Adsorption isotherms of caffeine on molecular imprinted polymer

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Abstract−A molecular imprinted polymer (MIP) using caffeine as the template and methacrylic acid (MAA) as the functional monomer was prepared. Acetonitrile was used as the porogen with ethylene glycol dimethacrylate (EGDMA) as the crosslinker and 2,2′-azobis(isobutyronitrile) (AIBN) as the initiator. By a linear and nonlinear regression analysis, the experimental parameters in the equilibrium isotherms were estimated. Then, the linear and quadratic equations for concentration and sorbents to adsorption amounts were expressed, and the adsorption equilibrium data were also correlated into the Freundlich isotherm model. Comparisons of caffeine adsorption isotherm on C_{18} particles as well as the molecular imprinted polymer were made. The results showed that the caffeine-imprinted polymer showed extraordinarily higher adsorption ability than C_{18} particles.

Key words: Adsorption Isotherm, Molecular Imprinted Polymer, Caffeine, HPLC

INTRODUCTION

The adsorption isotherm, which is a basic thermodynamic property of separation processes, is the relationship between the concentration of the solute in the stationary phase and that in the mobile phase. The parameters of the adsorption isotherm can be determined by fitting the model to the experimental data. By this way, it is possible to predict the individual band profile of separated sample components under various conditions and to optimize the separation condition [1]. The success of experiments and modeling are directly related to accurate adsorption isotherms and their parameters [2,3].

The technique of molecular imprinting consists of the self-assembly of a functional monomer and a template molecule in solution followed by the co-polymerization of the functional monomer and an excess of an appropriate crosslinking monomer. After removing of the small molecule, the resulting network polymer exhibits a significantly higher affinity for the molecule used as the template than for similar molecules, including closely related isomers [4]. MIP have been applied to chiral separation [5-8], solid extraction [4,9], biomimic sensor [10,11] and membrane separation [12,13]. MIP can be prepared by both the covalent and the non-covalent method, whereas the latter has been widely used in recent years because of the ease with which that method can be performed [14].

MIP is resistant not only to mechanical stress, high pressure and elevated temperature, but also to acids, bases, organic solvents and metal ions. The advantages that MIP possesses over biopolymers are low cost, good physical and chemical stability. More importantly, the functional groups in the resulting binding sites should be arranged in positions suitable for interaction with the template molecule; the molecule imprinting polymer can selectively recognize the template molecular among other structurally related molecules, after

removal of the template. Molecular imprinted polymers especially based on the non-covalent preparation as a new technique is attracting more and more interest. These kinds of materials show many advantageous features, such as, high selectivity, easily made, low cost for preparation and workability under different conditions, have been widely used [15,16].

In this work, the adsorption equilibrium data were correlated into the three isotherm models, the linear, quadratic and Freundlich types for the developed synthesis and manufacture of MIP, an imprinted polymer stationary phase by using caffeine as the template, MAA as the functional monomer and EGDMA as the crosslinker. Furthermore, the adsorption characteristics such as isotherms of caffeine on the caffeine-imprinted polymer and C_{18} particles were obtained.

EXPERIMENTAL

1. Materials

Caffeine and methacrylic acid (MAA) was bought from Sigma (St Louis, MO, U.S.A.). Ethylene glycol dimethacrylate (EGDMA) was purchased from Fluka (Buchs, Switzerland). 2,2′-azobis(isobutyronitrile) (AIBN) was produced by Junsei Chemical Co. Ltd. (Japan) and refined before use. Methyl alcohol and acetonitrile were from Pure Chemical Co., Ltd (Ansan, Korea). Acetic acid (analytical grade) was from Oriental Chemical Industries (Incheon, Korea). All the other solvents used in the experiment were HPLC or analytical grade. Distilled water was filtered with a vacuum pump (Division of Millipore, Waters, U.S.A.) and filter (HA-0.45, Division of Millipore, Waters, U.S.A.) before use. All the samples were filtered by using a filter (MFS-25, 0.2 μm TF, WHATMAN, U.S.A.) before injection into the HPLC system.

2. Instrumentation

The chromatography system consisted of Waters 600 s Multi solvent Delivery System and Waters 616 liquid chromatography (Waters Associates, Milford, MA, U.S.A.), Rhodyne injector (20 μL sam-

ple loop), a variable wavelength 2487 UV dual channel detector. Data processing was carried out with a Millenium 3.2 consisting of HP Vectra 500PC. The chromatographic conditions were a flow rate 0.5 μL/min, injection volume 0.5 μL, and UV wavelength 270 nm. The C_{18} column (25-35 μm particles, 100 Å pore sizes, 4.6×250 mm) from RS tech Corporation (Daejeon, Korea), methanol : water =40 : 60 (vol%) as the mobile phase, was used to determine the free concentration of the compound in the static method.

3. Methodology

The operating condition and preparation method for synthesis, polymerization and manufacture of molecular imprinted polymer were well cited in the referenced paper of Wang et al. [4]. Commercial SPE cartridges were emptied from their packing materials. Then the cartridge tube and frits were thoroughly cleaned and dried. About 200 mg of the corresponding polymers was packed dry in the cartridges and the upper first was placed on top. The used C_{18} SPE cartridge (with 200 mg packing material) was a product from Alltech (Deerfield, U.S.A.). Before the extraction, the MIP cartridges were treated with methanol : acetic acid (90 : 10, vol%), 4×3 mL (4 times per 3 mL), followed by methanol 4×3 mL. C_{18} particles and blank polymer were treated only by methanol 4×3 ml. Extraction experiments consisted of loading the SPE cartridges with a caffeine solution (the concentrations of 0.2, 1.0, 2.0, 3.5, 5.0, 7.0 and 8.0 mg/mL). Quantitative determination was based on the constructed calibration curve: y=1×10^{-6}×−2.1039 for caffeine. y is the volume of caffeine compounds in the methanol (mL) of injected samples, while x is the peak area (mAU*sec). 5, 10, 15 and 20 μL volumes of caffeine were injected into HPLC system to obtain the calibration curve. The regression coefficient of the straight line for the caffeine was 0.9763.

The adsorption method was performed on the manufactured molecular imprinted polymers and commercial C_{18} particles. 200 mg of the C_{18} particles and caffeine imprinted polymer were put into cartridge of the solid-phase extraction, respectively. Then 1.0 mL of caffeine solution with the concentration of 0.2 to 8.0 mg/mL was added. First, the elution was operated 5 times by 1.0 mL caffeine solution with the caffeine concentration of 0.2 mg/mL. The experiment was repeated 3 times by 1.0 mL caffeine solution at the different caffeine concentrations. The free amounts of caffeine in the solution were determined by the analytical RP-HPLC system at room temperature. The adsorbed caffeine on the molecular imprinted polymer and commercial C_{18} particles was calculated by subtracting the free amounts from the initial amounts of caffeine.

RESULTS AND DISCUSSION

Many isotherms have been reported in literature to successfully calculate the binding properties of MIPs: simple Langmuir, SIPs models, Bi-Langmuir models, Jovanovic, Bi-Jovanovic and Freundlich-Jovanovic [17]. In this work, in addition to linear and quadratic equations we have chosen to consider the nonlinear Freundlich model. The adsorption equilibrium data was fitted into the equilibrium models.

The amounts of caffeine by molecular imprinted polymer and C_{18} particles at the solid-phase cartridges were measured at the different concentrations, after the equilibrium adsorptions of caffeine on MIP and C_{18} particles were attained. Comparing the amount of

Table 1. Adsorption amounts of caffeine on MIP and C_{18} sorbents

Concentration (mg/ml)	Adsorption amounts (mg/g)	
	MIP	C_{18}
0.20	1.10	0.20
1.00	7.00	1.90
2.00	11.15	5.60
3.50	19.00	7.90
5.00	27.00	10.50
7.00	39.60	11.35
8.00	39.65	11.15

caffeine by MIP sorbent with that by C_{18} particles, it showed the similar trends that with the larger concentration of caffeine up to 5 mg/mL, the more caffeine was adsorbed on the stationary phases. Above the concentration, on the two stationary phases, the adsorption amounts were asymptotic to the saturated values. Table 1 illustrates the experimental results of the adsorption amounts of caffeine on the molecular imprinted polymer and C_{18} sorbents. The resulting experiment data were fitted to the following models of adsorption isotherm:

$$A=aC_m+b \tag{1}$$

$$A=aC_m^2+bC_m+c \tag{2}$$

$$A=aC_m^{1/c} \tag{3}$$

Where A and C_m were the adsorption amounts and the concentration of caffeine solution, respectively. And a, b and c were the parameters experimentally determined. These adsorption isotherms were the linear (1), quadratic (2) and Freundlich (3) isotherms. The parameters fitted by the three adsorption isotherm models are listed in Table 2, which indicated that the imprinted molecularly polymer showed higher affinity to the target molecule of caffeine than C_{18} particles. That is, the caffeine-imprinted polymer possessed higher saturation capacity by the template than that of C_{18} particles. Comparing the coincidences between the experimental data and the equilibrium isotherms, normally the regression coefficients of the MIP sorbents were larger than those of C_{18} particles. It is interesting to note that the equilibrium data by MIP was well fitted by the three isotherms; however, poor agreement was observed by the C_{18} particles. The comparison of experimental and calculated concentrations of caffeine on MIP is shown in Fig. 1. When the points were on the diagonal line, the adsorption isotherms well described the exper-

Table 2. Parameters in adsorption isotherm of caffeine on MIP and C_{18} particles

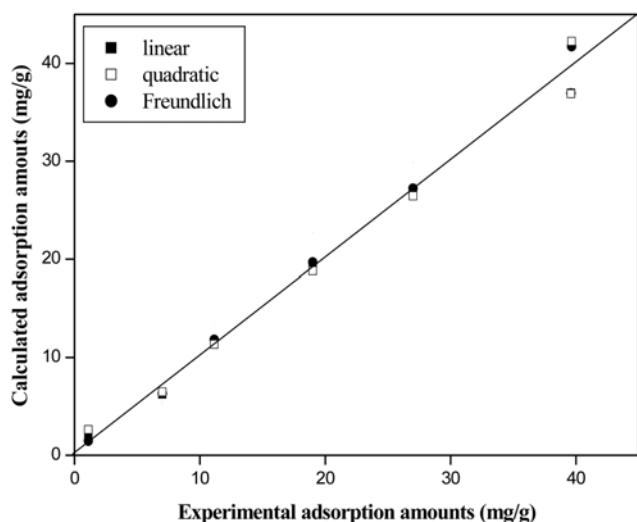| Adsorption isotherm		Parameters			Regression coefficient
		a	b	c	
MIP	Linear	5.1187	1.1287	-	0.9881
	Quadratic	0.0385	4.7644	1.6718	0.9897
	Freundlich	6.3091	-	0.9083	0.9873
C_{18}	Linear	1.4320	1.4700	-	0.8860
	Quadratic	0.0031	1.3990	1.5565	0.8889
	Freundlich	3.2887	-	0.6348	0.9454

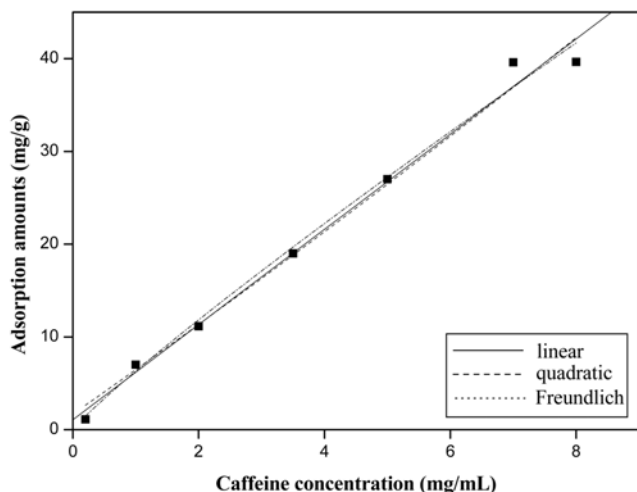**Fig. 1. Comparison of experimental and calculated concentrations of caffeine on MIP.**

Fig. 2. Adsorption isotherm of caffeine on MIP fitted by linear, quadratic and Freundlich equations.

imental data, and the more deviation from the line that occurred, the worse isotherm was used. This figure shows negligible deviation. Almost, the experimental and calculated data were on the diagonal neighborhood. This implies that these experimental data were well fitted to the three equations. Fig. 2 shows the plot of the experimental data of caffeine on the molecular imprinted polymer fitted by the linear (Eq. (1)), quadratic (Eq. (2)) and Freundlich (Eq. (3)) equation, respectively. The experimental data fitted to the three equations quite well. While in the Eqs. of (1) and (3), they have two parameters each, but in Eq. (2), in the quadratic form, there are three parameters. Form Table 2 and Fig. 2, Eqs. (1) and (2) were good enough to correlate the equilibrium experimental data in this experimental range of concentration. The regression coefficients of Freundlich adsorption isotherm of Eq. (3) were 0.9873 for caffeine-imprinted polymer and 0.9454 for C_{18} particles, respectively (refer to Table 2), whereas for quadratic adsorption isotherm of Eq. (2),

the regression coefficients are 0.9897 and 0.8889 for the MIP sorbent and C_{18} particles, respectively. The caffeine-imprinted polymer had significantly higher adsorption ability for the template than the C_{18} particles.

CONCLUSIONS

In this work, the adsorption amount of caffeine was experimentally measured to correlate the adsorption isotherm of caffeine for the prepared molecular imprinting polymer and C_{18} particles. The adsorption equilibrium data were fitted by the isotherm equations of linear, quadratic and Freundlich types. In the moderate range of concentration, the linear isotherm was the well-fit model to the experimental data. The regression coefficient was as high as 0.9881 in the MIP sorbent prepared by the template of caffeine. The large saturation capacity and high selectivity of the molecular imprinted polymer prepared in this work might be commercially implemented. Therefore, the polymer is able to be reproducibly synthesized and is an attractive feature for further applications.

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