

Competitive adsorption of protocatechuic acid and caffeic acid on C₁₈ particles

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Abstract—Comparisons of protocatechuic acid (PA) and caffeic acid (CA) adsorption isotherm on C₁₈ column and competitive adsorption of the two compounds were investigated. By linear and nonlinear regression analysis, the experimental parameters in the equilibrium isotherms were estimated. Adsorption equilibrium data of the two compounds were investigated using six different models including linear, Langmuir, Freundlich, Langmuir-Freundlich, competitive Langmuir and Quadratic. In the moderate range of concentrations, the competitive Langmuir isotherm proved to be the best model for these experimental data. The regression coefficients of the competitive Langmuir adsorption isotherms were 0.9860 for PA and 0.9898 for CA, respectively. The coefficients obtained for the six isotherm models confirmed the superiority of the competitive Langmuir isotherm for analyzing the competitive adsorption data of solutes.

Key words: Adsorption Isotherm, Competitive Adsorption, Protocatechuic Acid, Caffeic Acid

INTRODUCTION

The adsorption isotherm is a basic thermodynamic property of separation processes and it is the relationship between the concentration of the solute in the stationary phase and mobile phase. By fitting the experimental adsorption isotherm to a mathematical model, it is possible to estimate the binding properties of the system by using the corresponding fitted coefficients. After calculations of these mathematical models, the most conformable model for the experimental data is obtained. Using this method, it is possible to predict the individual band profile of separated samples under a variety of conditions as well as to optimize the conditions [1]. The experiments and modeling are related directly to the accurate adsorption isotherms and their parameters. The success of the experiments and modeling is associated with the accuracy of the adsorption isotherms and their parameters. Under such conditions, the competitive equilibrium isotherms between the two compounds are rarely linear [2,3]. The Langmuir model is the most popular equilibrium isotherm amongst the various nonlinear isotherm models. From the Langmuir model, interference of two compounds in competitive adsorption experiments cannot be applied, so the competitive Langmuir model appears. The competitive Langmuir isotherms for liquid chromatography have been studied by Juza [4,5] and Guiochon [6], and the multi-compound competitive isotherm has been shown to be better than the single compound isotherm [7,8]. A multi-compound Langmuir isotherm explains the competition of two compounds for available adsorption sites.

The nonlinear behavior of liquid chromatography under nonlinear conditions should be considered properly in an equilibrium isotherm. The successes of the experiments and modeling are immediately related to the exactness of the adsorption isotherms and their parameters [9]. Liquid chromatography is an excellent analysis method, which has had rapid development of applications in the chemical

industry, and has led to a recent renewal of interest in the fundamentals of nonlinear chromatography.

Protocatechuic acid (PA) and caffeic acid (CA) are present in edible plants, fruits and vegetables. PA and CA belong to the class of natural substances that have been proven to be of considerable medicinal value by modern pharmacological testing. They have been shown to have various therapeutic activities, e.g., antioxidative property [10], carcinogenesis in oral cavity [11], glandular stomach [12], colon [13], and liver [14]. The chemical structures of these two compounds are similar and shown in Fig. 1.

In this study, the adsorption equilibrium data of PA and CA on C₁₈ particles were correlated into the six isotherm models: the linear,

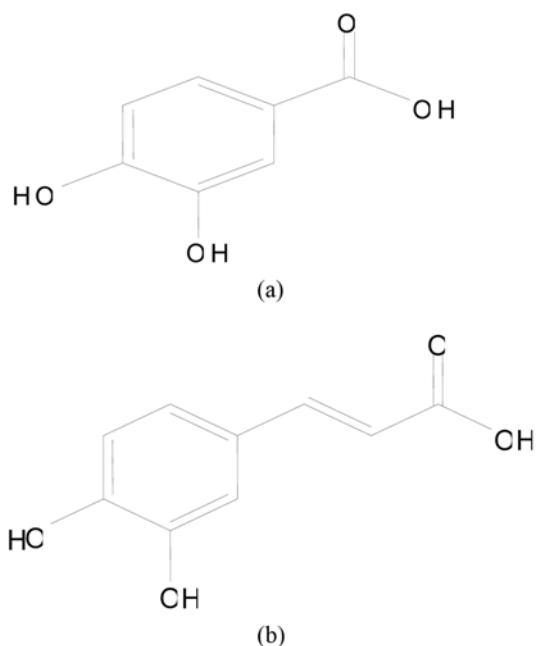


Fig. 1. Chemical structure of PA and CA.

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Langmuir, competitive Langmuir, Freundlich, Langmuir-Freundlich and Quadratic models. The competitive adsorption of PA and CA with similar chemical structures was also investigated.

EXPERIMENTAL

1. Reagents and Materials

Acetonitrile and acetic acid were purchased from Duksan Pure Chemical Co., Ltd (Ansan, Korea). 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. C_{18} particles were bought from Merck KGaA Co., Ltd (RP-18, 12 μ m, Darmstadt, Germany). PA was obtained from Aldrich (Milwaukee, WI, USA). CA (99%) was bought from Sigma (St Louis, MO, U.S.A.). All the other solvents used in the experiment were HPLC or analytical grade. All the samples were filtered by using a filter (MFS-25, 0.2 μ m TF, Whatman, U.S.A.) before injection into the HPLC system.

2. Apparatus

The chromatography system consisted of Waters 600s Multi solvent Delivery System and Waters 616 liquid chromatography (Waters Associates, Milford, MA, U.S.A.), Rheodyne injector (20 μ L sample loop), and a variable wavelength 2487 UV dual channel detector. Data processing was performed with a Millennium 3.2 consisting of HP Vectra 500PC. In the chromatographic condition, the flow rate 1.0 mL/min, injection volume 10 μ L, and UV wavelength 270 nm. The commercial C_{18} column (5 μ m, 150 \times 4.6 mm) was purchased from RS tech Corporation (Daejeon, Korea).

3. Static Adsorption Method

The static method was performed on the C_{18} particles. 0.02 g C_{18} particles were placed into 2.5 mL flasks, respectively, and then 1.0 mL PA solution, CA solution and mixture solution with the concentration of 0.01 to 0.30 g/L were added, respectively. The flasks were left alone at room temperature for 72 h and then the supernatant was collected and filtered (0.2 μ m). The concentrations of free PA and CA in the solution were determined on C_{18} commercial column by HPLC at room temperature. Absorbed PA and CA concentrations on C_{18} particles were calculated by subtracting the free concentrations from the initial concentrations of these compounds. Acetonitrile/water/acetic acid (30/70/0.05, v/v/v) was the mobile phase to determine the free concentration of the compound in the static method.

The experimental adsorption isotherms were fitted to the linear, Langmuir, competitive Langmuir, Freundlich, Langmuir-Freundlich and Quadratic models. This process was accomplished using the solver function in OriginPro 7.5 software (OriginLab Corporation, MA, USA) by varying the fitting parameters to reach a value of 1 for the squared correlation coefficient (r^2).

RESULTS AND DISCUSSION

1. Determination Concentrations of PA and CA

After mixing the PA solution, CA solution and the C_{18} particles for 72 hours, the adsorption equilibrium was obtained between the two phases. The unabsorbed concentrations of PA and CA were determined by HPLC, respectively. The calibration curves (peak area (Y) vs. concentration (X)) were determined from concentrations

Table 1. Calibration curve for the quantification of PA and CA

Compounds	Equation	r^2
PA	$Y = 2.647 \times 10^4 X + 179.72$	0.9981
CA	$Y = 2.026 \times 10^4 X + 103.29$	0.9982

of 0.01 to 0.3 g/L. The calibration equations were seen in Table 1.

The absorbed quantity (Q) of PA and CA on C_{18} particles was calculated as follows:

$$Q = \frac{(C_0 - C) \times V}{M} \quad (1)$$

where Q (mg/g) is the absorbed amount, C_0 (g/L) is the initiator concentration, C (g/L) is the unabsorbed concentration, and V (mL) is the volume of the sample solvent, M (g) is the mass of C_{18} particles. Q is computed from this procedure, and then the experimental parameters were approximated and compared with the equilibrium isotherms by linear and nonlinear regression analysis.

2. Analysis of Adsorption Isotherms

The experiment data of PA and CA were fitted to the following adsorption isotherm models:

$$Q = aC + b \quad (2)$$

$$Q = \frac{aC}{1 + bC} \quad (3)$$

$$Q = \frac{aC_1}{1 + bC_1 + cC_2} \quad (4)$$

$$Q = aC^{1/c} \quad (5)$$

$$Q = \frac{aC^c}{1 + bC^c} \quad (6)$$

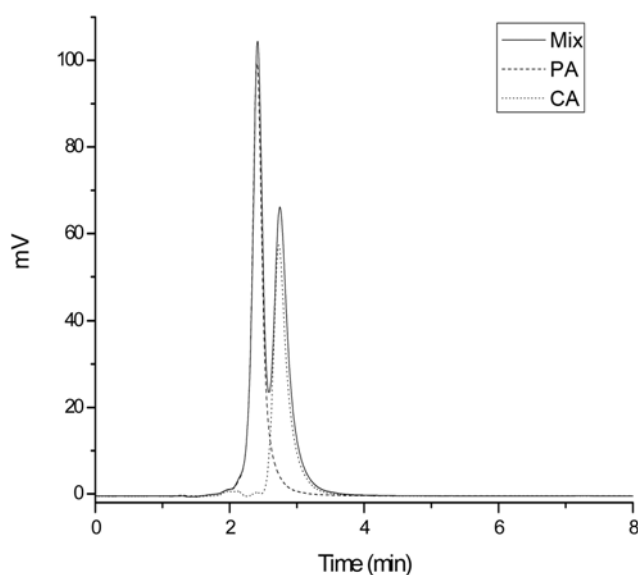
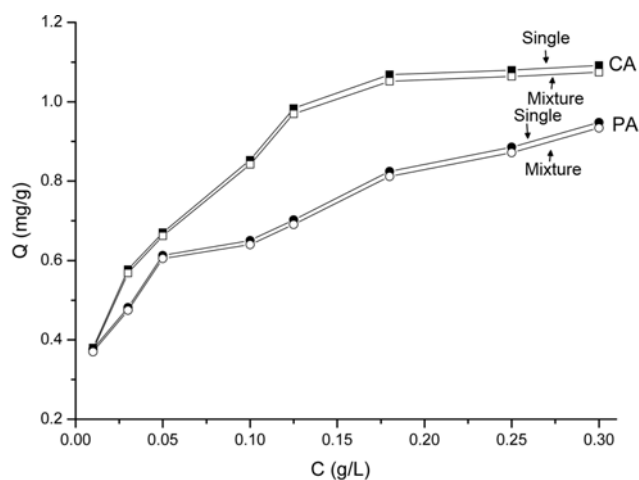
$$Q = aC^2 + bC + c \quad (7)$$

Where a, b and c are parameters, and C (g/L), C_1 (g/L) and C_2 (g/L) are the concentrations of component in the solution samples. These adsorption isotherm models are the linear (2), Langmuir (3), competitive Langmuir (4), Freundlich (5), Langmuir-Freundlich (6), and Quadratic (7), respectively. The numerical coefficient a in competitive Langmuir isotherm model was derived from the experimental values of the retention factors. The numerical coefficients b and c were obtained by fitting the experimental band profiles and the profiles given by the calculations of experimental data. The influence between the two compounds was obtained through the numerical coefficients b and c. The adsorption parameters by six adsorption isotherm models were seen in Table 2.

Among these adsorption isotherm models, the Langmuir equation is a more commonly used equation due to its simplicity and strong theoretical reasoning behind. Three essential propositions of the Langmuir isotherm [15] are monolayer coverage, adsorption site equivalence and independence. The competitive Langmuir and Langmuir-Freundlich adsorption isotherm are expansions of the Langmuir adsorption isotherm. From Table 2, the three parameter equations (competitive Langmuir Eq. (4), Langmuir-Freundlich Eq. (6) and Quadratic Eq. (7)) have better correlation results than that of two parameter equations of (linear Eq. (2), Langmuir Eq. (3) and Freundlich Eq. (5)). To better compare the competitive adsorption,

Table 2. Parameters in adsorption isotherm of PA and CA on the C₁₈ particles

	Compound	No. of adsorption isotherm	Parameters			r ²
			a	b	c	
Single	PA	(2)	1.8044	0.4496	-	0.9269
		(3)	36.9067	39.5733	-	0.8655
		(5)	1.2975	-	3.6373	0.9816
		(6)	1.4239	0.1133	0.2899	0.9809
		(7)	-4.8151	3.2706	0.3868	0.9689
	CA	(2)	2.2811	0.5401	-	0.8024
		(3)	37.4127	30.9569	-	0.9619
		(5)	1.6622	-	3.3875	0.9599
		(6)	9.0326	5.8540	0.6439	0.9821
		(7)	-13.6756	6.4454	0.3620	0.9858
Mixture	PA	(2)	1.7745	0.4429	-	0.9258
		(3)	36.4685	39.746	-	0.8650
		(4)	0.0449	-0.2797	-0.5582	0.9860
		(5)	1.2766	-	3.6423	0.9806
		(6)	1.7373	0.4262	0.3258	0.9778
		(7)	-4.7142	3.2101	0.3815	0.9674
	CA	(2)	2.2441	0.5326	-	0.8009
		(3)	36.9444	31.0437	-	0.9629
		(4)	0.0371	-0.3768	-0.4966	0.9898
		(5)	1.6365	-	3.3918	0.9597
		(6)	9.1071	6.0343	0.6489	0.9825
		(7)	-13.5197	6.3610	0.3565	0.9858

**Fig. 2. Chromatogram of the mixture and single compound of PA and CA on C₁₈ column (Mobile phase composition acetonitrile/water/acetic acid (30/70/0.05, v/v/v), flow rate: 1.0 mL/min, column: C₁₈ column (150×4.6 mm, i.d.), UV: 270 nm, injection volume: 10 μL).****Fig. 3. Adsorption isotherms of the mixture and single compound of PA and CA on the C₁₈ particles (Mobile phase composition acetonitrile/water/acetic acid (30/70/0.05, v/v/v), flow rate: 1.0 mL/min, column: C₁₈ column (150×4.6 mm, i.d.), UV: 270 nm, injection volume: 10 μL).**

the competitive Langmuir isotherm was used in this study. The regression coefficients (r^2) of competitive Langmuir of PA and CA are 0.9860 and 0.9898, respectively.

3. The Interactions between PA and CA

According to the literature [16], surface coverage of adsorbents can affect the degree of heterogeneity. The adsorption mechanism was determined mainly by the nature of the analytes, which can be more (strong adsorption) or less (weak adsorption) embedded into the surface of C₁₈ particles. At the composition of the mobile phase

Table 3. Differences in single and mixture adsorption of PA and CA on the C₁₈ particles

Compound	Concentration (g/L)	Q (mg/g)		
		Acetonitrile/water/acetic acid (v/v/v): 30/70/0.05		
		Single	Mixture	Difference
PA	0.010	0.3762	0.3701	0.0061
	0.030	0.4819	0.4747	0.0072
	0.050	0.6127	0.6053	0.0074
	0.100	0.6505	0.6401	0.0104
	0.125	0.7025	0.6908	0.0117
	0.180	0.8245	0.8117	0.0128
	0.250	0.8855	0.8714	0.0141
	0.300	0.9482	0.9338	0.0144
CA	0.010	0.3801	0.3739	0.0062
	0.030	0.5774	0.5687	0.0087
	0.050	0.6705	0.6620	0.0085
	0.100	0.8531	0.8419	0.0112
	0.125	0.9835	0.9692	0.0143
	0.180	1.0685	1.0516	0.0169
	0.250	1.0794	1.0639	0.0155
	0.300	1.0916	1.0744	0.0172

were acetonitrile/water/acetic acid (30/70/0.05, v/v/v), the unabsorbed concentrations of the two single compounds and mixture in the static method were determined, when the retention time of PA and CA were 2.45 min and 2.78 min (Fig. 2). At that time, these two compounds were not separated well, and there were some strong interactions between these. The adsorption isotherms of single compound and mixture on C₁₈ particles are shown in Fig. 3. From this figure, the adsorption isotherms of mixture were lower than the adsorption isotherms of single compound, and the absorbed quantity CA was higher than PA on the C₁₈ particles. Table 3 shows the differences in single and mixture adsorption of PA and CA on the C₁₈ particles.

Due to the different chemical structures of PA and CA, they had some different chromatographic behaviors on adsorbents. From Fig. 1, there are some hydroxyl groups in their chemical structures. The hydroxyl groups were active groups, and they have some interactions with the functional groups of C₁₈ particles. These interactions led to the competitive adsorption of PA and CA on C₁₈ particles. The calculations and analyses of the coefficients from the six models confirmed that the competitive Langmuir was the most suitable for the isotherm adsorption data of PA and CA, when the acetonitrile/water/acetic acid (30/70/0.05, v/v/v) was the mobile phase.

CONCLUSIONS

The adsorption equilibrium of PA and CA on C₁₈ particles was

investigated using six models: linear, Langmuir, Freundlich, Langmuir-Freundlich, competitive Langmuir and Quadratic. After the calculation and analysis by OriginPro 7.5 software, the adsorption isotherm parameters of these two compounds were obtained. The results showed the regression coefficients of mixture of competitive Langmuir adsorption isotherm were 0.9860 for PA and 0.9898 for CA, respectively. It indicated that the competitive Langmuir adsorption isotherm model was more suitable than other models for competitive adsorption of two compounds. It is expected that the competitive Langmuir isotherm model can become a helpful tool to understand the interactions between two compounds and to predict their separation conditions on stationary phase materials.

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