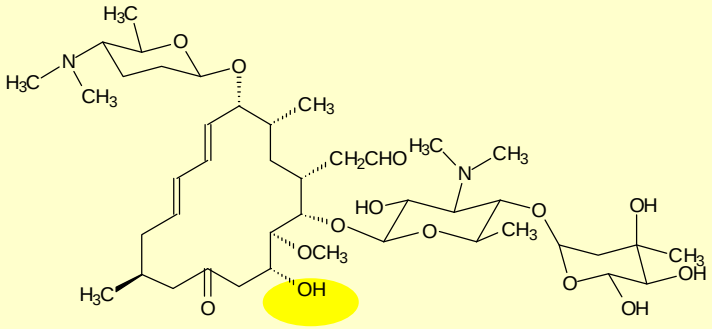
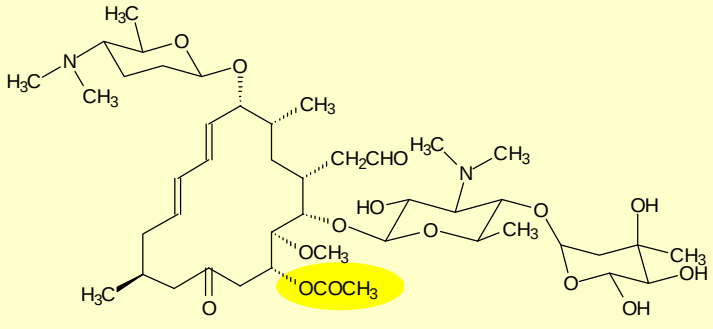
Separation of a Macrolide Antibiotic: Spiramycin

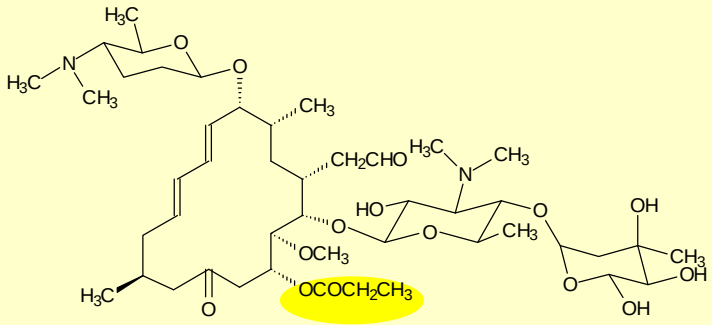
- Structure of Spiramycins



Spiramycin I



Spiramycin II



Spiramycin III

Analytical Separation of Spiramycins on a Polystyrenic Adsorbent

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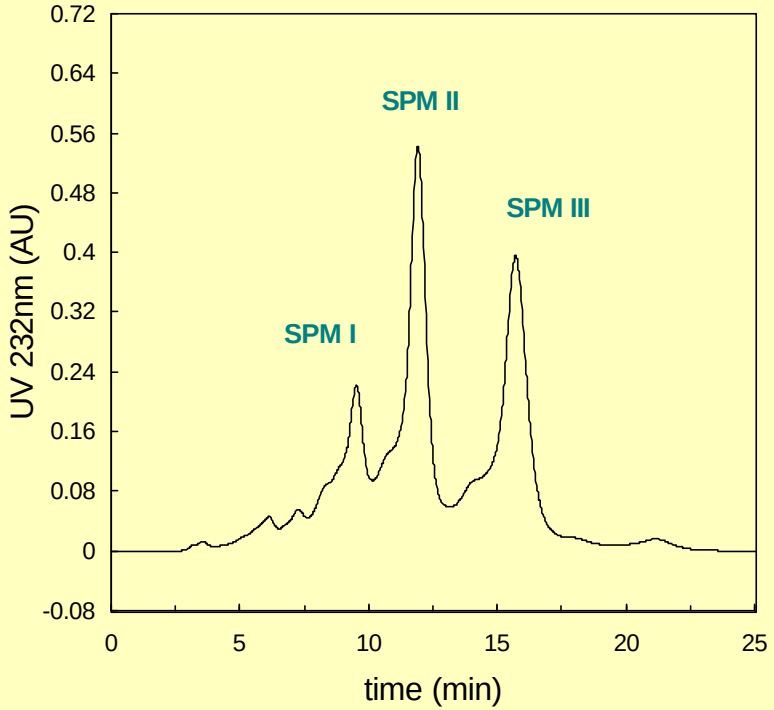

Figure. Separation of spiramycins on a polystyrenic adsorbent.
Conditions:
Adsorbent, CHP5C (10μm); Column size, 150mm x 4.6mm I.D.;
Eluent, AcCN/10mM Phosphate (pH9.2)=60/40; Flow rate, 0.5ml/min.
Sample: Spiramycin (1mg/ml). Injection: 30μl.

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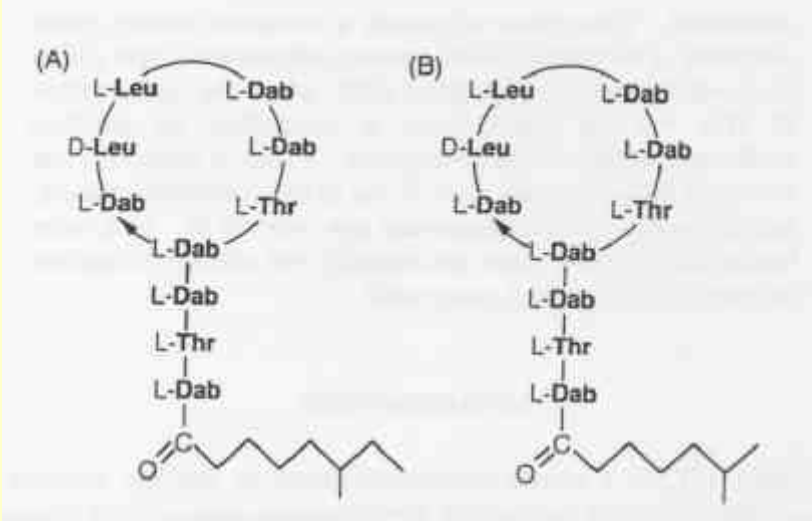
Separation of Peptide Antibiotics: Colistins

- Structure of Colistin A (Mw:1168) and B (Mw:1154)

  (Dab: α, γ – diaminobutyric acid)



- Separation Mode:
  - Reversed Phase Chromatography
    – Utilizing the difference in hydrophobicity
  - Ion Exchange Chromatography
    – Utilizing the difference in charge distribution

■ *Analytical Separations of Colistins on a Synthetic Adsorbent and an Ion Exchange Resin*

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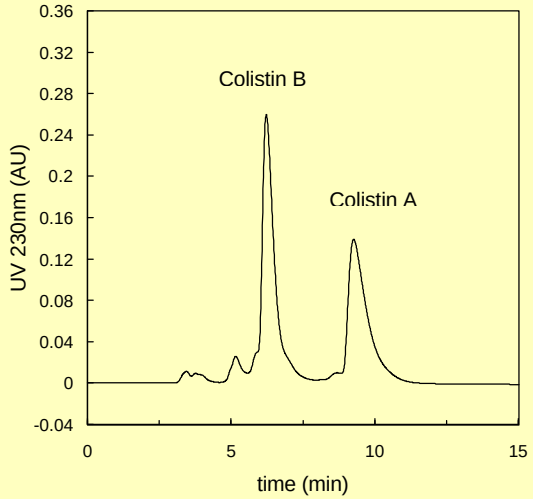
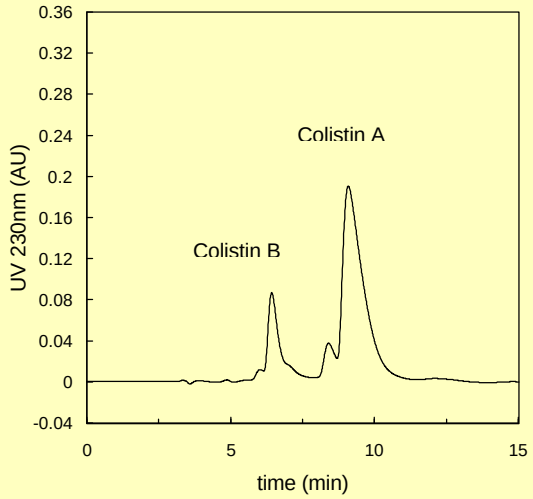
Polystyrenic Adsorbent

(Lot. 1 on CHP5C)

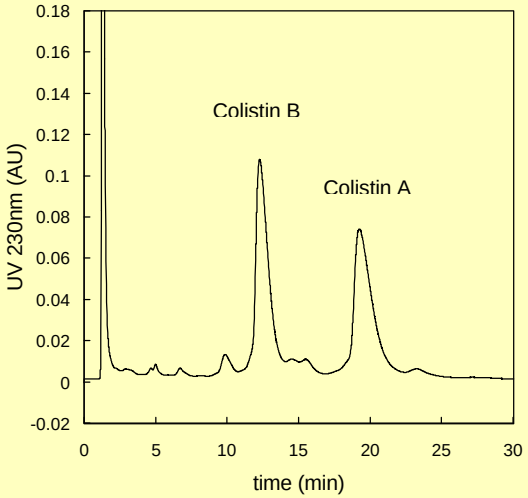


(Lot. 2 on CHP5C)

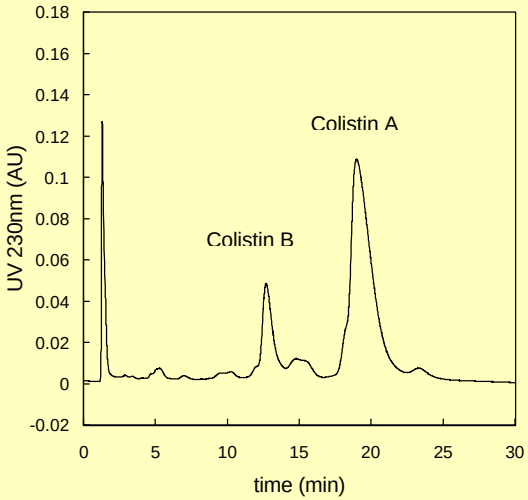


Ion Exchange Resin for Protein Separation

(Lot. 1 on ProtEx-SP)



(Lot. 2 on ProtEx-SP)



Analytical Separation of Vitamins on a Polymethacrylic Adsorbent

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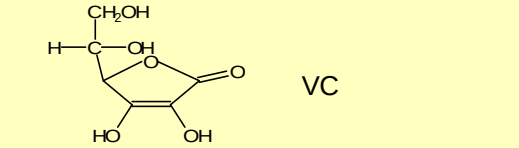
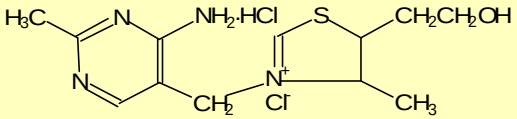
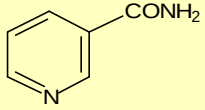


VC

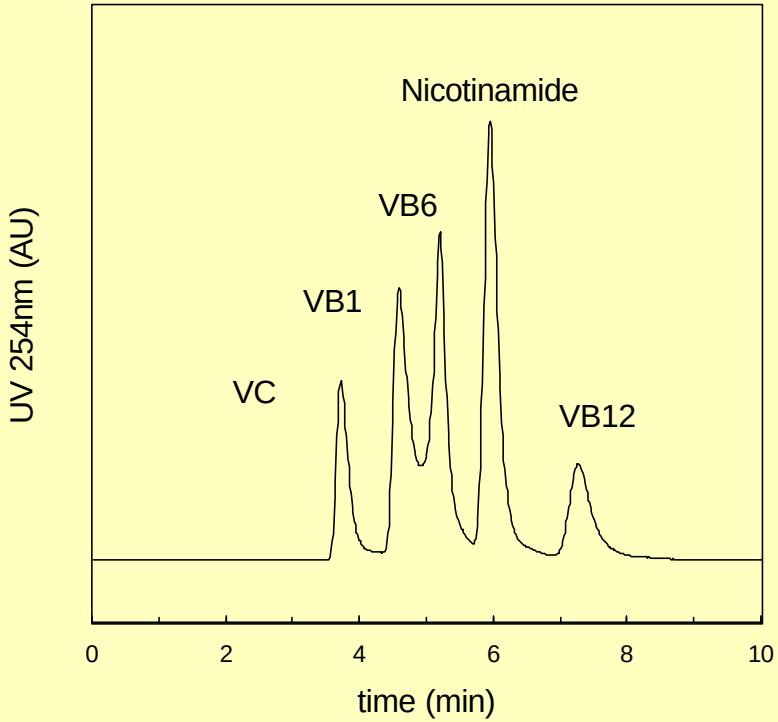


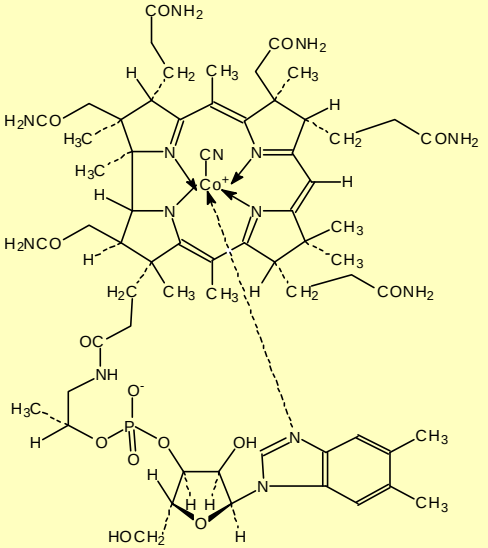
VB1

VB6



Nicotinamide





VB12

Figure. Separation of water-soluble vitamins on a polymethacrylic adsorbent.
Conditions:
Adsorbent, CHP2MGM (4μm); Column size, 150mm x 4.6mm I.D.;
Eluent, AcCN/16mM phosphate (pH7.0)=90/10; Flow rate, 0.5ml/min.
Flow rate, 0.5ml/min; Temperature, 55°C.

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List of Industrial Synthetic Adsorbents

Product Name	SP850	SP825	SP70	SP700	HP21	HP20	SP207	HP2MG
Water content	46-52%	52-62%	55-65%	60-70%	45-55%	55-65%	43-53%	55-65%
Particle size on 250μm	90% min.		-	-	90% min.			90% min. on 300μm
Effective size (mm)	0.25 min.		-	-	0.25 min.			0.35 min.
Uniformity coefficient	1.6 max.		-	-	1.6 max.			1.6 max.
Mean diameter (mm)	-	-	0.45	0.45	-	-	-	-
Specific surface area (m^2/g)	1000	1000	800	1200	570	600	630	470
Pore volume (ml/g)	1.2	1.4	1.6	2.3	1.1	1.3	1.3	1.2
Pore radius (Angstrom)	38	57	70	90	80	260	105	170
Cephalosporin C Adsorption (g/L)	85	76	60	76	48	38	119	<10

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Mitsubishi Chemical's Chromatographic Support Materials

	MCI GEL 3 - 10㎜	MCI GEL 10 - 50㎜	DIAION, SEPABEADS 50 - 250㎜	DIAION > 250㎜
Organic compounds	CHP10M, 5C CHP2MG	CHP55A,Y CHP20A,Y CHP2MGY	HP20SS (HP2MGSS)	HP21 HP20 HP2MG
Polysaccarides, Amino acids	CK series	CK series	UBK series	SK series
Proteins	ProtEx series	PrepEx series	FP-DA, FP-HG	

MCI GEL

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Synthetic Adsorbents for Chromatography

- Polystyrenic synthetic adsorbents

Product name	Particle size distribution	Average size (Ref.)	Surface area (Ref.)	Pore volume (Ref.)	Peak radius (Ref.)
MCI ® GEL CHP10M	4μm	4μm	640m^2/g	1.45ml/g	14.0nm
MCI ® GEL CHP5C	9-11μm	10μm	540m^2/g	1.39ml/g	14.0nm
MCI ® GEL CHP55A	15-20μm	18μm	580m^2/g	1.54ml/g	14.0nm
MCI ® GEL CHP55Y	25-35μm	30μm	590m^2/g	1.55ml/g	14.0nm
MCI ® GEL CHP20Y	25-35μm	30μm	560m^2/g	1.67ml/g	22.0nm
MCI ® GEL CHP20P	37-75μm	55μm	520m^2/g	1.17ml/g	30.0nm
SEPABEADS ® SP20SS	63-75μm	70μm	560m^2/g	1.40ml/g	29.0nm
DIAION ® HP20SS	63-150μm	100μm	540m^2/g	1.35ml/g	30.0nm
DIAION ® HP20	200-600μm	440μm	580m^2/g	1.30ml/g	30.0nm
DIAION ® HP21	200-600μm	440μm	630m^2/g	1.39ml/g	12.0nm

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Synthetic Adsorbents for Chromatography

- Polymethacrylic synthetic adsorbents

Product name	Particle size distribution	Average size (Ref.)	Surface area (Ref.)	Pore volume (Ref.)	Peak radius (Ref.)
MCI ® GEL CHP2MGM	4μm	4μm	460m^2/g	1.09ml/g	27.0nm
MCI ® GEL CHP2MG	9-11μm	10μm	590m^2/g	1.13ml/g	20.0nm
MCI ® GEL CHP2MGY	25-35μm	31μm	510m^2/g	1.15ml/g	23.0nm
DIAION ® HP2MG	200-600μm	490μm	560m^2/g	1.16ml/g	20.0nm

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Advantages of Using Synthetic Adsorbents

- Scalable from Analytical to Industrial Separations
  - Analytical: HPLC of Packed Columns
  - Preparative – Industrial: Batch or Column operations
  - The same separation conditions can be applied.
- Chemical Stability
  - Stable in wide pH: No restriction (Polystyrenic Adsorbents)
    2 – 12 (Polymethacrylic Adsorbents)
  - Caustic sterilization available
- Cost Effectiveness
  - Long life – No elimination of ligands
  - Rejuvenation with severe conditions available

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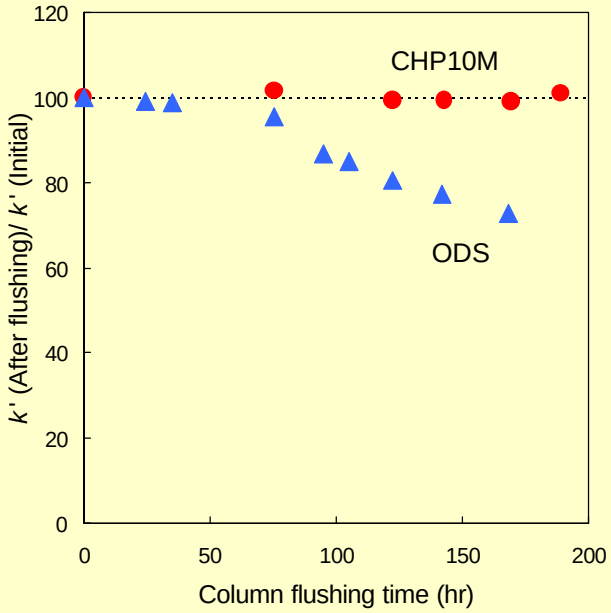
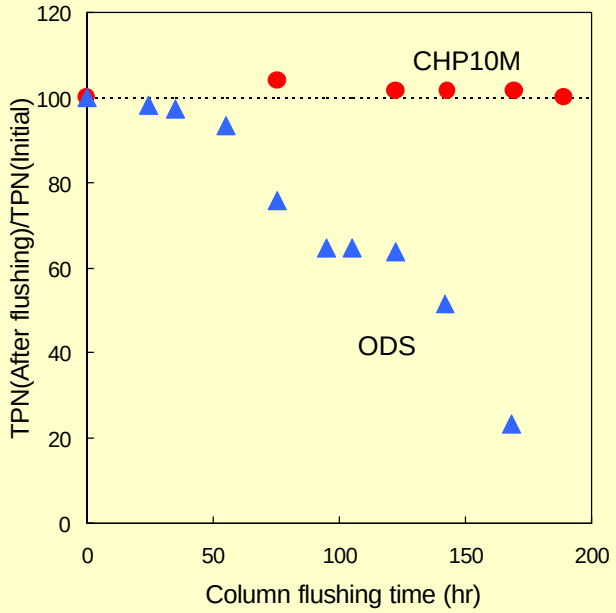
Alkaline Stability (pH12) of Synthetic Adsorbents

- Capacity Factor



- Theoretical Plate Number



Column, 150 x 4.6mmI.D.; Eluent, AcCN/20mM phosphate (pH12)=60/40;

Flow rate, 0.46ml/min; Temp., 25°C; Sample, Dimethylphthalate (1g/L), 5µl.

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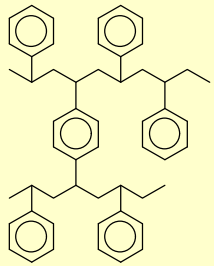
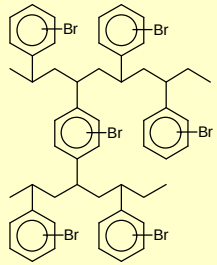
Chemical Structure of Synthetic Adsorbents

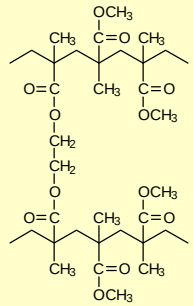
- Polystyrenic Adsorbents – Standard type
  - DIAION ® HP20, HP20SS, HP21
  - SEPABEADS ® SP70, SP700, SP825, SP850
  - MCI ® GEL CHP20A, Y, P, CHP55A, Y



- Chemically Modified Polystyrenic Adsorbent
  - SEPABEADS ® SP207
    Higher adsorption capacity
    High gravity (1.2)
    – Applicable to Expanded Bed Adsorption or Countercurrent operation



- Polymethacrylic Adsorbent
  - DIAION ® HP2MG
  - MCI ® GEL CHP2MG, CHP2MGY
    More polar than polystyrenic resins
    Applicable to normal-phase adsorption



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Chemical Structure of Synthetic Adsorbents

- Relationship between Chemical Structure and Adsorption Properties
  - Fundamental adsorption property – Van der Waars force
    - Solubility parameter and clogP of compounds may help estimation of adsorption strength.
  - Additional adsorption property for polystyrenic adsorbents
    - π-π interaction between benzene ring and compounds
  - Additional adsorption property for polymethacrylic adsorbents
    - hydrogen bonding interaction between ester group and compounds

Type of Synthetic Adsorbents	Solubility Parameter	Porarity
Polystyrenic synthetic adsorbents (SP850, SP825, SP70, SP700, HP21, HP20)	9.7	Small
Chemically modified synthetic adsorbent (SP207)	10.7	Small
Polymethacrylic synthetic adsorbent (HP2MG)	8.4	Medium

MITSUBISHI CHEMICAL

Pore Structure of Synthetic Adsorbents

- Specific surface area affects adsorption capacity.
  - Linear correlation between specific surface area and adsorption capacity of Cephalosporin C
- Pore size distribution gives size exclusion effect.
  - DIAION ® HP20, HP21
  - SEPABEADS ® SP207
  - DIAION ® HP2MG

    Relatively large pores

    – suitable for adsorption including large molecules
  - SEPABEADS ® SP825, SP850
  - SEPABEADS ® SP70, SP700 (New Products)

    Small pore and sharp distribution

    – suitable for selective adsorption of small molecules (Mw < 1000)

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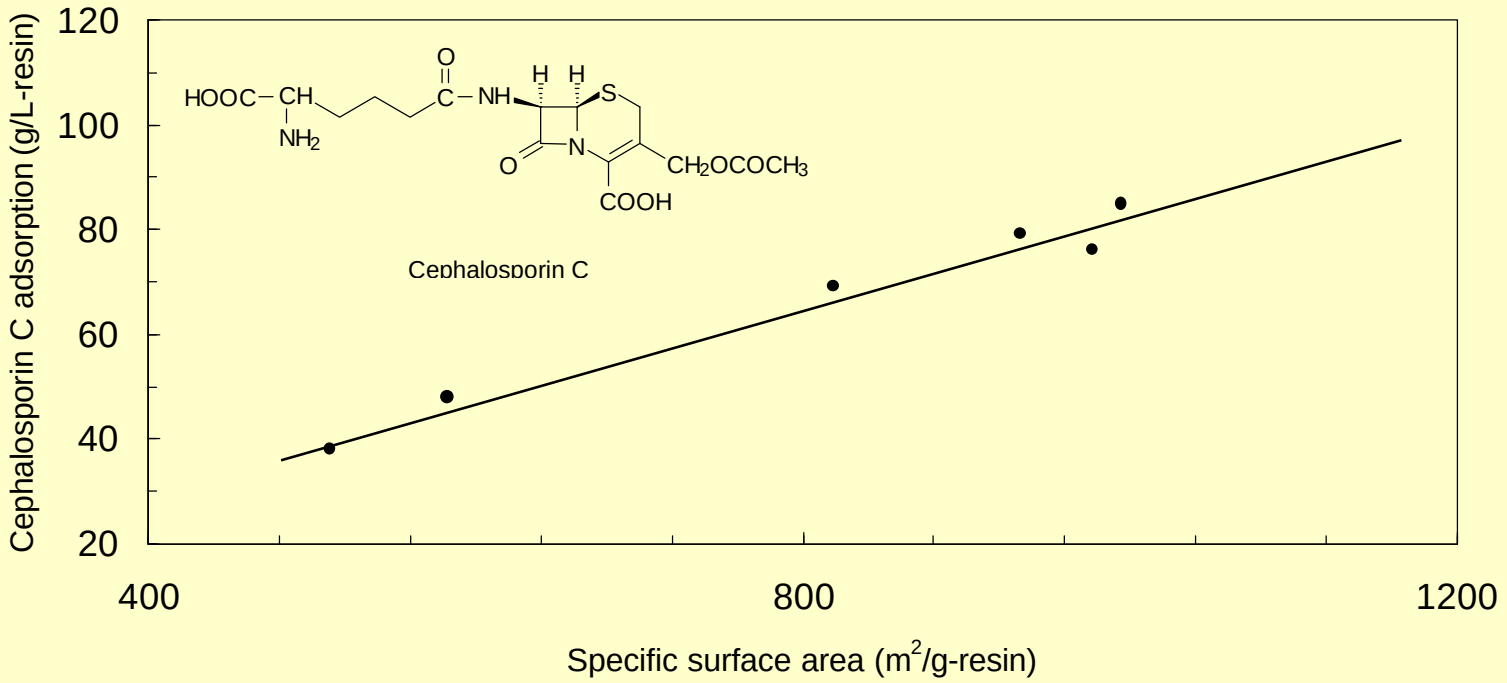
Pore Structure of Synthetic Adsorbents

- Relationship between Specific Surface Area of Polystyrenic Adsorbents and Adsorption Capacity of Cephalosporin C



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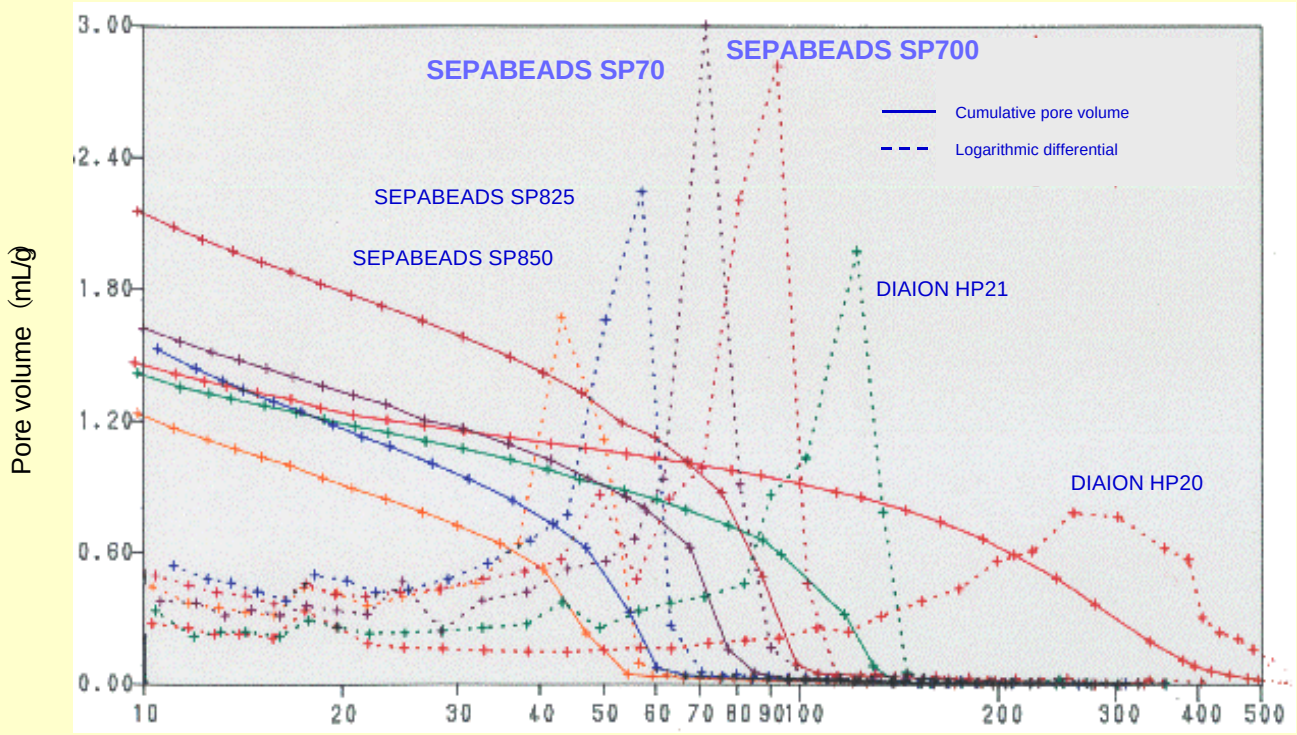
Pore Size Distribution of Synthetic Adsorbents



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Pore Structure of Synthetic Adsorbents

- Size Exclusion Effect of SEPABEADS® SP series

	SP850	SP825	SP700
Pore radius (Angstrom)	38.1	57.4	90.5
Cephalosporin C Mw = 415	85g/L-R	79g/L-R	80g/L-R
α-Lactalbumin Mw = 14,000	0g/L-R	2.9g/L-R	29.6g/L-R
Albumin Mw = 67,000	0g/L-R	0g/L-R	8.7g/L-R
γ-Globulin Mw = 160,000	0g/L-R	0g/L-R	2.1g/L-R

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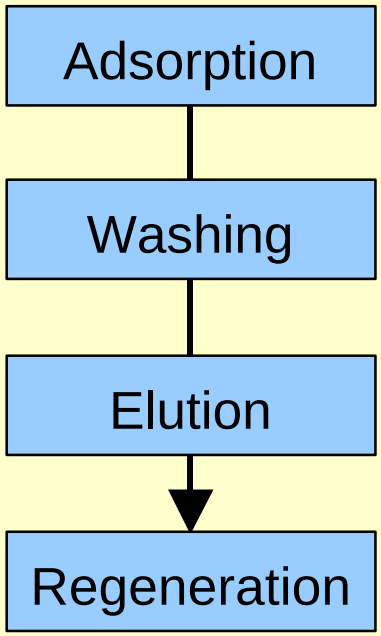
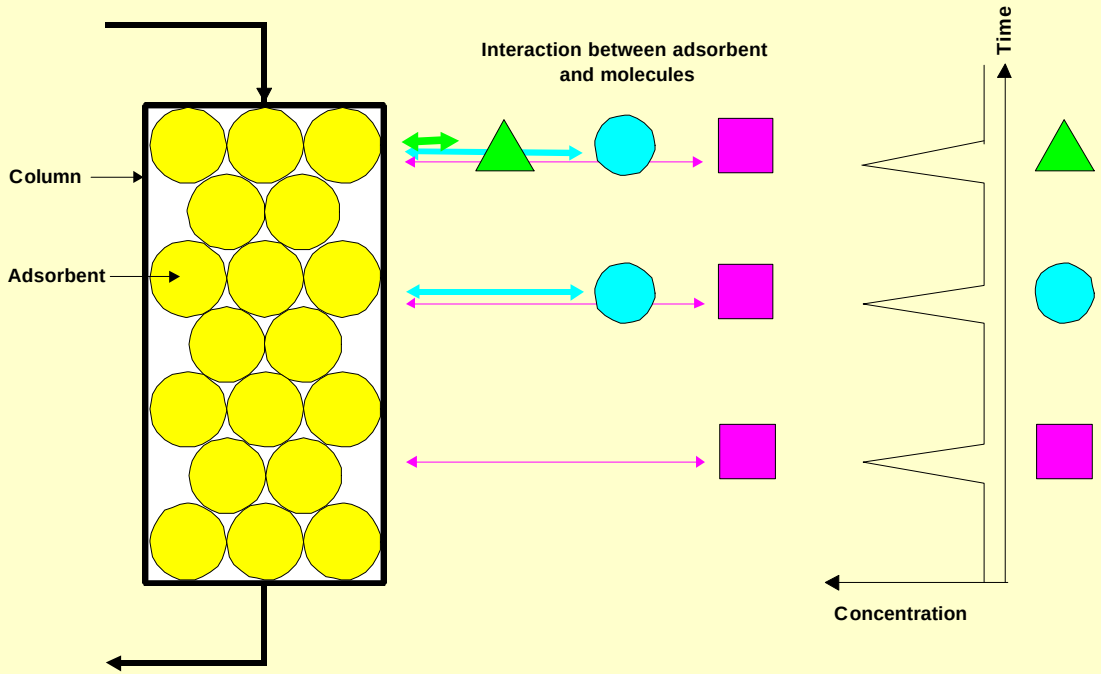
How to Use Synthetic Adsorbents

- Column Adsorption Method



- Chromatographic Operation



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Method Development of Chromatographic Separation

- HPLC separation using analytical columns
  - Selection of adsorbent type – polystyrenic or polymethacrylic
  - Selection of eluent system

    ↓
- Scale up separation using preparative columns
  - Effect of particle sizes on separation
  - Effect of loading amount on separation
  - Adsorbent life, Regeneration method, Feasibility study
  - Equipment design
    (Multi column system, ISMB system - if necessary)

    ↓
- Industrialization
  - Fine adjustment of operation conditions

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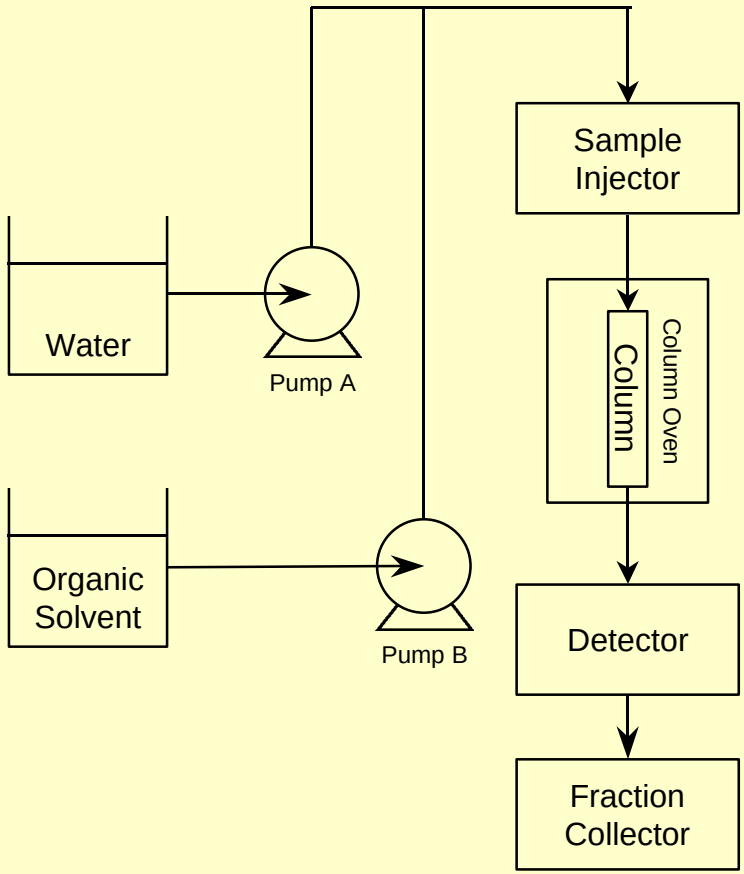
Example of HPLC Analysis Equipment

- Example of equipment:
  2 pumps system
  - Eluent concentration changeable
  - Regeneration with 100% organic solvent available

- Column size
  - CHP10M, CHP2MGM (4µm)
    150 x 4.6mm I.D.
    - Resin volume: 2.50ml
  - CHP5C, CHP2MG (10µm)
    250 x 4.6mm I.D.
    - Resin volume: 4.15ml

- Example of flow rate
  - 150 x 4.6mm I.D.
    - 0.5ml/min: LV=0.18m/h, SV=12
  - 250 x 4.6mm I.D.
    - 1.0ml/min: LV=0.36m/h, SV=14.4



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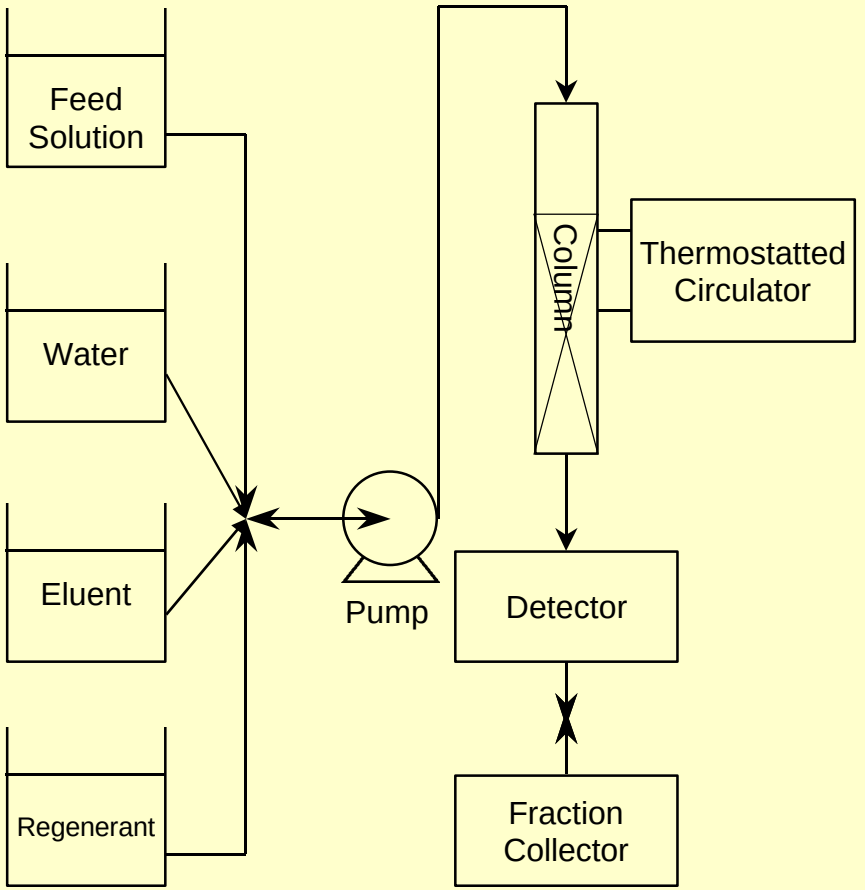
Example of Preparative Chromatography Equipment

- Example of preparative columns
  - 500 x 20mm I.D.
    - Resin volume: 157ml
  - 500 x 90mm I.D.
    - Resin volume: 3,180ml
- Example of flow rate
  - 500 x 20mm I.D.
    - 6.28ml/min:
      LV=1.2m/h, SV=2.4
  - 500 x 90mm I.D.
    - 63.6ml/min:
      LV=0.60m/h, SV=1.2



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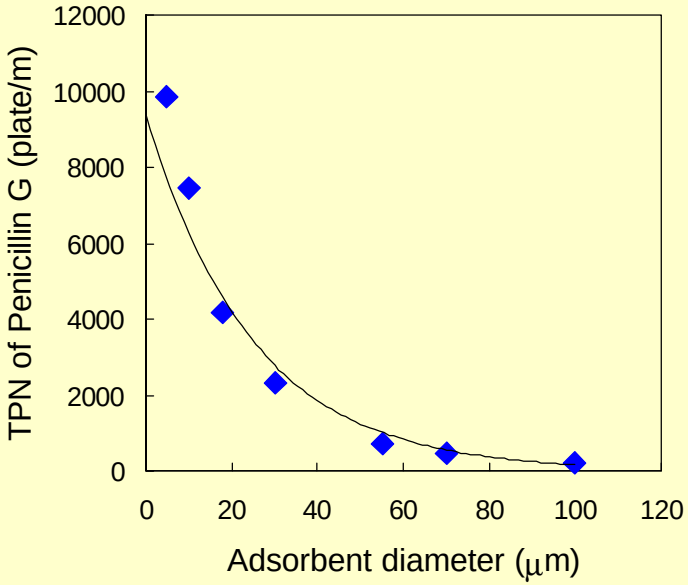
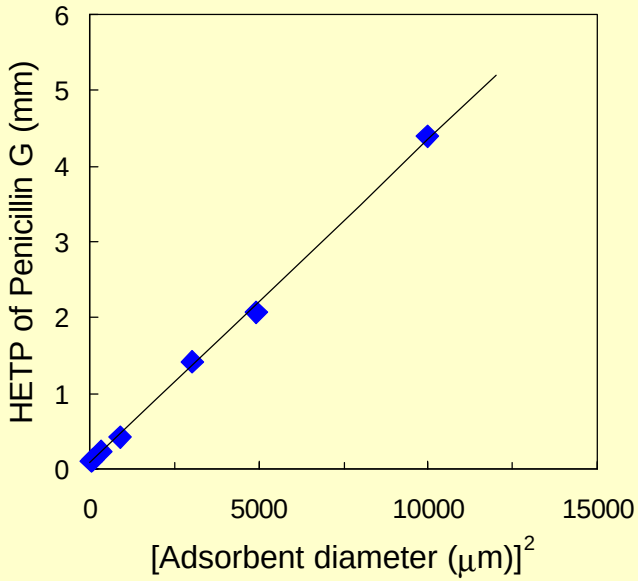
Effect of Adsorbent Diameter on Separation





- Separation comparison for Penicillin G on polystyrenic adsorbents of various particle sizes.
  - Column size; 150 x 4.6mm I.D. for CHP10M, CHP5C
    250 x 10mm I.D. for CHP55A, CHP55Y, CHP20P, SP20SS, HP20SS
  - Eluent; MeOH / 0.05M Phosphate (pH8)=60/40
  - Flow rate; LV=1.67m/hr
  - Detection; UV 254nm
  - Sample; Penicillin G (1g/L)
  - Loading amount; 0.005BV (5mg/L-R)

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Example of Theory for Scale-up Separation

- Yamamoto's Theory
  (Ion-exchange Chromatography of Proteins, Marcel Dekker, NY, 1988)

  - Linear gradient elution:

    $Rs^2 = (Constant)\ L\ LV^{-1}\ dp^{-2}\ GH^{-1}$,

    Rs is proportional to the square root of column length.
    Rs is inversely proportional to adsorbent diameter.
    Rs is inversely proportional to the square root of linear velocity.
    Rs is inversely proportional to the square root of
    GH (gradient slope normalized with respect to adsorbent volume).

  - Isocratic elution:

    Rs is proportional to the square root of column length.
    Rs is almost inversely proportional to adsorbent diameter.
    Rs is almost inversely proportional to the square root of linear velocity.

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Example of Application

- Separation of tea catechins
  - Adsorbent selection using HPLC columns (CHP5C, CHP2MG)
  - Optimization of elution conditions
  - Semi-preparative separation using CHP55A, CHP55Y
  - Preparative separation and fraction analysis
- Separation of soybean isoflavones
  - Exploration of separation possibility using an HPLC column
  - Optimization of elution conditions
  - Semi-preparative separation using CHP55A, CHP55Y
  - Preparative separation

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■ *Separation of tea extract on a column packed with analytical polystyrenic adsorbent*

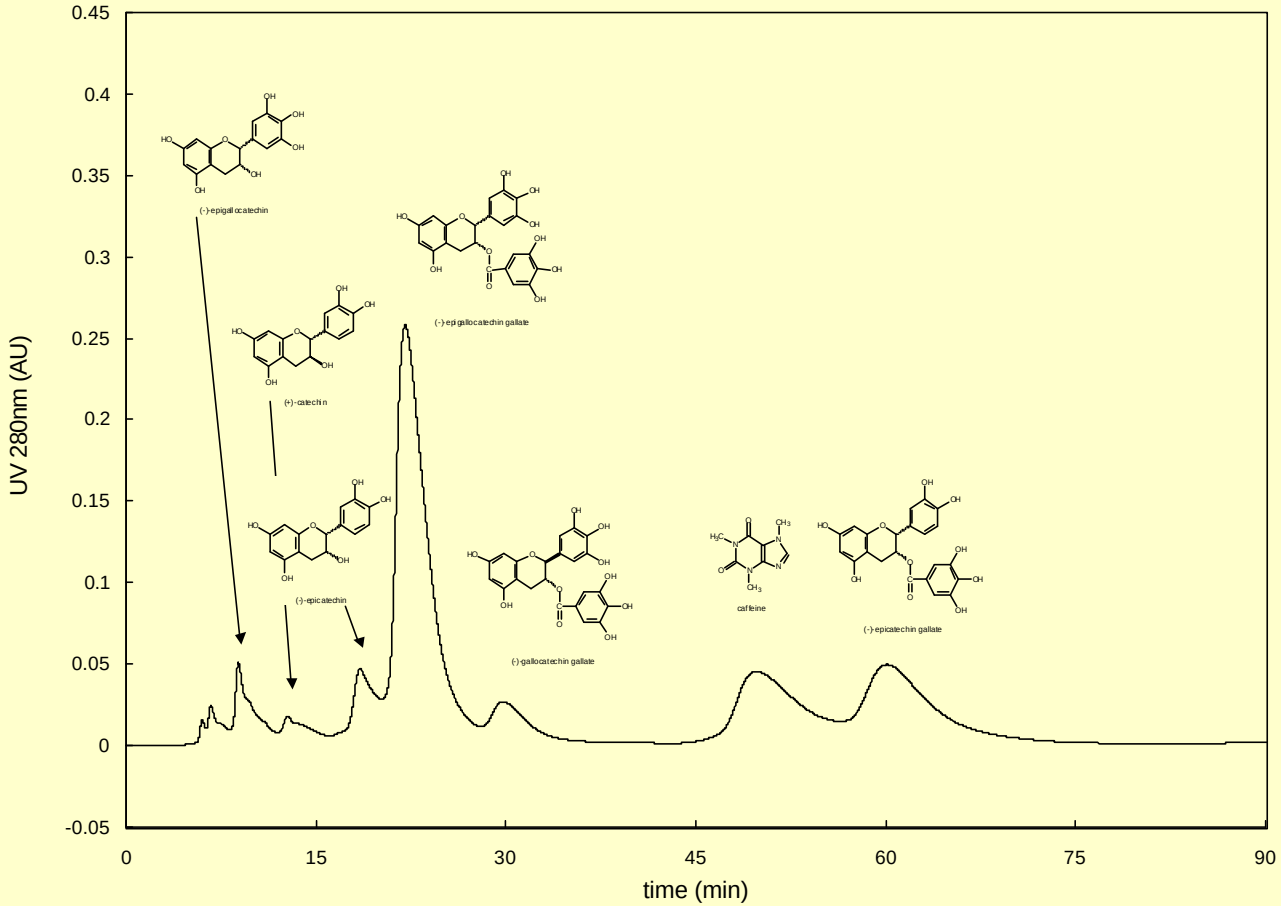

Figure. Separation of tea extract on a column packed with analytical polystyrenic adsorbent.
Conditions: Adsorbent, CHP5C (10μm); Column size, 150mm x 4.6mm I.D.;
Eluent, MeOH/0.01M Acetic acid=35/65; Flow rate, 0.46ml/min.
Sample: Polyphenon 60 (10mg/ml). Injection: 10μl.

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■ *Separation of tea extract on a column packed with analytical polymethacrylic adsorbent*

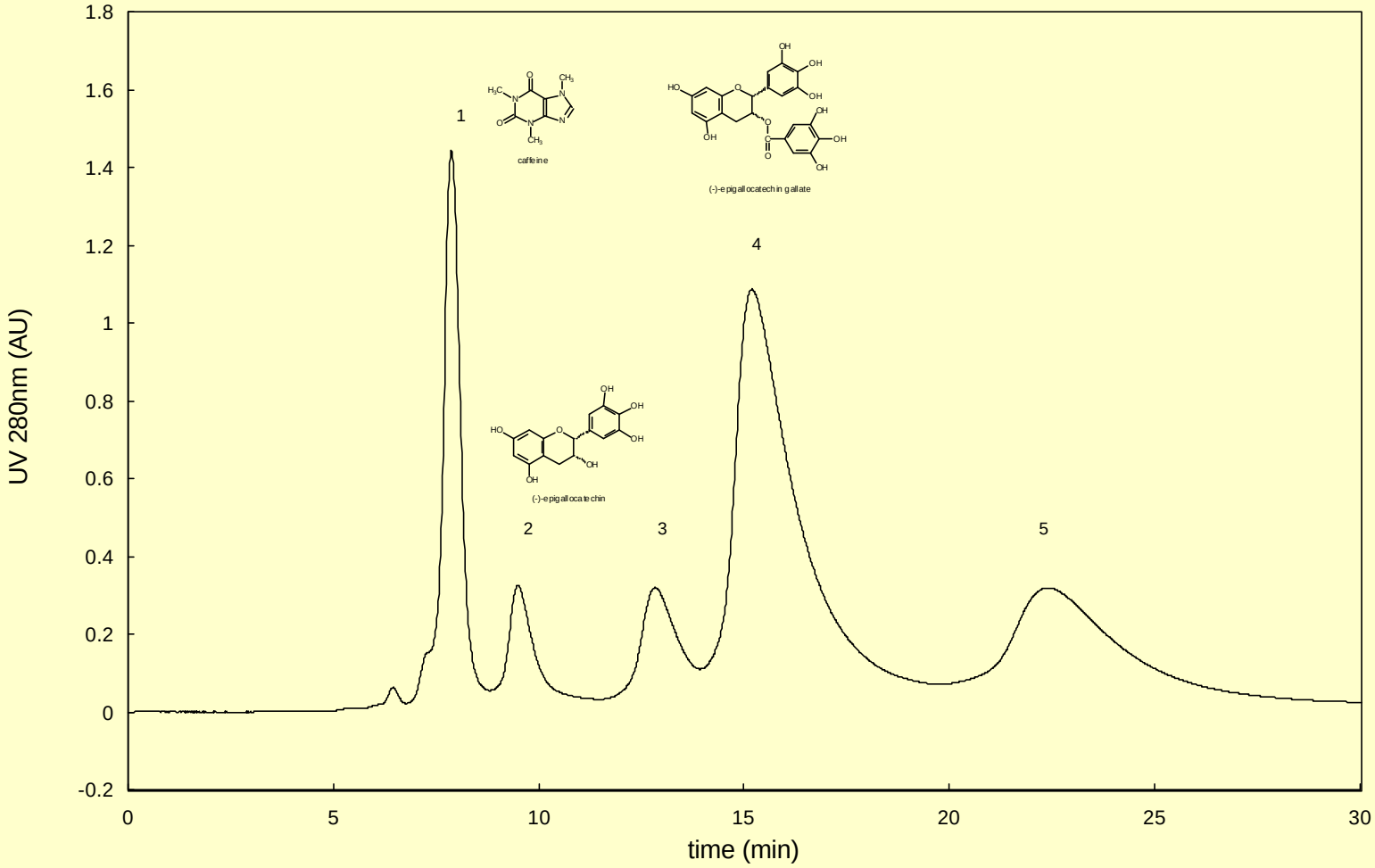

Figure 13. Separation of tea extract on a column packed with analytical polymethacrylic adsorbent of 10μm.
Conditions: Adsorbent, CHP2MG (10μm); Column size, 150mm x 4.6mm I.D.;
Eluent, MeOH/0.01M Acetic acid=60/40; Flow rate, 0.46ml/min.
Sample: Polyphenon 60 (10mg/ml). Injection: 12.5μl.
Peak identification: 1= caffeine; 2=(-)-epigallocatechin; 3=(+)-catechin and (-)-epicatechin;
4=(-)-epigallocatechin gallate; 5=(-)-gallocatechin gallate and (-)-epicatechin gallate.

MITSUBISHI CHEMICAL

■ *Chromatographic separation of tea extract on polystyrenic adsorbents with various particle sizes*

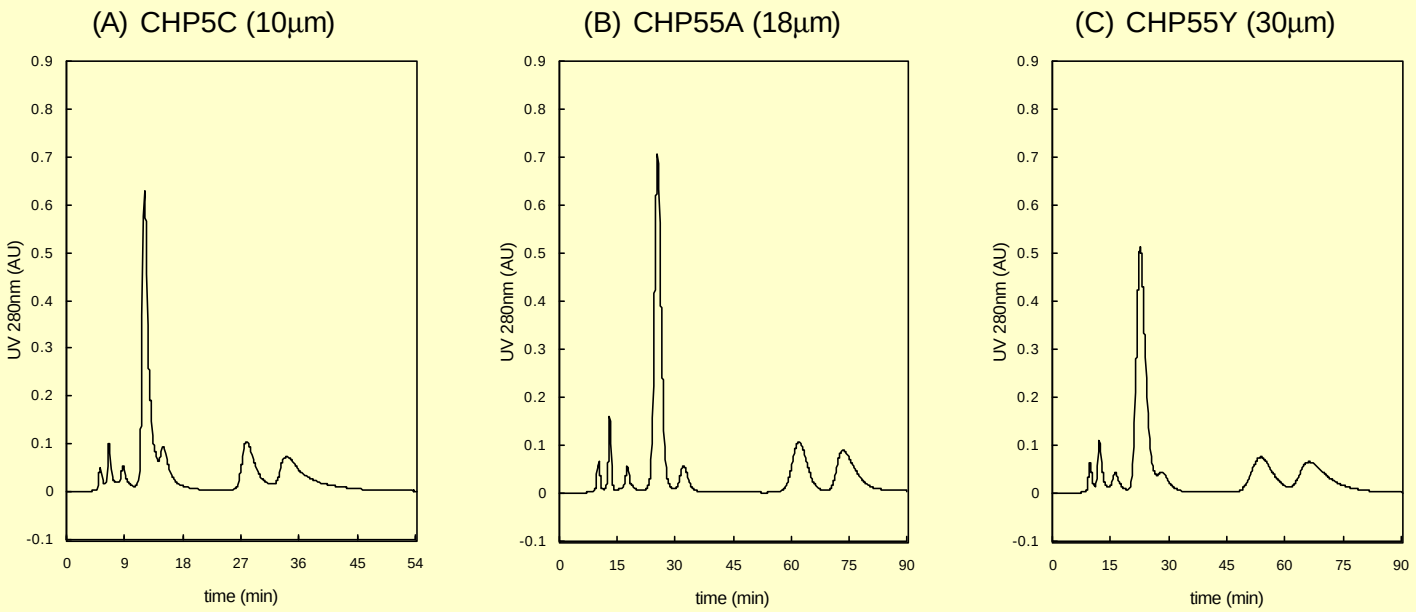

Figure. Chromatographic separation of tea extract on polystyrenic adsorbents with various particle sizes.

(A) Adsorbent, CHP5C (10μm); Column size, 150mm x 4.6mm I.D.; Eluent, MeOH/0.01M Acetic acid=40/60; Flow rate, 0.46ml/min. Sample: Polyphenon 60 (10mg/ml). Injection: 10μl.

(B) Adsorbent, CHP55A (18μm); Column size, 250mm x 10mm I.D.; Eluent, MeOH/0.01M Acetic acid=40/60; Flow rate, 2.18ml/min. Sample: Polyphenon 60 (10mg/ml). Injection: 47μl.

(C) Adsorbent, CHP55Y (30μm); Column size, 250mm x 10mm I.D.; Eluent, MeOH/0.01M Acetic acid=40/60; Flow rate, 2.18ml/min. Sample: Polyphenon 60 (10mg/ml). Injection: 47μl.

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■ *Preparative separation of tea extract on polystyrenic adsorbent of 18 μm*

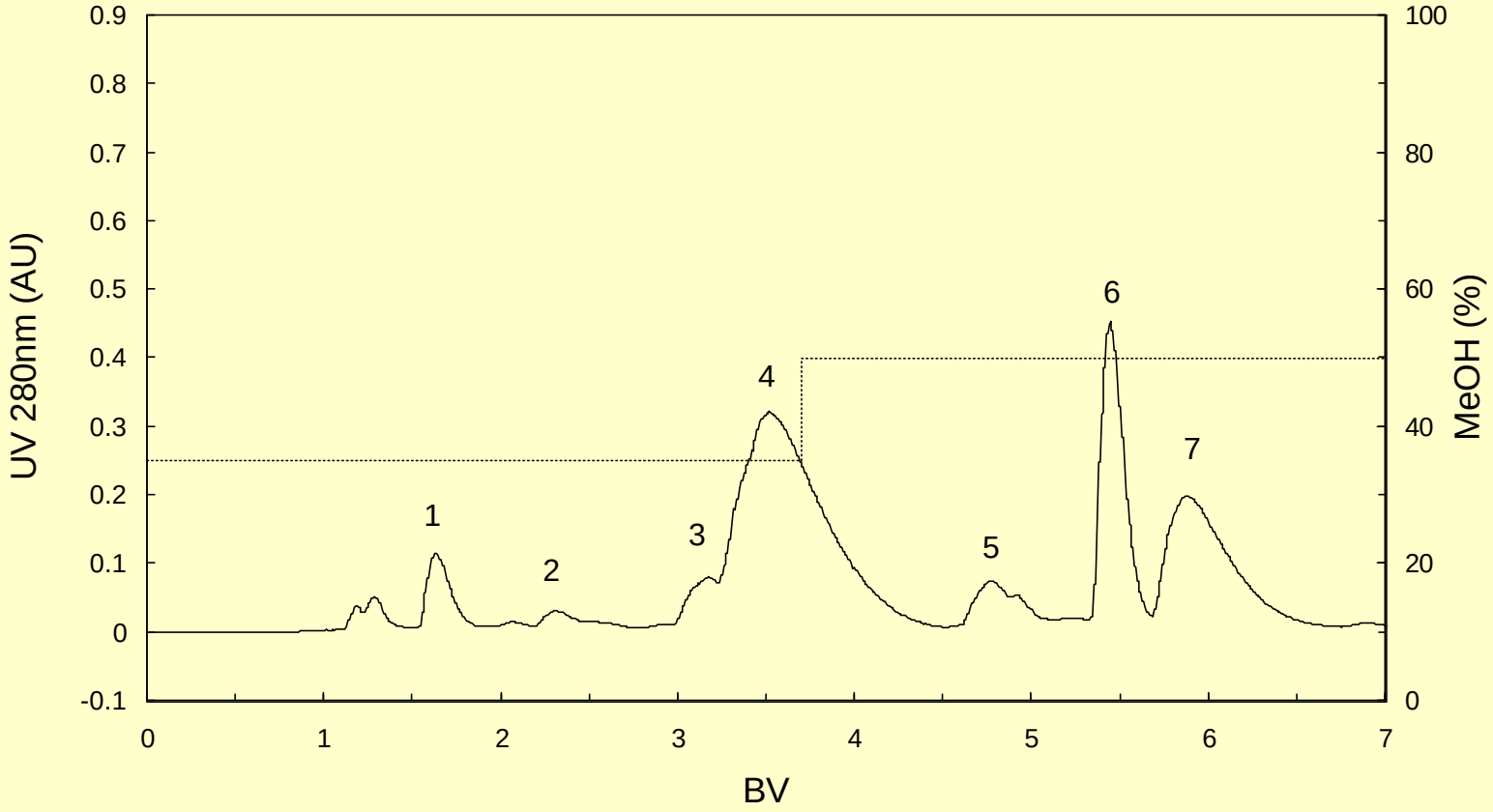

Figure. Preparative separation of tea extract on a column packed with polystyrenic adsorbent of 18μm.
Conditions: Adsorbent, CHP55A (18μm); Column size, 465mm x 32mm I.D. (374ml);
Eluent, 0-185min: MeOH/0.01M Acetic acid=35/65;
185-350min: MeOH/0.01M Acetic acid=50/50; Flow rate, 7.48ml/min (SV = 1.2).
Sample: Polyphenon 60 (10mg/ml). Injection: 18.7ml (0.05BV).
Peak identification: 1=(-)-epigallocatechin; 2=(+)-catechin; 3=(-)-epicatechin;
4=(-)-epigallocatechin gallate; 5=(-)-gallocatechin gallate; 6=(-)-epicatechin gallate; 7=caffeine.

Elution profile of each catechin derivative on CHP55A (18 μm)

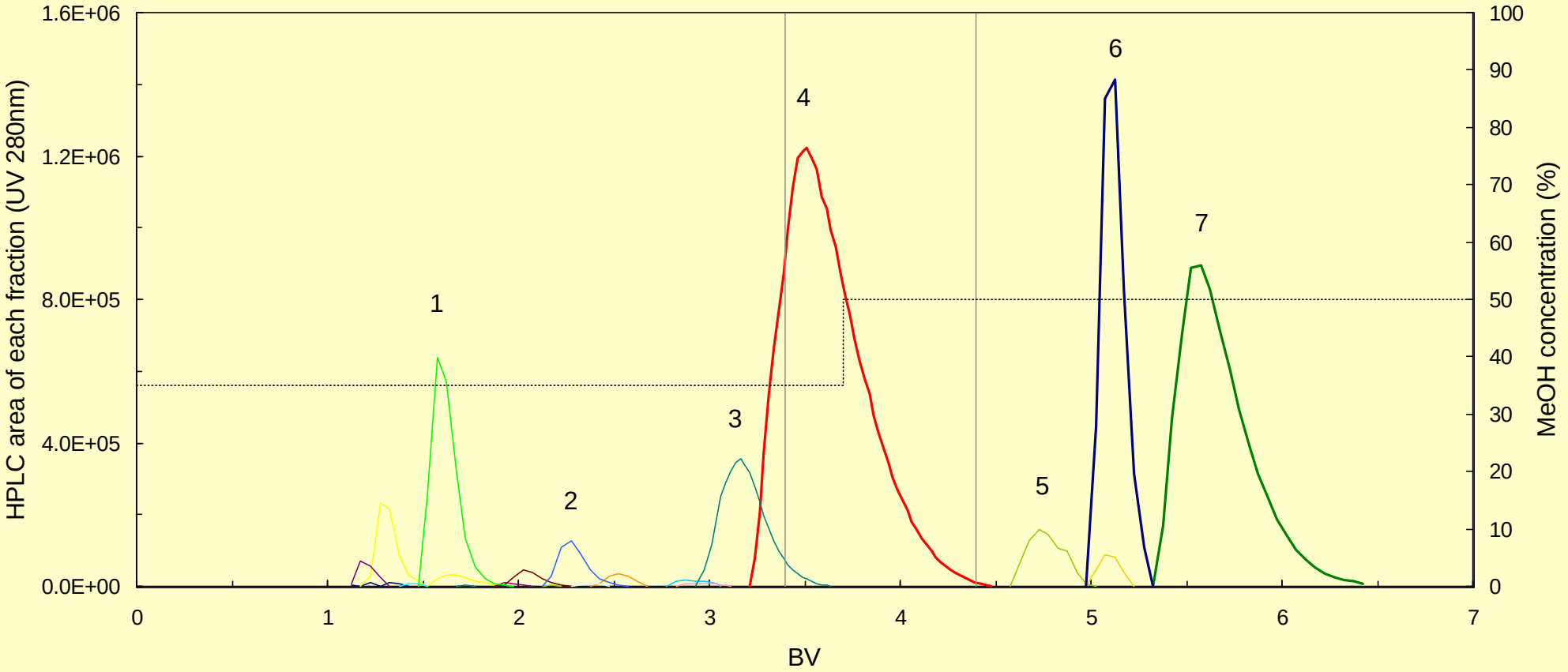

Figure. Elution profile of each catechin derivative determined by the fraction analysis.
Conditions: Adsorbent, CHP55A (18μm); Column size, 465mm x 32mm I.D. (374ml); Eluent, 0-185min: MeOH/0.01M Acetic acid=35/65;185-350min: MeOH/0.01M Acetic acid=50/50; Flow rate, 7.48ml/min (SV = 1.2). Sample: Polyphenon 60 (10mg/ml). Injection: 18.7ml (0.05BV).
Identification: 1=(-)-epigallocatechin; 2=(+)-catechin; 3=(-)-epicatechin; 4=(-)-epigallocatechin gallate; 5=(-)-gallocatechin gallate; 6=caffeine; 7=(-)-epicatechin gallate.

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■ *Scale-up preparative separation of tea extract on polystyrenic adsorbent of 30μm*

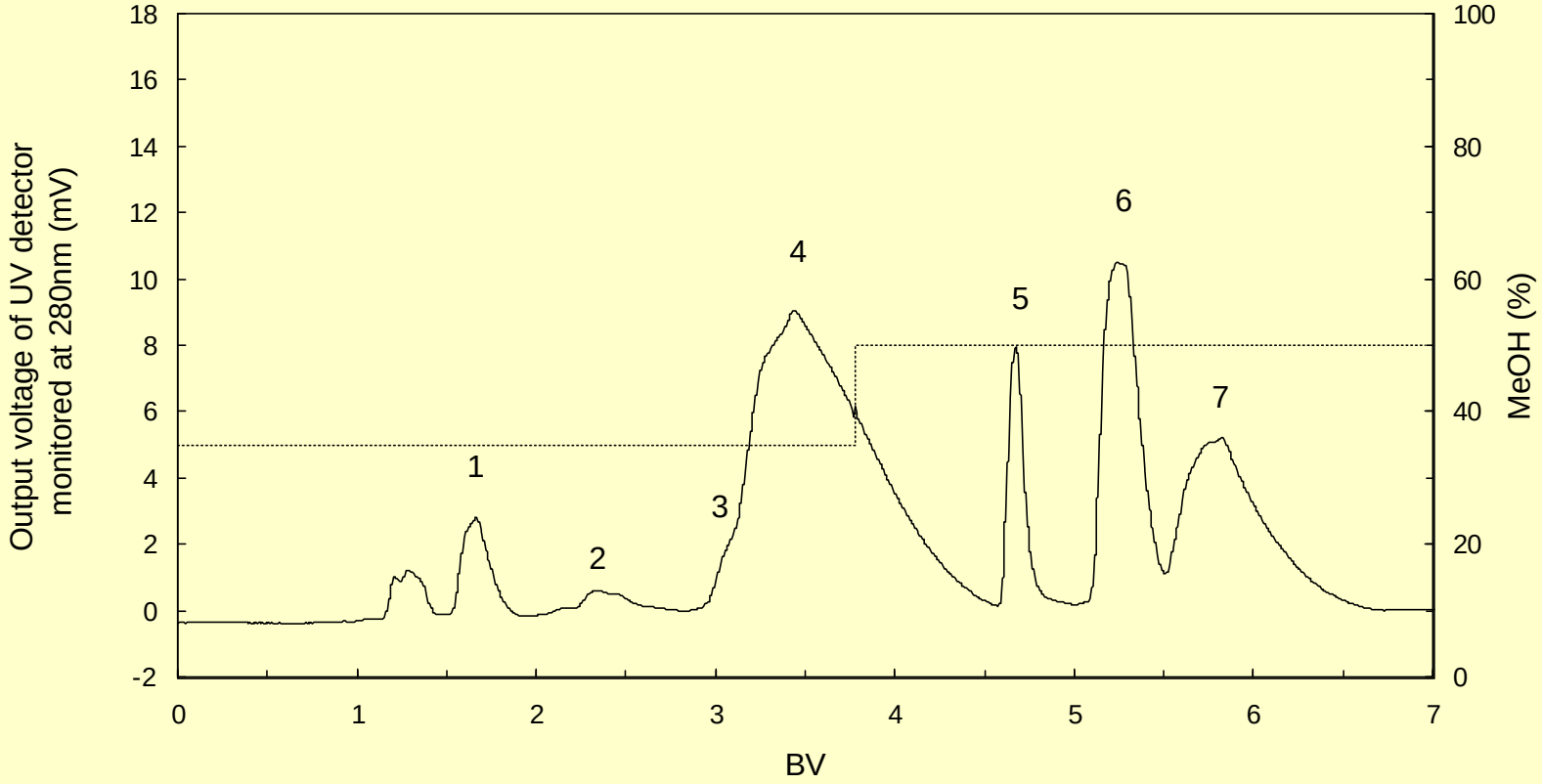

Figure. Scale-up preparative separation of tea extract on a column packed with polystyrenic adsorbent of 30μm.
Conditions: Adsorbent, CHP55Y (30μm); Column size, 435mm x 90mm I.D. (2770ml);
Eluent, 0-190min: MeOH/0.01M Acetic acid=35/65;
190-350min: MeOH/0.01M Acetic acid=50/50; Flow rate, 55ml/min (SV = 1.2).
Sample: Polyphenon 60 (20mg/ml). Injection: 140ml (0.05BV).
Peak identification: 1=(-)-epigallocatechin; 2=(+)-catechin; 3=(-)-epicatechin;
4=(-)-epigallocatechin gallate; 5=(-)-gallocatechin gallate; 6=(-)-epicatechin gallate; 7=caffeine.

Elution profile of each catechin derivative on CHP55Y (30 μm)

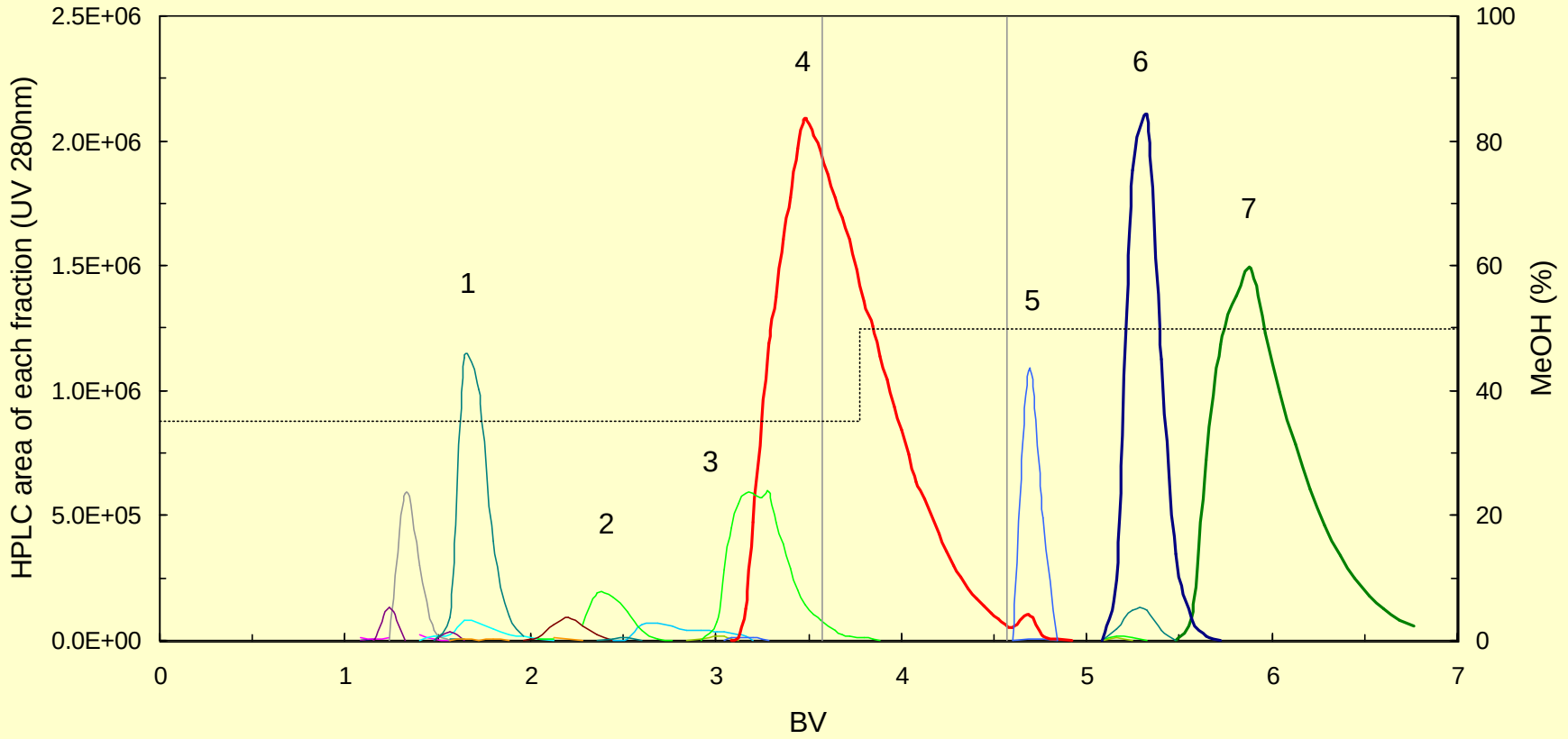

Figure. Elution profile of each catechin derivative determined by the fraction analysis.
Conditions: Adsorbent, CHP55Y (30μm); Column size, 435mm x 90mm I.D. (2770ml); Eluent, 0-190min: MeOH/0.01M Acetic acid=35/65; 190-350min: MeOH/0.01M Acetic acid=50/50; Flow rate, 55ml/min (SV = 1.2). Sample: Polyphenon 60 (20mg/ml). Injection: 140ml (0.05BV).
Identification: 1=(-)-epigallocatechin; 2=(+)-catechin; 3=(-)-epicatechin; 4=(-)-epigallocatechin gallate; 5=(-)-gallocatechin gallate; 6=caffeine; 7=(-)-epicatechin gallate.

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Example of Scale-up Separation

- Separation of tea catechins
  - Optimization of elution conditions using CHP5C
  - Semi-preparative separation using CHP55A, CHP55Y
  - Preparative separation and fraction analysis
    - Purity and recovery of (-)-epigallocatechin gallate

Resin	Column size	Resin volume	Loadability	Loading amount	Purity	Recovery
CHP5C (10μm)	150 x 4.6mmI.D.	2.50ml	0.05g/L	125μg	-	-
CHP55A (18μm)	465 x 32mmI.D.	374ml	0.50g/L	187mg	99%	82%
CHP55Y (30μm)	435 x 90mmI.D.	2770ml	1.01g/L	2800mg	99%	61%

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Example of Application

- Separation of tea catechins
  - Adsorbent selection using HPLC columns (CHP5C, CHP2MG)
  - Optimization of elution conditions
  - Semi-preparative separation using CHP55A, CHP55Y
  - Preparative separation and fraction analysis
- Separation of soybean isoflavones
  - Exploration of separation possibility using an HPLC column
  - Optimization of elution conditions
  - Semi-preparative separation using CHP55A, CHP55Y
  - Preparative separation

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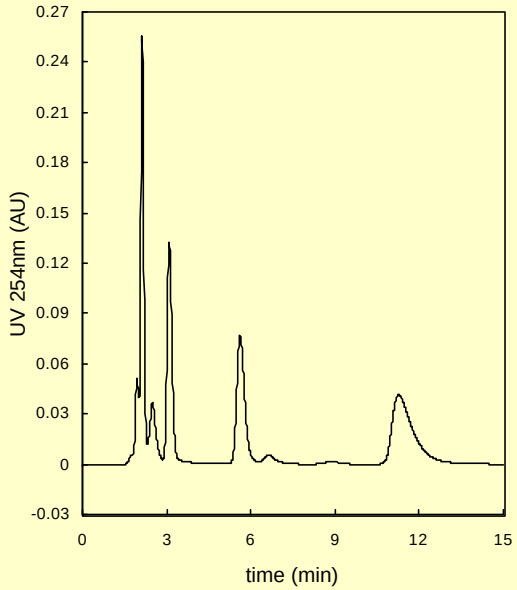
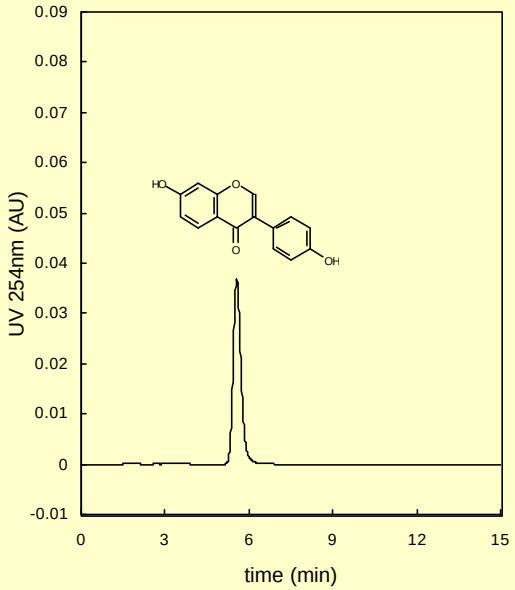
Chromatograms of soybean crude extract and isoflavones on analytical polystyrenic adsorbent

(A) Soybean crude extract



(B) Daidzein



(C) Genistein

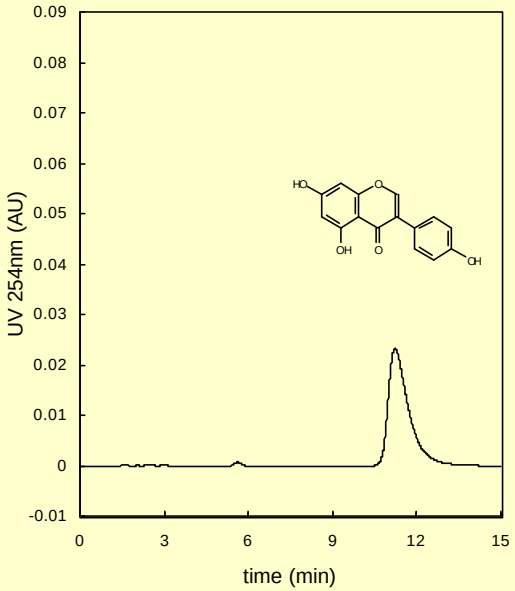


Figure. Chromatograms of soybean crude extract and isoflavones on analytical polystyrenic adsorbent.

Adsorbent, CHP5C (10μm); Column size, 150mm x 4.6mm I.D.; Eluent, MeOH/0.1M ammonium acetate=80/20; Flow rate, 1.00ml/min.

(A) Sample: Soybean crude extract. Injection: 10.0μl.

(B) Sample: Daidzein (100μg/ml). Injection: 1.0μl.

(C) Sample: Genistein (100μg/ml). Injection: 1.0μl.

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■ *Preparative separation of soybean crude extract on polystyrenic adsorbent*

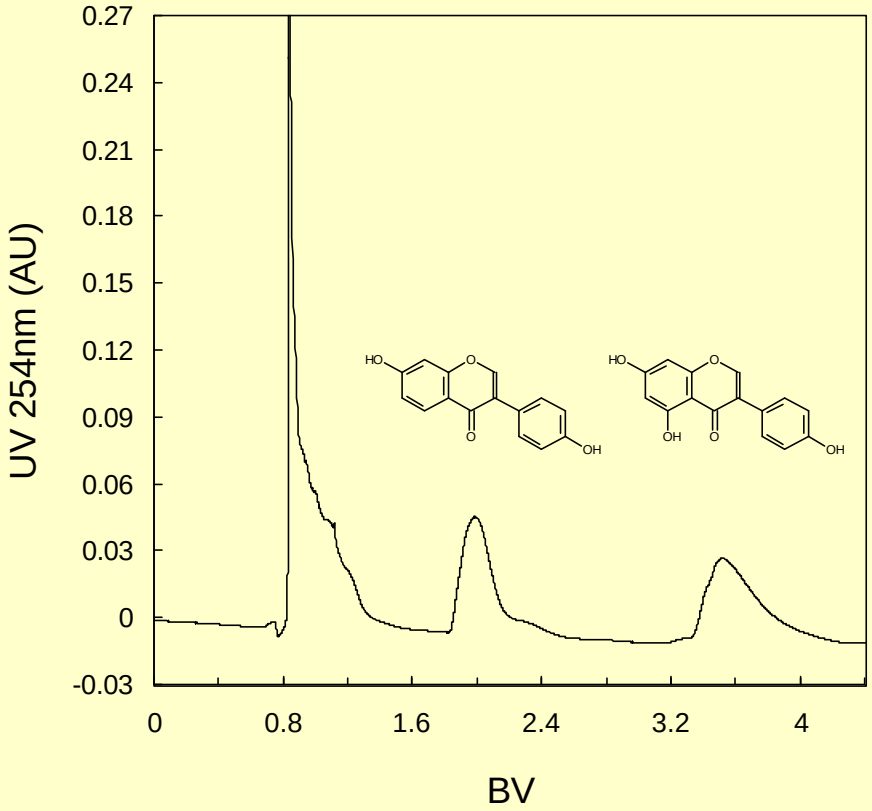

Figure. Preparative chromatographic separation of soybean crude extract on polystyrenic adsorbent with fine grade particle size of 18µm.

Conditions:
Adsorbent, CHP55A (18µm); Column size, 465mm x 32mm I.D.;
Eluent, MeOH/0.1M ammonium acetate=80/20; Flow rate, 7.48ml/min.
Sample: Soybean crude extract. Injection: 37.4ml (0.1BV).

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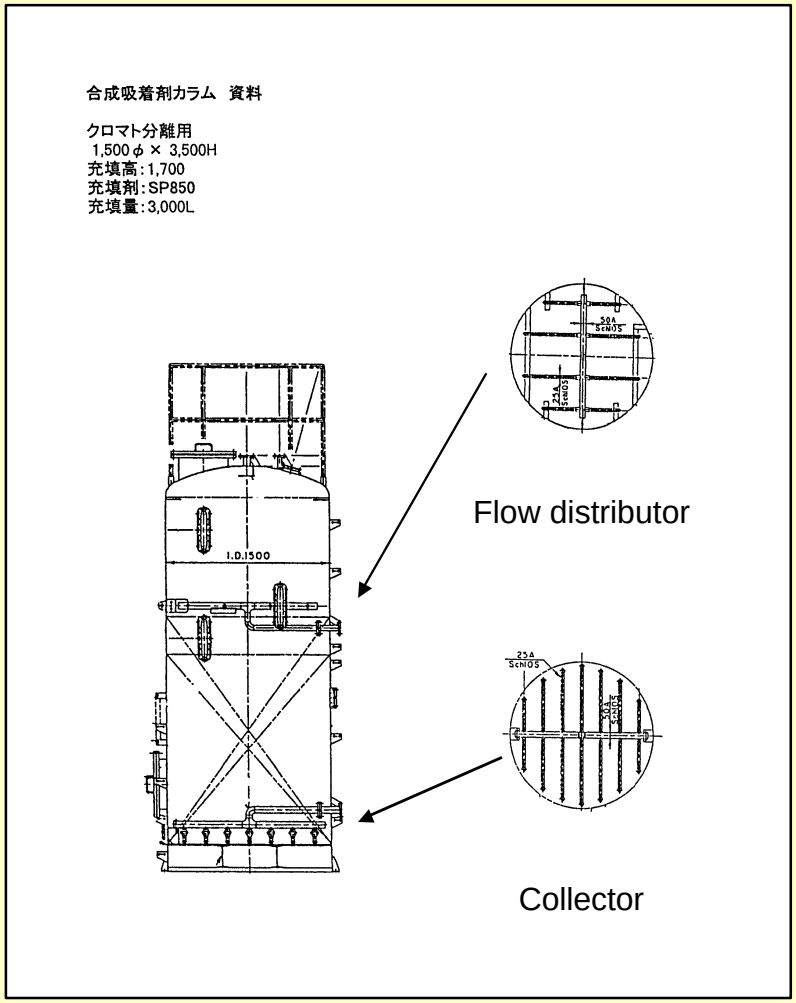
Example of a Column for Production

- Example of a Column for Production
  - 350 x 150cm I.D.
    - Resin volume of 3000L with 170cm height
  - Fundamental concept for scale-up
    - Enlarge diameter rather than bed height expansion
- Structure of flow distributor and collector affects the separation.
  - Nippon Rensui Co. and Rensui Asia Co. can supply columns suitable for synthetic adsorbents.



Typical Operation Procedure of a Column System

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	Procedure	Flow rate	Flow volume	Remarks
Packing and Conditioning	Packing	-	-	
	Backwash	-	-	Removal of small and broken particles
	Pretreatment	SV: 1 – 5	5 – 10 BV	Alcohol or aqueous alcoholic solution
	Washing	SV: 1 – 5	3 – 4 BV	Water or Buffer solution (the same pH as feed solution)
Cyclic Operation	Adsorption	SV: 0.5 – 3	Depends on Adsorption Amount	Loading amount should be lower than maximum capacity.
	Washing	SV: 1 – 5	0.5 – 1 BV	Removal of feed solution
	Elution	SV: 0.5 – 3	2 – 10BV	(Aqueous) solvent elution (MeOH, Acetone), pH elution (Acid, Alkali, Buffer solution), and the use of both
	Washing	SV: 1 – 5	3 – 4 BV	Water or Buffer solution (the same pH as feed solution)
Regeneration	Regeneration	SV: 0.5 – 3	3 – 4 BV	Operated every several – several tens cycles. Alcohol, Acetone, Alkali + Alcohol, etc.
	Washing	SV: 1 – 5	3 – 4 BV	In case of alkali rejuvenation, neutralization with acid solution will be added.

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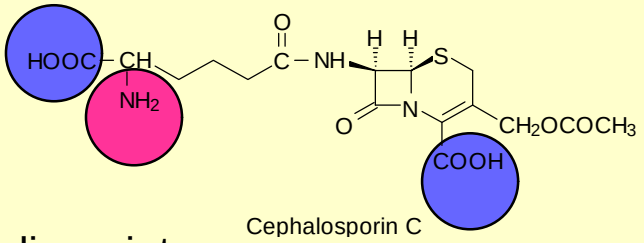
Methods of Adsorption and Elution

- Solvent Composition Change
  - Adsorption Process

    Water solution or Aqueous solution with low solvent composition
  - Elution Process

    Neat solvent or Aqueous solution with high solvent composition

    Selection of solvent and gradient elution



Cephalosporin C

- pH Change
  - Adsorption Process

    pH region in which ionic functionality does not dissociate

    (For zwitterionic compounds, pH region near pI)
  - Elution Process

    pH region in which ionic functionality dissociates

    Combinational use of solvent composition change

  Example: Cephalosporin C, Adsorption pH=3, Elution pH=6-8

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How to Use Synthetic Adsorbents

- Rejuvenation Methods of Synthetic Adsorbents

Rejuvenation Method	Specific Surface Area (m^2/g)
HP20 New Resin	Ca. 700
HP20 Used Resin	96
99% Methanol (3BV)	246
99% Isopropanol (3BV)	406
95% Propanone (2BV)	563
75% Isopropanol + 4% Sodium hydroxide (4BV)	562

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Important Notice

- Column Length – Min. 50cm is required for process operation.
  - Max. 3m to avoid resin compression
- Loading Capacity – Considering the balance with separation
  - several g/L – several tens g/L
- Flow Rate – SV = 0.5～3
- Bed Expansion / Shrinkage – Raise of backpressure
  - Not so serious with large size columns
  - In case of medium or small size columns,
    short time backwash will release the backpressure.
- Temperature – Effect on separation and purity
  - Temp↑ – Separation↑, Pressure drop↓
  - Care for decomposition or bioactivity loss should be taken.

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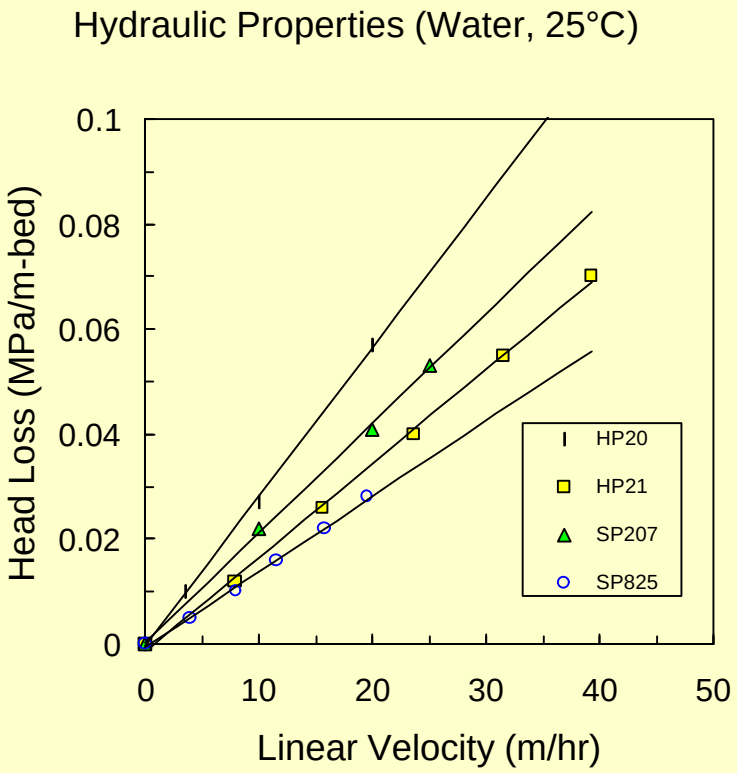
Pressure Drop of Synthetic Adsorbents

- Typical Flow Rate:
  - SV = 0.5 – 3
- LV = SV x H
  - H = 1 – 3,
  - LV = 0.5 – 9 < 10,
  - Synthetic adsorbents don't compress at the flow rate under 10m/hr.
- Compression due to the bed expansion during solvent change
  - Especially in the case of polystyrenic adsorbents



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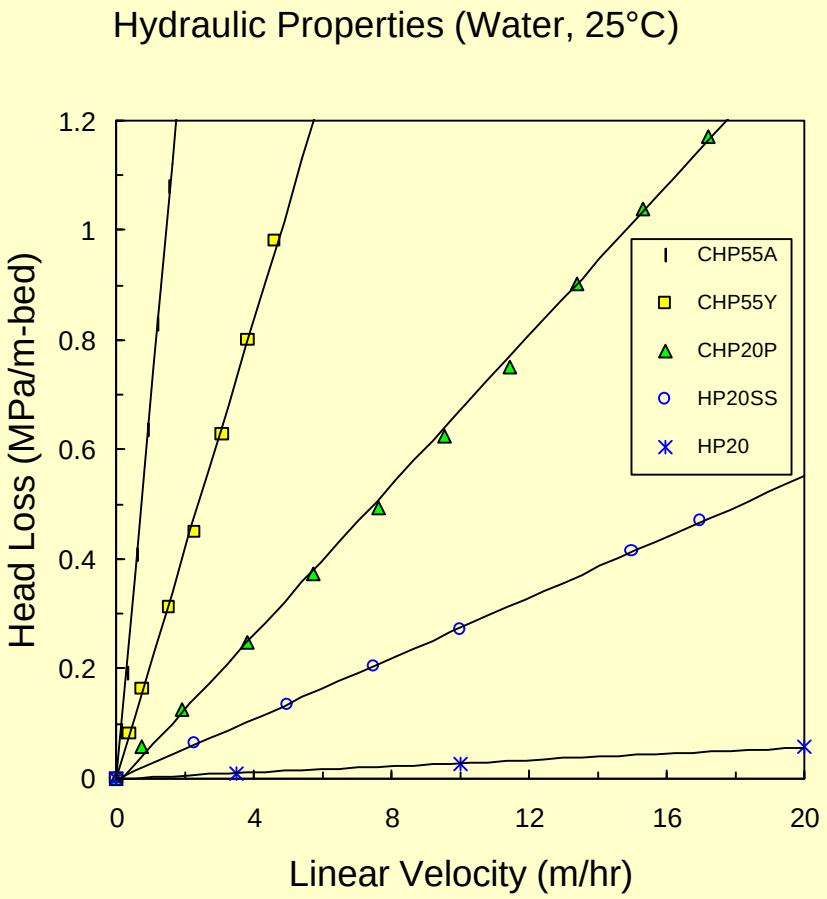
Pressure Drop of Synthetic Adsorbents for Preparative Chromatography

- Linear relationship between LV and head loss up to 1MPa/m-bed height (10.2kgcm^{-2}/m-bed) is achieved for CHP55A (20µm) and CHP55Y (30µm)
- Pressure drop approximation for CHP55A
  - P = 0.69 x LV x H
- Pressure drop approximation for CHP55Y
  - P = 0.21 x LV x H



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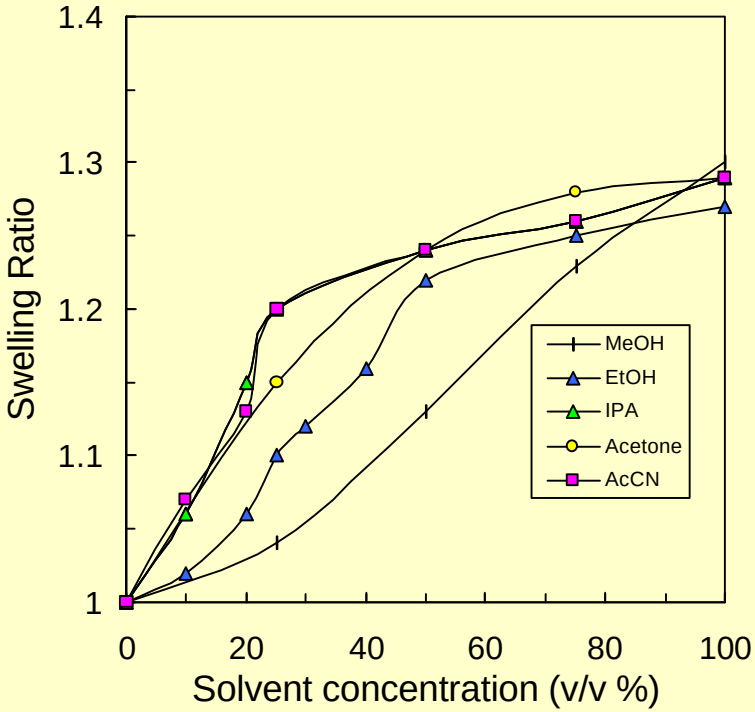
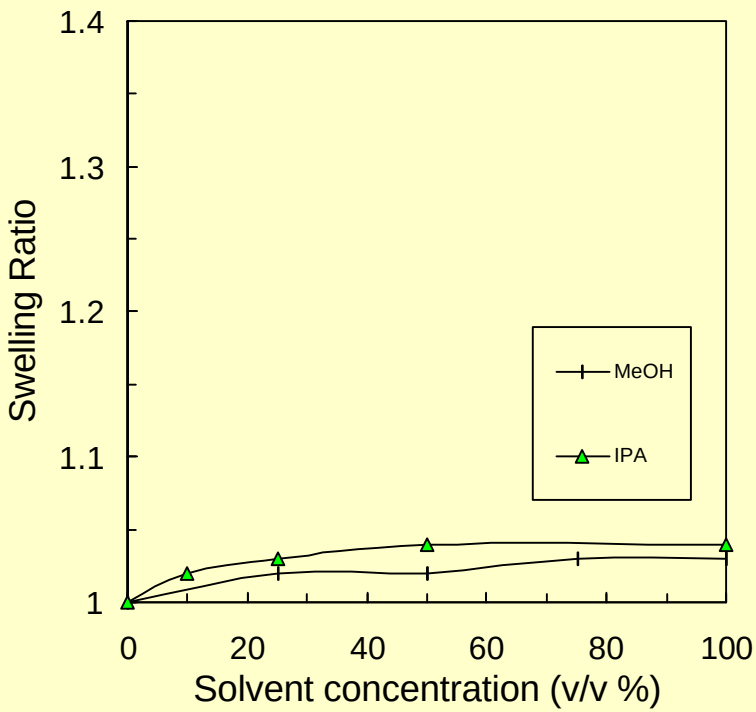
Swelling / Shrinkage of Synthetic Adsorbents

Swelling ratios of HP20 for various aqueous solvents (25°C)



Swelling ratios of HP2MG for various aqueous solvents (25°C)



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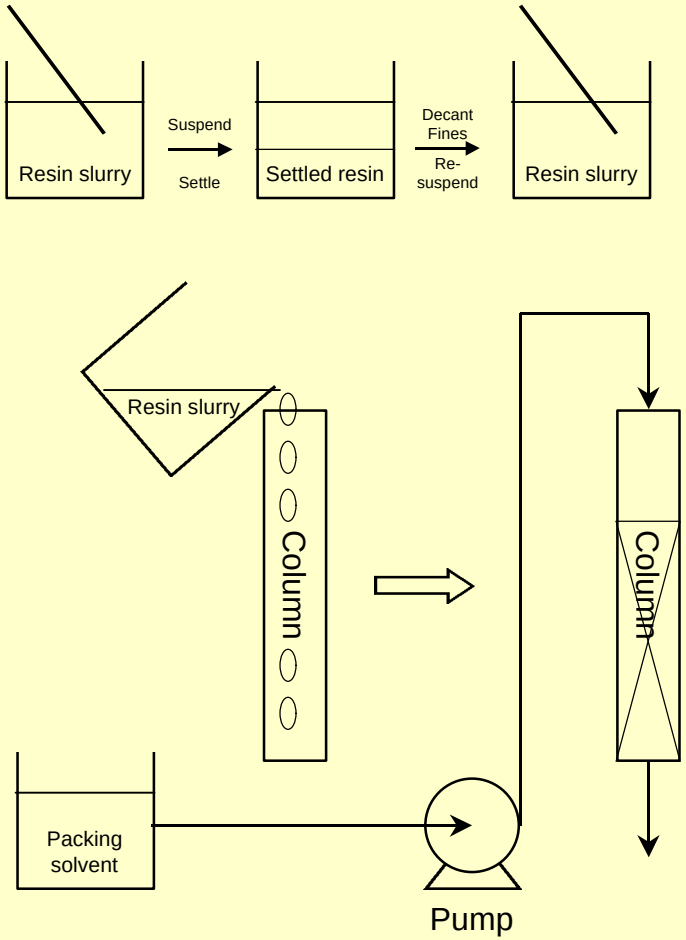
Example of Packing Procedure

- Column recommendation
  - Adjustable columns
- Resin slurry formation
  - Example of solvent:
    50% Alcohol or AcCN
    Polystyrenic resins don't disperse in 100% water.
  - De-fining by means of decantation is recommended.
- Packing using a pump
  - Example of packing solvent:
    20% Alcohol or AcCN
  - Flow rate:
    2-4 times of the operating flow rate
  - Don't exceed column maximum pressure.
  - Avoid air entrapment in the column.



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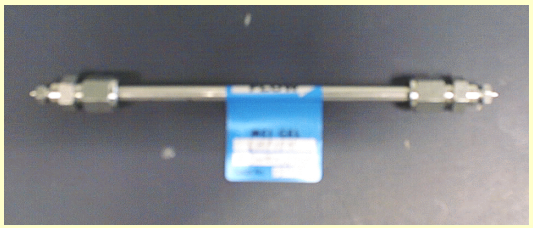
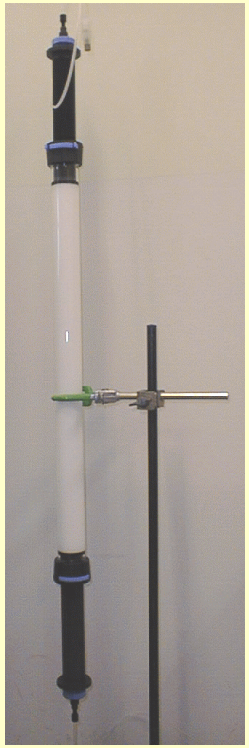
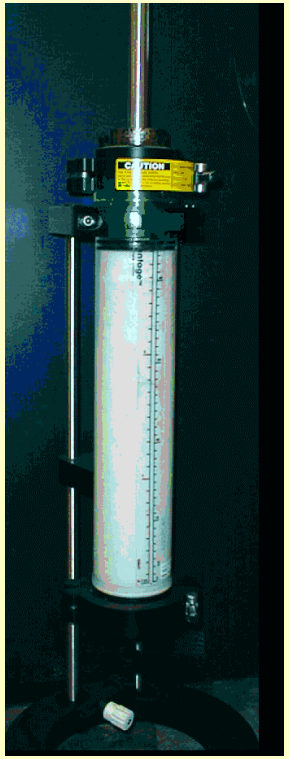
Example of Packed Columns



Analytical column
CHP10M
150 x 4.6mm I.D.



Preparative column 1
CHP55A
500 x 32mm I.D.
(Max. Vol.: 400mL)



Preparative column 2
CHP55Y
500 x 90mm I.D.
(Max. Vol.: 3L)

Applications from Patents – Separation of Herbal Drugs

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- Examples of HP20
  - β-Glucuronidase inhibitor from “KEIGAI” used for antitumor agent
    - Jpn. Kokai Tokkyo Koho JP 05043469
    (Tsumura and Co.; Yakult Honsha Co., Ltd., Japan)
  - Saikosaponin from “MISHIMASAIKO” (Bupleurum falcatum L.) for anti-inflammatory
    - Jpn. Kokai Tokkyo Koho JP 09067388 (Shiseido Co Ltd, Japan)
  - Pilocarpine from Pilocarpus jaborandi (Citrus) used for perspiration promoter
    - Jpn. Kokai Tokkyo Koho JP 09188628 (Tsumura and Co., Japan)
  - Mutation inhibitors comprising condensed tannins from Diospyros Kaki Thunb (persimmon)
    - Jpn. Kokai Tokkyo Koho JP 09315985 (Kanebo, Ltd., Japan)
  - Anti-HIV agent from A. hypogaea (peanut)
    - Jpn. Kokai Tokkyo Koho JP 11246431
    (Agency of Industrial Sciences and Technology; Tokiwa Shokubutsu Kagaku Kenkyusho Ltd., Japan)

Applications from Patents – Separation of Herbal Drugs

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- Example of HP21
  - Food for tooth decay prevention from Oolong Tea
    - Jpn. Kokai Tokkyo Koho JP 04178320 (Suntory, Ltd., Japan)
- Example of SP207
  - Xanthone glycosides for improvement of brain functions from “ENSHI”
    - Jpn. Kokai Tokkyo Koho JP 07179487 (Tsumura &; Co, Japan)

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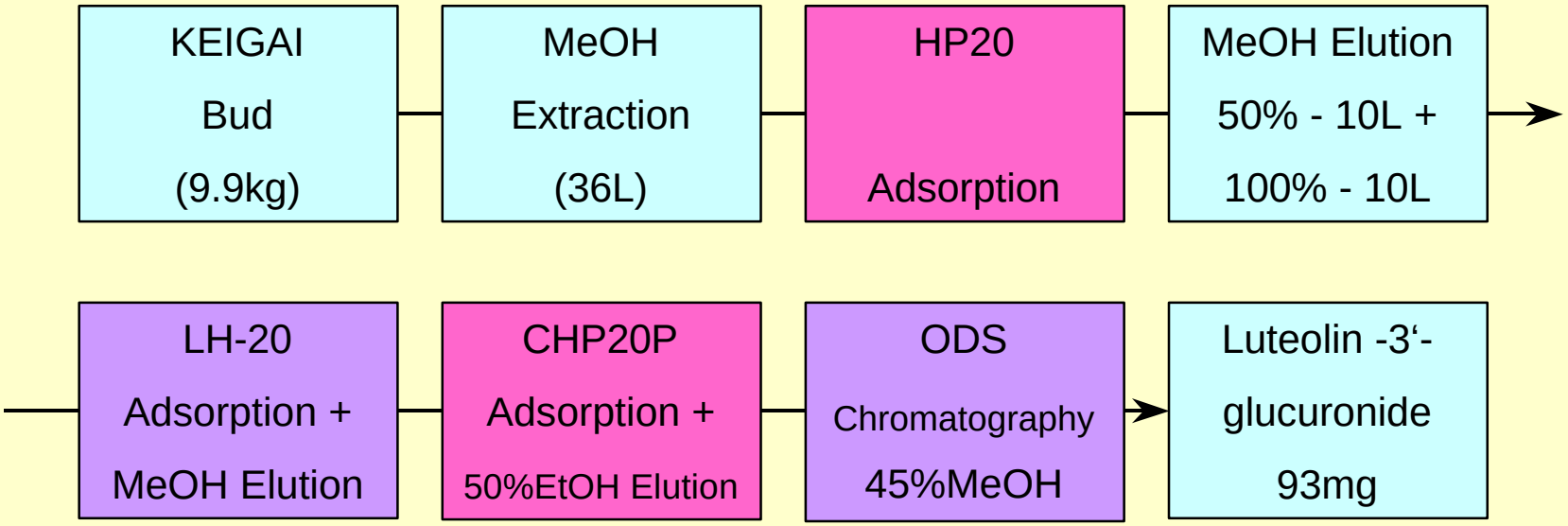

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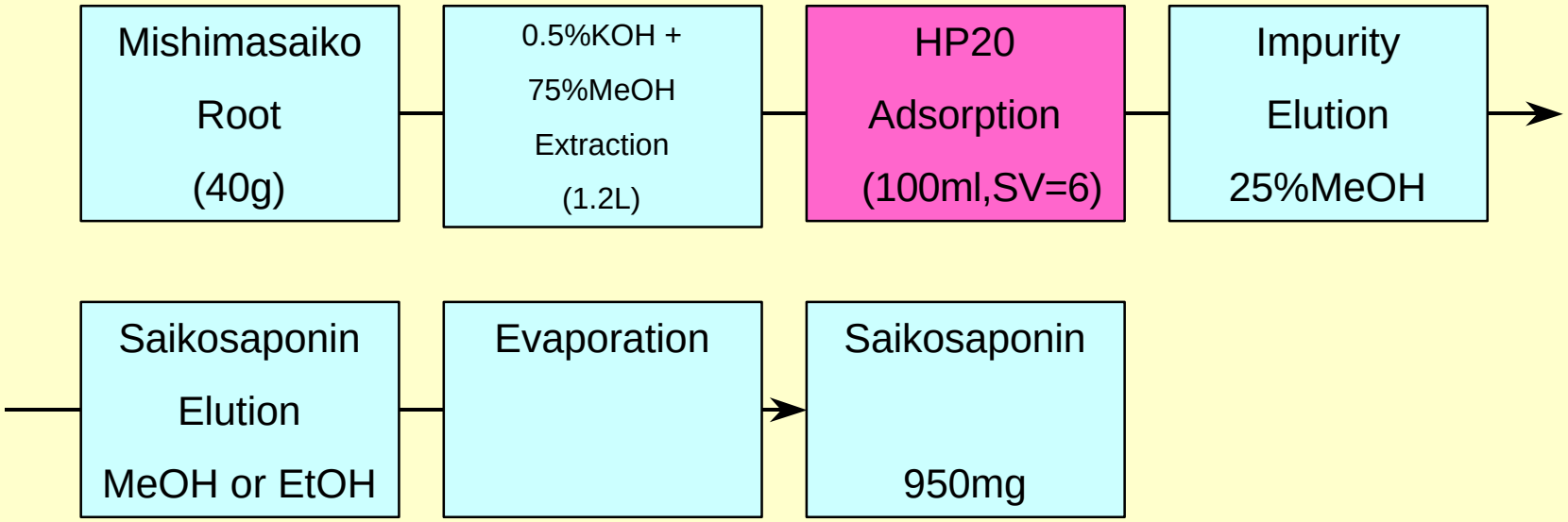
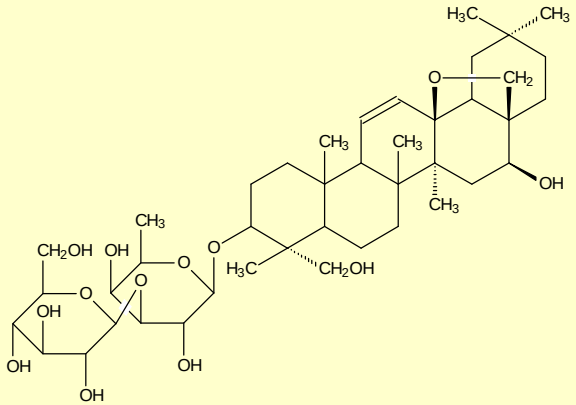
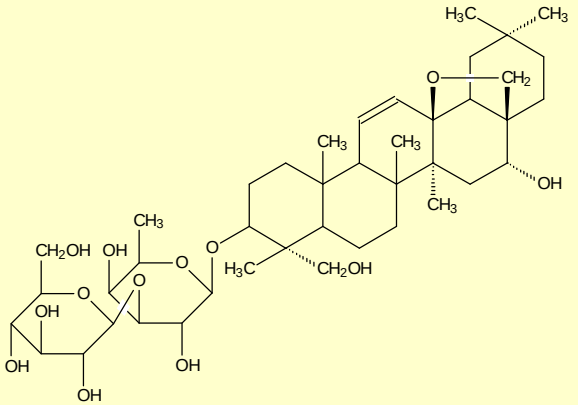


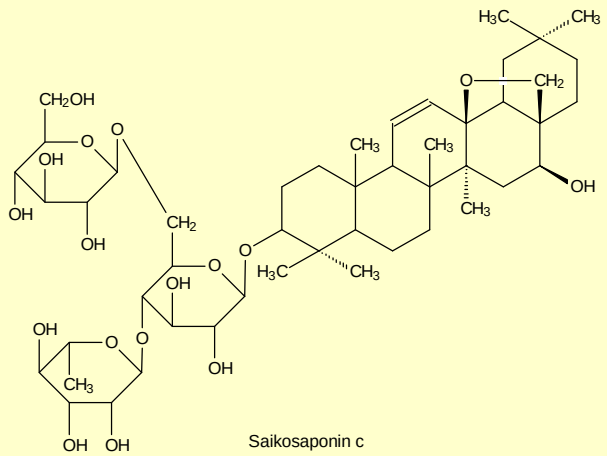
Applications from Patents – Separation of Saikosaponins



Saikosaponin a



Saikosaponin d



Saikosaponin c

Applications from Patents – Separation of Herbal Drugs

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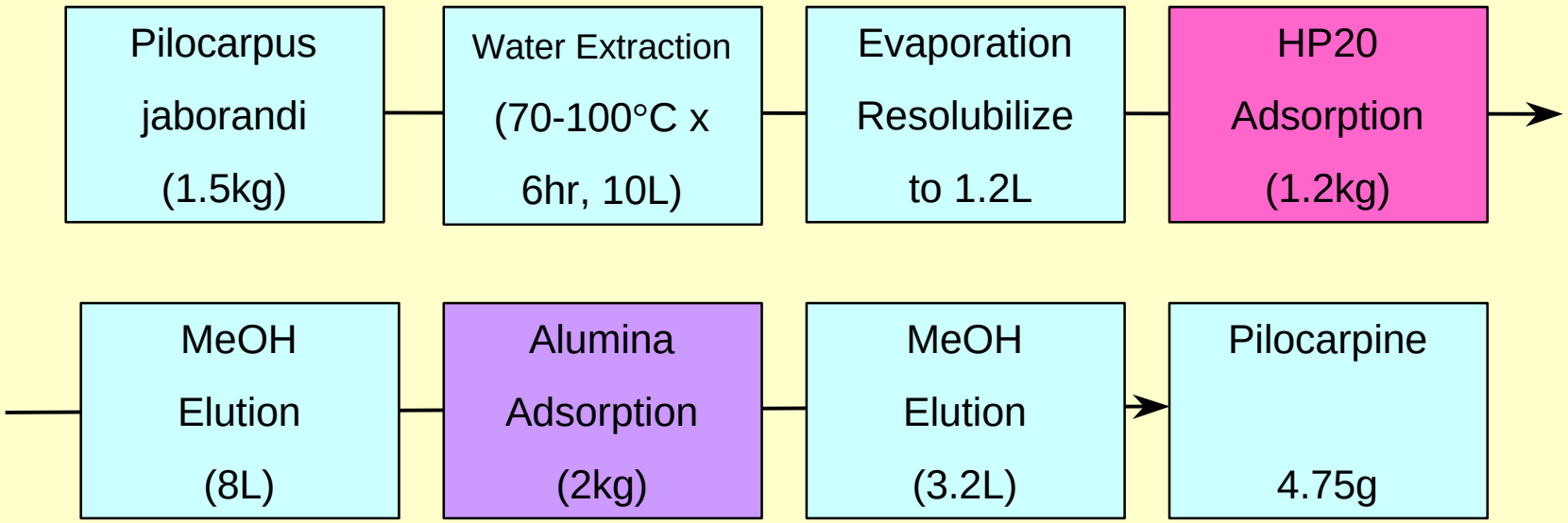

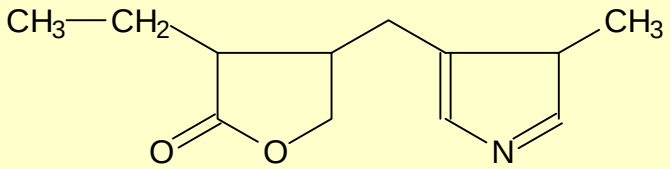

Applications from Patents – Separation of Herbal Drugs

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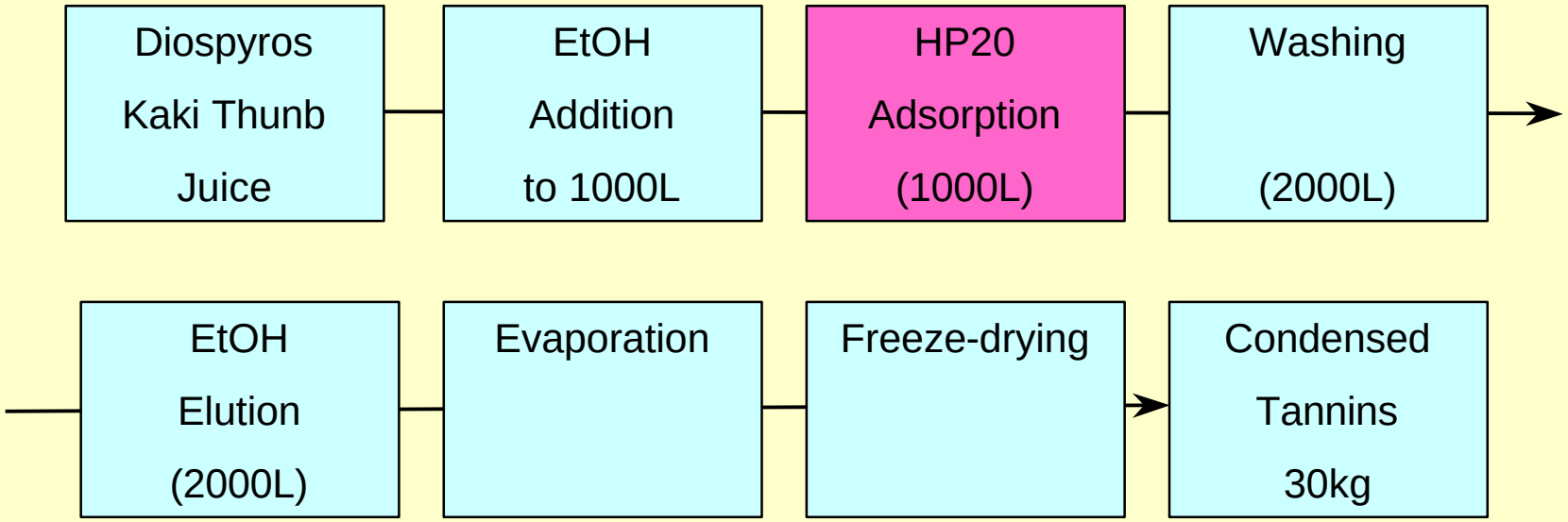
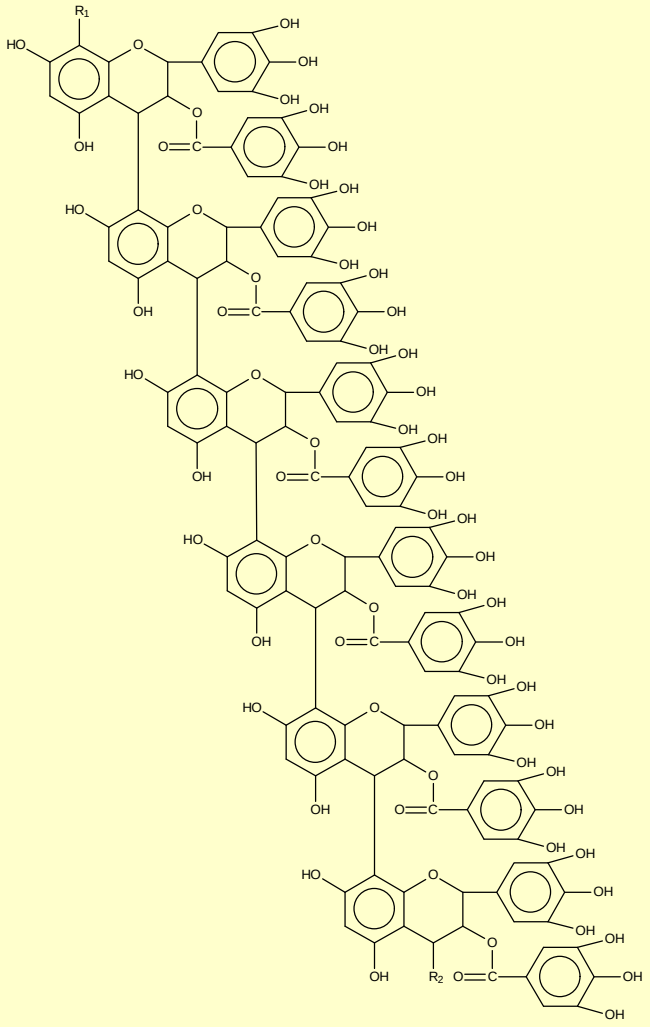


Applications from Patents – Separation of Persimmon Tannins



Applications from Patents – Separation of Herbal Drugs

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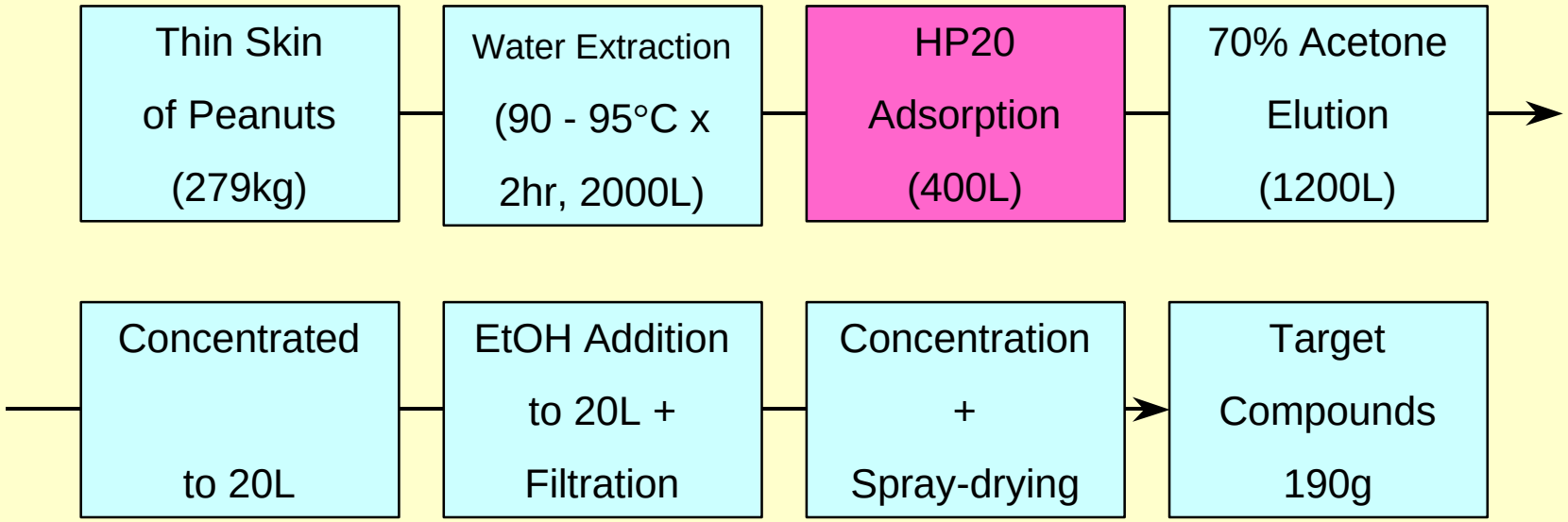

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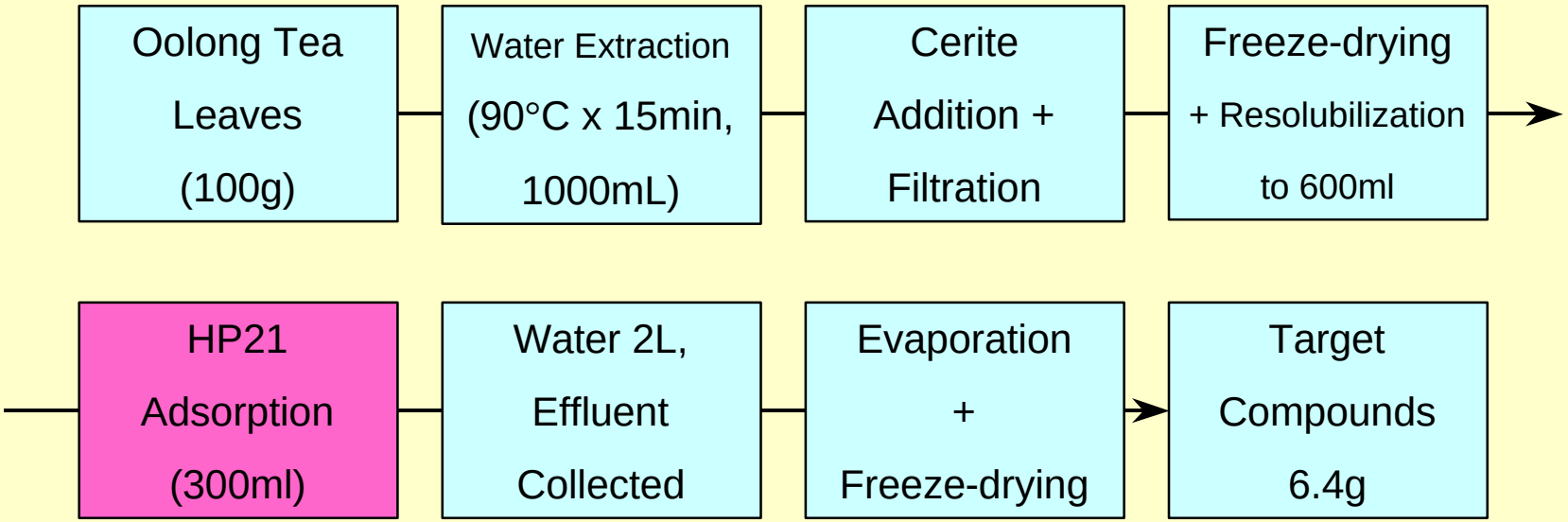

Applications from Patents – Separation of Herbal Drugs

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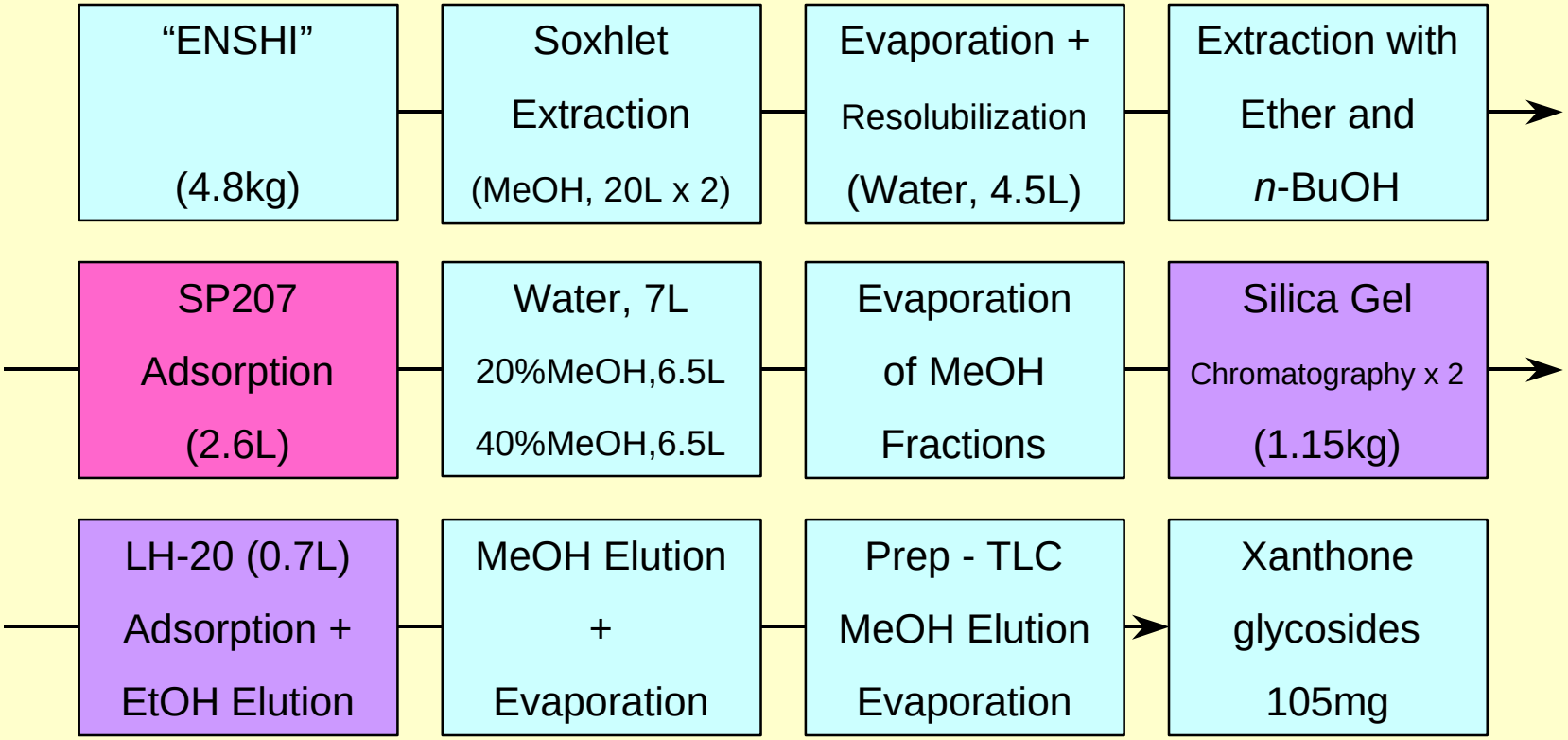
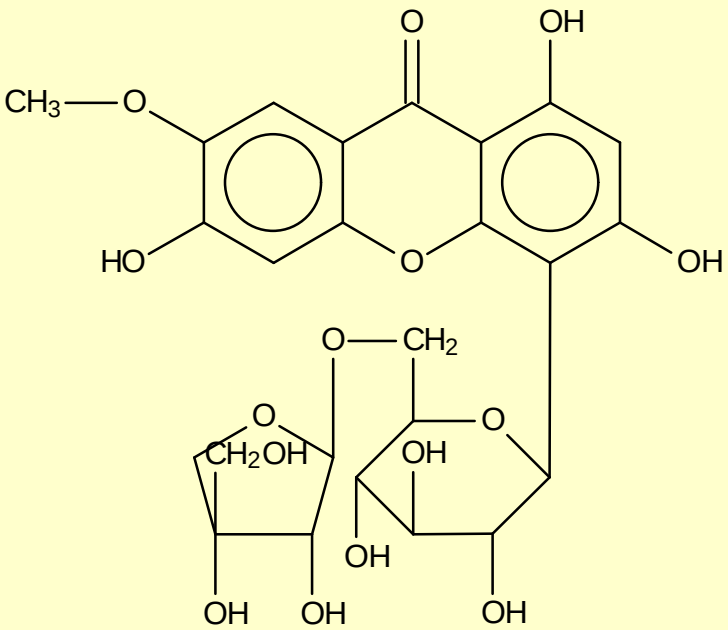


Applications from Patents – Separation of Xanthone Glycoside



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Leachables from Synthetic Adsorbents

- Solvent Extraction
  - GC-MS Analysis
    - Monomers and Impurities of Monomers
    - After MeOH Treatment (5-10BV)
      - No Leachables Detected
- Sepabeads ® SP70
  - FDA Approved Resin (DVB <50ppb)
    - Suitable for Pharmaceutical Use and Food Processing

- Analysis of MeOH Extract from HP20
  - Resin 30ml + MeOH 60ml
  - Extraction: r.t. – 19hr

	m/z	Non-Treated Resin	MeOH Treatment (5BV)	Supposed Structure of the Compound
1	104	5.75mg/L	N.D.	Styrene
2	134	4.58mg/L	N.D.	Diethylbenzene
3	134	8.01mg/L	N.D.	Diethylbenzene
4	134	2.49mg/L	N.D.	Diethylbenzene
5	132	0.62mg/L	N.D.	Ethylvinylbenzene
6	132	0.68mg/L	N.D.	Ethylvinylbenzene
7	132	0.17mg/L	N.D.	Ethylvinylbenzene
8	106	0.35mg/L	N.D.	Benzaldehyde

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References

- ***DIAION ® Manuals I, II***

  - Explanation of Products and Applications
  - Japanese, English and Chinese Version Available

- ***Natural Products Isolation***
  (Edited by R. J. P. Cannell, 1998, Humana Press Inc., Totowa, NJ)
  (ISBN: 0-89603-362-7)

  - Methods of Extraction and Purification from Natural Products
  - Many Applications using DIAION ® or SEPABEADS ®

- ***http://www.diaion.com***

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Information Available on the Web

- ***http://www.diaion.com***

  - Introduction of products from general data to applications

  - You can get more information through E-mail

  - English information available