

## Single and Competitive Isotherms of Phenol and *o*-Cresol by Pulsed-Input Method

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**Abstract**—There is a considerable industrial interest in both the scale-up and optimization of chromatographic operations used in the purification of fine chemicals and biomolecules. One of the major factors affecting the adsorption operation is the adsorption isotherm. Reversed-phase high-performance liquid chromatography (RP-HPLC) was used to measure the adsorption isotherm of phenol and *o*-cresol. From the experimental results, the retention times were decreased with increasing sample sizes, and the front of the peak was very stiff, so Langmuir adsorption isotherm was applied. Also Early-eluting component, phenol, in a mixture of the two components elutes faster than that in a pure component. Pulsed-injection method (PIM) was used to determine the two parameters of the Langmuir isotherm. The resulting parameters, *a* and *b*, were used to calculate the elution profiles of phenol and *o*-cresol in pure and mixed state. The agreement between the experimental data and calculated elution profiles was fairly good in a mixture as well as a pure component.

Key words: Competitive Isotherm, Phenol, *o*-Cresol, Pulsed-Input Method

### INTRODUCTION

High-performance liquid chromatography (HPLC) is the most widely used tool for analyzing and separating natural compounds [Jung and Row, 1998; Row and Lee, 1999a]. With good sensitivity, HPLC can calculate the exact amount of the components and also separate the volatile or heat-sensitive components. Preparative HPLC with a large amount of sample and separation condition is quite different from analytical HPLC [Row and Lee, 1999b].

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 phases and that in the mobile phases. The parameters of the adsorption isotherm can be determined by fitting the model to the experimental data. If the model is adequate and the form and the isotherm equation are measured, it is possible to predict the individual band profiles of separated sample components under various working conditions and to optimize the separation condition. In analytical chromatography, the injected amounts of sample are so low that linear isotherm is frequently used, which means that the retention factors of the analytes are independent of their concentrations in the sample and the retention data can be calculated in a straightforward manner. However, in preparative chromatography high concentrations of the components in the sample should be injected and the separation is usually run under overloaded column conditions controlled by a non-linear isotherm [Jandera et al., 1996]. Normally, the nonlinear isotherms are divided into two parts, Langmuir and Freundlich isotherms. The former one is characterized by a decrease in retention time with increase in sample size, while in the latter one, the retention time is longer with sample size. In many cases, the static method [Fritz and

Schluender, 1974] and frontal analysis [Jacobson et al., 1984] in dynamic methods are used to determine the coefficients of the isotherms, but these methods consume a great deal of sample and time.

The dynamic methods of measuring adsorption isotherm by HPLC were frontal analysis (FA) [Choi et al., in press], frontal analysis by characteristic point (FACP) [Guan et al., 1994], elution by characteristic point (ECP) [Guiochon et al., 1994] and pulsed-input method (PIM), by which the Langmuir isotherm of a single component of 5'-GMP was found by the authors [Choi et al., 1998]. In this work, by using the PIM, single and competitive Langmuir adsorption isotherms of phenol and *o*-cresol were determined by experimental elution profile, and the calculated profiles with the isotherms were compared with experimental data.

### THEORETICAL

Several models have been suggested to describe the distribution of one or multi-components between a stationary and mobile phases. The dependencies of the coefficients of the isotherms should be known to allow numerical calculations of the peak profiles and optimization of the effects of the mobile phase on the yield, purity and production rate under overloaded isocratic and gradient elution conditions [Jandera and Komers, 1997]. The following Langmuir isotherm is commonly used because of its simplicity.

$$q = \frac{ac}{1+bc} \quad (1)$$

*q* is the concentration of the solute in the stationary phase and *c* is the concentration of the solute in the mobile phase; *a* and *b* are the parameters of the Langmuir isotherm. When a solute passes through a chromatographic column, the concentration of a solute between the stationary phase and mobile phase can be expressed as the following first-order partial differential equation from the mass bal-

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ance of solute in a chromatographic column. For a component  $i$ ,

$$u \frac{\partial c_i}{\partial x} + \varepsilon \frac{\partial c_i}{\partial t} + (1 - \varepsilon) \frac{\partial q_i}{\partial t} = 0 \quad (2)$$

$u$  is the linear velocity of the mobile phase and  $\varepsilon$  is void fraction. Eq. (2) does not account for the axial dispersion term. Finite difference method (FDM) is used to solve Eqs. (1) and (2). It does not have an analytical solution, especially when two simultaneous partial differential equations are involved. Therefore, Eq. (2) is rewritten to Eq. (3) by FDM.

$$u \Delta t (c_{x+\Delta x, t} - c_{x, t}) + \varepsilon (c_{x+\Delta x, t+\Delta t} - c_{x+\Delta x, t}) + (1 - \varepsilon) (q_{x+\Delta x, t+\Delta t} - q_{x+\Delta x, t}) = 0 \quad (3)$$

An increment of the column direction,  $\Delta x$ , is assumed to be a height equivalent to a theoretical plate (HETP) (9).

To find the solution of Eq. (2), the parameters of the Langmuir isotherm,  $a$  and  $b$ , should be known. The parameter  $a$  is calculated from the retention time of the small injection.

$$q = Kc \quad (4)$$

$$k' = K\varepsilon \quad (5)$$

$K$  is the equilibrium constant and  $k'$  is the retention factor. To obtain the parameter  $b$  the retention time calculated in real-time domain is adjusted by a numerical solution of the mass balance. By the pulsed-injection method (PIM) used in this work, the parameter  $b$  of the Langmuir adsorption isotherm is obtained by an optimization method. The elution profile was calculated by an assumed value of  $b$  and compared with the experimental data based on the retention time and the column efficiency of phenol and *o*-cresol. Calculations were repeated until a good coincidence between the two profiles was reached.

In a binary mixture with large concentration, an early-eluting component interferes with a late-eluting one in a limited adsorption site, so the competitive interaction between the two components should be considered. To describe the competitive equilibrium between the components  $i, j$  of a binary sample mixture, the following competitive Langmuir isotherm is used. For an earlier component (phenol),

$$q_1 = \frac{a_1 c_1}{1 + b_1 c_1 + b_2 c_2} \quad (6)$$

For a slower component (*o*-cresol),

$$q_2 = \frac{a_2 c_2}{1 + b_1 c_1 + b_2 c_2} \quad (7)$$

## EXPERIMENTAL

### 1. Chemicals and Instrumentation

Phenol and *o*-cresol were obtained from Hayashi Pure Chemical Industries, Japan. Methanol (HPLC grade) was obtained from J. T. Baker, USA. All solvents were filtered through a filter (HA-0.45  $\mu\text{m}$ , FH-0.5  $\mu\text{m}$ ) by using a vacuum pump (Division of Millipore, Waters).

RP-HPLC was used to acquire the experimental data necessary for the determination of the equilibrium isotherm: equipped with a 600S solvent delivery and control system (Waters Co.), 2487 dual

$\lambda$  absorbance detector and Millennium32 data acquisition system and solvent reservoirs continuously stripped with helium to degas the mobile phase. A stainless steel column (0.39  $\times$  30 cm) was packed with 15  $\mu\text{m}$  C<sub>18</sub> packing (Lichrosphere, Merck). Sufficient time was allowed for the stabilization of the column and detector signal after each injection. The mobile phase composition, water/methanol (50/50 vol%), was constant throughout the experiments. The wavelength was fixed at 295 nm. The flow rate was 1 ml/min and all the experiments were performed at room temperature.

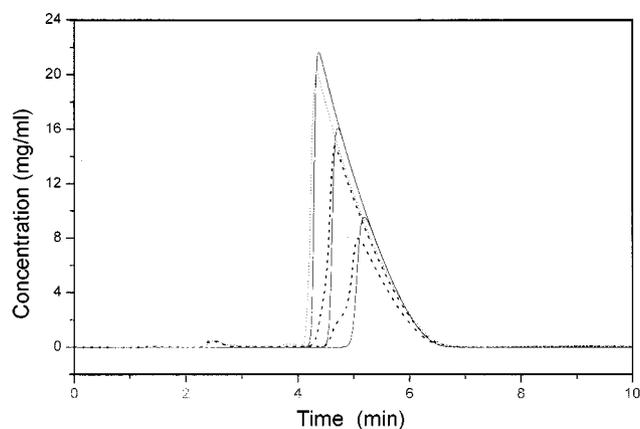
### 2. Single and Competitive Isotherm

Single isotherms were determined for both *o*-cresol and phenol. The constant injection volume of the phenol or *o*-cresol was 0.05 ml. Both the samples were well dissolved into methanol. The concentrations of a sample were 130, 260, and 390 mg/ml.

The competitive isotherms were determined with sample mixtures containing phenol and *o*-cresol in the concentration ratio, 130 : 390, 260 : 260 and 390 : 130 mg/ml, with the sample size 6.5 : 19.5, 13 : 13 and 19.5 : 6.5 mg, respectively. The parameters of  $a$  and  $b$  in the competitive isotherm were used by the same values in the adsorption isotherm of pure components.

## RESULTS AND DISCUSSION

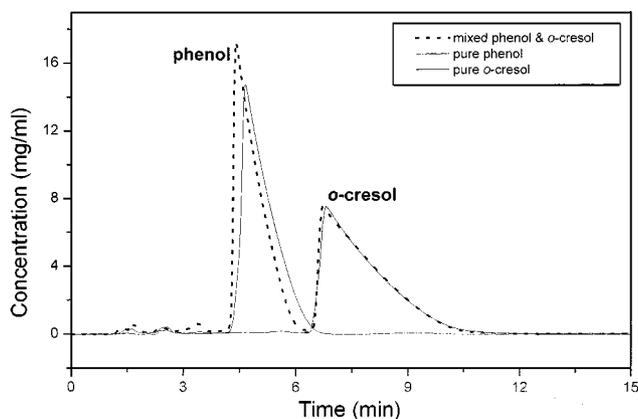
The elution profiles of phenol in Fig. 1 show that the isotherm is Langmuir type because the retention time is decreased with increasing sample size. This figure also indicates that the calculated elution profiles by Eq. (3) fit well with the experimental data. For the elution profile of *o*-cresol, similar matches were observed. The parameters used in the Langmuir isotherm obtained by PIM are shown in Table 1. To investigate the interference effect of phenol and *o*-cresol, three experimental runs were performed. Two experimental runs were done by each pure sample of phenol and *o*-cresol



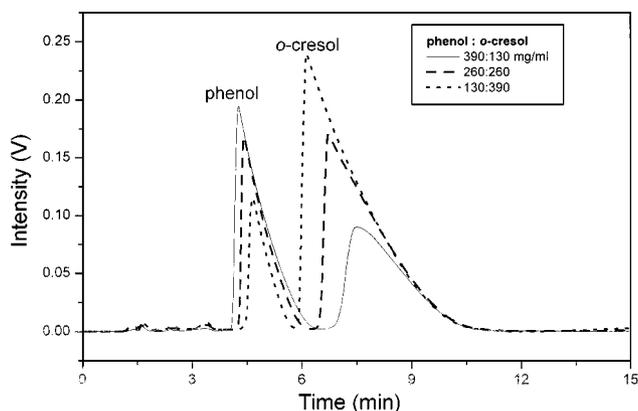
**Fig. 1. Comparison of the experimental data (dotted) and calculated elution profiles of phenol (solid).** (130, 260, 390 mg/ml concentration, 0.05 ml injection volume, and 1 ml/min)

**Table 1. Parameters of Langmuir isotherm**

	$a$ [-]	$b$ [ml/mg]
Phenol	2.129	0.012
<i>o</i> -cresol	3.805	0.026



**Fig. 2. Experimental profiles in pure and mixture of phenol and *o*-cresol.**  
(260 mg/ml concentration, 0.05 ml injection volume, and 1 ml/min)

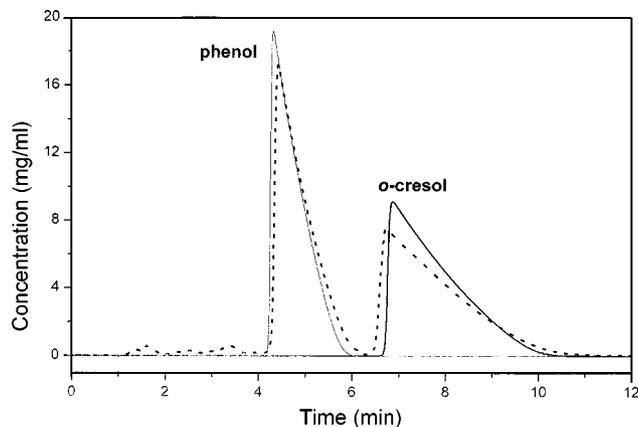


**Fig. 3. Experimental profiles of phenol and *o*-cresol with the concentration ratio.**  
(0.05 ml injection volume, and 1 ml/min)

(solid lines in Fig. 2). Some deviation of the elution profiles of phenol was observed. The elution profile of the mixture was expressed with a dotted profile, as shown in Fig. 2. For the late-eluting component, *o*-cresol, the shape and retention time of the peak were almost identical, while the early-eluting component in the mixture, phenol, came out of the column faster than pure phenol.

The experimental elution profiles of the mixture are shown in Fig. 3. The rear parts of the peak of *o*-cresol were almost coincident with the increased amount, while those of phenol were apart. This means that *o*-cresol interferes with phenol in the adsorbent phase; consequently, the peak of phenol elutes earlier.

In the case of a mixture of phenol and *o*-cresol, the mass balance, Eq. (2), with the competitive Langmuir isotherm of Eqs. (6), (7), was formulated to predict the calculated profile of the components. The competitive Langmuir parameters,  $a_i$  and  $b_i$ , were used as previously obtained and listed in Table 1. The parameter  $b$  of phenol was  $b_1$ , while that of *o*-cresol was  $b_2$  in the competitive Langmuir isotherm. The good agreement between the experimental data and the calculated elution profiles is shown in Fig. 4. The competitive isotherms of phenol and *o*-cresol were as follows. The units of  $q$  and  $c$  were mg/ml.



**Fig. 4. Experimental and calculated profiles in mixture of phenol and *o*-cresol.**  
(260 mg/ml concentration, 0.05 ml injection volume, and 1 ml/min)

for phenol (1),

$$q_1 = \frac{2.129c_1}{1 + 0.012c_1 + 0.026c_2}$$

for *o*-cresol (2),

$$q_2 = \frac{3.805c_2}{1 + 0.012c_1 + 0.026c_2}$$

## CONCLUSIONS

Compared to the conventional static method, the main advantage of PIM used in this work is its simplicity. With a couple of injections, the parameters of Langmuir isotherm,  $a$  and  $b$ , were calculated by the optimization method based on the retention time and column efficiency. In the mass balance, axial dispersion term was not included and a common numerical scheme of FDM was utilized. Nevertheless, the interference effect in the binary mixture of phenol and *o*-cresol was well explained by the mathematical modeling and the competitive Langmuir isotherm, Eqs. (7), (8). A study on the more general form of the adsorption isotherm, including Freundlich equation and various classes of mixtures, will be published in the near future.

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## NOMENCLATURE

- $a$  : the parameter of Langmuir isotherm [-]
- $b$  : the parameter of Langmuir isotherm [ml/mg]
- $c$  : concentration of solute in mobile phase [mg/ml]
- $K$  : equilibrium constant [-]
- $k'$  : retention factor [-]
- $q$  : concentration of solute in stationary phase [mg/ml]

t : time [min]  
 u : the linear velocity of the mobile phase [m/sec]  
 ε : void fraction [-]

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