Adsorption Isotherms of Catechin Compounds on (+)Catechin-MIP

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A molecular imprinted polymer (MIP) using (+)catechin ((+)C) as a template and acrylamide (AM) as a 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. The adsorption isotherms in the MIP were measured and the parameters of the equilibrium isotherms were estimated by linear and nonlinear regression analyses. The linear equation for original concentration and adsorpted concentrations was then expressed, and the adsorption equilibrium data were correlated into Langmuir, Freundlich, quadratic, and Langmuir Extension isotherm models. The mixture compounds of (+)C and epicatechin (EC) show competitive adsorption on specific binding sites of the (+)catechin-MIP. The adsorption concentrations of (+)C, epicatechin (EC), epicatechin gallate (ECG), and epigallocatechin gallate (EGCG), on the (+)catechin-molecular imprinted polymer were compared. Through the analysis, the (+)catechin-molecular imprinted polymer showed higher adsorption ability than blank polymer which was synthesized molecular imprinted polymer without (+)catechin. Furthermore, the competitive Langmuir isotherms were applied to the mixture compounds of (+)C and EC.

Key Words : Adsorption isotherm, Molecular imprinted polymer, (+)C, Catechin compounds, Competitive Langmuir

Introduction

The adsorption isotherm is a basic thermodynamic property of a separation processes and 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 experimental data to the model. In this manner, it is possible to predict the individual band profile of separated sample components under various conditions and to optimize the separation conditions.[1] The success of experiments and modeling are directly related to the accuracy of the 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 a solution followed by co-polymerization of the functional monomer and an appropriate cross-linking monomer. After removal of template molecules, the resulting network polymer exhibits significantly higher affinity for the template molecules than for the other similar molecules, including closely related isomers.[4] MIP(molecular imprinted polymer) have been applied to chiral separation,[5-7] solid extraction,[8,9] biomimic sensor,[10,11] and membrane separation.[12,13] While MIP can be prepared by both the covalent and the non-covalent method, the latter has been widely used in recent years owing to its relatively simple procedure.[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 offers over biopolymers include low cost, and 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 so that the molecule imprinted polymer can selectively recognize the template molecular among other structurally related molecules after removal of the template. Molecular imprinted polymers based on non-covalent preparation have attracted a great deal of attention. Notably, they show many advantageous features, such as high selectivity, ease of manufacture, low cost for preparation, and workability under different conditions.[15,16] Most liquid chromatography is carried out under nonlinear conditions, and the nonlinear behavior should be considered properly in the equilibrium isotherms. The Langmuir model is the most popular equilibrium isotherm among the various nonlinear isotherm models. A multi-compounds Langmuir isotherm explains the competition of two compounds for available adsorption sites.[17,18] The competitive Langmuir isotherms for liquid chromatography has been studied by Juza[17,19] and Guiochon.[20] and the multi-compounds competitive isotherm was shown to be better than that of the single compound isotherm.[21-23] According to reports in the literature, many isotherms have been successfully employed to calculate the binding properties of MIPs: simple Langmuir, SIPs models, Bi-Langmuir models, Jovanovic model, Bi-Jovanovic model, and Freundlich-Jovanovic model.[22]

In this work, the adsorption equilibrium data of a single compound were correlated into five isotherm models: linear, Langmuir, Freundlich, quadratic, and Langmuir Extension isotherms. For the developed synthesis and manufacture of MIP, a molecular imprinted polymer stationary phase was produced using (+)C as a template, AM as a functional

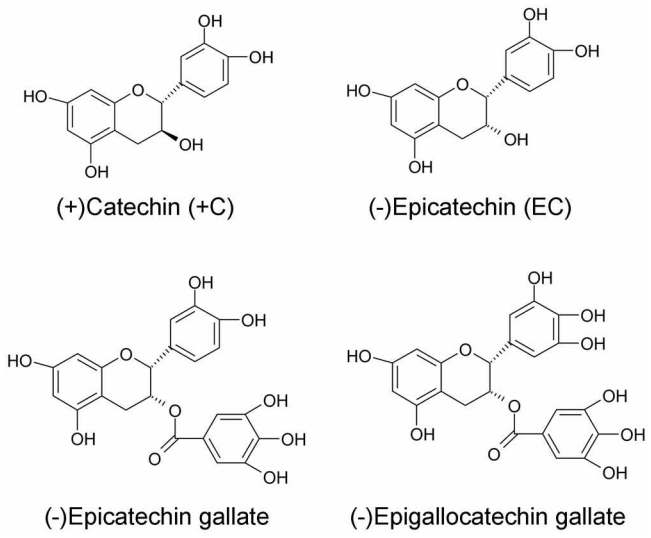

Figure 1. Chemical structures of (+)C, EC, ECG and EGCG.

monomer, and EGDMA as a cross-linker. The adsorption characteristics such as isotherms of similar chemical structures (Figure 1) of (+)C, EC, ECG and EGCG on the (+)catechin-molecular imprinted polymer were obtained. Furthermore, isotherms of (+)C on the imprinted and blank polymers were determined using static method. Competitive Langmuir isotherms were also applied to the mixture compounds of (+)C and EC.

Materials and Methods

Materials. (+)C, EC, ECG, and EGCG and AM were purchased from Sigma (St Louis, MO, U.S.A.). EGDMA was purchased from Fluka (Buchs, Switzerland). AIBN was produced by Junsei Chemical Co. Ltd. (Japan) and refined before use. Methanol 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 (MFS-25, 0.2 μm TF, WHATMAN, U.S.A.) before injection into the HPLC system.

Instrumentation. The chromatography system consisted of a Waters 600s Multi solvent Delivery System and a Waters 616 liquid chromatography (Waters Associates, Milford, MA, U.S.A.), a Rheodyne injector (20 μL sample loop), and a variable wavelength 2487 UV dual channel detector. Data processing was carried out with Millenium 3.2 using a HP Vectra 500PC. The flow rate was 0.5 mL/min, the injection volume was 5 μL, and the UV wavelength was 270 nm. A C18 column (5 μm particles, 100 Å pore sizes, 4.6 × 250 mm) from RS tech Corporation (Daejeon, Korea), where water/methanol = 60/40 (vol. %) was the mobile phase, was used to determine the free concentration of the compound *via* the static method.

Polymer Preparations. The following were added to a 250 mL two-neck glass flask: 5 mmol of the monomer (AM), 30 mmol of the crosslinker (EGDMA), 0.12 g of the initiator (AIBN), 9 mL of acetonitrile of the porogen and 0.25 mmol of the template ((+)C). The reaction mixture was subjected to supersonication for 10 min, sparged with helium for 10 min to remove oxygen, and then vacuumed for 10 min and sealed under vacuum. Polymerization was performed in a water bath that was held at 60 °C for 24 hr. After the polymerization, the bulk polymer was removed from the reaction flask and put into an oven for drying. The dried polymer was grounded into particles and passed through a 32 μm sieve; small particles were removed by repeated sedimentations with water. By these procedures, particles of 25-32 μm size were collected. The dried particles were packed into a 3.9 × 150 mm stainless steel HPLC column. First, a solution of methanol/acetic acid = 90/10 (vol. %) was used to remove the template molecule; then the residual acetic acid was removed with methanol. For comparison, blank polymer was prepared with the same procedure but in the absence of the template.

Static Method. The static method was performed on the manufactured polymer particles. Ten shares of 30 mg of the (+)catechin-imprinted polymers were placed into 10 mL flasks, respectively. And then 3.0 mL of (+)C, EC, ECG and EGCG solution with a concentration of 0.15 to 2 mmol/L was added. More than, mixture solutions of (+)C and EC with different concentrations were added. The mixture was left at room temperature for 72 hr and then the supernatant was collected and filtered (0.2 μm). The concentrations of free (+)C, EC, ECG and EGCG in the solution were determined using a C18 column at room temperature. Absorbed (+)C, EC, ECG and EGCG on the molecular imprinted polymers were calculated by subtracting the free concentrations from the initial concentrations of these compounds.

Results and Discussion

In this work, in addition to a linear equation, we have chosen to consider the nonlinear Langmuir, Freundlich, quadratic, and Langmuir Extension models. The adsorption equilibrium data was fitted into the equilibrium models.

The concentrations of (+)C, EC, ECG, and EGCG using (+)catechin-molecular imprinted polymer at the flasks were measured at different concentrations, after the equilibrium adsorptions of these compounds on MIP were attained, respectively. Comparing the concentration of (+)C, EC, ECG, and EGCG on the MIP sorbent, similar trends with that with the larger concentration of (+)C, EC, ECG, and EGCG up to 2 mmol/L were observed, and the more (+)C was adsorbed on the stationary phases. Above 2 mmol/L, the adsorption concentrations were asymptotic to the saturated values. Quantitative determination was based on the constructed calibration curve (Table 1).

Figure 2 shows the experimental results of the adsorption concentrations of catechin compounds on the (+)catechin-molecular imprinted polymer. The adsorption concentrations

Table 1. Calibration equations of caffeine and catechin compounds

Compounds	Equation	r^2
(+)C	$y = 2 \times 10^{-6}x - 0.8001$	0.9855
EC	$y = 2 \times 10^{-6}x - 0.9117$	0.9845
ECG	$y = 3 \times 10^{-7}x + 2.8526$	0.9867
EGCG	$y = 3 \times 10^{-6}x + 4.5698$	0.9950

x: peak area (mAU*Sec), y: injection volume of catechin compounds in the methanol (μL)

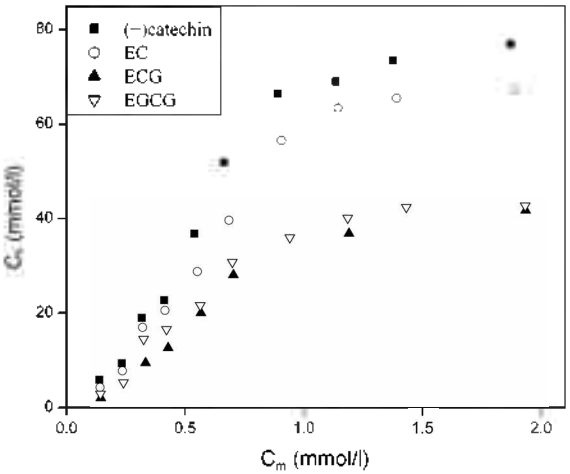

Figure 2. The adsorption concentrations of catechin compounds on (+)catechin-MIP.

of (+)C, EC, ECG, and EGCG increased with increased concentrations of solutions. The adosption concentration of (+)C was higher than the adsorption concentrations of another catechin compounds. The imprinted polymer surface is often regarded as heterogeneous and there are two kinds of binding sites on the imprinted polymer surface, one is selective or has high affinity with high binding energy and the other is nonselective or has low affinity with low binding energy. In the low concentration range, the adsorption on selective binding sites is stronger than that on nonselective binding sites.[23]

The resulting experiment data were fitted to the following models of adsorption isotherm:

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

$$C_s = \frac{aC_m}{1 + bC_m} \tag{2}$$

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

$$C_s = aC_m^2 + bC_m + c \tag{4}$$

$$C_s = \frac{1}{a + bC_m^{c-1}} \tag{5}$$

where C_s and C_m are the adsorption and non-adsorption concentrations of (+)C, EC, ECG, and EGCG solutions, respectively; a, b, and c are experimentally determined parameters experimentally determined. These adsorption isotherms are the linear (1), Langmuir (2), Freundlich (3), quadratic (4), and Langmuir Extension (5) isotherms.

Table 2. Parameters in adsorption isotherm of single compound on (+)catechin-MIP

Adsorption isotherm		Parameters			Regression coefficient
		a	b	c	
(+)C	Linear	45.6352	8.5187	–	0.8454
	Langmuir	90.2411	0.5254	–	0.9267
	Freundlich	56.6032	–	1.3787	0.8902
	Quadratic	−36.3721	115.7683	−13.5278	0.9820
	Langmuir Extension	0.0123	0.0027	−1.5523	0.9911
EC	Linear	40.8294	5.6494	–	0.8689
	Langmuir	71.7872	0.4219	–	0.9330
	Freundlich	48.4219	–	1.3065	0.9029
	Quadratic	−29.5887	98.3586	−12.6442	0.9880
	Langmuir Extension	0.0133	0.0044	−1.3250	0.9882
ECG	Linear	22.0474	4.7732	–	0.8611
	Langmuir	44.3381	0.4884	–	0.9402
	Freundlich	28.3014	–	1.3863	0.9072
	Quadratic	−16.8078	57.5409	−7.0323	0.9888
	Langmuir Extension	0.0229	0.0062	−1.4722	0.9960
EGCG	Linear	23.8459	6.3975	–	0.8369
	Langmuir	54.9385	0.6433	–	0.9384
	Freundlich	31.8590	–	1.4801	0.8956
	Quadratic	−19.5974	62.7166	−6.1630	0.9889
	Langmuir Extension	0.0215	0.0057	−1.1701	0.9891

The parameters fitted by the five adsorption isotherm models are listed in Tables 2, and Figure 2 indicates that the molecular imprinted polymer showed higher affinity to the target molecule of (+)C than the catechin compounds. That is, the (+)catechin-imprinted polymer possessed higher saturation capacity by as a result of the template than that of EC, ECG, and EGCG. One can also see that the 3 parameter equations (quadratic and Langmuir Extension), Eq. (4) and (5), have the better correlation results than that of 2 parameter equations of (Langmuir and Freundlich), Eq. (2) and Eq. (3). The regression coefficients of quadratic and Langmuir Extension adsorption isotherm models Eq. (4) and Eq. (5) are above 0.98 for these catechin compounds, respectively. All of these, to a higher degree, suggest that the polymer surface shows higher homogeneity and the non-specific binding sites adsorption is small in the tested concentration. Moreover, the (+)catechin-imprinted polymer had significantly higher adsorption capacity for the template than EC, ECG, and EGCG.

The experimental and calculated data are shown in Figure 3. The known C_m and C_s were fitted to obtain the five equilibrium equations. Almost the experimental and calculated data were on the diagonal neighborhood. This indicates that these experimental data is well fitted to the five equations. From the parameters listed in Table 2 and 3, it can be seen

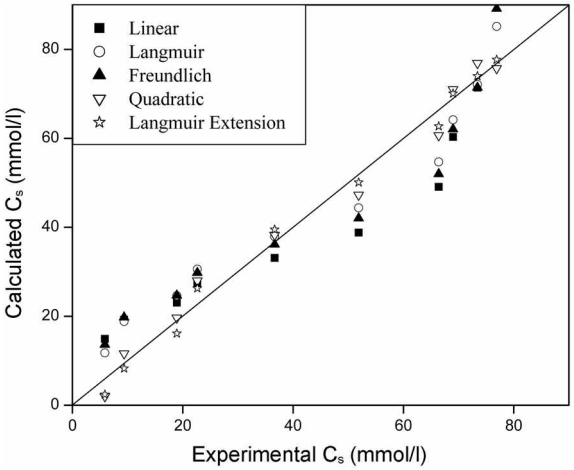

Figure 3. Comparison of experimental and calculated concentrations of (+)C on the molecular imprinted polymer.

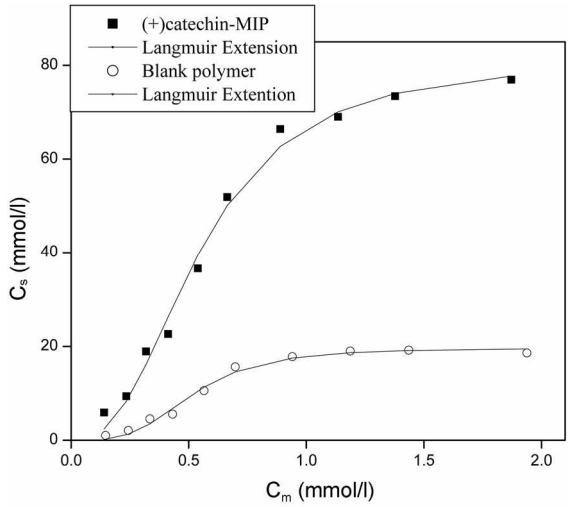

Figure 4. Adsorption isotherm of (+)C on (+)catechin-MIP and blank polymer fitted by Langmuir Extension equations.

that the (+)catechin-imprinted molecularly polymer shows higher affinity to the target molecule than blank polymer. The (+)catechin-imprinted polymer shows higher saturation capacity for the template than that of blank polymer (Figure 4). Figure 4 shows the plot of the experimental data of (+)C on the (+)catechin-molecular imprinted polymer and blank polymer fitted by the Langmuir Extension equation (5). It

Table 3. Parameters in adsorption isotherm of (+)C on blank polymer

Adsorption isotherm		Parameters			Regression coefficient
		a	b	c	
(+)C	Linear	11.2674	2.4827	–	0.7589
	Langmuir	24.6764	0.6151	–	0.8697
	Freundlich	14.5128	–	1.4421	0.8186
	Quadratic	−11.4430	34.1157	−4.9757	0.9712
	Langmuir Extension	0.0509	0.0050	−2.5293	0.9870

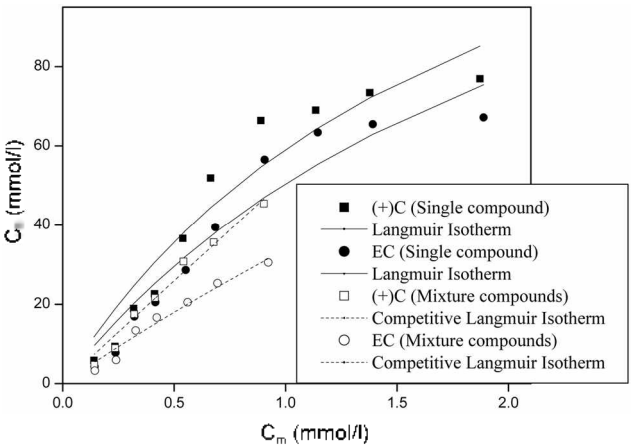

Figure 5. The adsorption concentrations of single and mixture compounds of (+)C and EC on (+)catechin-MIP.

shows that the experimental data fits to the Langmuir Extension equation quite well. The adsorbed concentration of (+)C and EC for a single compound was smaller than the adsorbed concentrations of (+)C and EC for the mixture compounds, respectively. This indicates that the mixture compounds were adsorbed competitively on the specific binding sites of (+)catechin-MIP. The effect of competitive adsorption was investigated using the parameters of the adsorption isotherm model with a single compound.

For a multi-compounds system, the isotherm of compounds i is:

$$C_{s,i} = \frac{a_i C_{m,i}}{1 + \sum_j b_j C_{m,j}} \quad j = 1, 2, \ldots, N \tag{6}$$

where a_i and b_i are parameters for compounds i, with the subscript j being the number of compounds in the mixture. A multi-compounds analysis was performed with different compositions of the two compounds. For example, the parameters of the mixture compounds from the single compounds Langmuir isotherms were used in the static method, as follows:

$$C_{s(-)C} = \frac{52.2002 C_{m(+)C}}{1 + 0.0126 C_{m(-)C} + 0.1344 C_{m(EC)}} \quad r^2 = 0.9879 \tag{7}$$

$$C_{s(EC)} = \frac{38.4184 C_{m(EC)}}{1 + 0.0126 C_{m(+C)} + 0.1344 C_{m(EC)}} \quad r^2 = 0.9732 \tag{8}$$

Figure 5 shows the plot of the experimental data of single and mixed solutions of (+)C, and EC on the molecular imprinted polymer fitted by the Langmuir (Eq. 2) and competitive Langmuir (Eq. 6) equation, respectively. Good agreement was obtained between the experimental data and the calculated values. The experimental data were compared with the values calculated using each adsorption isotherm model. In the single Langmuir adsorption isotherm model, because another adsorption sites were not considered, the calculated values were in good agreement (Table 4). In the case, these compounds with a relationship of competitive adsorption, because the samples were competitively adsorb-

Table 4. Parameters in adsorption isotherm of mixture compounds on (+)catechin-MIP

Adsorption isotherm		Parameters			Regression coefficient
		a	b	c	
(+)C	Linear	54.5895	−1.6830	–	0.9820
	Langmuir	52.2002	0.0126*	–	0.9786
	Freundlich	5.3810	–	0.9790	0.9732
	Quadratic	−26.9204	82.5632	−7.2340	0.9945
	Langmuir Extension	0.0147	0.0062	−0.7896	0.9952
EC	Linear	35.7165	−0.3708	–	0.9644
	Langmuir	38.4184	0.1344*	–	0.9675
	Freundlich	34.6409	–	1.0335	0.9648
	Quadratic	−24.3457	61.5370	−5.5915	0.9888
	Langmuir Extension	0.0246	0.0073	−0.8918	0.9889

*The other b_i's are also considered as competitive forms in Eqs. (6), (7), and (8).

ed in the same adsorption site, the adsorption concentrations of the sample were varied. The parameters of the competitive adsorption models were obtained from the parameters of the adsorption isotherm model of a single component. The competitive adsorption isotherm model used showed good agreement for the single compounds. Good agreement was noted when the experimental data was compared with the calculated values using the adsorption isotherm model. According to these results, there were several adsorption sites for catechin compounds, which were competitively adsorbed.

Conclusion

The characteristics of adsorption of catechin compounds on the stationary phase of a molecular imprinted polymer were investigated by a five adsorption isotherm models. In this work, the static method was experimentally implemented to measure the adsorption isotherms of (+)C, EC, ECG, EGCG, and mixtures of solutions of (+)C and EC by the prepared (+)catechin-molecular imprinting polymer. The adsorption equilibrium data were fitted by the isotherm equations of linear, Langmuir, Freundlich, quadratic and Langmuir Extension isotherms. In a moderate range of concentration, the five isotherms agreed well with the experimental data. The Langmuir Extension isotherm was the best-fit model to the experimental data among the five isotherm models. The regression coefficient was as high as 0.98 in the (+)catechin-MIP sorbent prepared using a template of (+)C. The (+)catechin-molecular imprinted polymer showed extraordinarily higher adsorption ability than the blank polymer. Moreover, the competitive Langmuir isotherms were well-fit to the experimental data. The large saturation capacity and high selectivity of the molecular imprinted polymer prepared in this work indicate that the proposed MIP could be commercially implemented. The association of competitive adsorption was examined using the parameters of the adsorption isotherm model using a single component and good agreement was obtained between the experimental data and the calculated values. Therefore, the polymer can be reproducibly synthesized and offers attractive feature for further applications.

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