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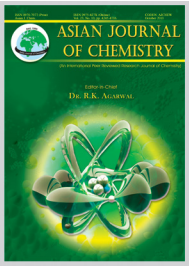

Adsorption Isotherms of Tryptophan Enantiomer on D-Tryptophan Molecular Imprinted Polymer

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A molecular imprinted polymer using D-tryptophan as a template and methacrylic acid and acrylamide as a functional co-monomer was prepared. Water and methanol were used as the hydrophilic porogen with ethylene glycol dimethacrylate as the crosslinker and 2,2'-azobis(isobutyronitrile) as the initiator. Comparison of D and L form of tryptophan adsorption isotherm on molecular imprinted polymer was made by the static method. The experimental parameters in the equilibrium isotherms were estimated by linear and nonlinear regression analyses. The linear equation for concentration and sorbents to adsorption concentrations was then expressed and the adsorption equilibrium data were correlated into Langmuir, Freundlich, quadratic, Allometric, Langmuir and Freundilich extension isotherm models. The calculated data is well fitted with the experimented data. The D-tryptophan imprinted polymer showed extraordinarily higher adsorption ability for D-tryptophan than the L-tryptophan compounds that were also assessed. Furthermore, the competitive Langmuir isotherms were applied to the mixture compounds of D and L form tryptophan.

Key Words: Adsorption isotherm, Molecular imprinted polymer, Tryptophan, Enantiomer, Hydrophilic porogen.

INTRODUCTION

Enantiomers were called chiral compounds, which have same chemical formula and structure, but they are each other's mirror image. They share the same physical and chemical properties but different in optical property. The increasing need for pure enantiomer is due to that the pharmaceutical activities of the enantiomers in human body are always different and sometimes reverse[1].

L-Tryptophan *i.e.*, L-2-amino-3-(indol-3-yl)propionic acid, is a vital constituent of proteins and is indispensable for establishing and maintaining a positive nitrogen balance in human nutrition. As an essential amino acid, L-tryptophan can not be formed endogenously and is instead considered the precursor of the neurotransmitter serotonin[2], which plays an important role in brain function and related regulatory mechanisms[3-5]. Although D-tryptophan shows nospecial biological functions, it has been extensively used as a pharmaceutical intermediate. Due to this difference in nutritional and biological functions of these enatiomers, their chiral separation is a critical, yet challenging task.

The adsorption isotherm is a basic thermodynamic property of a separation processes and it 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 various kinds of model to the experimental data. 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[6]. The success of experiments and modeling are directly related to the accuracy of the adsorption isotherms and their parameters[7,8].

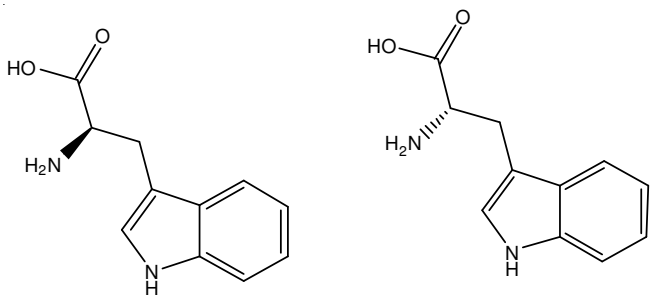
The technique of molecular imprinting consists of the self-assembly of a functional monomer and a template molecule in solution followed by co-polymerization of the functional monomer and an excess of an appropriate crosslinking monomer. After removal of small molecules, the resulting network polymer exhibits significantly higher affinity for the molecules used as the template than for similar molecules, including closely related isomers[9]. While molecular imprinted polymer 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[10].

Molecular imprinted polymer 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 molecular imprinted polymer offer 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 imprinting 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[11,12].

Most of the liquid chromatography is carried out under non-linear conditions and the nonlinear behaviour 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[13,14]. The competitive Langmuir isotherms for liquid chromatography has been studied by Juza[13,15] and Guiochon[14,16] and the multi-compounds competitive isotherm was shown to be better than that of the single compound isotherm[5,17].

In this work, the adsorption equilibrium data of a single compound were correlated into nine isotherm models, linear, Langmuir, Freundlich, Allometric1, Allometric2, quadratic, LangmuirEXT1, LangmuirEXT2 and FreundlichEXT isotherms. For the developed synthesis and manufacture of molecular imprinted polymer, an molecular imprinted polymer stationary phase was produced using D-tryptophan as a template, methacrylic acid + acrylamide (MMA + AM) as a functional comonomer, water and methanol as a hydrophilic porogen and ethylene glycol dimethacrylate (EGDMA) as a crosslinker. The adsorption characteristics such as isotherms of enantiomer chemical structures (Fig. 1) of D and L forms tryptophan on the D-tryptophan-molecular imprinted polymer were obtained. Competitive Langmuir isotherms were also applied to the mixture compounds of D and L form tryptophan.



L-tryptophan D-tryptophan

Fig. 1. Chemical structures of tryptophan enantiomers

EXPERIMENTAL

D-Tryptophan, L-tryptophan, acrylamide (AM) and methacrylic acid (MAA) were purchased from Sigma (St Louis, MO, USA). 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. Methanol was from Pure Chemical Co., Ltd. (Ansan, 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, USA) and filter (HA-0.45, Division of Millipore, Waters, USA) before use. All the samples were filtered (MFS-25, 0.2 μm TF, WHATMAN, USA) before injection into the HPLC system.

The chromatography system consisted of a Waters 600s Multi solvent delivery system and a waters 616 liquid chromatography (Waters Associates, Milford, MA, USA), 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. In the chromatographic condition, the flow rate was 0.5 mL/min, the injection volume was 5 μL and the UV wavelength was 280 nm. A C_{18} column (5 μm particles, 100 Å pore sizes, 4.6 mm × 250 mm) from RS tech Corporation (Daejeon, Korea), where water/methanol = 10/90 (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: 8 mmol of the co-monomers (4 mmol of the methacrylic acid + 4 mmol of the acrylamide), 30 mmol of the crosslinker (ethylene glycol dimethacrylate), 0.056 g of the initiator [2,2'-azobis(isobutyronitrile)], 3 mL of H_2O and 12 mL of methanol of the porogen and 1 mmol of the template (D-tryptophan). 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 ºC for 24 h. 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.

Static method: The static method was performed on the manufactured polymer particles. Ten share of 30 mg of the quercetin imprinted polymers was placed into 10 mL flasks, respectively. And then 3.0 mL of D and L forms tryptophan solution with a concentration of 0.15-2.00 mmol/L was added. More than, mixture solutions of D form and L form tryptophan with different concentrations were added. The mixture was left at room temperature for 72 h and then the supernatant was collected and filtered (0.2 μm). The concentrations of free D-tryptophan and L-tryptophan in the solution were determined using a C_{18} column at room temperature. Absorbed D-tryptophan and L-tryptophan on the molecular imprinted polymers were calculated by subtracting the free concentrations from the initial concentrations of these compounds.

RESULTS AND DISCUSSION

According to literature, many isotherms have been successfully employed to calculate the binding properties of molecular imprinted polymers: these include, simple Langmuir, SIPs models, Bi-Langmuir models, Jovanovic, Bi-Jovanovic and Freundlich-Jovanovic[18]. In this work, in addition to a linear equation, we have chosen to consider the

nonlinear Langmuir, Freundlich, Allometric1, Allometric2, quadratic, LangmuirEXT1, LangmuirEXT2 and FreundlichEXT models. The adsorption equilibrium data was fitted into the equilibrium models.

The concentrations of D and L forms of tryptophan using D-tryptophan molecular imprinted polymer at the flasks were measured at different concentrations, after the equilibrium adsorptions of these compounds on molecular imprinted polymer were attained, respectively. Comparing the concentration of D and L forms of tryptophan on the molecular imprinted polymer sorbent, similar trends with the larger concentration of D-tryptophan and L-tryptophan up to 2 mmol/L were observed and the more D-tryptophan was adsorbed on the stationary phases. Above 2 mmol/L, on the stationary phases, the adsorption concentrations were asymptotic to the saturated values. Quantitative determination was based on the constructed calibration curve: $y = 8 \times 10^{-7}x$, $r^2 = 0.9980$ (regression coefficient) for D-tryptophan; $y = 9 \times 10^{-7}x$, $r^2 = 0.9585$ for L-tryptophan. y is the concentration of D-tryptophan (or L-tryptophan) compounds in the methanol (mmol/L) of injected samples, while x is the peak area (mAU × s). 0.15 and D-tryptophan concentrations of 0.45, 1.00, 1.50 and 2.00 mmol/L were assessed in the HPLC system so as to obtain the calibration curve.

Fig. 2 illustrates the adsorption isotherm plots of D-tryptophan and L-tryptophan on molecular imprinted polymer. It is clear from the figure that the D-tryptophan-imprinted polymer shows significantly higher adsorption ability for the template than the L-tryptophan. 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[19]. In the low concentration range, the adsorption on selective binding sites is stronger than that on nonselective binding sites[19].

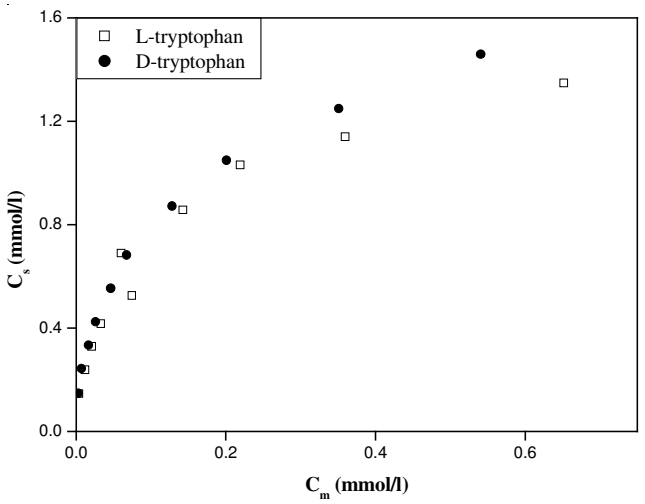


Fig. 2. Adsorption concentrations of tryptophan on D-tryptophan-molecular imprinted polymer

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

$$C_s = aC_m + b \quad (1)$$

$$C_s = \frac{aC_m}{1 + bC_m} \quad (2)$$

$$C_s = aC_m^{1/c} \quad (3)$$

$$C_s = aC_m^{b} \quad (4)$$

$$C_s = a + bC_m^{c} \quad (5)$$

$$C_s = aC^2{}_m + bC_m + C \quad (6)$$

$$C_s = \frac{abC_m^{1-c}}{1 + bC_m^{1-c}} \quad (7)$$

$$C_s = \frac{1}{a + bC_m^{c-1}} \quad (8)$$

$$C_s = aC_m^{bC_m^{-c}} \quad (9)$$

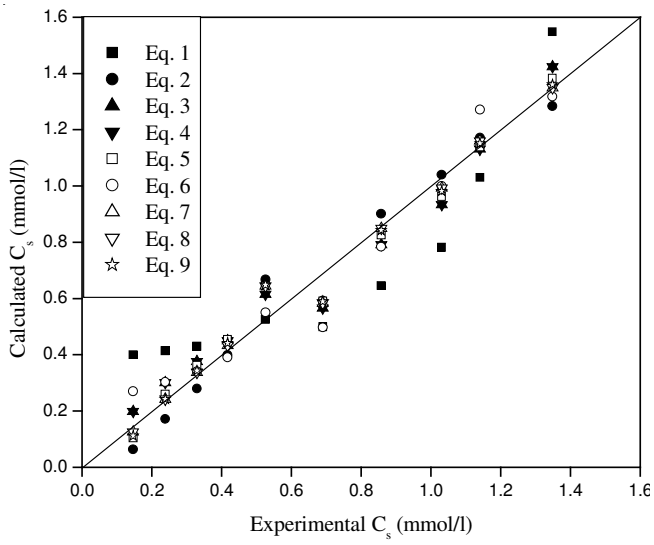
where C_s and C_m are the adsorption concentrations of D-tryptophan and L-tryptophan, respectively. a, b and c are experimentally determined parameters. These adsorption isotherms are the linear (1), Langmuir (2), Freundlich (3), Allometric1 (4), Allometric2 (5), quadratic (6), LangmuirEXT1 (7), LangmuirEXT2 (8) and FreundlichEXT (9) isotherms. The parameters fitted by the nine adsorption isotherm models are listed in Table-1 and indicate that the molecular imprinted polymer showed higher affinity to the target molecule of D-tryptophan than the L-tryptophan. That is, the D-tryptophan-imprinted polymer possessed higher saturation capacity as a result of the template than that of L-tryptophan. Comparing the coincidences between the experimental data and the equilibrium isotherms, generally the regression coefficients of the molecular imprinted polymer sorbents are larger than 0.94 in nonlinear isotherms. It is worth noting that the equilibrium data by molecular imprinted polymer was well fitted by the nonlinear isotherms.

Almost all the experimental and calculated data were on the diagonal neighborhood. This indicates that these experimental data are well fitted to the four equations (Fig. 3). From Table-1 and Fig. 3, eqns. (1-9) could adequately correlate the equilibrium experimental data in this experimental range of concentration. The regression coefficients of the Freundlich adsorption isotherm of eqn. 3 were 0.9950 and 0.9651 for D-tryptophan and L-tryptophan, respectively (refer to Table-1), whereas for the Langmuir adsorption isotherm of eqn. 2, the regression coefficients are 0.9710 and 0.9663 for D-tryptophan and L-tryptophan, respectively. Moreover, the regression coefficients of the linear adsorption isotherm of eqn. 1 were 0.8723 and 0.8001 for D-tryptophan and L-tryptophan, respectively. All of these, to a higher degree, indicate that the polymer surface shows higher homogeneity and the nonspecific binding sites adsorption is small in the tested concentration. Moreover, the D-tryptophan-imprinted polymer had significantly higher adsorption capacity for the template than L-tryptophan.

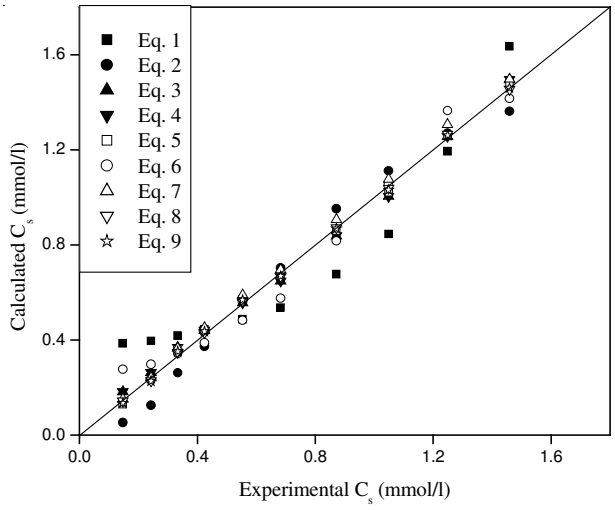
It is also seen that the three parameter equations (Allometric2, LangmuirEXT1, LangmuirEXT2 and FreundlichEXT), eqns. 5 and 7-9, except quadratic equation (eqn. 6), have the better correlation results than that of two parameter equations of (Langmuir, Freundlich and Allometric2), eqns. 2-4. The regression coefficients of FreundlichEXT adsorption isotherm model eqn. 9 are 0.9990 for D-tryptophan and 0.9802 for L-tryptophan, respectively.

TABLE-1
PARAMETERS IN ADSORPTION ISOTHERM OF TRYPTOPHAN ON D-TRYPTOPHAN-MOLECULAR IMPRINTED POLYMER

Adsorption isotherm		Parameters			Regression coefficient
		a	b	c	
L-Tryptophan	Linear	1.7727	0.3926	–	0.8001
	Langmuir	16.5460	11.3523	–	0.9663
	Freundlich	1.6809	–	2.5826	0.9651
	Allometric1	1.6807	0.3872	–	0.9651
	Allometric2	-0.4152	1.9948	0.2438	0.9766
	Quadratic	-4.1032	4.3075	0.2526	0.9454
	LangmuirEXT1	2.0288	2.6439	0.3313	0.9815
	LangmuirEXT2	0.4929	0.1864	0.3313	0.9815
	FreundlichEXT	1.5132	0.2352	0.1264	0.9802
D-Tryptophan	Linear	2.3209	0.3793		0.8723
	Langmuir	18.8728	12.0142	–	0.9710
	Freundlich	1.9140	–	2.4874	0.9950
	Allometric1	1.9139	0.4020	–	0.9950
	Allometric2	-0.1737	2.0053	0.3227	0.9982
	Quadratic	-5.3114	5.0055	0.2619	0.9685
	LangmuirEXT1	3.2896	1.1670	0.4541	0.9993
	LangmuirEXT2	0.3040	0.2746	0.4541	0.9993
	FreundlichEXT	1.7763	0.3070	0.0617	0.9990



(a)



(b)

Fig. 3. Comparison of experimental and calculated concentrations of L-tryptophan (a) and D-tryptophan (b) on the molecular imprinted polymer

Fig. 4 shows the plot of the experimental data of solution of D-tryptophan on the molecular imprinted polymer fitted by the Freundlich (eqn. 3). 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 Freundlich adsorption isotherm model, because other adsorption sites were not considered, the calculated values were in good agreement.

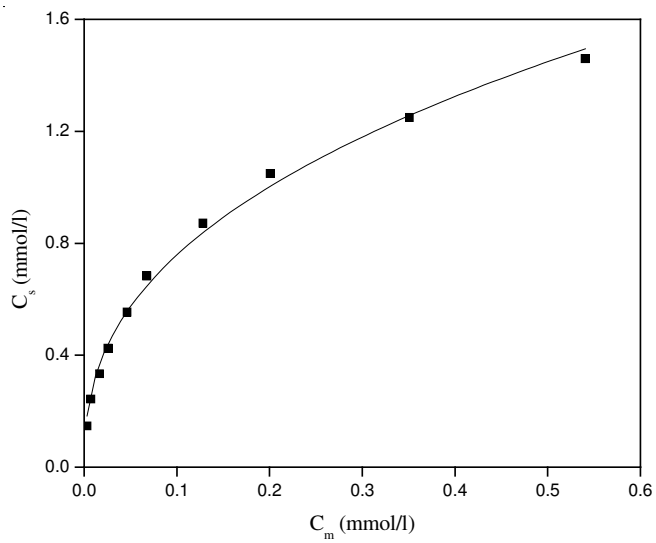


Fig. 4. Adsorption isotherm of D-tryptophan fitted by Freundlich equation

The adsorption concentration of D and L forms of tryptophan for a single compound was smaller than the adsorption concentrations of D and L forms of tryptophan for the mixture compounds, respectively. This indicates that the mixture compounds were adsorbed competitively on the specific binding sites of D-tryptophan-molecular imprinted polymer. 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:

$$C_{s,i} = \frac{a_i C_{m,i}}{1 + \sum_j b_j C_{m,j}} \qquad j = 1, 2, \ldots, N \qquad (10)$$

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:

$$C_{s(L-tryptophan)} = \frac{16.5460 C_{m(L-tryptophan)}}{1 + 11.3523 C_{m(L-tryptophan)} + 12.0142 C_{m(D-tryptophan)}} \qquad r^2 = 0.9840 \qquad (11)$$

$$C_{s(D-tryptophan)} = \frac{18.8728 C_{m(D-tryptophan)}}{1 + 11.3523_{m(L-tryptophan)} + 12.0142 C_{m(D-tryptophan)}} \qquad r^2 = 0.9830 \qquad (12)$$

In the case, these compounds with a relationship of competitive adsorption, because the samples were competitively adsorbed 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 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 tryptophan, which were competitively adsorbed.

Binding parameters of caffeine on the imprinted microgel spheres were determined by Scatchard analysis[20,21]. The Scatchard equation is as follows:

$$\frac{Q}{[Tryptophan]} = \frac{(Q_{max} - Q)}{K_D} \qquad (13)$$

where Q is the amount of D and L forms of tryptophan bound to the polymer, Q_{max} is the maximum binding capacity, K_D is the equilibrium dissociation constant, tryptophan represents the equilibrium concentration of D-tryptophan and L-tryptophan. Fig. 5 is the plot according to the Scatchard equation. There is one distinct section within the plot that can be regarded as straight lines, which indicates that there exist one classes of binding sites in the imprinted polymer. This is consistent with the common description about molecular imprinted polymer. It is known that the imprinted polymer surface and also the imprinted polymer are often regarded as homogeneous and there are one kinds of binding sites on the imprinted polymer surface: For D-tryptophan, the selective or high affinity with high binding energy; for L-tryptophan, the nonselective or low affinity with low binding energy. In low concentration range, the adsorption on selective binding sites is stronger than that on nonselective binding sites[19]. From the slope and intercept of its Scatchard plot, the dissociation constants (K_D) and the maximum binding capacity of the imprinted polymer were $K_D = 1.7114 \times 10^{-4}$ mol/L, $Q_{max} = 2.9664$ μmol/g ($r^2 = 0.9529$) for D-tryptophan and $K_D = 2.1948 \times 10^{-4}$ mol/L, $Q_{max} = 2.3193$ μmol/g ($r^2 = 0.9001$) for L-tryptophan, respectively.

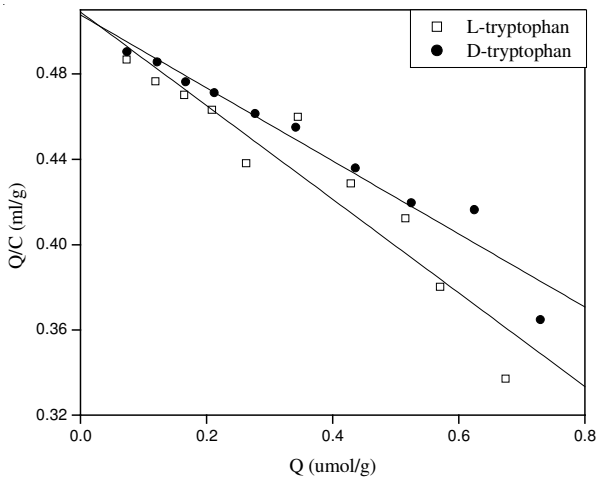


Fig. 5. Scatchard plot of D-tryptophan imprinted polymer for D and L forms of tryptophan

Conclusion

The characteristics of adsorption of D and L forms of tryptophan on the stationary phase of a molecular imprinted polymer using analytical chromatography were investigated by a nine adsorption isotherm models. In this work, the static method was experimentally implemented to measure the adsorption isotherms of D-tryptophan and L-tryptophan of solutions by the prepared D-tryptophan molecular imprinting polymer. The adsorption equilibrium data were fitted by the isotherm equations of linear, Langmuir, Freundlich, Allometric1, Allometric2, quadratic, LangmuirEXT1, LangmuirEXT2 and FreundlichEXT isotherms. In a moderate range of concentration, the nine isotherms agreed well with the experimental data. The regression coefficient was as high as 0.94 in the molecular imprinted polymer sorbent prepared using a template of tryptophan in nonlinear isotherms. The Freundlich isotherm was the best fitted model to the experimental data among the two parameters isotherm models. The regression coefficient is 0.9950 and 0.9651 for D-tryptophan and L-tryptophan, respectively. The large saturation capacity and high selectivity of the molecular imprinted polymer prepared in this work indicate that the proposed molecular imprinted polymer 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 using hydrophilic porogen and offers attractive feature for further applications.

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