Available online at www.sciencedirect.com
SCIENCE DIRECT®
Journal of Chromatography A, 1036 (2004) 33–44
JOURNAL OF CHROMATOGRAPHY A
www.elsevier.com/locate/chroma

Fundamental characteristics of synthetic adsorbents intended for industrial chromatographic separations

Tadashi Adachi*, Eiji Isobe

Separation Materials Laboratory, Specialty Chemicals Research Center, Mitsubishi Chemical Corporation, 1000 Kamoshida-cho, Aoba-ku, Yokohama 227-8502, Japan

Abstract

With the aim of obtaining comprehensive information on the selection of synthetic adsorbents for industrial applications, effect of pore and chemical structure of industrial-grade synthetic adsorbents on adsorption capacity of several pharmaceutical compounds was investigated. For relatively low molecular mass compounds, such as cephalexin, berberine chloride and tetracycline hydrochloride, surface area per unit volume of polystyrenic adsorbents dominated the equilibrium adsorption capacity. On the contrary, effect of pore size of the polystyrenic adsorbents on the equilibrium adsorption capacity was observed for relatively high molecular mass compounds, such as rifampicin, Vitamin B_{12} and insulin. Polystyrenic adsorbent with high surface area and small pore size showed small adsorption capacity for relatively high molecular mass compounds, whereas polystyrenic adsorbent with relatively small surface area but with large pore size showed large adsorption capacity. Effect of chemical structure on the equilibrium adsorption capacity of several pharmaceutical compounds was also studied among polystyrenic, modified polystyrenic and polymethacrylic adsorbents. The modified polystyrenic adsorbent showed larger adsorption capacity for all compounds tested in this study due to enhanced hydrophobicity. The polymethacrylic adsorbent possessed high adsorption capacity for rifampicin and insulin, but it showed lower adsorption capacity for the other compounds studied. This result may be attributed to hydrogen bonding playing major role for the adsorption of compounds on polymethacrylic adsorbent. Furthermore, column adsorption experiments were operated to estimate the effect of pore characteristics of the polystyrenic adsorbents on dynamic adsorption behavior, and it is found that both surface area and pore size of the polystyrenic adsorbents significantly affect the dynamic adsorption capacity as well as flow rate.

Keywords: Adsorbents; Pore structure; Equilibrium capacity; Dynamic capacity; Pharmaceutical analysis; Vitamin; Antibiotics; Berberine chloride; Proteins

1. Introduction

Synthetic adsorbents are widely used as polymeric media for recovery and separation of pharmaceuticals or their intermediates, foods, nutraceuticals, etc. [1–3]. For example, they are used for separation of antibiotics such as penicillin, cephalosporin and their derivatives, because of their high adsorption capacity, mechanical strength and chemical stability suitable for industrial operations [4,5].

Column operations are commonly adopted for those applications. In this sense, the synthetic adsorbents are used as chromatographic separation media. Therefore, both pore and chemical characteristics of the synthetic adsorbents will affect the separation and adsorption capacity of target compounds. But except the cases of β-lactam compounds [6–15], there seems to be few comprehensive reports concerning the effect of porosity and chemical structure of the synthetic adsorbents on the separation and adsorption capacity, especially in dynamic operations.

In this study, effect of chemical and pore structure of industrial-grade synthetic adsorbents on equilibrium adsorption capacity of several pharmaceutical compounds was comprehensively investigated. Effect of chemical structure on the adsorption capacity was also studied among polystyrenic, modified polystyrenic and polymethacrylic adsorbents. In addition, column adsorption behavior was also carried out on these synthetic adsorbents.

* Corresponding author. Tel.: +81-45-963-3223; fax: +81-45-963-3953.
E-mail address: 1104883@cc.m-kagaku.co.jp (T. Adachi).

0021-9673/$ – see front matter © 2003 Elsevier B.V. All rights reserved.
doi:10.1016/j.chroma.2003.09.018

Table 1
Characteristics of synthetic adsorbents

Number	Adsorbent name	Type	Specific surface area of a referential lot (m^2/g)	Pore radius of a referential lot (nm)	Specific pore volume of a referential lot (ml/g)	Average particle diameter of a referential lot (μm)	Apparent density of a referential lot (g/l)	Water content of a referential lot (%)	Specific surface area of a referential lot (m^2/ml)
1	SEPABEADS SP850	Polystyrenic	894	4.9	1.11	450	698	51.9	300
2	SEPABEADS SP825	Polystyrenic	908	6.4	1.56	450	676	55.9	271
3	SEPABEADS SP700	Polystyrenic	1123	8.2	2.15	529	691	65.9	265
4	SEPABEADS SP70	Polystyrenic	885	7.3	1.65	527	675	61.8	228
5	DIAION HP21	Polystyrenic	658	10.7	1.45	440	682	54.9	202
6	DIAION HP20	Polystyrenic	570	30.0	1.36	440	682	58.9	160
7	SEPABEADS SP207	Modified polystyrenic	565	12.1	1.12	460	789	50.7	220
8	DIAION HP2MG	Polymethacrylic	557	19.8	1.16	490	732	64.1	146

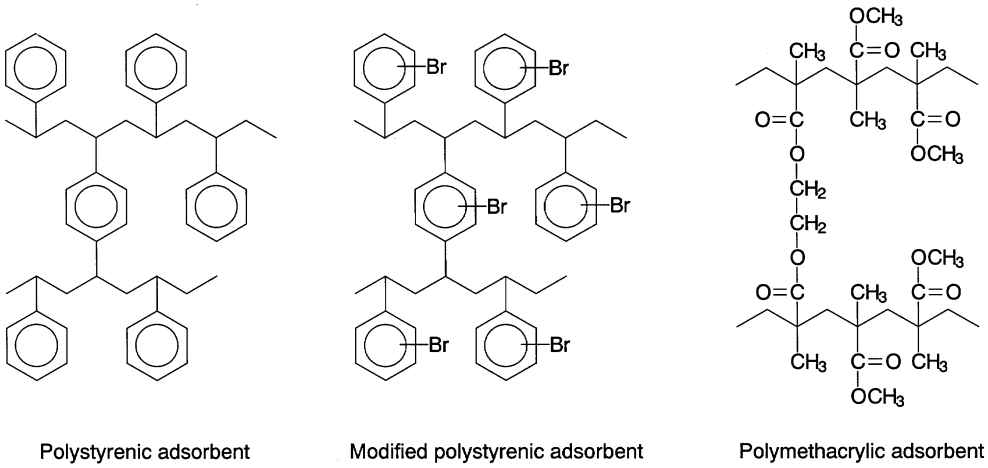

Fig. 1. Chemical structure of synthetic adsorbents.

2. Experimental

2.1. Materials

All polystyrenic and polymethacrylic synthetic adsorbents listed in Table 1 were from Mitsubishi Chemical (Tokyo, Japan). All the adsorbents have spherical shape. Chemical structure of each adsorbent is shown in Fig. 1. Fundamental characteristics of polystyrenic and polymethacrylic adsorbents were described elsewhere [4,5,16,17].

2.2. Reagents and chemicals

Cephalexin, berberine chloride, tetracycline hydrochloride and rifampicin were purchased from Sigma (St. Louis, MO, USA). Bovine insulin was purchased from Wako (Tokyo, Japan). Vitamin B_{12} was purchased from Kishida (Osaka, Japan). Chemical structure of them (except insulin) is shown in Fig. 2. Other reagents and chemicals were of the highest quality available, and were purchased from various suppliers. Demineralized water was

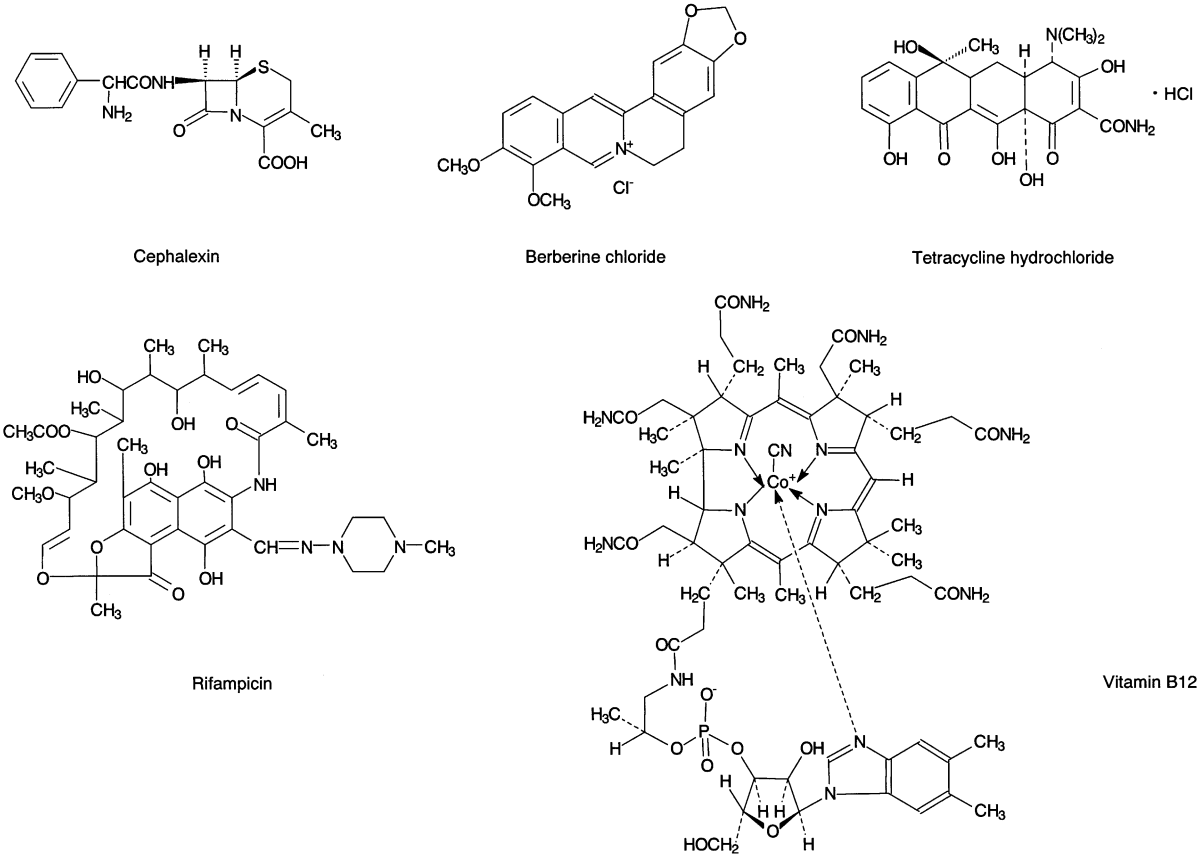

Fig. 2. Chemical structure of pharmaceutical compounds investigated in this study.

Table 2
Molecular weight, solution condition and detection condition of each pharmaceutical compound

Compound name	Molecular mass	Solution condition	Detection condition (nm)
Cephalexin	347	Demineralized water	UV 260
Berberine chloride	372	Demineralized water	Vis 416
Tetracycline hydrochloride	481	Demineralized water	Vis 356
Rifampicin	823	20 mM Sodium citrate buffer, pH 4.0	Vis 474
Vitamin B_{12}	1355	Demineralized water	Vis 360
Bovine insulin	5734	50 mM Sodium citrate buffer, pH 2.5	UV 275

prepared by Milli-Q system (Millipore, Bedford, MA, USA).

2.3. Procedures

2.3.1. Porosity analysis

Surface area measurement of the synthetic adsorbents was operated by use of a Micromeritics (Norcross, GA, USA) FlowSorb 2300 with a singlepoint BET method. Pore size distribution of the synthetic adsorbents was measured using a Micromeritics ASAP 2400. N_2 was used for both the above measurements.

2.3.2. Adsorption equilibrium

Equilibrium isotherms were obtained by introducing 5 ml of each adsorbent into solutions of each compound with various concentrations, shaking in a thermostatic bath shaker (100 strokes per minute) at 5 °C for overnight (at least 12 h). Equilibration time of over 12 h is sufficient for the adsorbents tested.[1] Then concentrations of the compound in the solutions were measured by using a Shimadzu (Kyoto, Japan) UVmini-1240 UV-Vis spectrophotometer. The adsorption capacity was calculated by mass balance.

2.3.3. Column adsorption

A jacketed glass column of 500 mm × 16 mm i.d. was used and 40 ml of each adsorbent was packed. Solution with fixed concentration of compound was pumped into the column at various space velocities. Concentration of the compound in effluent from the column outlet was directly monitored by using a Shimadzu SPD-6AV UV-Vis detector with a preparative cell, or determined by measuring absorbance of fractions collected by a Hitachi (Tokyo, Japan) L-5200 fraction collector. Operating temperature was 5 °C.

Molecular mass, solution condition and UV-Vis detection condition of each compound is listed in Table 2.

3. Results and discussion

3.1. Porosity analysis

Specific surface area and pore radius of each synthetic adsorbent as well as other characteristics are listed in Table 1. Adsorbents 1–6 are made of poly(styrene-divinylbenzene) and have a variety of specific surface areas and pore radii. Adsorbent 7 is made of chemically modified poly(styrene-divinylbenzene) and the porosity of it is relatively similar to that of adsorbents 5 and 6. Adsorbent 8 is a polymethacrylic adsorbent and the porosity of it also resembles that of adsorbents 5 and 6.

From the porosity analysis and other characteristics, specific surface area of each adsorbent per unit volume was calculated; the results are listed in Table 1. From Table 1, it is found that the polystyrenic adsorbent with smaller pore radius has larger surface area per unit volume.

3.2. Adsorption equilibrium

3.2.1. Effect of surface area of synthetic adsorbents on equilibrium adsorption capacity

Adsorption isotherms for each pharmaceutical compound on various synthetic adsorbents are shown in Figs. 3–8.

Among the polystyrenic adsorbents 1–6, the adsorbent with larger specific surface area per unit volume gives the larger adsorption amount of relatively low molecular mass compounds, such as cephalexin, berberine chloride and tetracycline hydrochloride at the same equilibrium concentration. From Figs. 3–5, the adsorption amount of each compound on each adsorbent at equilibrium concentration of 0.5 g/l was calculated using interpolation and plotted versus surface area per unit volume of each adsorbent in Fig. 9. Fig. 9 clearly demonstrates that the surface area per unit volume of the adsorbents with the same chemical structure dominates the equilibrium adsorption capacity of these pharmaceutical compounds.

3.2.2. Effect of pore size of synthetic adsorbents on equilibrium adsorption capacity

When the molecular masses of compounds become larger, the molecular sizes of them also become larger. Therefore, diffusion of those compounds into smaller pores of the synthetic adsorbents than the sizes of those compounds is restricted. This phenomenon affects the equilibrium adsorption capacity of the synthetic adsorbents with different porosity. In Figs. 6–8, the adsorbents with higher surface areas per unit volume do not always possess higher equilibrium adsorption capacity. From Figs. 6–8, the adsorption amount of each compound on each adsorbent at equilibrium concentration of 0.5 g/l was also calculated and plotted in Fig. 10.

[1] Gouseikyuutyakuzai no sentaku (technical bulletin, in Japanese), Mitsubishi Chemical Corporation.

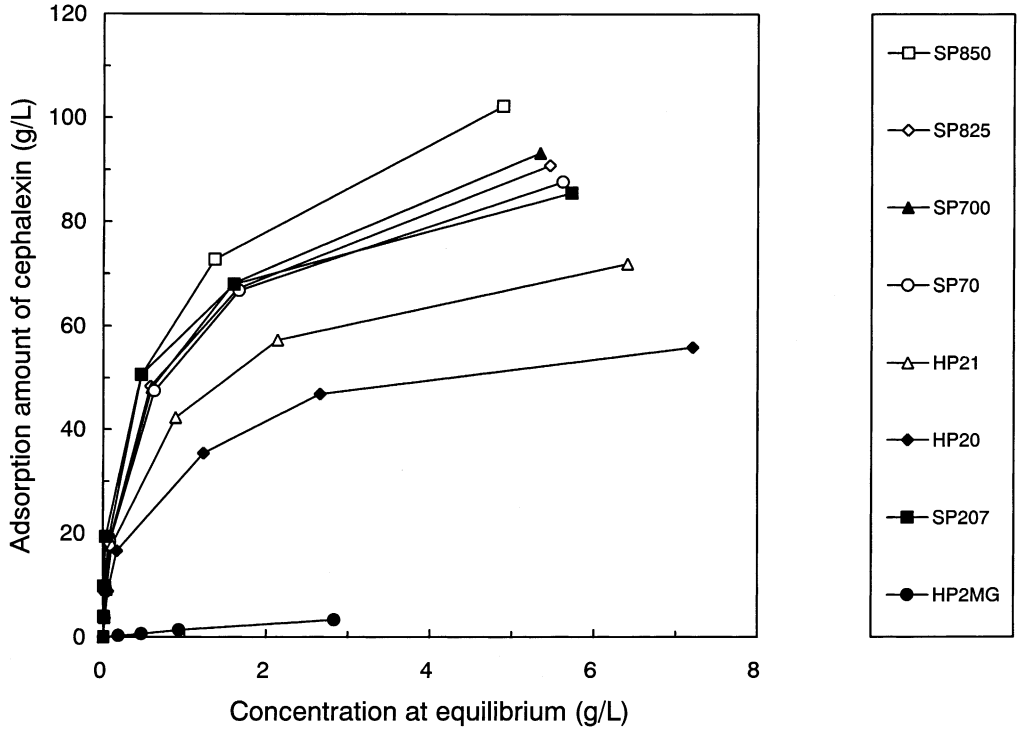

Fig. 3. Adsorption isotherms of cephalexin on various synthetic adsorbents at 5 °C.

For rifampicin and Vitamin B_{12}, the adsorption capacity increases as increasing surface area per unit volume of the adsorbent to around 260 m^2/ml. But over 270 m^2/ml, the adsorption capacity goes down as increasing surface area. On the other hand, the adsorption capacity of bovine insulin decreases as increasing surface area of the adsorbent. This result is due to the large molecular size of insulin compared to the pore size of the adsorbents with higher surface areas.

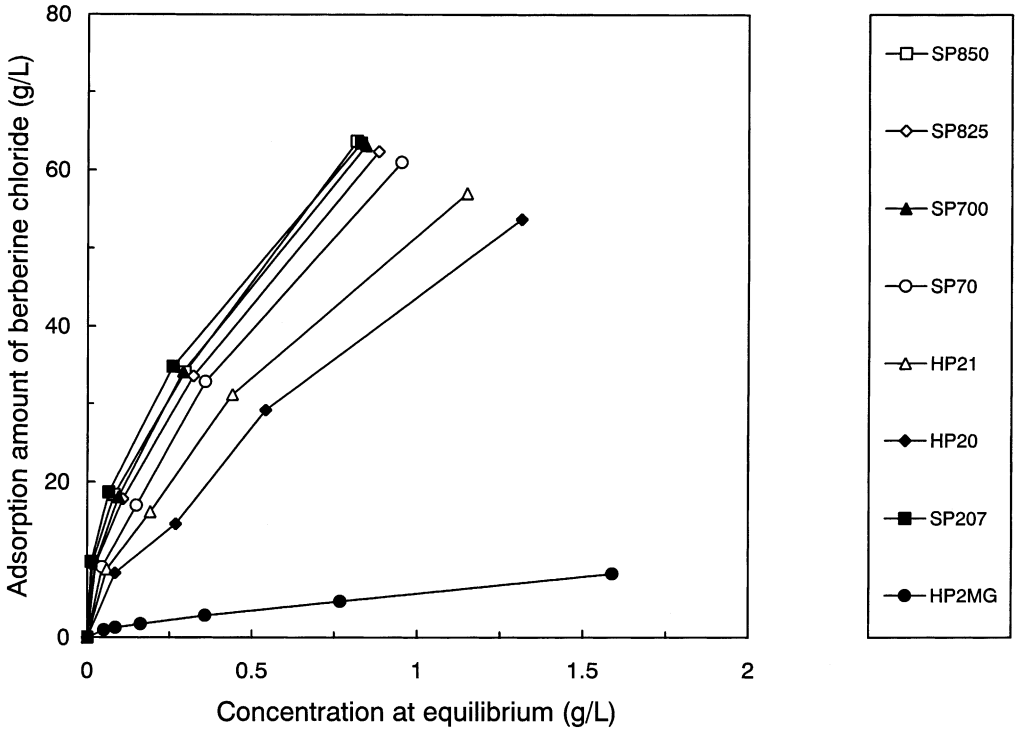

Fig. 4. Adsorption isotherms of berberine chloride on various synthetic adsorbents at 5 °C.

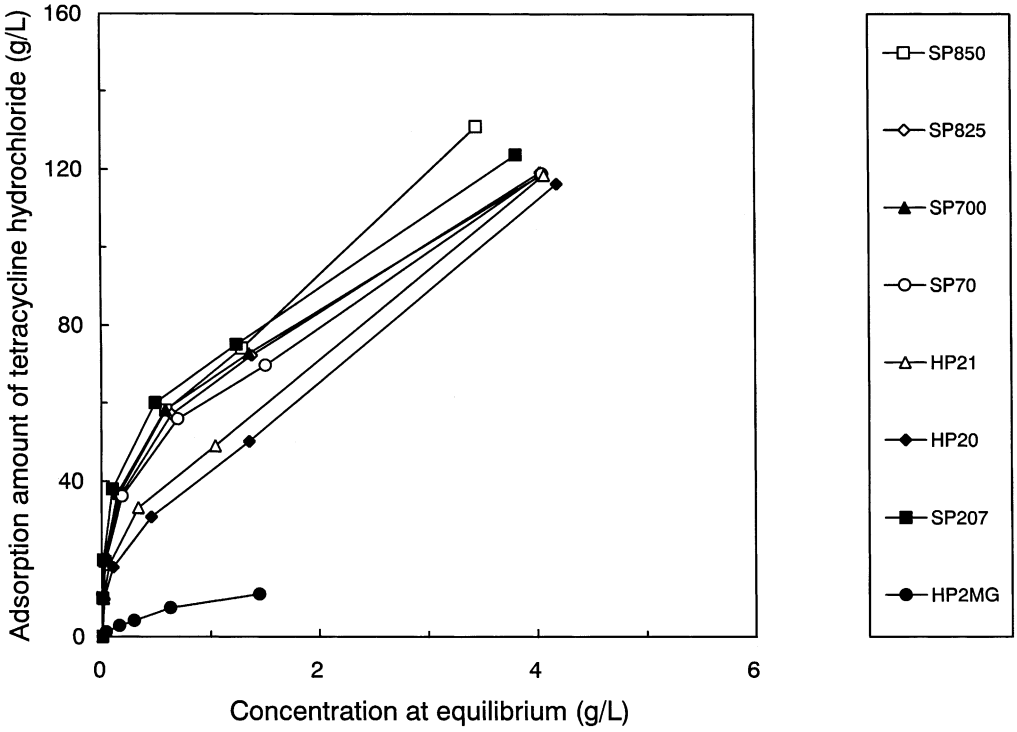

Fig. 5. Adsorption isotherms of tetracycline hydrochloride on various synthetic adsorbents at 5 °C.

Furthermore, the adsorption amount at equilibrium concentration of 0.5 g/l is plotted versus pore radius of the adsorbents in Fig. 11. Fig. 11 also shows the effect of pore size of the synthetic adsorbents on the equilibrium adsorption capacity of relatively large molecular weight compounds.

3.2.3. Effect of chemical structure of synthetic adsorbents on equilibrium adsorption capacity

Effect of chemical structure on equilibrium adsorption amount of the synthetic adsorbents with relatively similar porosity is also depicted in Figs. 3–8. In Figs. 3–7,

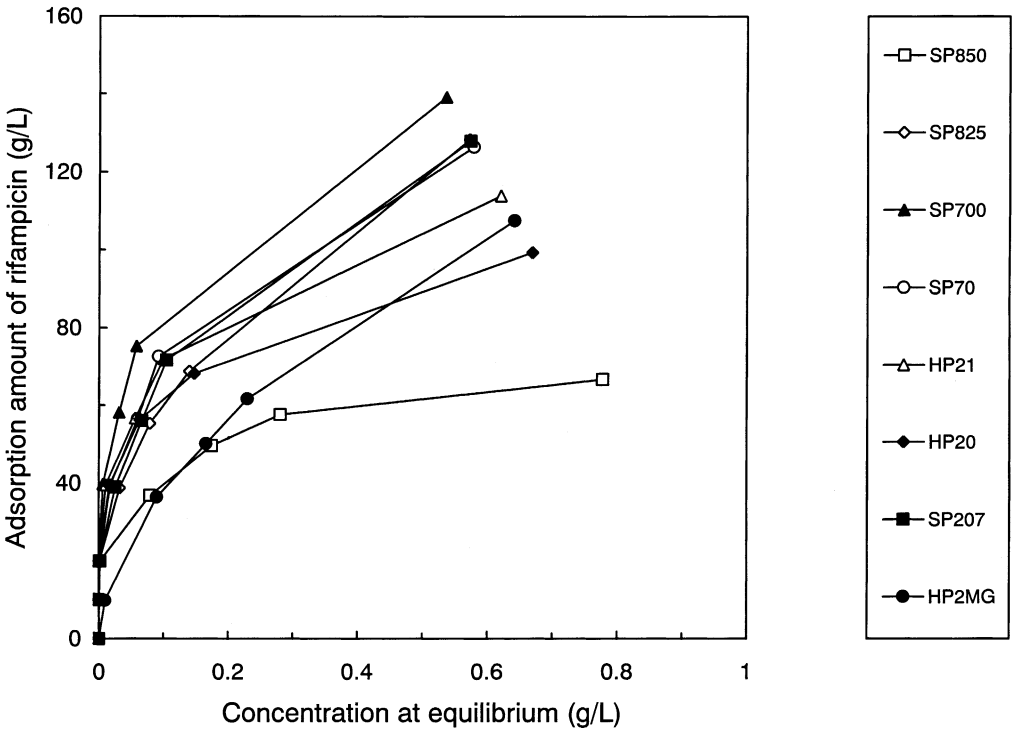

Fig. 6. Adsorption isotherms of rifampicin on various synthetic adsorbents at 5 °C.

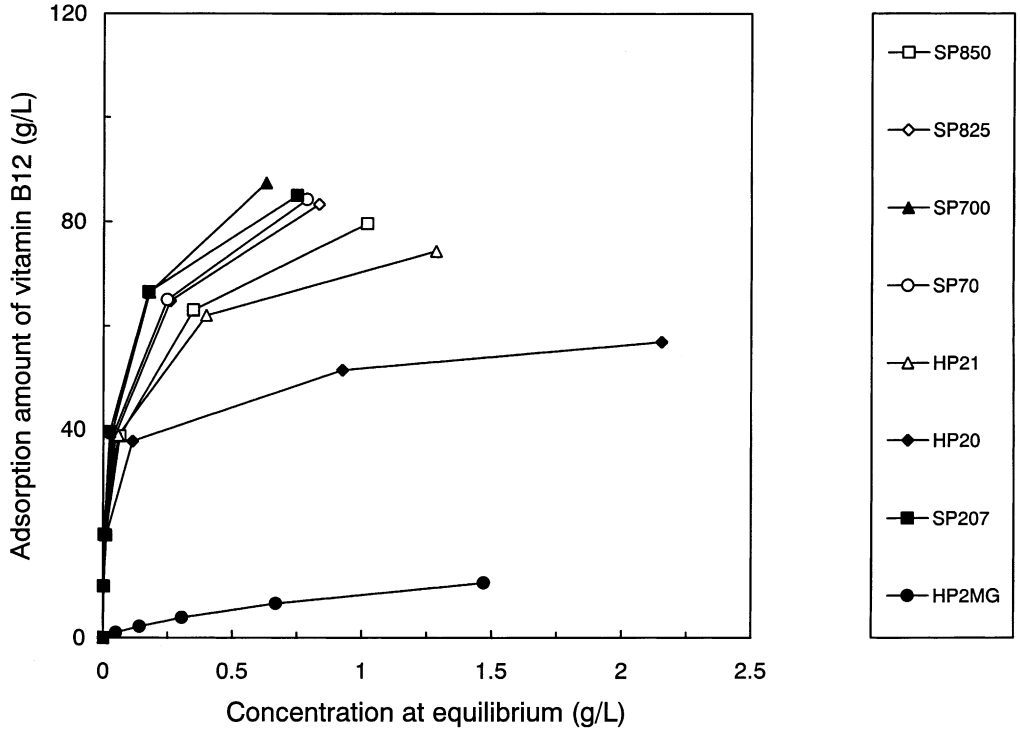

Fig. 7. Adsorption isotherms of Vitamin B_{12} on various synthetic adsorbents at 5 °C.

adsorbent 7 shows higher equilibrium adsorption amounts of the compounds than adsorbents 5 and 6. Higher equilibrium adsorption capacity of adsorbent 7 is probably attributed to enhanced adsorption force due to introduction of hydrophobic functionality described in Fig. 1.

On the other hand, adsorption behavior of adsorbent 8 is different. In Figs. 3–5 and 7, adsorbent 8 shows lower

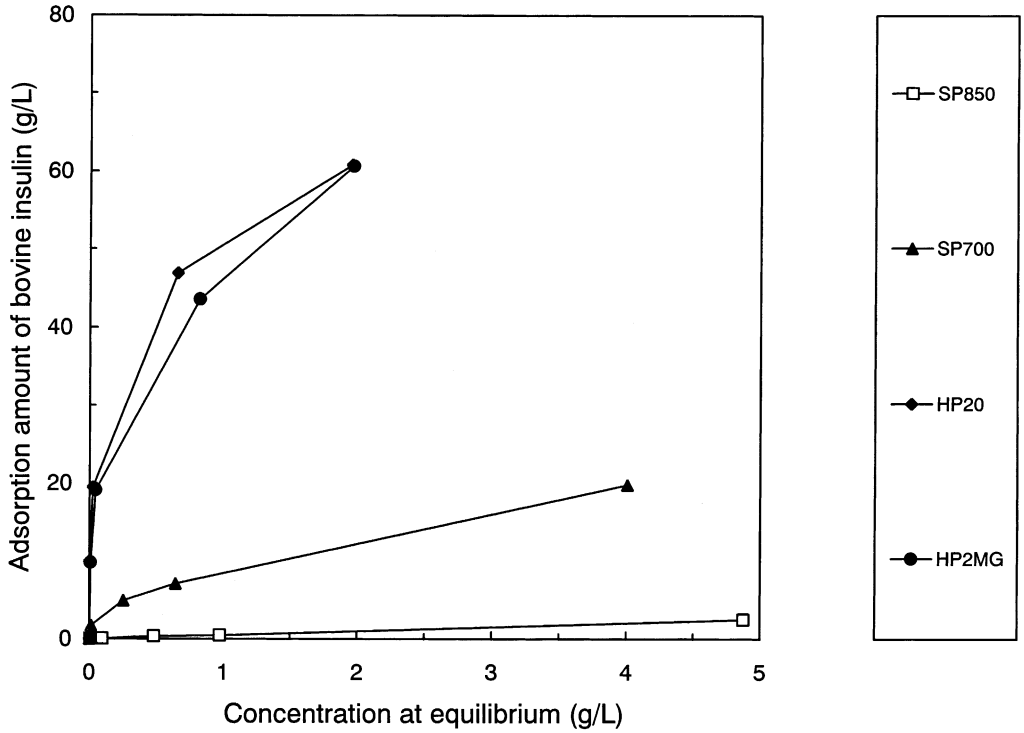

Fig. 8. Adsorption isotherms of bovine insulin on various synthetic adsorbents at 5 °C.

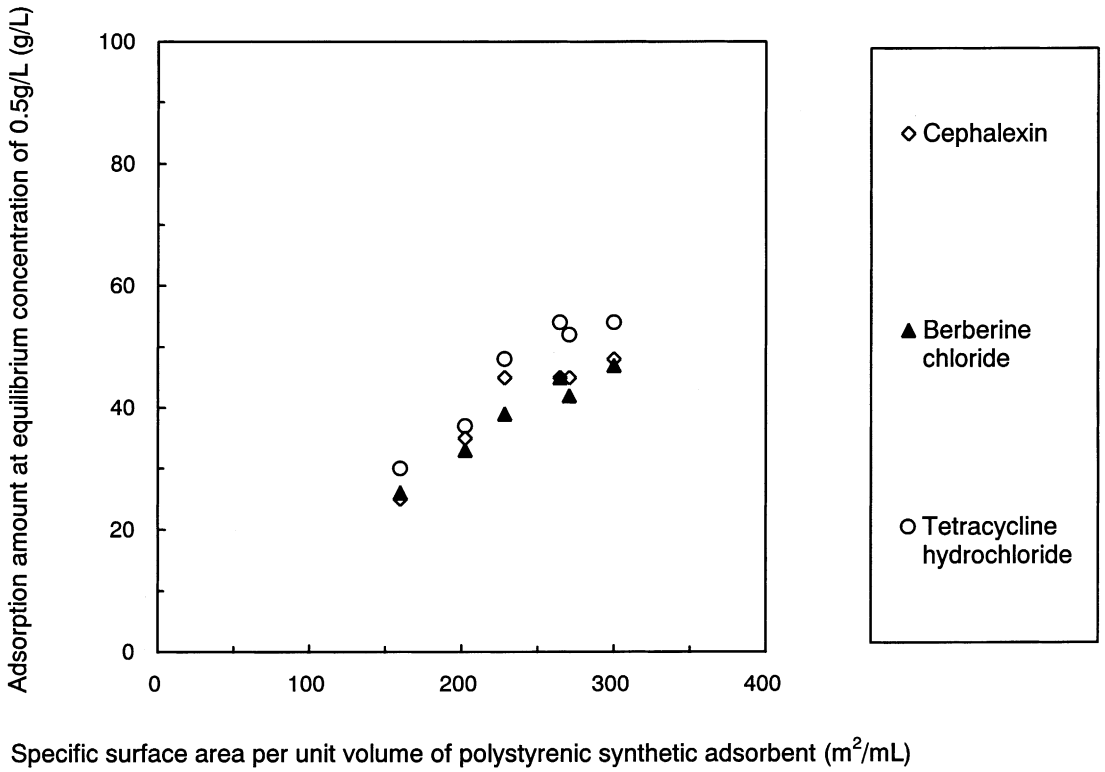

Fig. 9. Effect of specific surface area per unit volume of the polystyrenic synthetic adsorbents on the adsorption amount of cephalexin, berberine chloride and tetracycline hydrochloride at equilibrium concentration of 0.5 g/l.

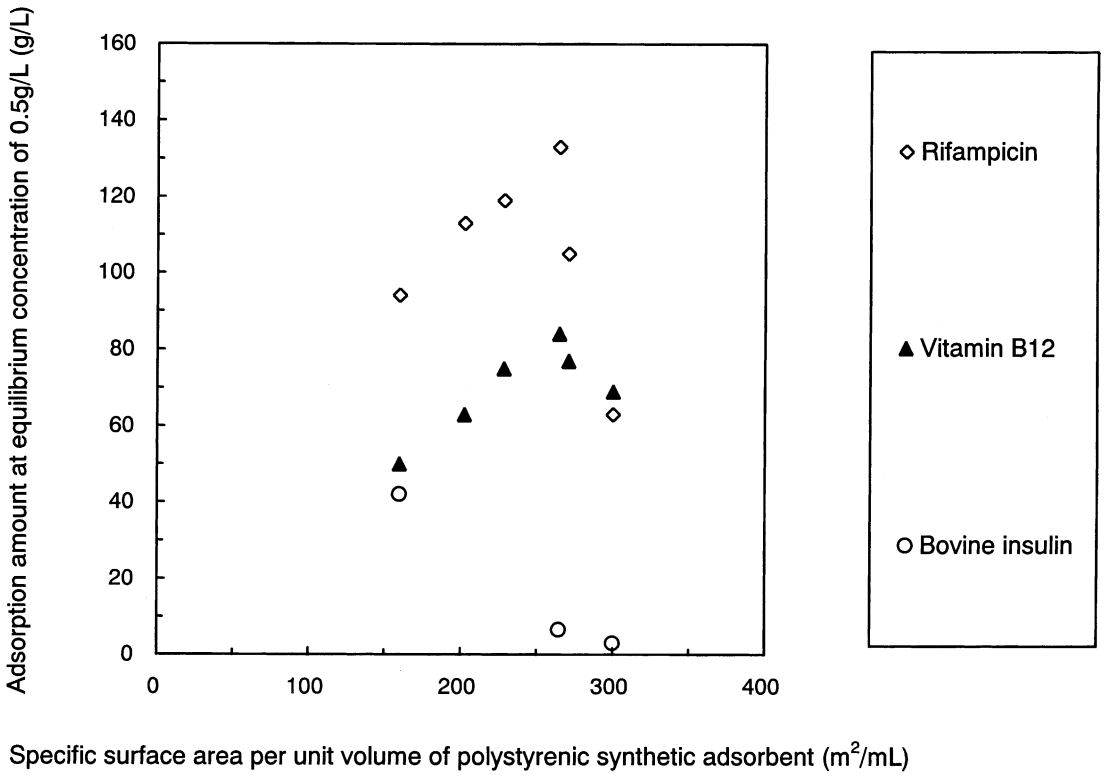

Fig. 10. Effect of specific surface area per unit volume of the polystyrenic synthetic adsorbents on the adsorption amount of rifampicin, Vitamin B_{12} and bovine insulin at equilibrium concentration of 0.5 g/l.

equilibrium adsorption amounts of compounds than adsorbents 5 and 6. But in Figs. 6 and 8, the equilibrium adsorption amount of rifampicin and bovine insulin on the adsorbents 8 is comparable to that of adsorbent 5 and 6. Insulin is a polypeptide and has a lot of functionalities that can create hydrogen bonding. The polymethacrylic adsorbent has esters that can also create hydrogen bonding. In the case of normal-phase adsorption, it was found that polymethacrylic adsorbent shows higher equilibrium adsorption amount than polystyrenic adsorbent due to hydrogen bonding [18]. Relatively high equilibrium adsorption capacity of bovine insulin onto polymethacrylic adsorbent may also be explained by hydrogen bonding effect.

3.3. Column adsorption

3.3.1. Column adsorption behavior of cephalexin onto polystyrenic synthetic adsorbents

Column adsorption experiments were operated to estimate the effect of pore characteristics of the polystyrenic adsorbents on the adsorption efficiency from the industrial point of view. Fig. 12 shows breakthrough curves of cephalexin ($M_w = 347$) on the polystyrenic synthetic adsorbents with various porosities under the space velocity of 4.0 h^{-1} and feed concentration of 6.0 g/l.

Adsorbent 1 gives the highest total dynamic adsorption capacity of cephalexin among the three adsorbents tested, this result corresponds to the highest specific surface area per unit volume of adsorbent 1. But the shelving breakthrough curve was observed for adsorbent 1. Adsorbent 1 has the smallest pore radius among the three, so the shelving breakthrough curve suggests the slower diffusion of cephalexin into the small pores of the adsorbent. On the contrary, adsorbent 6 shows the sharpest breakthrough curve and gives the lowest dynamic adsorption capacity. The largest pore radius of adsorbent 6 enables the fastest pore diffusion of cephalexin among the three adsorbents, thus the sharpest breakthrough curve is obtained. But the larger pore creates the smaller specific surface area per unit volume of the adsorbent, so the dynamic adsorption capacity of adsorbent 6 becomes smaller than the others. Intermediate breakthrough profile and dynamic adsorption capacity was observed for adsorbent 2.

Dynamic adsorption capacity at low leakage is also crucial for industrial applications. Five percent breakthrough capacity of cephalexin was calculated from Fig. 12 and found as 67 g/l for adsorbent 1, but adsorbent 2 showed superior capacity of 70 g/l. These results probably come from the slower diffusion of cephalexin into the small pores of the adsorbent 1 compared to the case of adsorbent 2.

3.3.2. Column adsorption behavior of Vitamin B_{12} onto polystyrenic synthetic adsorbents

Effect of adsorbate molecular size on dynamic adsorption behavior was also investigated. In Fig. 13, breakthrough curves of Vitamin B_{12} ($M_w = 1355$) on the polystyrenic

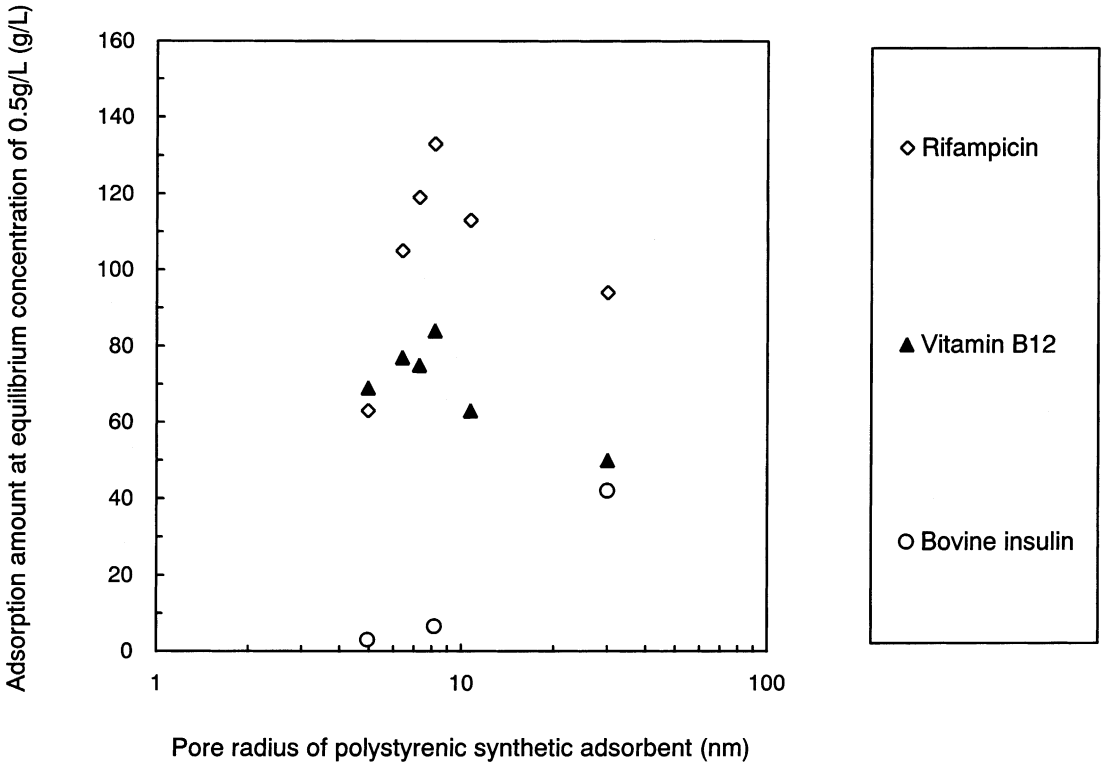

Fig. 11. Effect of pore radius of the polystyrenic synthetic adsorbents on the adsorption amount of rifampicin, Vitamin B_{12} and bovine insulin at equilibrium concentration of 0.5 g/l.

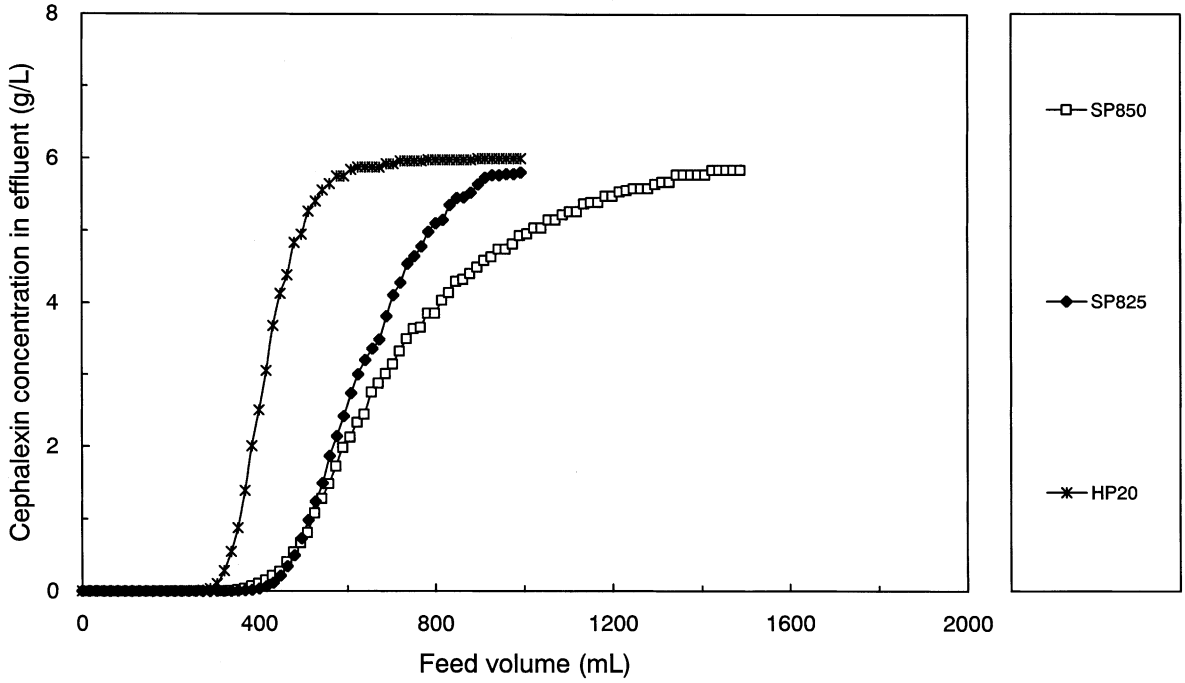

Fig. 12. Breakthrough curves of cephalexin on the polystyrenic synthetic adsorbents with various porosities. Conditions: adsorbent volume, 40 ml; column diameter, 1.6 cm; bed length, 20 cm; space velocity, 4.0 h^{-1}; feed concentration of cephalexin, 6.0 g/l; temperature, 5 °C.

synthetic adsorbents with various porosities at the space velocity of 4.0 h^{-1} and feed concentration of 6.0 g/l are described. In the case of Vitamin B_{12}, breakthrough curve of adsorbent 2 is more shelving than the case of cephalexin. This result is probably attributed to the slower pore diffusion of Vitamin B_{12} due to its larger size compared to that of cephalexin.

And the adsorption profile comparison between adsorbents 2 and 3 reveals that adsorbent 3 with larger pore radius shows steeper breakthrough curve and higher total

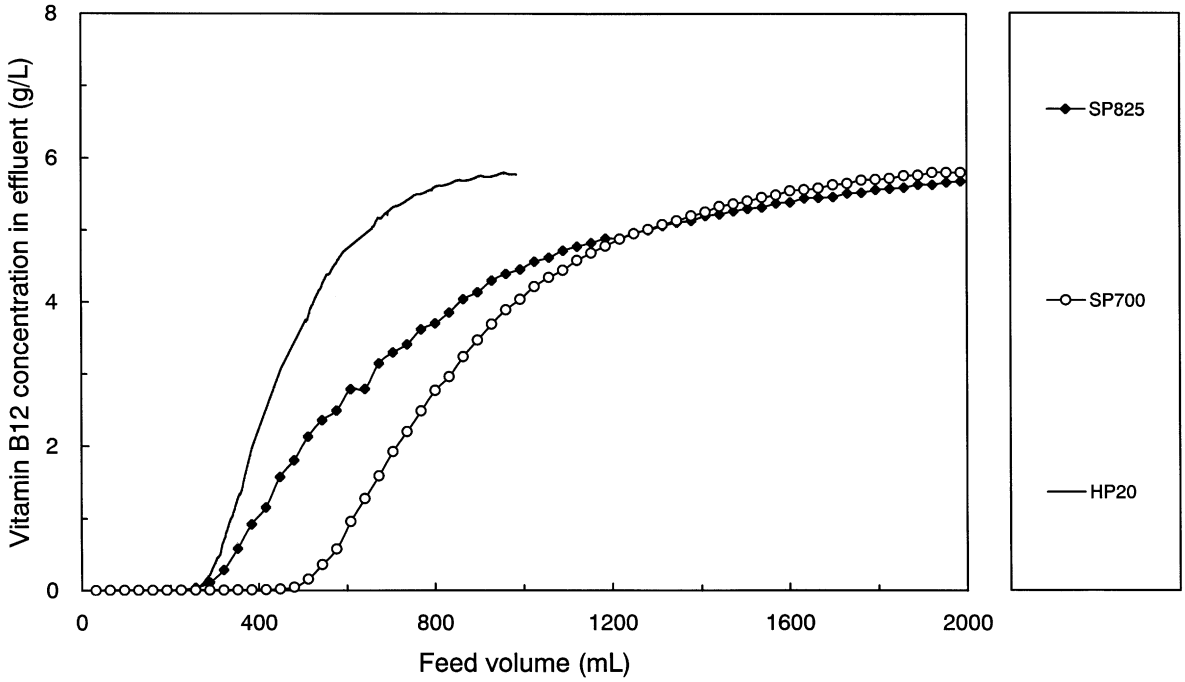

Fig. 13. Breakthrough curves of Vitamin B_{12} on the polystyrenic synthetic adsorbents with various porosities. Conditions: adsorbent volume, 40 ml; column diameter, 1.6 cm; bed length, 20 cm; space velocity, 4.0 h^{-1}; feed concentration of Vitamin B_{12}, 6.0 g/l; temperature, 5 °C.

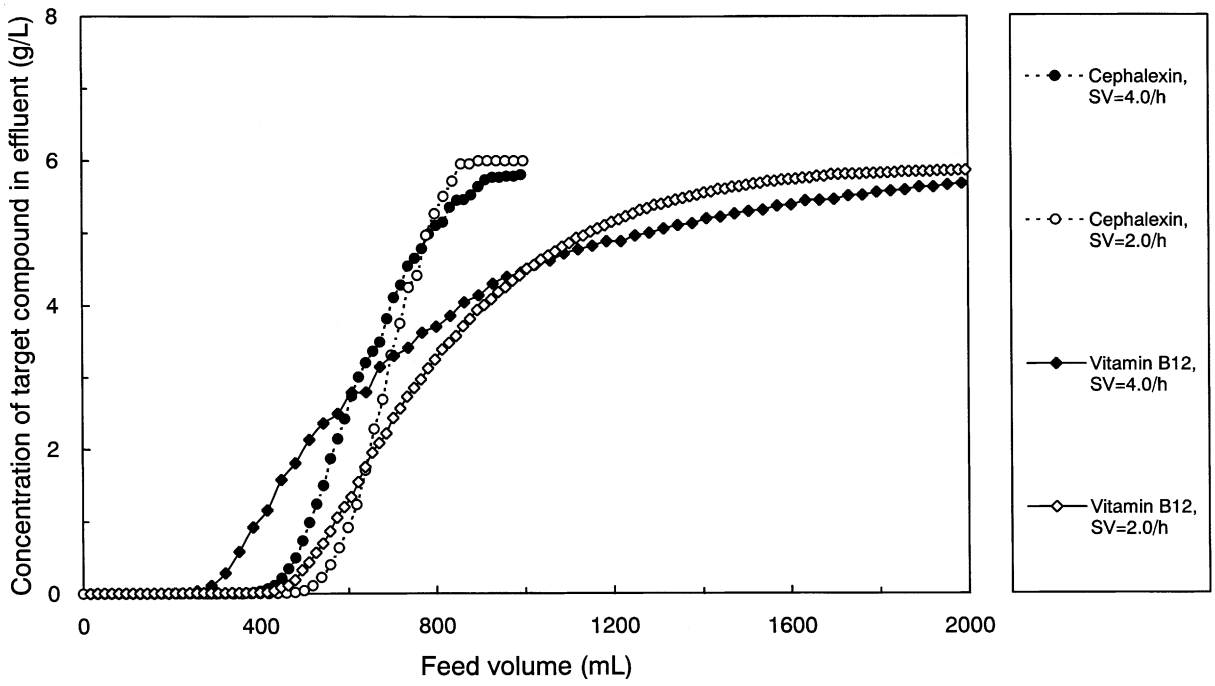

Fig. 14. Breakthrough curves of cephalexin and Vitamin B_{12} on polystyrenic synthetic adsorbent 2 at different space velocities. Conditions: adsorbent volume, 40 ml; column diameter, 1.6 cm; bed length, 20 cm; feed concentration, 6.0 g/l; temperature, 5 °C.

dynamic adsorption capacity than adsorbent 2 despite of almost the same specific surface areas per unit volume of those adsorbents. These results presumably imply the differences between the two adsorbents both in accessible surface area for Vitamin B_{12} and in pore diffusion of Vitamin B_{12}.

Five percent breakthrough capacity of Vitamin B_{12} was calculated from Fig. 13 and found as 48 g/l for adsorbent 2 and 82 g/l for adsorbent 3. The results obtained revealed that the pore size significantly affects the productivity of column chromatographic separation of relatively large molecules using synthetic adsorbents.

3.3.3. Effect of flow rate on column adsorption behavior onto polystyrenic synthetic adsorbents

Effect of flow rate on dynamic adsorption behavior is one of the most important factors for industrial applications. Breakthrough curves of cephalexin and Vitamin B_{12} on polystyrenic adsorbent 2 at different space velocity are depicted in Fig. 14. For both compounds, breakthrough profiles at the space velocity of 2.0 h^{-1} show sharper shape compared to those at the space velocity of 4.0 h^{-1}. But the effect of flow rate on column adsorption behavior is more significant in the case of Vitamin B_{12} than in the case of cephalexin. Furthermore, 5% breakthrough capacity of Vitamin B_{12} calculated from Fig. 14 improves by 54% (from 48 to 74 g/l) with lowering the space velocity from 4.0 to 2.0 h^{-1}, whereas 5% breakthrough capacity of cephalexin only gives 17% increase (from 70 to 82 g/l) with the same space velocity change. From these results, it can be mentioned that slower flow rate is industrially more favorable for column adsorption of relatively large compounds such as Vitamin B_{12} on synthetic adsorbents.

4. Conclusions

Effect of pore and chemical structure of industrial grade synthetic adsorbents on adsorption capacity of several pharmaceutical compounds was comprehensively investigated. For relatively low molecular mass compounds, surface area per unit volume of polystyrenic adsorbents dominated the equilibrium adsorption capacity. On the contrary, pore size affected the equilibrium adsorption capacity of relatively high molecular mass compounds on the synthetic adsorbents with the same chemical structure.

Effect of chemical structure on the equilibrium adsorption capacity of several pharmaceutical compounds was also studied among polystyrenic, modified polystyrenic and polymethacrylic adsorbents. Hydrophobic substituent of the modified polystyrenic adsorbent enhanced the equilibrium adsorption capacity for all compounds tested in this study compared to the polystyrenic adsorbent with relatively similar porosity. The polymethacrylic adsorbent gave high equilibrium adsorption capacity for rifampicin and insulin, but it showed lower equilibrium adsorption capacity for the other compounds studied. This result may be attributed to the effect of hydrogen bonding.

Column adsorption tests were also operated and they revealed that both surface area and pore size of the polystyrenic

adsorbents significantly affect the dynamic adsorption capacity as well as flow rate.

These empirical results will provide fundamental information both on the selection of the synthetic adsorbents and on the operation conditions for industrial separations of pharmaceuticals or their intermediates, foods, nutraceuticals, etc. But further investigation including numerical approach or adsorption selectivity study should be done for more versatile information.

References

[1] W. Voser, J. Chem. Tech. Biotechnol. 32 (1982) 109.
[2] R.E. Schwartz, D.F. Sesin, H. Joshua, K.E. Wilson, A.J. Kempf, K.A. Goklen, D. Kuehner, P. Gailliot, C. Gleason, R. White, E. Inamine, G. Bills, J. Antibiotic 45 (1992) 1853.
[3] C. Robinson, Spec. Publ. R. Soc. Chem. 196 (1997) 199.
[4] H. Takayanagi, J. Fukuda, E. Miyata, in: M. Verrall (Ed.), Downstream Processing of Natural Products, Wiley, Chichester, 1996, Chapter 11, p. 159.
[5] DIAION Manual, I and II, Mitsubishi Chemical Corporation.
[6] J.L. Casillas, M. Martinez, F. Addo-Yobo, J. Aracil, Chem. Eng. J. 52 (1993) 1371.
[7] M. Hickketier, K. Buchholz, Appl. Microbiol. Biotechnol. 32 (1990) 680.
[8] J.-W. Lee, H.-J. Jung, H. Moon, Korean J. Chem. Eng. 14 (1997) 277.
[9] J.-W. Lee, H.-C. Park, H. Moon, Sep. Purif. Technol. 12 (1997) 1.
[10] J.-W. Lee, H. Moon, Adsorption 5 (1999) 381.
[11] M. Dutta, N.N. Dutta, K.G. Bhattacharya, Sep. Purif. Technol. 16 (1999) 213.
[12] M. Dutta, N.N. Dutta, K.G. Bhattacharya, J. Chem. Eng. Jpn. 33 (2000) 303.
[13] Y. Xie, E. Van de Sandt, T. de Weerd, N.-H.L. Wang, J. Chromatogr. A 908 (2001) 273.
[14] E. Firouztale', J.J. Maikner, K.C. Deissler, P.G. Cartier, J. Chromatogr. A 658 (1994) 361.
[15] S.A. Yang, D.L. Pyle, J. Chem. Technol. Biotechnol. 74 (1999) 216.
[16] T. Adachi, S. Ando, J. Watanabe, J. Chromatogr. A 944 (2002) 41.
[17] T. Adachi, E. Isobe, J. Chromatogr. A 989 (2003) 19.
[18] M.V. Chaubal, G.F. Payne, Biotechnol. Prog. 11 (1995) 468.