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Application of Mesoporous MCM-48 and SBA-15 Materials for the Separation of Biochemicals Dissolved in Aqueous Solution

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Abstract−The application of molecular sieves for the separation and purification of biological molecules has been limited because of their available pore size (<1.3 nm). With the development of synthetic mesoporous materials, the available pore size range (1.5-10 nm) has been extended. They have pores large enough to allow access for a number of biological molecules. This work investigated the use of two mesoporous materials (MCM-48 and SBA-15) with different pore sizes and structures for adsorption of cephalosporin C as a model compound of biological molecules in terms of ethanol concentration, solution pH, and temperature. It was found that these materials have the potential for the separation and purification processes of biochemicals including antibiotics, amino acids, and proteins.

Key words: Adsorption Equilibrium, MCM-48, SBA-15, Biochemicals

INTRODUCTION

Biochemicals can be classified into many groups such as amino acids, antibiotics, proteins and others [Smith, 1985]. One of the antibiotics, cephalosporin C, has been widely used as an important starting material to make medical and pharmaceutical products. It has been produced by fermentation technology using *cephalosporium acremonium*. The key process for the production of cephalosporin C is the question of separation and purification from its fermentation broths that contain many components such as cephalosporin C, deacetoxycephalosporin C, penicillin N, methionine and its decomposition product, 2-hydroxy-4-methylmercapto-butyric acid etc. [Abraham, 1986]. Liquid chromatography and cyclic adsorption processes have been employed extensively in large scale for biochemical recovery and purification [Asenjo, 1983; Lee et al., 1997a, b; Lee and Moon, 1999; Yang et al., 2003].

For the separation of biological molecules, high selectivity is often needed to separate these molecules from mixtures containing impurities with similar chemical and physical properties. Mesoporous silicates, denoted M41S, have been the subject of much interest since they were first reported by Mobil researchers [Beck et al., 1992]. The mesoporous silicates, M41S, are classified into several members including MCM-41, MCM-48, MCM-50, and others. Recently, other mesoporous materials such as SBA-1, SBA-15, and KIT-1 have been synthesized [Seo et al., 1998]. SBA-15 has an hexagonal array of unidirectional pores while MCM-48 has a bicontinuous cubic pore system [Oh et al., 2003; Ryoo et al., 1999]. The synthesis and utilization of these materials have been investigated by many researchers because of their peculiar characteristics such as a highly regular pore structure, uniform pore sizes (15-100 Å), high surface areas (1,000 m^2/g), and high thermal stability. These mesoporous materials may be useful as adsorbents, supports, catalysts, and sensors. For adsorption studies, many works have been mainly focusing on gas-phase adsorption. However, there are few papers concerning the application of mesoporous materials for the liquid-phase adsorption. Recently, Kisler et al. [2001] addressed the separation of biological molecules (trypsin, lysozyme, and riboflavin) using mesoporous materials (MCM-41 and MCM-48). More recently, Deere et al. [2002] reported on the mechanical and structural features of liquid-phase adsorption and desorption of various proteins onto mesoporous materials with cyano-functional groups. So far, there has been no report on adsorption properties of biochemicals on mesoporous materials in terms of various operating conditions.

We investigated the adsorption equilibrium of cephalosporin C as a model compound of biochemicals under key operating conditions of adsorption and chromatography, such as ethanol concentration, solution pH, and temperature. In addition, hydrothermal stability of two mesoporous materials (MCM-48 and SBA-15) before and after immersion in water was examined through XRD data.

EXPERIMENTAL

MCM-48 mesoporous sample was synthesized by using cethyltrimethylammonium bromide (CTMABr, $C_{19}H_{42}BrN$, Aldrich), as the template, and Ludox AS-40 (Du Pont, 40 mass% colloidal silica in water) as the silicon source according to the method described in previous reports [Lee et al., 2003; Oh et al., 2003; Ryoo et al., 1999]. The template was removed by calcination in air at 873 K for 10 h at a heating rate of 1 K/min. The resultant white powder was stored in a sealed bottle. SBA-15 silica was synthesized as reported by Zhao et al. [1998] using Pluronic P123 triblock copolymer ($EO_{20}PO_{70}EO_{20}$). A sample of Pluronic P123 was dissolved in water and 2 M HCl solution. Then, TEOS was added, and the resulting mixture was stirred for 5 min and then kept at 308 K for 20 h with stirring. This low-temperature preparation was followed by aging for 1 day at 353 K. The solid product was filtered, washed with water and dried in an oven for 24 h at 373 K (SBA-15 (#1)), and subsequently calcined in air for 4 hrs at 823 K (SBA-15 (#2)). To compare with MCM-48 and SBA-15, a commercialized non-

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ionic polymeric sorbent used in this study was SP850, a macroreticular and spherical polystyrene resin cross-linked with DVB (divinylbenzene) supplied by Mitzubishi Chemical Co. (Japan). The arithmetic average particle diameter was determined by sorting wet resin particles with an optical microscope. It was about 380 µm. Prior to experiments, the sorbent was leached with isopropyl alcohol for 24 h to wet internal pores. Sorbent particles were loaded in a 0.02 m ID glass column, and a ten-bed volume of sodium hydrocide (0.1 N) and HCl (0.1 N) was passed through the column successively at a flow rate of 1.0×10^{-4} m^3/min to remove impurities. Finally, a twenty bed-volume of distilled and deionized water was passed at the same flow rate to rinse off HCl.

We used cephalosporin C as a model compound of biochemicals, which was supplied by Cheil Food and Chemicals Inc. (Korea). The chemical formula and molecular weight are $C_{16}H_{21}N_3O_8S$ and 415.44, respectively. All chemicals used in the experimental work were analytical reagent grade. Distilled and deionized water was used to prepare the test solutions. The solution pH was adjusted by using HCl and NaOH. Equilibrium data were obtained by introducing a given amount of mesoporous sorbents (MCM-48, SBA-15) into an aqueous solution of cephalosporin C with a known concentration, shaking the solution in a constant temperature incubator at 293.15 K for 5 h, which was long enough to establish equilibrium, and measuring the remaining adsorbate concentration in the solution. After equilibrium was reached, the amount of cephalosporin C left in the solution was analyzed with a UV spectrometer (Varian, model DMS 100S) at 260 nm. The adsorption capacity of cephalosporin C on mesoporous sorbents was determined in terms of ethanol concentration (1 and 5 vol%), initial pH of solution (2.50, 3.00, 5.30), and temperature (283.15, 293.15, and 303.15 K).

RESULTS AND DISCUSSION

The quality of the MCM-48 and SBA-15 (#2) prepared in this study was examined by nitrogen adsorption and X-ray diffraction (XRD) techniques. Fig. 1 shows nitrogen adsorption and desorption isotherms measured at 77 K by using a Micromeritics ASAP 2010 automatic analyzer. BET surface areas and the pore size determined by BJH (Barrett, Joyner, and Halenda) method for the synthesized mesoporous sorbents (MCM-48 and SBA-15 (#2)) were determined by the adsorption branches of the isotherms. The two mesoporous materials yield a type IV isotherm. The isotherm of MCM-48 is reversible and does not exhibit hysteresis between adsorption and desorption. The maxima in the pore size distribution curves indicated uniform mesopores of approximately 32 Å. Unlike MCM-48, SBA-15 (#2) shows the isotherm with H_1-type hysteresis. This result mainly depends on the pore size and shape for the silica materials. Table 1 summarizes the important physical properties of mesoporous sorbents. The surface areas of MCM-48 and

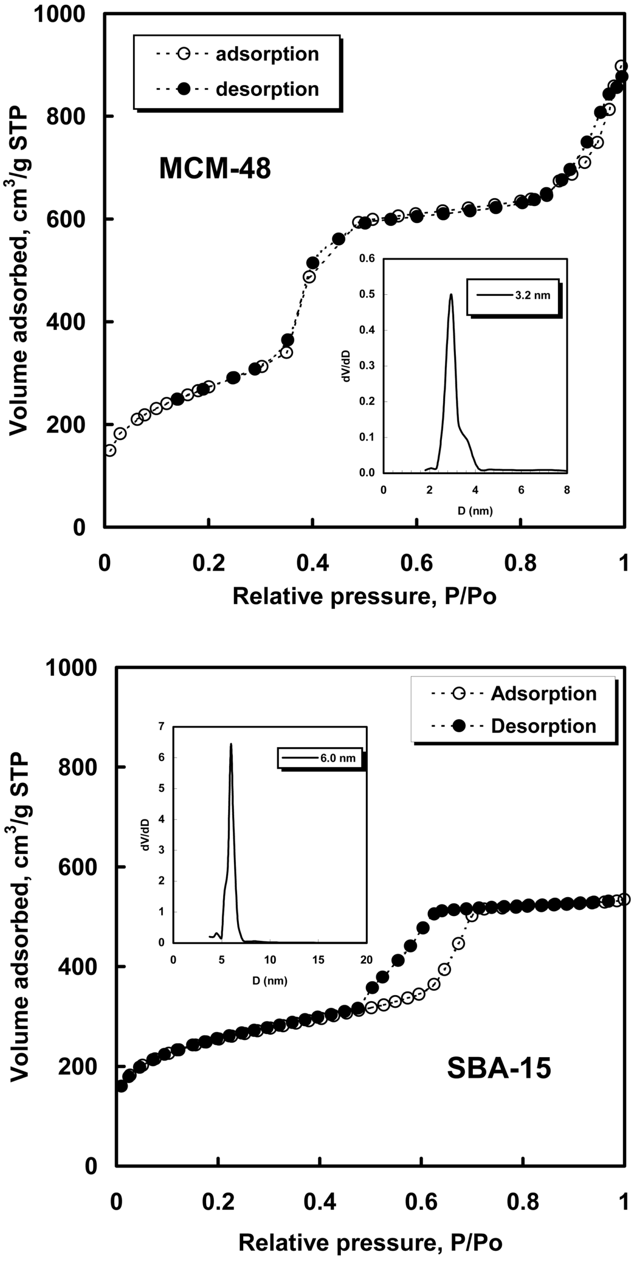

Fig. 1. Nitrogen adsorption and desorption on MCM-48 and SBA-15 at 77 K.

Table 1. Physical properties of mesoporous adsorbents

Properties	MCM-48	SBA-15 (#1)	SBA-15 (#2)	SP850
Pore shape	cubic	hexagonal	hexagonal	amorphous
Surface area (m^2/g)	993	610	645	1,000
Pore volume (cc/g)	1.08	0.80	0.83	0.89
Mean pore size (Å)	32	56	60	38

SBA-15 (#1): without calcination
SBA-15 (#2): calcination for 4 hrs at 823 K.

two SBA-15 samples were 993, 610 and 645 m^2/g, and their average pore size were 32, 56 and 60 Å, respectively. It was found that the physical properties for two SBA-15 materials with and without calcinations are highly different. A commercialized nonionic polymeric sorbent, SP850 was chosen to compare the mesoporous materials. The physical property of SP850 is close to MCM-48 except the pore structure as listed in Table 1.

In order to check the structural degradation after immersion in water, XRD data of MCM-48 and SBA-15 (#2) were obtained on a Phillips PW3123 diffractometer equipped with a graphite monochromator and Cu Kα radiation of wavelength 0.154 nm. Fig. 2 shows the XRD peaks of the samples before and after immersion in water in terms of contact time. The XRD patterns of original MCM-48 showed the two characteristic (211) and (220) reflections in the 2θ range from 2° to 10°, indicating a well-ordered cubic pore. Like MCM-48, the XRD patterns of the original SBA-15 showed three diffraction peaks that can be indexed to (100), (110), and (200) in the 2θ range from 0.8° to 10°, representing well-ordered hexagonal pores. The observation data from the original samples of MCM-48 and SBA-15 (#2) are in good agreement with that previously reported [Ryoo et al., 1999; Zhao et al., 1998]. On the other hand, XRD data were obtained to check the structural degradation of mesoporous samples before and after immersion in water for as little as 48 h at 293.15 K. There was no significant change before and after immersion. This result implies that significant structural degradation did not occur for samples after immersion. However, the XRD peaks of MCM-48 and SBA-15 (#2) very slightly reduced intensity in (211) and (100) peaks, respectively, indicating loss of structural order. In order to overcome this problem, a hydrophobic coating has been applied to siliceous MCM-48 and SBA-15 using

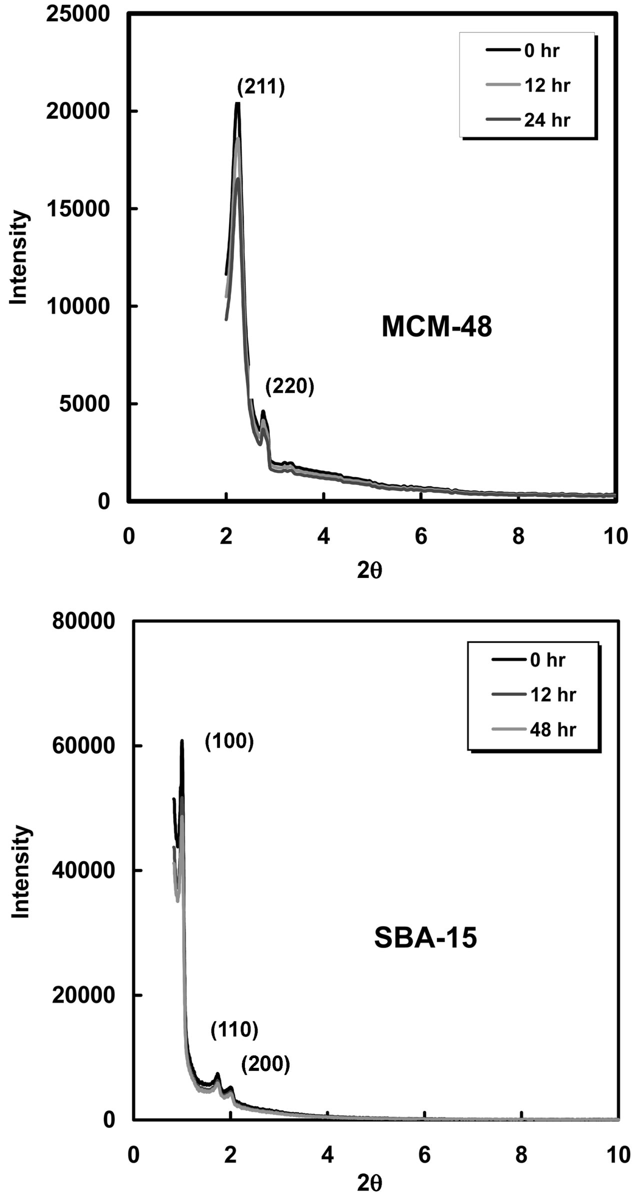

Fig. 2. XRD peaks of MCM-48 and SBA-15 (#2).

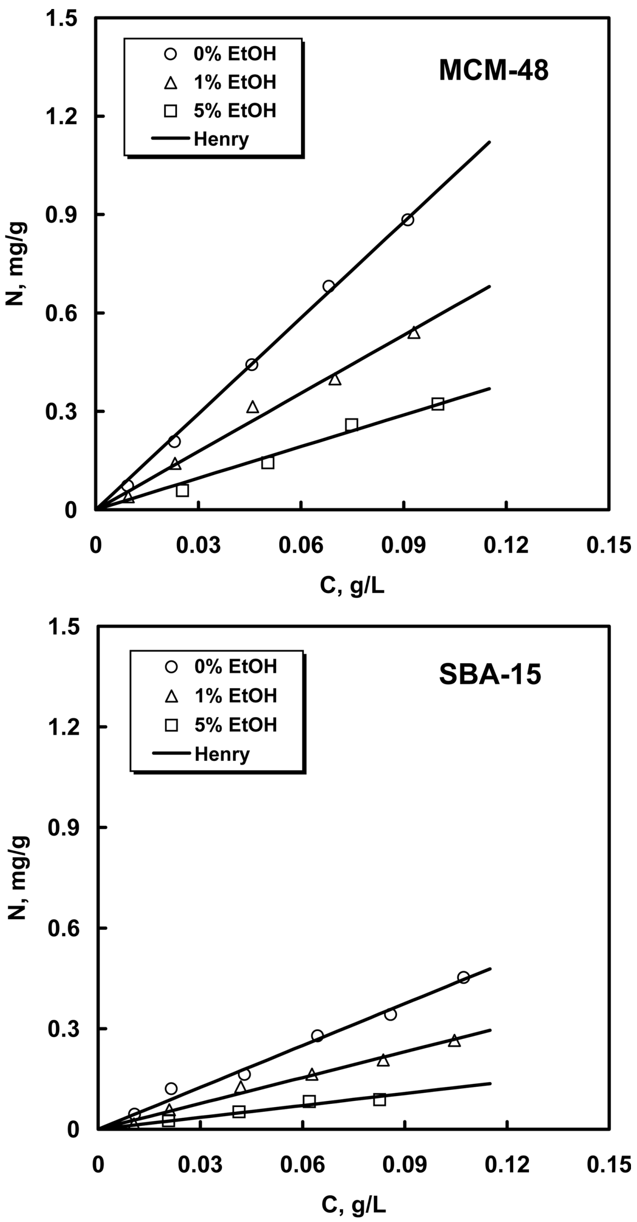

Fig. 3. Effect of EtOH concentration on the adsorption isotherms of cephalosporin C on MCM-48 and SBA-15 (#1) (pH 5.30 and 293.15 K).

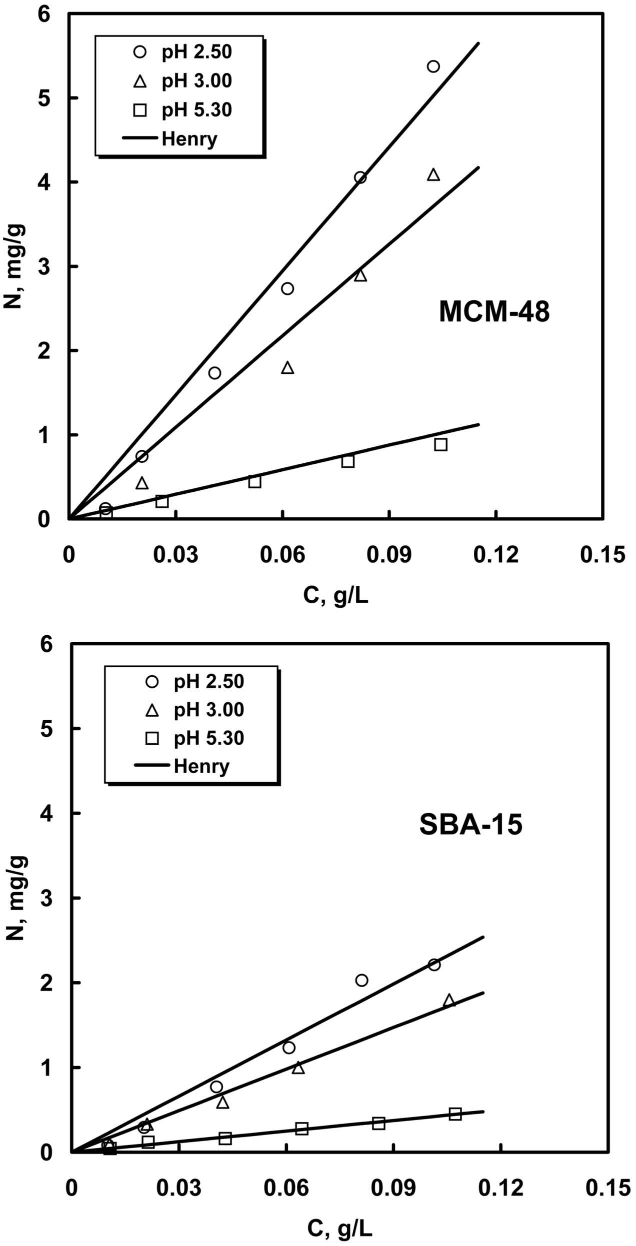

Fig. 4. Effect of solution pH on the adsorption isotherms of cephalosporin C on MCM-48 and SBA-15 (#1) (EtOH 0% and 293.15 K).

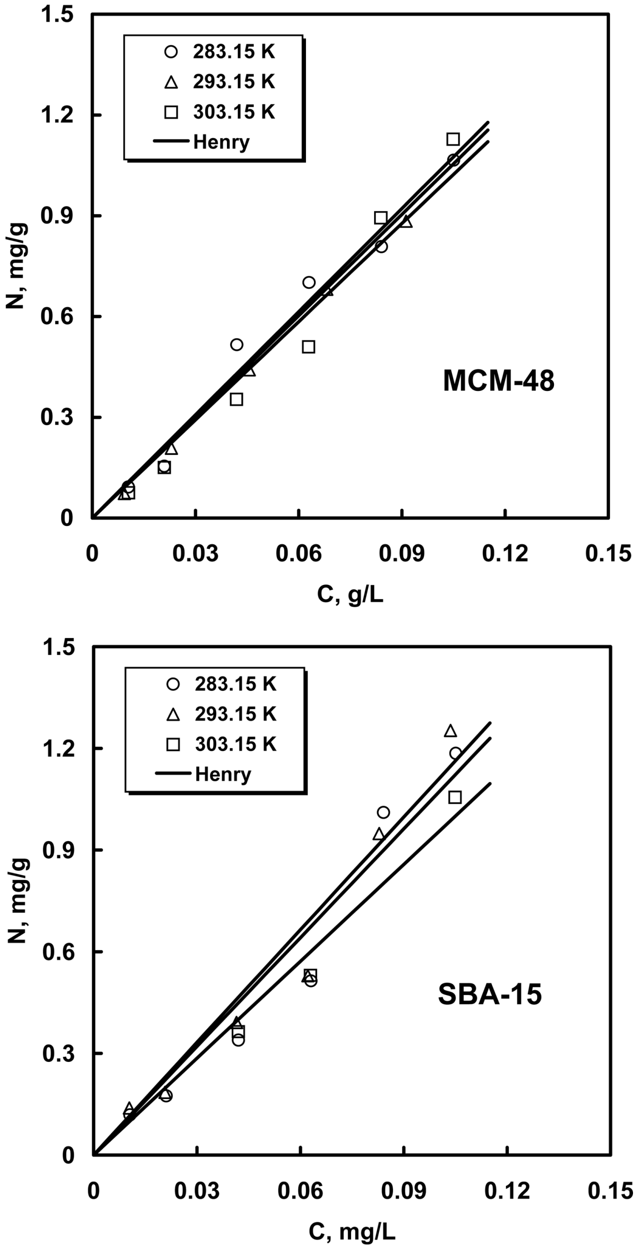

Fig. 5. Effect of temperature on the adsorption isotherms of cephalosporin C on MCM-48 and SBA-15 (#2) (EtOH 0% and pH 5.30).

hexamethyldisilazane to reduce the degradation of the structure by hydrolysis and so to increase their stability in water [Kisler et al., 2001].

Isotherms play a crucial role in model prediction and design of adsorption process and chromatography. Figs. 3-5 show the adsorption isotherms of cephalosporin C on MCM-48 and SBA-15 in terms of key operating conditions such as ethanol concentration, solution pH, and temperature. The adsorption amount of MCM-48 is higher than that of SBA-15 (#1) in terms of ethanol concentration and solution pH. This result may come from the different physical properties of surface area for two mesoporous materials listed in Table 1. The adsorption amounts were highly dependent on the two operating conditions such as ethanol concentration and solution pH. This result is quite meaningful in adsorption and chromatography because the adsorption affinity can be easily controlled by adjustment of mobile phase using these operating conditions. For low concentration (<0.12 g/L), adsorption isotherms of cephalosporin C were almost linear. Thus, the equilibrium data were correlated with the Henry isotherm. The isotherm parameters were determined by minimizing the mean percent deviations between experimental and predicted amounts adsorbed, and they are listed in Table 2. The adsorption amount for SBA-15 (#1) and SBA-15 (#2) samples prepared with and without calcinations was highly different. Fig. 3 shows the adsorption isotherms of cephalosporin C as a function of ethanol concentrations (0, 1, and 5 vol%). As expected, the adsorption amount was greatly decreased with a small increase in the ethanol concentration. This result comes from the fact that the two mesoporous silica materials have organophilic surfaces [Lee et al., 2003]. Fig. 4 shows the adsorption isotherms of cephalosporin C at different solution pH (2.50, 3.00, and 5.30). The adsorption amount greatly decreases with pH. The difference in the amount adsorbed might be due to the change of its ionic forms, which strongly depends on the solution pH [Lee and Moon, 1999]. They calculated the fraction of various ionic forms of cephalosporin C from the association-dissociation equilibria with pH values to quantitatively analyze adsorption dynamics. Here, we do not consider ion form with solution pH. Unlike the great effect of ethanol concentration and solution pH on the adsorption amount, the influence of temperature was small as shown in Fig. 5. To compare the adsorption amount with the two mesoporous materials, the adsorption isotherm on the commercialized non-ionic polymeric sorbent, SP850, was obtained as shown in Fig. 6. The adsorption amount of the two mesoporous materials (MCM-48 and SBA-15 (#2)) and SP850 was almost similar on a mass basis. Further work is being conducted for the adsorption and desorption of proteins and vitamin on MCM-48 and SBA-15 adjusted by the attachment of hexamethyldisilazane to the surface to form hydrophobic coatings to reduce degradation of the structure by hydrolysis, and at the same time to increase separation selectivity. Our experimental results for adsorption capacity and hydrothermal stability investigated in this work indicate that mesoporous materials can be extensively applicable for the separation and purification of biochemicals.

Table 2. Henry isotherm parameters of cephalosporin C on MCM-48, SBA-15 and SP850

Sorbent	EtOH (vol%)	Initial pH	Temperature (K)	K
MCM-48	0	5.30	293.15	9.745
	1	5.30	293.15	5.918
	5	5.30	293.15	3.210
	0	2.50	293.15	48.940
	0	3.00	293.15	36.261
	0	5.30	283.15	10.240
	0	5.30	303.15	10.043
SBA-15 (#1)	0	5.30	293.15	4.162
	1	5.30	293.15	2.567
	5	5.30	293.15	1.182
	0	2.50	293.15	22.072
	0	3.00	293.15	16.354
SBA-15 (#2)	0	5.30	283.15	10.694
	0	5.30	303.15	9.529
SP850	0	5.30	293.15	9.847
	1	5.30	293.15	9.193
	5	5.30	293.15	8.936

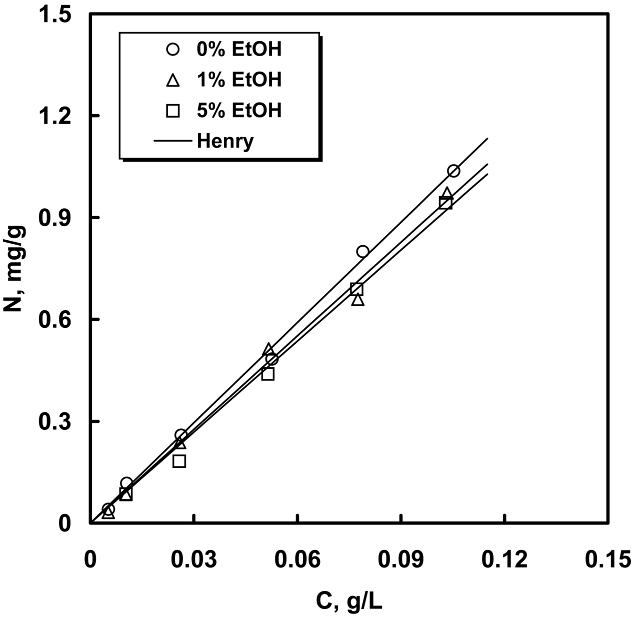

Fig. 6. Adsorption isotherms of cephalosporin C on SP850 in terms of EtOH concentration (pH 5.30 and 293.15 K).

CONCLUSION

High hydrothermal stability of MCM-48 and SBA-15 under our experimental conditions was observed from XRD data. It was also observed that the effect of ethanol concentration and solution pH on adsorption capacity was considerably higher than that of temperature. Compared with the commercialized polymer resin, SP850, the adsorption capacity of MCM-48 and SBA-15 was as high as that of the resin, SP850, on a mass basis. It was found that mesoporous materials have a potential role in the separation and purification processes of biochemicals including antibiotics, amino acids, and proteins based on both size exclusion and chemical selectivity. In order to develop this potential, further work is underway for the adsorption and desorption of proteins and vitamin on MCM-48 and SBA-15 modified by the attachment of ligands to the surface to form functional coatings to increase hydrothermal stability and separation selectivity.

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REFERENCES

Abraham, E. P., “Biosynthesis of Penicillins and Cephalosporins, in *β*-Lactam Antibiotics for Clinical Use,” Marcel Dekker Inc., New York (1986).

Asenjo, J. A., “Separation Processes in Biotechnology,” Marcel Dekker Inc., New York (1983).

Beck, J. S., Vartuli, J. C., Roth, W. J., Leonowicz, M. E., Kresge, C. T., Schmitt, K. D., Chu, C. T.-W., Olson, D. H., Sheppard, E. W., McCullen, S. B., Higgins, J. B. and Schlenker, J. L., “A New Family of Mesoporous Molecular Sieves Prepared with Liquid Crystal Templates,” *J. Am. Chem. Soc.*, **114**, 10834 (1992).

Deere, J., Magner, E., Wall, J. G. and Hodnett, B. K., “Mechanical and Structural Features of Protein Adsorption into Mesoporous Silicates,” *J. Phys. Chem. B*, **106**, 7340 (2002).

Kisler, J. M., Dahler, A., Stevens, G. W. and O’Connor, A. J., “Separation of Biological Molecules Using Mesoporous Molecular Sieves,” *Microporous and Mesoporous Materials*, **44-45**, 769 (2001).

Lee, J. W., Jung, H. J. and Moon, H., "Effect of Operating Conditions on Adsorption of Cephalosporin C in a Column Adsorber," *Korean J. Chem. Eng*., **14**(4), 277 (1997a).

Lee, J. W., Park, H. C. and Moon, H., "Adsorption and Desorption of Cephalosporin C on Nonionic Polymeric Sorbents," *Separation and Purification Technology*, **12**, 1 (1997b).

Lee, J. W. and Moon, H., "Effect of pH on Adsorption of Cephalosporin C by a Nonionic Polymeric Sorbent," *Adsorption*, **5**, 381 (1999).

Lee, J. W., Lee, J. W., Shim, W. G., Suh, S. H. and Moon, H., "Adsorption of Chlorinated Volatile Organic Compounds on MCM-48," *J. Chem. Eng. Data*, **48**, 381 (2003)

Oh, J. S., Shim, W. G., Lee, J. W., Kim, J. H., Moon, H. and Seo, G., "Adsorption Equilibrium of Water Vapor on Mesoporous Materials," *Chem. Eng. Data*, **48**, 1458 (2003).

Ryoo, R., Joo, S. H. and Kim, J. M., "Energetically Favored Formation of MCM-48 from Cationic-Neutral Surfactant Mixtures," *J. Phys. Chem. B*, **103**, 7435 (1999).

Seo, G., Kim, T. J., Lim, S. K., Ko, C. H. and Ryoo, R., "The Reduction of Dissolved Oxygen by Hydrazine over Platinum Catalyst Supported on Disordered Mesoporous Materials," *Korean J. Chem. Eng*., **15**(6), 611 (1998).

Smith, A., "Cephalosporics, in Comprehensive Biotechnology," Pergamon Press, **3**, 163 (1985).

Yang, W. C., Shim, W. G., Lee, J. W. and Moon, H., "Adsorption and Desorption Dynamics of Amino Acids in a Nonionic Polymeric Sorbent XAD-16 Column," *Korean J. Chem. Eng*., **20**(5), 922 (2003).

Zhao, D., Huo, Q., Feng, J., Chmelka, B. F. and Stucky, G. D., "Nonionic Triblock and Star Diblock Copolymer and Oligomeric Surfactant Syntheses of Highly Ordered, Hydrothermally Stable, Mesoporous Silica Structures," *J. Am.Chem. Soc*., **120**, 6024 (1998).