DIAION™ Technical Manual

Synthetic adsorbents
DIAION™ & SEPABEADS™

Mitsubishi Chemical Corporation

MITSUBISHI CHEMICAL

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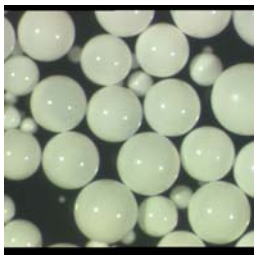
1. What are synthetic adsorbents?

Synthetic adsorbents are made of spherical crosslinked polymer particles that have a porous structure. Adsorption of compounds to synthetic adsorbents mainly results from hydrophobic interactions between the compounds and the synthetic adsorbents. Thanks to special manufacturing technologies, synthetic adsorbents have a highly porous structure, enabling the adsorption of a broad range of target compounds in large quantities.



DIAION™ HP20

Because of similarities in pore structure and adsorption mechanism, synthetic adsorbents are often compared to activated carbons. However, there are significant differences between synthetic adsorbents and activated carbons. First, synthetic adsorbents are produced by polymerization, so pore sizes ranging from several tens to several hundreds of angstrom can be precisely controlled. This feature enables the production of synthetic adsorbents with the ability to selectively adsorb a target compound by size exclusion. In addition, synthetic adsorbents can more easily elute the adsorbed compounds than activated carbons; furthermore, synthetic adsorbents are not only used for removal of target compounds but also widely used for separation processes. Moreover, synthetic adsorbents usually have a longer useful lifespan compared to that of activated carbons.

Synthetic adsorbents are often compared to alkyl-bonded silica gels; however, synthetic adsorbents are very resistant to acidic and caustic conditions. Because of this characteristic, a variety of separation conditions can be applied to synthetic adsorbents, and unlike alkyl-bonded silica gels, almost no contamination of dissolved matter from synthetic adsorbents occurs. Moreover, synthetic adsorbents can tolerate caustic sanitization that cannot be applied to alkyl-bonded silica gels.

2. Application areas of synthetic adsorbents

Many compounds can be separated by use of synthetic adsorbents, for example, extracts from plants such as herbal drugs and natural pigments, fermented antibiotics such as penicillins and cephalosporins, peptides such as insulin, proteins, vitamins, nutraceuticals such as polyphenols, and more.

One characteristic of the target compound to be adsorbed to synthetic adsorbents is that it must be moderately soluble in water. For example, many amino acids and saccharides are highly soluble in water, so only a small amount of the target compound is adsorbed to the synthetic adsorbent. In this case, the target compounds are likely to be subject to ion exchange, or size exclusion separation. In contrast, compounds that have poor solubility in water can be treated with organic solvents, so solvent extraction or normal phase separation processes can be applied. In some cases of normal phase separation, polymethacrylic adsorbents can be used.

3. Fundamental characteristics of synthetic adsorbents

Mitsubishi Chemical Corporation produces industrial synthetic adsorbents that have the basic features listed in the table below.

Product Name	SP850	SP825L	SP70	SP700	HP21	HP20	SP207	HP2MGL
Water content	46-52%	52-62%	57-67%	60-70%	50-60%	55-65%	43-53%	55-65%
Particle size on 250 μm	90% min.	95% min.			90% min.			99% min. on 355 μm
Effective size (mm)	0.25 min.							0.40 min.
Uniformity coefficient	1.6 max.							1.6 max.
Specific surface area (m^2/g)	930	930	870	1200	640	590	600	570
(m^2/mL)	320	290	220	280	190	160	250	150
Pore volume (mL/g)	1.1	1.4	1.5	2.1	1.3	1.3	1.0	1.3
Pore radius (Angstrom)	45	70	70	90	110	290	110	240
Cephalosporin C Adsorption (g/L)	86	76	60	76	48	38	118	<10

4. Chemical structures of synthetic adsorbents

There are three types of synthetic adsorbents produced by Mitsubishi Chemical Corporation; these are shown in Fig. 1.

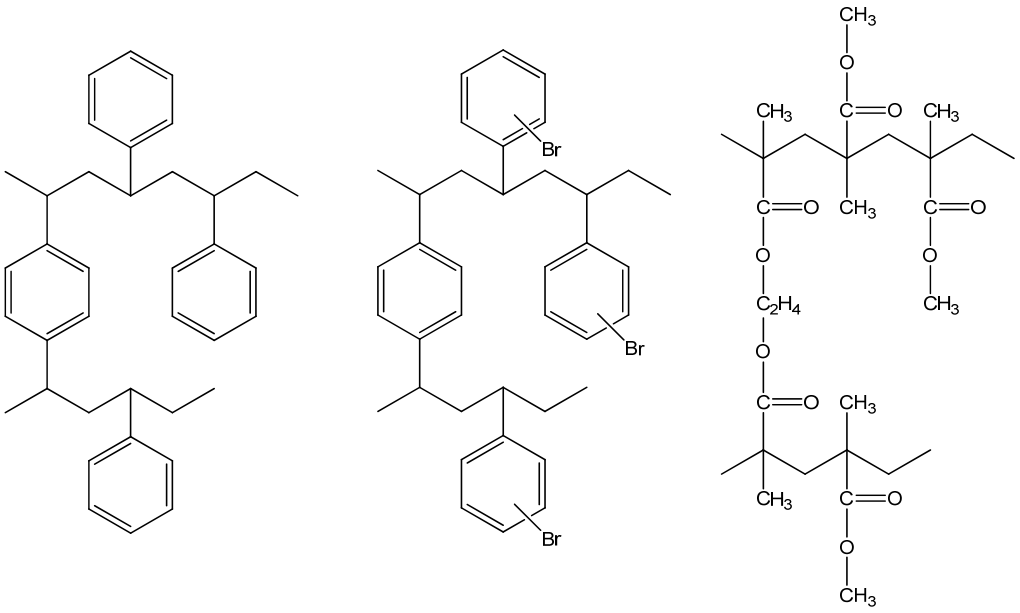


Fig. 1. Chemical structures of synthetic adsorbents.

4.1 Polystyrenic synthetic adsorbents

Synthetic adsorbents made from poly(styrene-divinylbenzene)* are the most widely used. For industrial purposes, the products are DIAION™ HP20 and HP21 and SEPABEADS™ SP70, SP700, SP825, and SP850. DIAION™ HP20SS and SEPABEADS™ SP20SS that have small particle sizes are used for precise separation at an industrial scale. Furthermore, other synthetic adsorbents include MCI GEL™ CHP20/C04, CHP20/C10, CHP20/P20, 30, 50, and CHP50/P20 and 30, and these are typically used for analytical and preparative chromatographic separation. Therefore, the most suitable grade with optimum particle size and a pore structure tailored to the separation demands can be chosen. The following tables show particle size grades of synthetic adsorbents that have similar pore characteristics.

Grade	Type	Size distribution	Referential average diameter	Referential peak radius
CHP20/C10	Polystyrenic	monodisperse	10 μm	17.0 nm
CHP20/P20	Polystyrenic	15-25 μm	18 μm	26.0 nm
CHP20/P30	Polystyrenic	25-35 μm	30 μm	22.0 nm
CHP20/P50	Polystyrenic	35-75 μm	55 μm	30.0 nm
SP20SS	Polystyrenic	63-75 μm	70 μm	29.0 nm
HP20SS	Polystyrenic	63-150 μm	100 μm	30.0 nm
HP20	Polystyrenic	250-600 μm	440 μm	30.0 nm

Grade	Type	Size distribution	Referential average diameter	Referential peak radius
CHP20/C10	Polystyrenic	monodisperse	10 μm	17.0 nm
CHP50/P20	Polystyrenic	15-25 μm	18 μm	14.0 nm
CHP50/P30	Polystyrenic	25-35 μm	30 μm	14.0 nm
HP21	Polystyrenic	250-600 μm	440 μm	12.0 nm

4.2 Chemically modified polystyrenic synthetic adsorbents

SEPABEADS™ SP207, and MCI GEL™ CHP07/C04 and CHP07/C10 have bromine functionality attached to the poly(styrene-divinylbenzene)** main chain. The electron accepting effect of the added bromine increases the hydrophobicity of the chemically modified synthetic adsorbents; consequently, they adsorb many compounds very strongly. In contrast, due to the strong adsorption, elution from the chemically modified synthetic adsorbents can be difficult, and either a greater quantity of eluent or a larger proportion of organic solvents must be used in the elution processes.

Another feature of chemically modified synthetic adsorbents is their high specific gravity. The

specific gravity of SEPABEADS™ SP207 is ca. 1.2. This suppresses floatation of the resin during feeding of high-density liquids, maintaining separation performance. Furthermore, in the case of feed solutions such as fermentation broths that contain suspended matter, countercurrent flow operation using chemically modified synthetic adsorbents eliminates suspended material from the resin tower. This method is known as “expanded bed adsorption.” [1)]

4.3 Polymethacrylic synthetic adsorbents

Polymethacrylic synthetic adsorbents have an entirely different chemical structure from the above polystyrenic synthetic adsorbents. Among the adsorbents of this type, DIAION™ HP2MG and SEPABEADS™ SP2MGS are used for industrial separation, while MCI GEL™ CMG20/C04, C10, and CMG20/P30 are used for analytical and preparative chromatographic separations. As shown in Fig. 1, polymethacrylic synthetic adsorbents contain ester groups, and these adsorbents are more polar than polystyrenic synthetic adsorbents. Consequently, the adsorption characteristics of polymethacrylic synthetic adsorbents toward compounds with functional groups that can form hydrogen bonds are expected to be different from those of polystyrene based adsorbents. For example, DIAION™ HP2MG shows high adsorption capacity to insulin (p34, Fig. 22).

Grade	Type	Size distribution	Referential average diameter	Referential peak radius
CMG20/C10	Polymethacrylic	9-11 μm	10 μm	20.0 nm
CMG20/P30	Polymethacrylic	25-35 μm	31 μm	23.0 nm
SP2MGS	Polymethacrylic	monodisperse	146 μm	25.0 nm
HP2MGL	Polymethacrylic	355-850 μm	490 μm	20.0 nm

5. Pore structure of synthetic adsorbents

The specific surface area, pore size, and pore volume are typically used as indicators of pore structure of synthetic adsorbents,

5.1 Specific surface area

Specific surface area is measured by nitrogen adsorption. Compounds are adsorbed onto the surface of synthetic adsorbents due to hydrophobic interactions, and the adsorption amount is proportional to the specific surface area. For example, the relationship between the specific surface area of polystyrenic synthetic adsorbents and equilibrium binding capacity of cephalosporin C (M_w = 415) is plotted in Fig. 2.

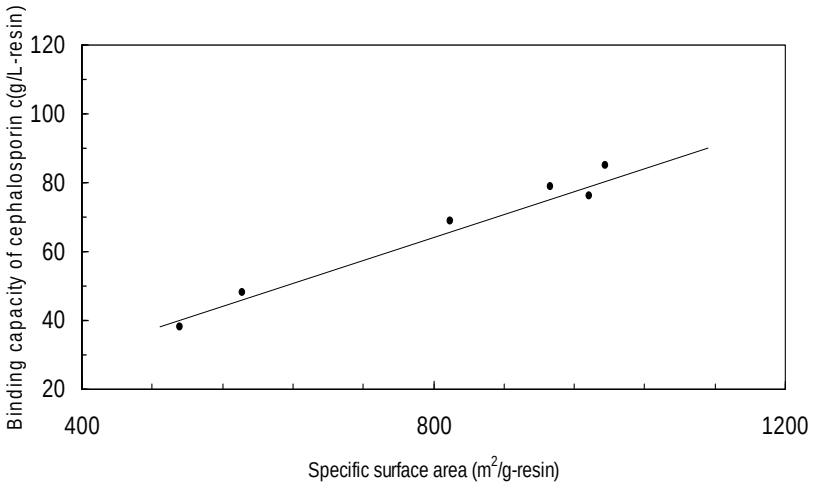


Fig. 2. The relationship between the specific surface area of polystyrenic synthetic adsorbents and adsorption amount of cephalosporin C.

5.2 Pore size and pore size distribution

In addition to the fundamental relationship between specific surface area and adsorption amount,

the pore size of synthetic adsorbents greatly affects adsorption amount of compounds. Pore size and pore size distribution are measured by nitrogen adsorption method or mercury intrusion method. Examples of pore size distributions of synthetic adsorbents are shown in Fig. 3.

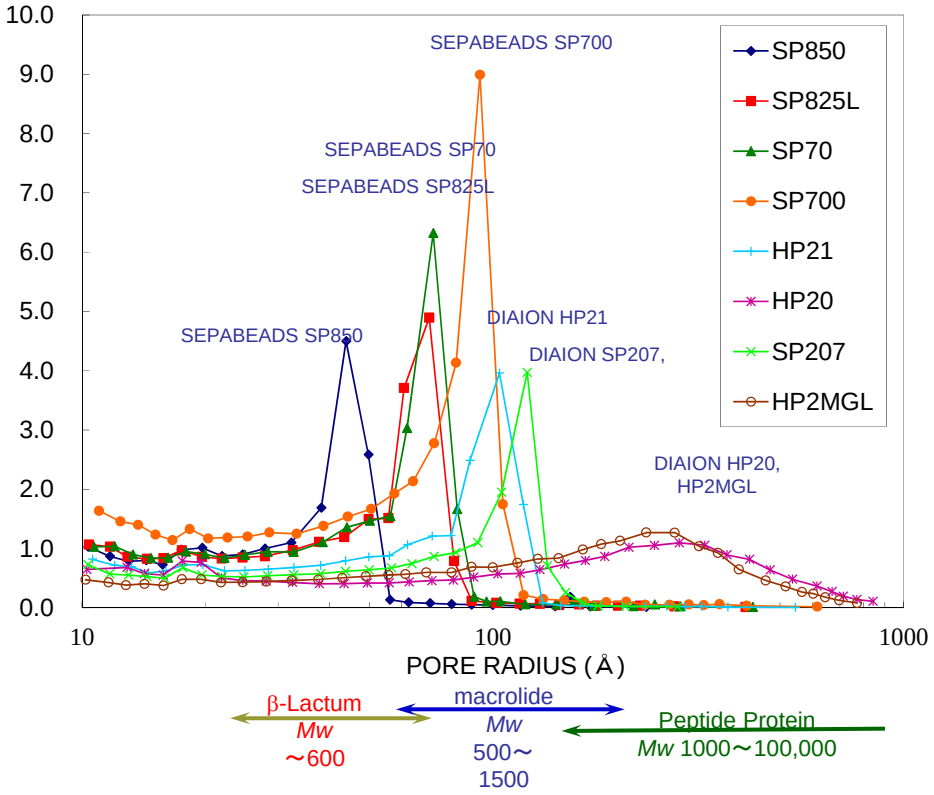


Fig. 3. Pore size distributions of several synthetic adsorbents.

As described before, the pore sizes of synthetic adsorbents can be precisely controlled and range between several tens and several hundreds of angstroms. Therefore, by optimizing the pore size according to the molecular weight of target compounds, we can maximize adsorption amount of the target compounds. Moreover, by using small pore size synthetic adsorbents, selective adsorption of small molecular weight compounds from feed solutions containing large molecular weight impurities such as proteins is possible.

As an example, the relationship between the adsorption of tetracycline (M_w = 481), rifampicin (M_w = 823), and insulin (M_w = 5734) at an equilibrium concentration of 500 ppm and the peak pore radius of polystyrenic synthetic adsorbents is plotted in Fig. 4. In the case of low molecular weight

compounds such as tetracycline, the adsorption amount is proportional to the specific surface area of the synthetic adsorbents. As the pore size of a synthetic adsorbent increases, its specific surface area decreases. Therefore, synthetic adsorbents with large pore sizes adsorb lower amounts of smaller molecular weight compounds. In contrast, in the case of larger molecular weight compounds such as insulin, their diffusion into the smaller pore of synthetic adsorbents is restricted (size exclusion effects). Thus, adsorption of insulin by adsorbents with small pore size is almost zero. However, synthetic adsorbents with larger pore sizes have a significant number of pores that insulin can diffuse into; consequently, they can adsorb large quantities of insulin. In the case of compounds such as rifampicin, which is moderately sized, as the pores in the adsorbent become larger, the amount of the target material adsorbed increases because the number of accessible pores also increases. However, when the pore size exceeds a certain value, the effect of the decrease in the specific surface area becomes significant. Therefore, the adsorption profile of moderate molecular size compounds such as rifampicin is parabolic.

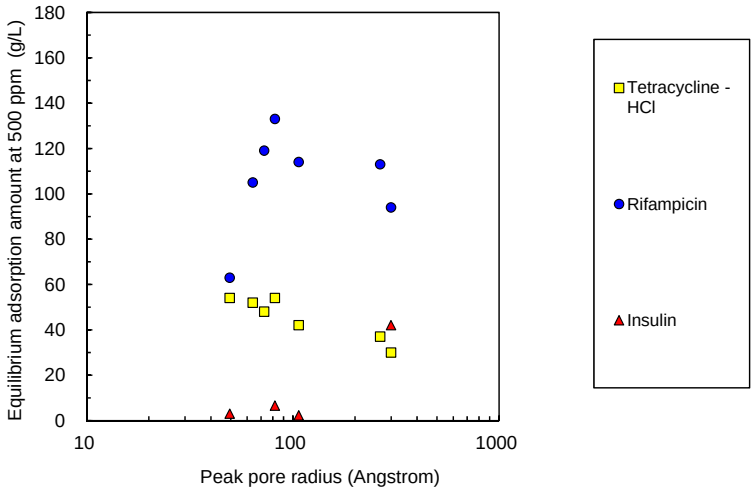


Fig. 4 Relationship between peak pore radius of polystyrenic synthetic adsorbents and equilibrium adsorption amounts of various pharmaceutical compounds (5℃, equilibrium concentration: 500 ppm)

From above results, if the selective separation of smaller size molecules from feed solutions containing proteins is required, synthetic adsorbents with smaller pore sizes such as SEPABEADS™ SP series are recommended. In contrast, synthetic adsorbent with a larger pore size, such as the DIAION™ HP series, will be suitable for the separation of larger molecules.

In summary, highly porous synthetic adsorbents have large pore volumes, but in the case of synthetic adsorbents with only small pores, a large specific surface area is available despite the relatively small pore volumes.

6. Particle sizes of synthetic adsorbents

Even if synthetic adsorbents with similar chemical and pore structures are used, there remain some effects of the particle size on the material dynamic properties. In the case of batch adsorption processes, the time required for diffusion of adsorbates into the core of the synthetic adsorbents becomes shorter as particle size decreases, and the usage of smaller synthetic adsorbent particles enables shorter operation times. In the case of separation by column chromatography, theoretical plate number is inversely proportional to the square of particle size and resolution is inversely proportional to particle size; As a consequence, precise separation can be achieved by the use of synthetic adsorbents with small particle sizes.

For the precision separation required by industry, synthetic adsorbents such as DIAION™ HP20SS (63–150 μm) and SEPABEADS™ SP20SS (63–75 μm) are used. In addition, the MCI GEL™ series is used for analytical and preparative chromatographic separations. In particular, HPLC packed columns with 4–μm uniformly sized synthetic adsorbents such as CHP20/C04 and CMG20/C04 offer high theoretical plate numbers and suitable for analysis.

In contrast, when smaller sized synthetic adsorbents are used for batch operations, time for sedimentation or filtration will be longer. Furthermore, in the case of column chromatographic separations, the pressure drop will be greater. Pressure drop at identical operation conditions is inversely proportional to the square of particle sizes, so high-pressure-durable equipment such as pumps, columns, and tubing must be used. Consequently, this results in higher separation cost at scale-up due to higher equipment costs. Therefore, the particle size of synthetic adsorbent should be optimized by conducting a feasibility study considering the separation scale, target compound purity, separation cost, and so on.

7. How to use synthetic adsorbents

7-1. Introduction

In the case of actual separation, it is rare that there are only two compounds to be separated, an exception being chiral separation. In most cases, there are more than three compounds, and in some cases, several tens of compounds may be contained in the feed solution. Furthermore, the compounds to be separated have a range of molecular weights and hydrophobicities, making separation quite difficult. In addition, inorganic ions may also be present, but they are typically not adsorbed by synthetic adsorbents, and their removal is easy.

When designing separation systems using synthetic adsorbents, you must determine whether the target compounds should be adsorbed to synthetic adsorbents or not (in the case of a column separation system, selection of an adsorption-desorption system or flow-through system). When the mass of the target compounds is high, a large quantity of synthetic adsorbent will be needed for adsorption processes. In contrast, in the case of selective adsorption of impurities only, the required amount of synthetic adsorbents will be lower, and a cost effective separation process can be developed. However, in this case, it will be difficult to remove impurities that have a lower affinity for the synthetic adsorbents than the target compounds; therefore, precise separation will be difficult.

When the concentration of the target compounds in the feed solutions is low, the target compounds are first adsorbed onto the synthetic adsorbents, followed by desorption from the adsorbents with high purity and high concentration. Furthermore, when the target compounds are adsorbed onto synthetic adsorbents, the target compounds can be separated from hydrophilic and hydrophobic compounds by optimizing separation conditions. Either the relatively hydrophilic compounds are not adsorbed onto the synthetic adsorbents at the feed step, or they are eluted from the synthetic adsorbents earlier. Then, relatively hydrophobic compounds are eluted from the synthetic adsorbents after elution of the target compounds. In fact, this method is used widely without intention. For example, in the case of adsorption/desorption process with aqueous systems, organic solvents are often used for regeneration after the desorption step. This method can be used intentionally, and eluent solutions with different concentrations of organic solvent can be fed into the system stepwise, allowing separation of several target compounds.

When high purity target compounds are required, feed solution conditions are set not to adsorb

both the target compounds and impurities onto the synthetic adsorbent, separating the target compounds and impurities by utilizing small hydrophobicity differences among the target compounds and the impurities. This method is called as elution chromatographic separation method. Elution chromatographic separation method can offer extremely high purity separation, but throughput will be lower.

7-2. Typical operation procedures

A typical operation procedure for the column process is shown in the table below.

	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	2–10 BV	(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.

An example of the operation procedure of the column purification process.

7-3. Methods of adsorption

In the case of adsorption/desorption systems, adsorption conditions should be set to increase the hydrophobicity of the target compounds so that they can be adsorbed onto the synthetic adsorbents.

For example, the hydrophobicity of ionic compounds increases when the pH of the feed solution is such that dissociation is suppressed In the case of zwitterionic compounds, hydrophobicity will increase around the isoelectric point. Moreover, adsorption of compounds will be stronger when salts are added to the feed solution to increase the salting out effect.

In the case of the target compounds that are poorly soluble in water, aqueous-organic solvent mixtures can be used for adsorption, but the concentration of organic solvent should be kept as low as possible to maximize the amount of adsorbed target compound.

7-4. Methods of elution

Elution of compounds from synthetic adsorbents is done by applying opposing conditions to those of the adsorption step. In the case of ionic compounds, pH changes that induce dissociation of functional groups causes an increase in hydrophilicity, leading to desorption from the synthetic adsorbents. In addition, elution by applying aqueous organic solvents mixture with a high concentration of organic solvents or elution by applying pure organic solvents can also be used. In some cases, combinational elution, where both pH and organic solvent concentration are changed, can be used for specific applications.

The optimization of pH, aqueous organic solvent concentration, and type of organic solvents used (among other factors) enables the selective elution of several compounds. In the case of laboratory scale separation, linear gradient elution systems are commonly used. In contrast, in the case of industrial scale separation, stepwise gradient elution systems with several eluents are mainly used due to limitations in equipment design, the reproducibility of process operation, and economics.

In the following example, the elution profiles of cephalosporin C with 30% aqueous isopropanol solution from DIAION™ HP20 and SEPABEADS™ SP207 are shown (see Fig. 5.)

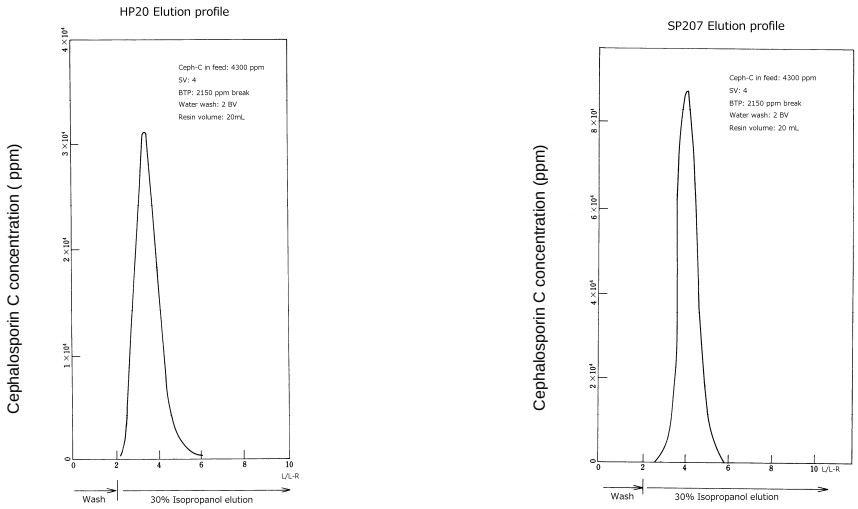


Fig. 5 Elution profiles of cephalosporin C (ceph-C) from synthetic adsorbents (30% aqueous isopropanol solution).

Elution profiles of cephalosporin C (ceph-C) and its derivatives from SEPABEADS™ SP825 and SP850 with 50 mM sodium acetate are also shown in Fig. 6.

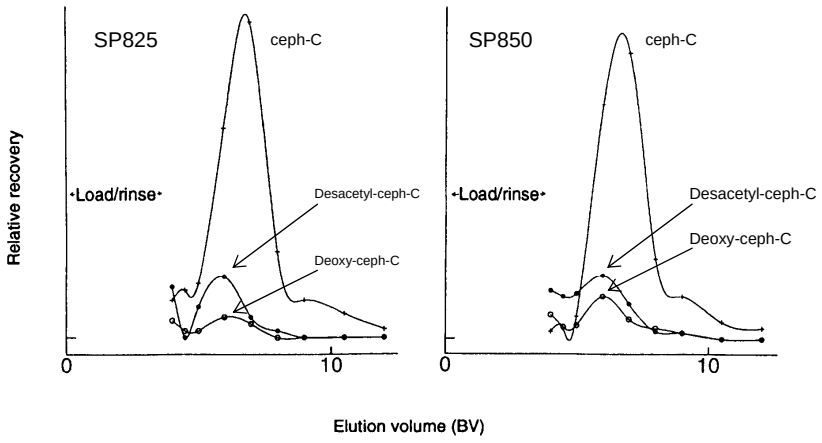


Fig. 6. Elution profiles of cephalosporin C (ceph-C) and its derivatives from SEPABEADS™ SP825 and SP850 with 50 mM sodium acetate

8. Test methods of synthetic adsorbents

8-1. Pretreatment

If synthetic adsorbents have been dried during storage, they may float on water at processing time. To avoid floatation and to effectively wash the adsorbent, they should be immersed in a >50% aqueous solution of alcohol or acetone of more than twice the volume of adsorbent and stirred periodically. A magnetic stirrer should not be used to avoid breakage of synthetic adsorbent particles by the stirrer bar; instead, a swizzle stick should be used. In addition, small particulate matter or broken particles should be removed by decantation. Then, the pretreated synthetic adsorbent is poured into the column, and a volume of water (five times the volume of the adsorbent) should be fed through the column at a space velocity (SV) of five (i.e., five times volume of the synthetic adsorbent volume per hour).

8-2. Batch process test methods

Batch process adsorption tests are an easy method to check whether a target compound can be adsorbed onto a synthetic adsorbent and how much target compound can be adsorbed. Batch process adsorption tests are used for the screening of chemically and physically different types of synthetic adsorbents or screening of adsorption/desorption conditions. However, in some cases, a batch process is used for industrial separations.

In detail, a fixed amount of synthetic adsorbent is placed into a flask(s), and the solution containing the compound(s) is added. Then, the flask(s) is shaken for a fixed period. After shaking, the concentration of the target compound(s) is measured to calculate the adsorption amount. When shaking time is sufficient, the equilibrium adsorption amount can be determined. In addition, dynamic adsorption characteristics can be determined by measuring the change in target compound(s) concentration with time. Furthermore, recovery at desorption can be determined by removing the feed solution from the synthetic adsorbent and adding the eluent solution; then, the flask is shaken for a set period of time, and the concentration of the desorbed target compound(s) is measured. Use of a thermostatic shaker is recommended to avoid the effects of temperature changes on the rate or amount of adsorption.

8-3. Column process test methods

Concerning industrial applications, column test can be scaled-up easily. In the case of column tests, the selection of column size in consideration of actual industrial process is necessary. First, column diameter greater than 20 mm is recommended to avoid turbulence in the adsorption and desorption processes. Second, the column should be as long as feasible (close to the actual length) so that more precise data can be obtained. However, in the case of small-scale experiments, long columns with small diameters may become broken if volume expansion of synthetic adsorbents occurs on contact with organic solvents. Therefore, to avoid column breakage, a column bed height of 50–100 cm is recommended.

In addition, the effective utilization ratio to equilibrium adsorption capacity of synthetic adsorbents decreases on using shorter length columns. Resolution is also reduced when using shorter length columns, but when significant separation is achieved by laboratory testing using shorter length columns, better separation and effective utilization ratios can be expected in actual operations when using longer length columns. An example of column test equipment is shown in Fig. 7.

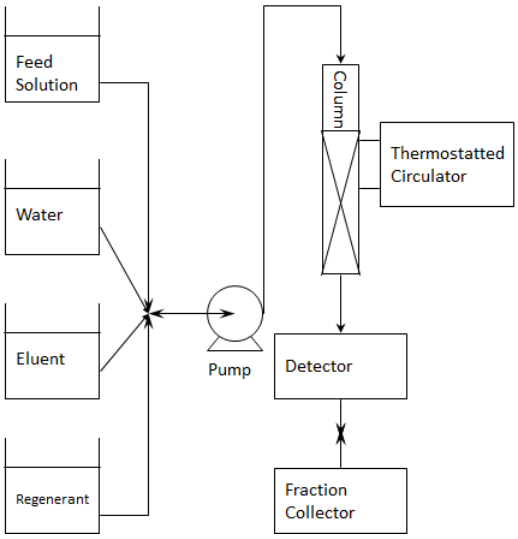


Fig. 7. Example column test equipment.

8-4. Utilization of HPLC

Previously, method development of separation processes using synthetic adsorbents was done by small scale tests using the same industrial synthetic adsorbents with smaller columns. In this case, due to the low resolution obtained with the larger particle sizes of industrial synthetic adsorbents, relatively large columns should be used. Therefore, a large quantity of both sample and eluent will be consumed, and much effort and investment will be required for method development.

To minimize these burdens, Mitsubishi Chemical Corporation provides synthetic adsorbents with small particle sizes but with the same chemical and pore structures as industrial adsorbents; in addition, packed columns are available. HPLC equipment is now widely used, so method development by use of HPLC columns packed with small particle sized synthetic adsorbents is recommended. Utilization of HPLC enables drastic reduction of method development periods. Furthermore, preparative scale purification can be performed by applying the law of similitude.

Both polystyrenic adsorbents CHP20/C04 (4 μm) or CHP20/C10 (10 μm) and polymethacrylic adsorbents CMG20/C04 (4 μm) or CMG20/C10 (10 μm) in packed columns are available for HPLC equipment. Samples containing the target compounds are injected either automatically or manually by a sample injector and separated by the HPLC packed column. To increase the sample load, concentrated sample injection, a large volume sample loop, and/or a sample feed pump should be used. For a two pump gradient HPLC system, both linear gradient elution and stepwise gradient elution can be studied. Confirmation of separation can be carried out by using various chromatographic detection systems such as UV, RI, or fractional analysis of the eluent.

8-5. Regeneration and rejuvenation methods

During actual cyclic operations of synthetic adsorbents, compounds that do not elute from synthetic adsorbents may accumulate. Accumulation of such non-eluted compounds leads to a decrease in both the amount of adsorbed target compound and purity; therefore, a regeneration process must be carried out every cycle or every few cycles so that accumulated compounds can be washed out from the synthetic adsorbents. Furthermore, a harsher rejuvenation processes rather than regeneration process might be applied for a longer period (for example, every tens of cycles or one hundred cycles) than a period that the regeneration process might be applied for.

The table below shows a comparison of the rejuvenation effect in terms of specific surface area. Organic solvents or mixture of aqueous caustic solutions and organic solvents can recover their original specific surface area. In the case of industrial use of synthetic adsorbents, periodical rejuvenation may be important to prolong adsorbent lifetime.

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

Effect of various rejuvenation conditions on the recovery of the original specific surface area of deteriorated synthetic adsorbents.

9. Scale-up methods

9-1. Fundamentals of scale up

In the case of column system equipment designs, the conditions optimized by small scale testing are scaled up by applying the law of similitude. In terms of flow rate, the same SV is used when considering industrial productivity.

In addition, a column length that is too long may cause of compression of the synthetic adsorbent at the base of the column. This compression can reduce the flow rate or increase the pressure drop; consequently, the maximum column height should be less than 4 m. Furthermore, a higher ratio (L/D) of column length (L) and column diameter (D) should not be chosen. Therefore, if a lot of synthetic adsorbents is to be used, a column height should be selected that allows the desired resolution or separation, then an appropriate column diameter should be chosen to allow the required amount of synthetic adsorbent to be used.

9-2. Effects of flow rate

Generally speaking, the flow rate of adsorption/desorption using synthetic adsorbents is set to a SV of 0.5 to 2.0, considering the effective utilization ratio of equilibrium adsorption amount. In the case of column processes, if the flow rate is raised to increase throughput, the diffusion of compounds into the core of the synthetic adsorbents becomes insufficient and effective utilization ratio to equilibrium adsorption amount decreases. The larger the molecular weight of the target compound, the shorter column length, and the smaller the pore size of the synthetic adsorbents, the more evident this tendency becomes.

In the following example, Fig. 8 shows adsorption profiles of vitamin B12 (M_w: 1,355) on SEPABEADS™ SP825 at various flow rates. SP825 has a relatively smaller pore size of the variety of synthetic adsorbents, so when relatively large molecular weight (>1,000) compounds such as vitamin B12 are adsorbed, the effect of flow rate is large, and at a SV of four, the breakthrough capacity at 5% is only 44% of equilibrium adsorption capacity. However, when a SV

of two is used, the effective utilization rate increases to 62%. Throughput itself is better at a SV of four, so flow rate should be optimized in consideration of both purity and productivity.

To demonstrate the effect of flow rate obviously, in this case, synthetic adsorbent with relatively small pore size was used and a compound with a relatively large molar mass was fed at a high flow rate (SV = 4) for a short column length of 20 cm; therefore, the effective utilization ratio to equilibrium adsorption amount was lower. For practical industrial processes, in cases of adsorption of compounds with a molecular weight greater than 1,000, synthetic adsorbents with larger pore sizes such as DIAION™ HP20, HP21, and SEPABEADS™ SP207 should be used selected and longer column heights are necessary so that effect of flow rate on adsorption amount can be minimized.

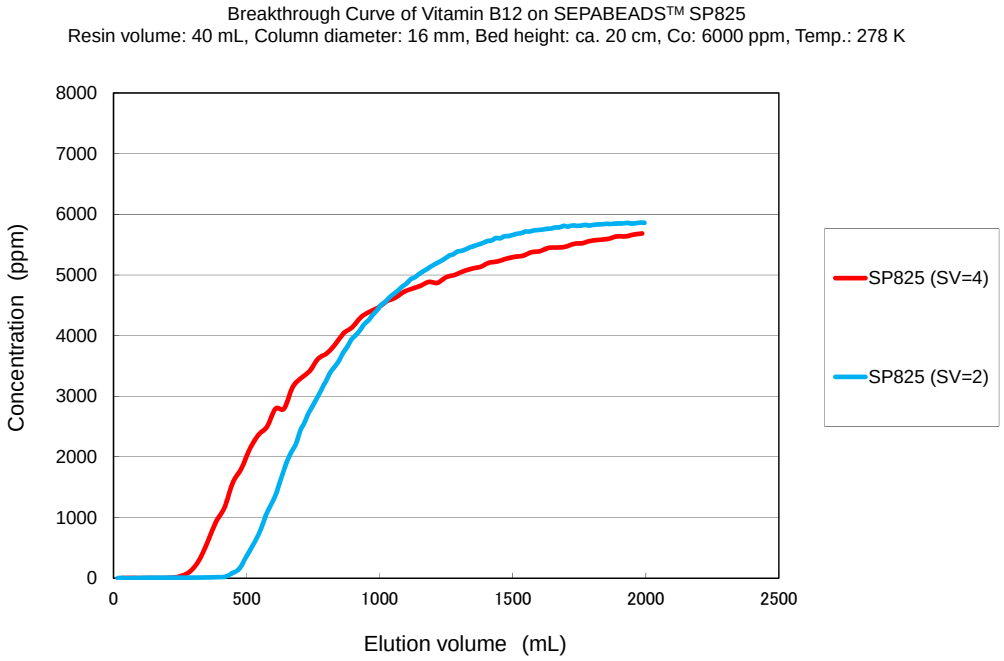


Fig. 8. Effect of flow rate on column adsorption profiles of Vitamin B12 on SEPABEADS™ SP825

Column adsorption profiles of Cephalexin (M_w = 347) on various synthetic adsorbents are shown in Fig. 9. At SV of 4, the breakthrough curve shape becomes steeper for the adsorbents in the following order HP20 > SP700 > SP850 > SP825. SEPABEADS™ SP series show shelving

breakthrough curves due to the smaller diffusion rate of Cephalexin in smaller pores. However, in terms of the dynamic adsorption capacity, the SP series adsorbents have larger values than that of HP20. In addition, in the case of SP825 at a SV of two, a large dynamic adsorption capacity is observed, the largest of the synthetic adsorbents tested at various flow rates. Thus, it is vital that the chosen grade of the synthetic adsorbent is appropriate for the operation conditions, allowing the total throughput of the separation process to be maximized.

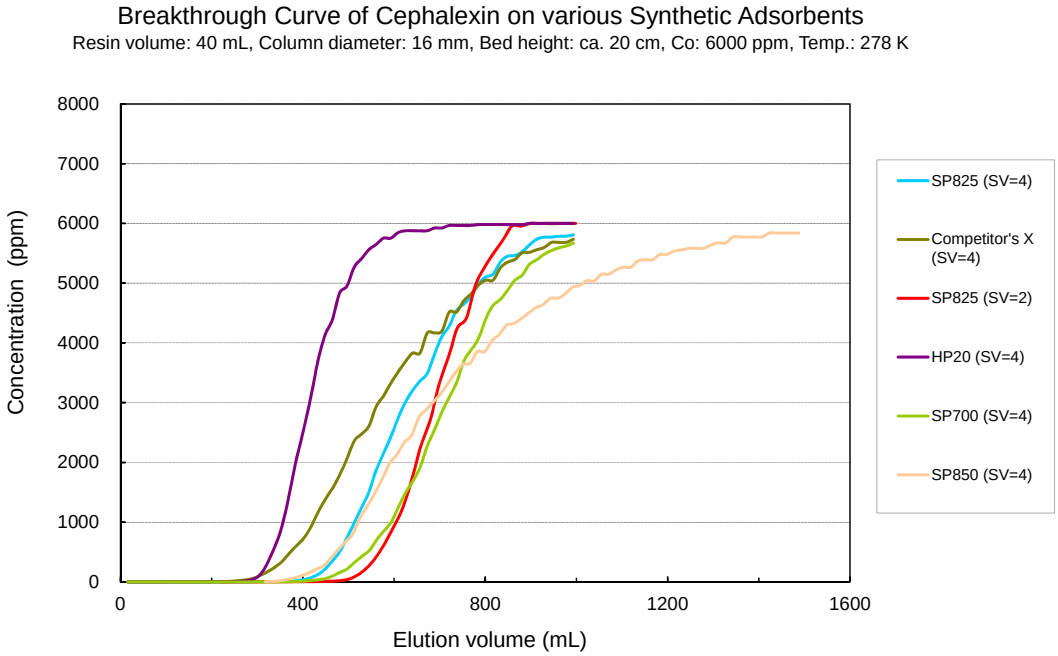


Fig. 9. Column adsorption profiles of Cephalexin on various synthetic adsorbents.

9-3. Effects of particle sizes

As the particle size of synthetic adsorbents becomes smaller, the time required for compounds to diffuse into the core of the synthetic adsorbents decreases, and the utilization ratio to equilibrium adsorption amount of the compounds increases. In addition, in the case of column chromatographic separation, theoretical plate height is inversely proportional to the square of particle size and resolution is inversely proportional to particle size. The theoretical equation of linear gradient elution chromatography[2)] shown below says that in the case of scale up using a synthetic adsorbent with twice the particle size of that used in small scale test, a column length four times longer or

one-quarter of the linear velocity of the flow rate should be applied to obtain the same resolution.

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

Rs: resolution, L: column length,

LV: linear velocity, dp: particle diameter,

GH: gradient slope per unit column volume

9-4. Pressure drop

In actual industrial processing using synthetic adsorbents, pressure drop significantly affects equipment costs. Although synthetic adsorbents are made of a hard polymer matrix and are, thus, resistant to compression at high flow rates, careful attention should be paid when small sized synthetic adsorbents are used at high flow rates of viscous solutions because the pressure drop is inversely proportional to square of particle size and proportional to flow rate and viscosity of the solution. Figure 10 shows hydraulic properties of typical synthetic adsorbents.

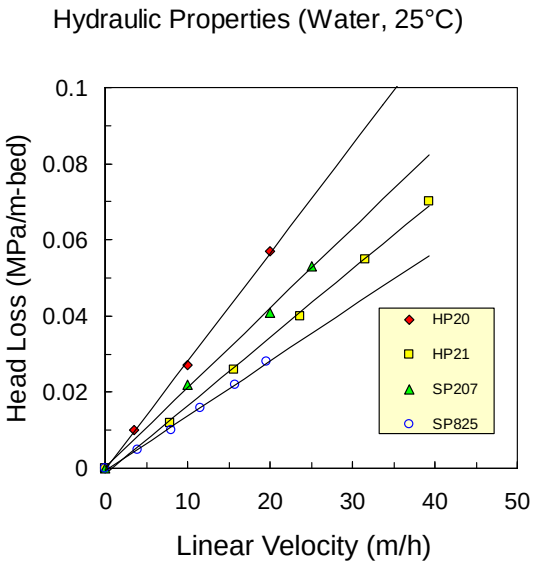


Fig. 10. Hydraulic properties of various synthetic adsorbents (water at 25 °C).

9-5. Effects of resin swelling and shrinkage

Due to the affinity of synthetic adsorbents for organic solvents, swelling of the absorbent particles occurs in organic solvents. Therefore, elution with organic solvents leads to bed height expansion; consequently, the column length should be set in consideration of bed height expansion (in addition, extra column length is also required for backwashing). Furthermore, volume changes affect the level of liquid in the column and may affect separation performance; therefore, the control of eluent feed position and liquid level is important.

In cases where fixed packed bed columns are used, it is important to use columns that can resist the high pressures generated by the swelling of synthetic adsorbents.

These days, height adjustable columns, in which the top of the column moves in accordance with the volume change of synthetic adsorbents, are available. Using this type of column, extra diffusion created by the space between resin top and liquid collector and uneven flow across the column are eliminated, enabling greater separation.

In Fig. 11, the swelling characteristics of synthetic adsorbents for various organic solvents are depicted.

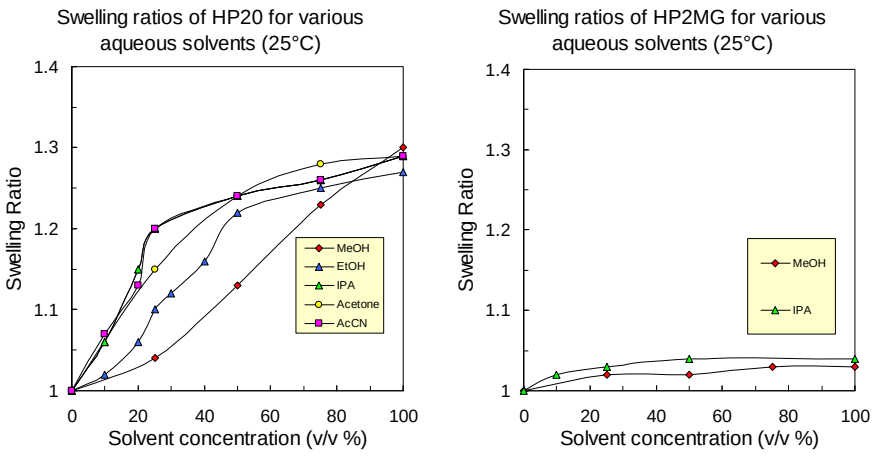


Fig. 11. Swelling characteristics of polystyrenic (DIAION™ HP20) and polymethacrylic (DIAION™ HP2MG) synthetic adsorbents in various organic solvents solutions (25 °C, HP2MG is a different particle size grade of HP2MGL).

10. Specific usage of synthetic adsorbents

10-1. Normal phase applications

In previous chapters, separation with aqueous solution systems has been mentioned. However, in some cases, target compounds are extracted with non-aqueous solvents and separated by use of silica gels. This is known as “normal phase separation.” Normal phase separation is widely used in laboratory scale separation, but due to the significant fluctuations in adsorption or retention due to changes in the water content of silica gels and the lack of reproducibility of silica gels, it is not widely used for industrial applications.

When synthetic adsorbents are used in nonpolar solvents such as hexane, the polarity of the synthetic adsorbent is greater than that of the solvents and a greater quantity of polar compounds can be adsorbed onto the synthetic adsorbents. The adsorbed compounds can be desorbed by feeding polar solvents such as ethanol through the adsorbent bed.

In the case of normal phase adsorption, polymethacrylic synthetic adsorbents have ester groups with greater polarities than those of polystyrenic adsorbents; consequently, the adsorption force is greater in polymethacrylic synthetic adsorbents.

Figure 12 shows capacity factors of alkyl phthalates chromatographed on MCI GEL™ CMG20/C10 HPLC column packed with a polymethacrylic synthetic adsorbent, MCI GEL™ CHP5C HPLC column packed with polystyrenic synthetic adsorbent (substitute is CHP20/C10) and HPLC column packed with silica gel. The polymethacrylic synthetic adsorbent shows greater retention than both polystyrenic synthetic adsorbent and silica gel.

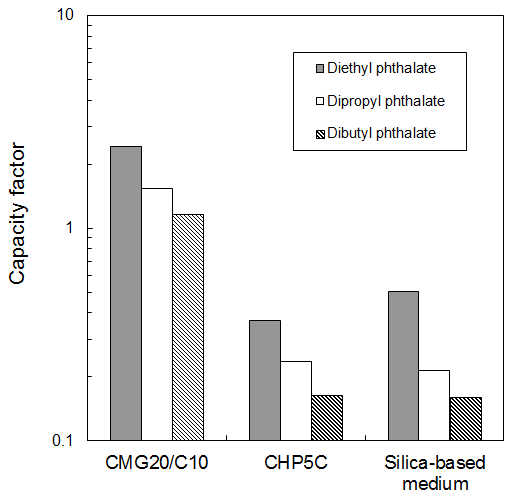


Fig. 12. Capacity factors of alkyl phthalates on HPLC columns packed with various adsorbents (hexane/*iso*-PrOH = 90/10)

As an example of an application, the preparative separation of tocopherols and tocotrienols on polymethacrylic synthetic adsorbent MCI GEL™ CMG20/P30 is shown in Fig. 13. When polymethacrylic synthetic adsorbents are used for normal phase chromatography, separation utilizing the differences in polarity between tocopherols and tocotrienols is possible. By using synthetic adsorbents for normal phase separations, fluctuations in adsorption or retention due to the change in water content or problems in batch to batch reproducibility can be eliminated; therefore, their use seems highly suited to industrial applications.

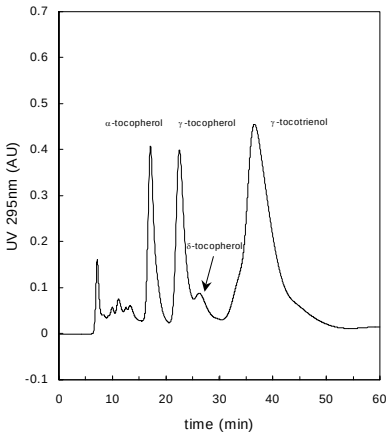


Fig. 13. Preparative separation of tocopherols and tocotrienols on polymethacrylic synthetic adsorbent MCI GEL™ CMG20/P30 (hexane/EtOH = 98/2).

10-2. Gas phase applications

The specific surface areas of synthetic adsorbents are quite large, and they can adsorb volatile organic compounds in the gas phase. Please ask for details.

11. Examples of synthetic adsorbent applications

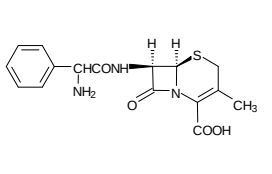
Examples of synthetic adsorbent applications from patent information are listed in the table below. Synthetic adsorbents are used in a broad range of application areas.

Grade	Target compounds	Reference
HP20	Glycyrrhizic acid (licorice root)	Shipin Yu Fsjio Gongye, 25, p40 (1999).
HP20	Saponin (Korean ginseng)	J. Ginseng Res., 22, p155 (1998).
HP20	Cephalexin (Fermentation broth)	Lizi Jiaohuan Yu Xifu, 14, P18 (1998).
HP20	Isoflavone glycoside (Soy bean)	JP H11-255792.
HP20	Cancer cell growth-inhibiting factor (Citrus)	J. Agric. Food Chem., 47, P2509 (1999).
HP20	Novel macrolide (Fermentation broth)	Proceedings of Natural Organic Compounds Conference, 39, P595 (1997).
HP20	Phenethyl alcohol (Fermentation broth)	USP 5,919,991.
HP20	Ginsenosides (Korean ginseng)	J. Ginseng Res., 22, p284 (1998).
HP20	Novel nucleoside	Sanop Misaengmul Hakhoechi, 26, p558 (1998).
HP20	Daunomycin (Fermentation broth)	WO 9911650.
HP20	Novel macrolide (Fermentation broth)	JP H11-35578.
HP20	Novel cyclic peptide (Fermentation broth)	JP H11-29595.
HP20	Novel antifungal (Fermentation broth)	J. Antibiot., 51, p1081 (1998).
HP20	Tannin (Herbal drug)	Tianran Chanwu Yanjiu Yu Kaifa, 10, p14 (1998).
HP20	Flavonoid (Citrus)	J. Agric. Food Chem., 47, p128 (1999).
HP20	Hydroxy naphthalene sulfonic acid	JP H10-265434.
HP20	Triterpene	JP H10-245394.
HP20	Saponin derivatives (Barley)	WO 9838198.
HP20	Phytohormone (Corn)	JP H10-194906.
HP20	Fluorostatin (Fermentation broth)	J. Antibiot., 51, p553 (1998).
HP20	Tyrosinase inhibitor (Herb)	J. Food Sci. Nutr., 2, p285 (1997).
HP20	Epostation (Fermentation broth)	J. Antibiot., 51, p253 (1998).
HP20	Novel anthracycline (Fermentation broth)	J. Antibiot., 51, p130 (1998).
HP20	Aroma chemical (Coffee)	JP H10-77496.
HP20	Polyphenol (Grape)	WO 9812189.
HP20	Yucca extract	JP H10-45566.
HP20	Calcium from pilgrim shell (Removal of impurity)	JP H10-14535.
HP20	β-lactamase inhibitor (Fermentation broth)	Yakhak Hoechi, 41, p658 (1997).
HP20	Cellular alteration inhibitor (Persimmon)	JP H9-315985.
HP20	Naphthopyrandione derivatives (Fermentation broth)	JP H9-176160.
HP20	Anti-HIV compound	JP H9-176160.
HP20	Pilocarpine (Herb)	JP H9-188628.
HP20	Novel cyclic peptide (Fermentation broth)	JP H9-227594.
HP20	Food color (Red cabbage)	JP H9-255888.
HP20	Plant saponin	JP H9-175988.
HP20	Phenethyl glucoside	JP H9-87294.
HP20	Curonostatin (Fermentation broth)	J. Antibiot., 50, p105 (1997).
HP20	Dicephalosterol (Fermentation broth)	JP H9-67393.
HP20	Novel polysaccharide (Fermentation broth)	Sanop Misaengmul Hakhoechi, 25, p82 (1997).
HP20	Novel phenolic compound (Herb)	Chin. Pharm. J., 48, p331 (1996).
HP20	Acetyl isoflavone glycoside	JP H8-291191.
HP20	Mitomycin (Fermentation broth)	JP H8-245626.
HP21	Aurapten (Citrus oil)	JP H11-29565.
SP850	Cephalosporin C (Fermentation broth)	Korean J. Chem. Eng., 14, P277 (1997).
SP850	Quinolone carboxylic acid derivative	JP H8-259541.
SP825	Novel peptide	WO 9634011.
HP2MG	Tea polyphenol	JP H5-306279.
HP2MG	Proinsulin	USP 5,952,461

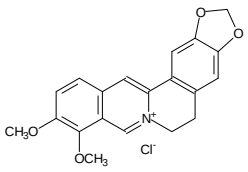
12. Equilibrium adsorption data of synthetic adsorbents

Equilibrium adsorption data of various synthetic adsorbents for various pharmaceutical compounds listed in Fig. 14 are plotted in Figs. 15–22.

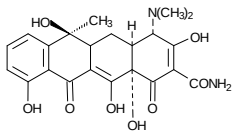
Compound name	Molecular weight	Solution condition	Detection condition
Cephalexin	347	Demineralized water	UV 260nm
Berberine chloride	372	Demineralized water	VIS 416nm
Tetracycline hydrochloride	481	Demineralized water	VIS 356nm
Rifampicin	823	20mM Sodium citrate buffer, pH4.0	VIS 474nm
Vitamin B12	1355	Demineralized water	VIS 360nm
Bovine insulin	5734	50mM Sodium citrate buffer, pH2.5	UV 275nm



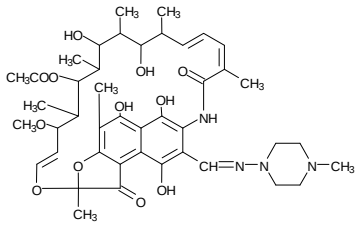
Cephalexin



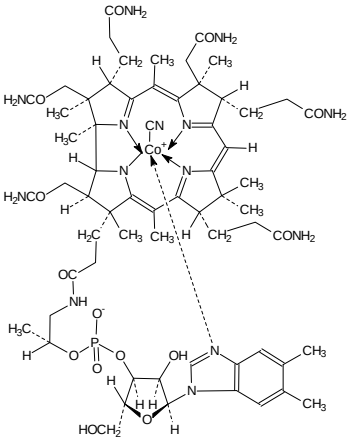
Berberine chloride



Tetracycline hydrochloride



Rifampicin



Vitamin B12

Fig. 14. Chemical structures and adsorption conditions of the pharmaceutical compounds tested.

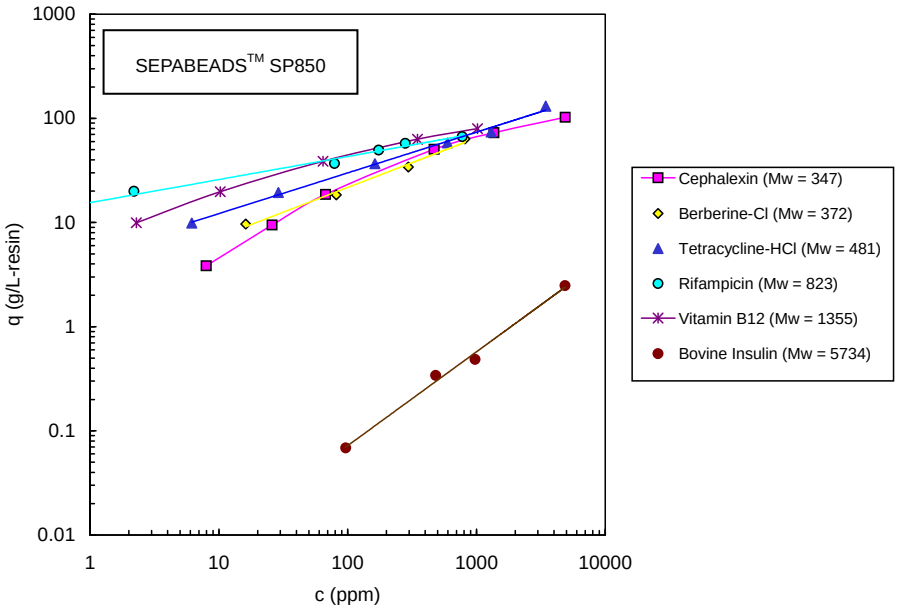


Fig. 15. Equilibrium adsorption data for SEPABEADS™ SP850 (5 °C).

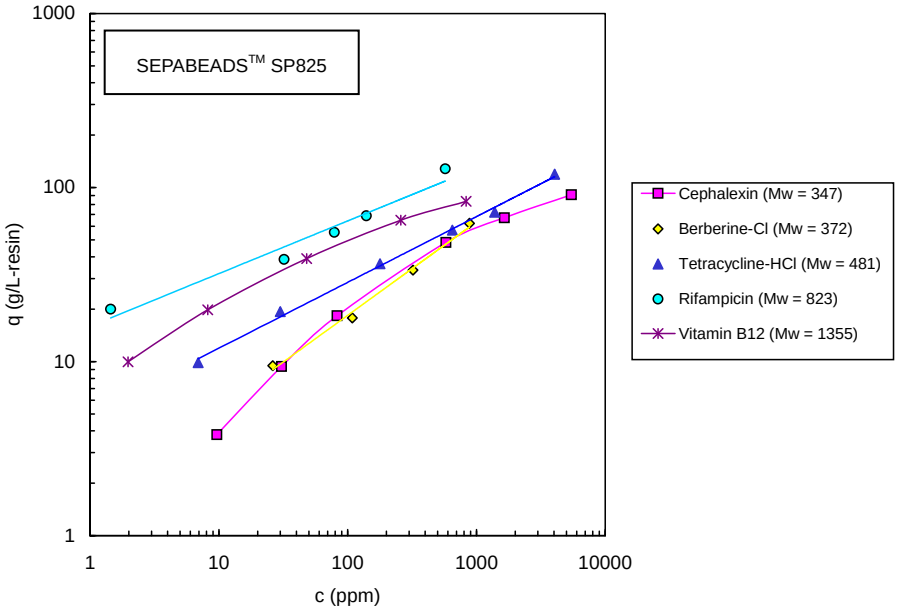


Fig. 16. Equilibrium adsorption data for SEPABEADS™ SP825 (5 °C).

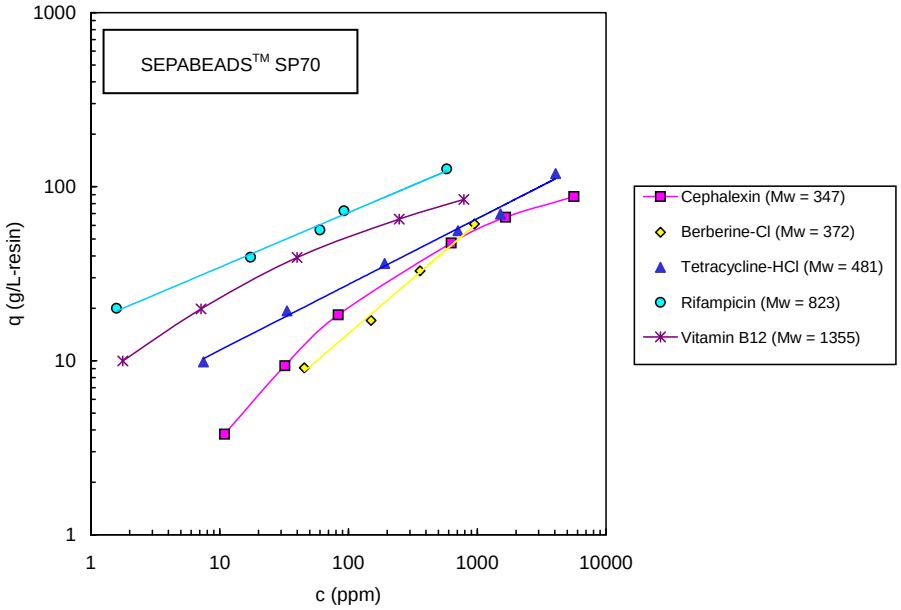


Fig. 17. Equilibrium adsorption data for SEPABEADS™ SP70 (5 °C).

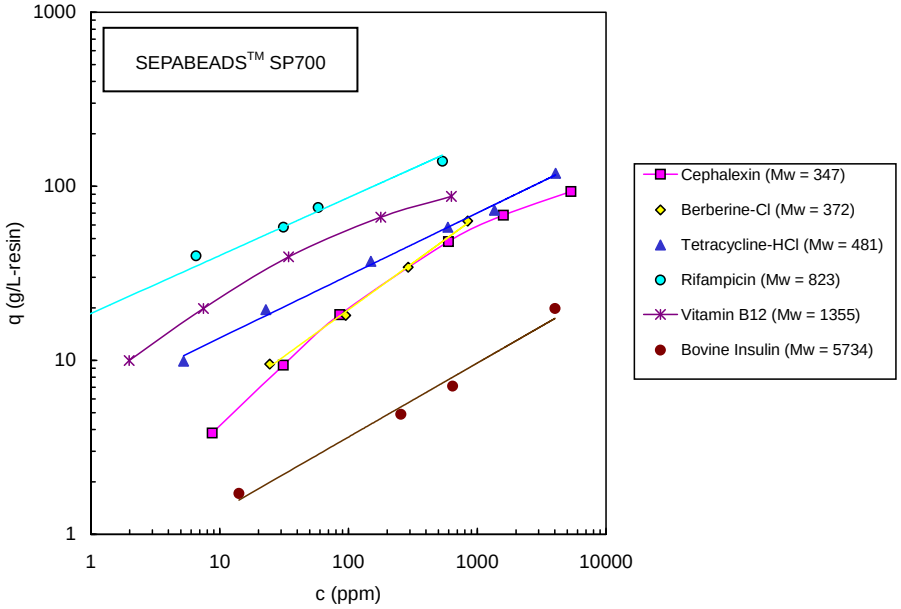


Fig. 18. Equilibrium adsorption data for SEPABEADS™ SP700 (5 °C).

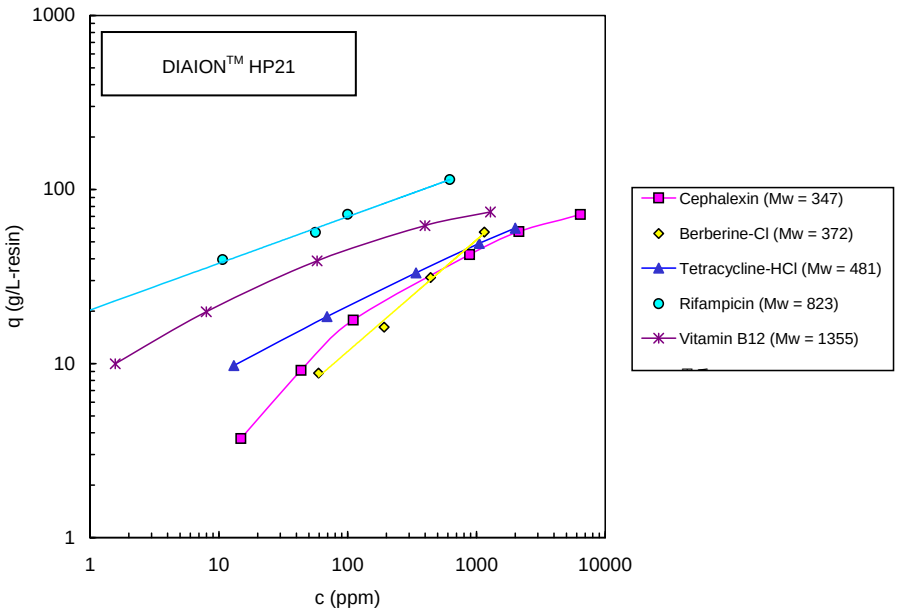


Fig. 19. Equilibrium adsorption data for DIAION™ HP21 (5 °C)

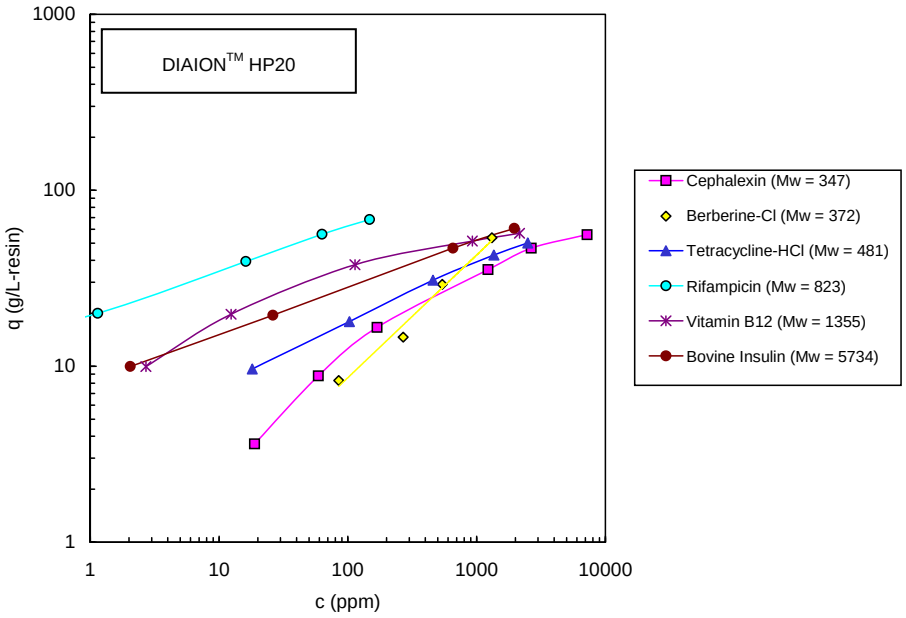


Fig. 20. Equilibrium adsorption data of DIAION™ HP20 (5 °C).

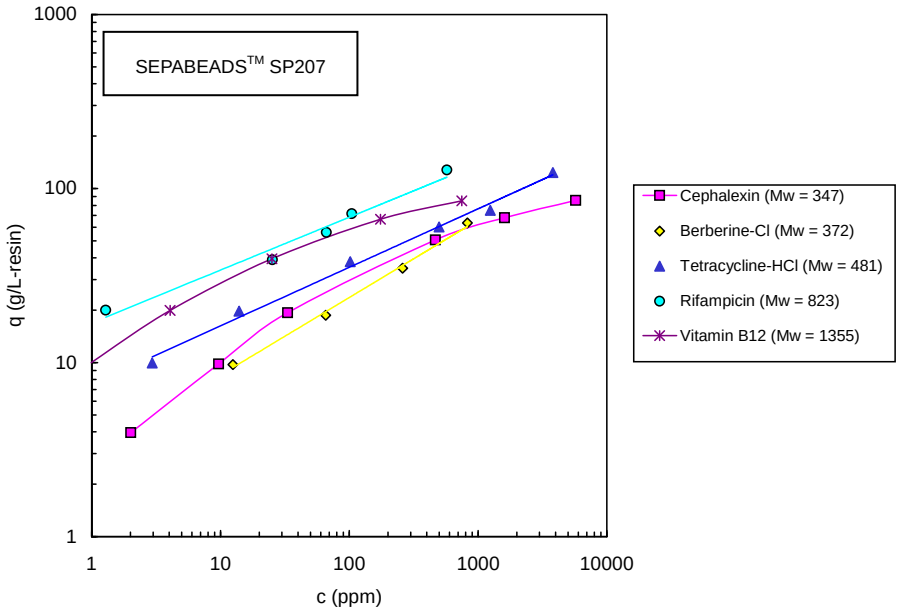


Fig. 21. Equilibrium adsorption data for SEPABEADS™ SP207 (5 °C).

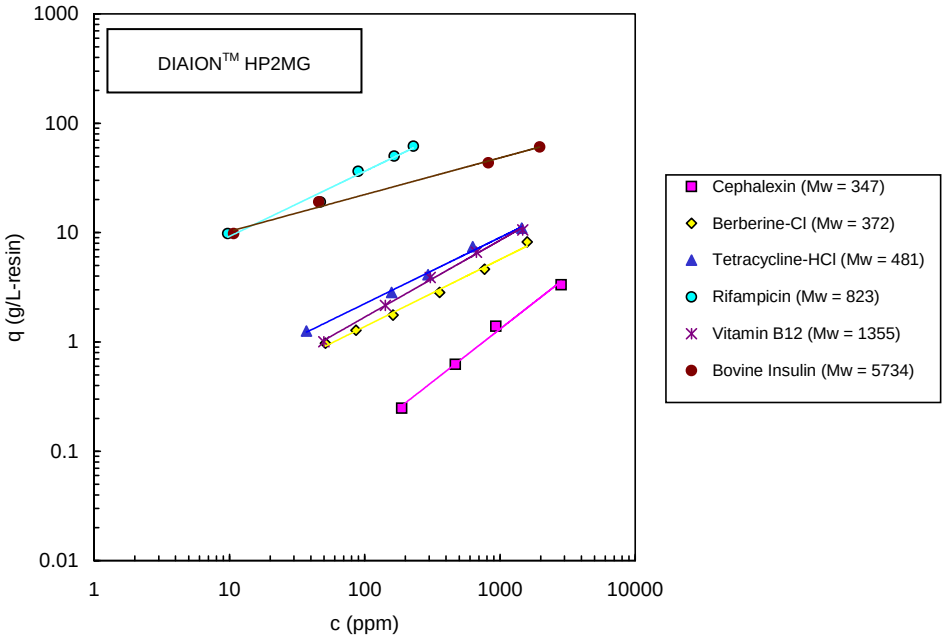


Fig. 22. Equilibrium adsorption data for DIAION™ HP2MG (5 °C, HP2MG is a different particle size grade of HP2MGL).

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Notes

*‘Poly(styrene-divinylbenzene)’ is common nomenclature. however, industrial-grade divinylbenzene contains ethylstyrene, so the correct chemical structures are ‘poly(styrene-ethylstyrene-divinylbenzene)’ and ‘poly (ethylstyrene-divinylbenzene)’ (SEPABEADS™ SP70, SP700).

**The correct chemical structure is ‘brominated poly (styrene-ethylstyrene-divinylbenzene)’.

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