Kinetic modeling for chromatographic separation of cytosine monophosphate and uracil monophosphate

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Abstract−Kinetic modeling for preparative chromatography is a topic of present interest in the fine chemicals and pharmaceutical industries. In this study, chromatographic separation of the two nucleotides CMP and UMP was simulated by the equilibrium-dispersive (ED) model, and the adsorption isotherms in the ED model were determined by the inverse method. Prediction performance of the model was validated under three different kinds of conditions and the importance of selecting isotherms was discussed in detail. Excellent agreement was achieved with the experimental band profiles and the prediction of the ED model. The ED model with bi-Langmuir isotherm was especially suitable for simulating chromatographic separation of CMP and UMP. The error of prediction by the ED model with bi-Langmuir isotherm was about 9.4 times smaller than that with Langmuir isotherm.

Key words: Kinetic Modeling, Chromatographic Separation, Equilibrium-Dispersive Model, Adsorption Isotherms, CMP and UMP

INTRODUCTION

Chromatography is now a powerful purification technique in the life science and pharmaceutical industry, where many problems cannot be solved by traditional methods. An accurate kinetic model of chromatography means that we can perform computer-assisted optimization and scale-up from analytical chromatography to large-scale preparative chromatography conveniently, which saves considerable time and money. However, the mechanism of chromatographic separation is quite complex because many factors need to be considered, including axial dispersion, interfacial film mass transfer and intraparticle diffusion. Fortunately, there are presently some models available for this subject, and numerical solutions for these mathematical equations [Rosen, 1952; Guiochon, 2002], as well as application of the models [Choi et al., 2004], have become realizable since the fast development of computer technology. The equilibrium-dispersive (ED) model is one of the most widely applied models for simulation and optimization research of chromatographic separation, because of its high efficiency and relatively low computation time. When column efficiency is high and mass transfer is fast, precision of chromatographic simulation by the ED model is satisfactory [Kaczmarski and Antos, 1996]. The band profiles are calculated by the ED model [Guiochon et al., 1994], which has been successfully applied in non-linear chromatography.

Adsorption isotherms are the fundamental thermodynamic property of chromatographic separation models, which are very important for accurate prediction of the individual band profiles. There are several dynamic methods available to measure adsorption isotherms, including frontal analysis, elution by characteristic point, pulsed input method [Chois, 2000] and inverse method [Seidel-Morgenstern, 2004]. Among these methods above, the inverse method

is becoming more and more attractive because it needs relatively small amounts of sample and solvent [Felinger et al., 2003a, b], which is particularly advantageous for those expensive samples.

Nucleotide is an extremely important kind of biochemistry compound for metabolism, which includes AMP, UMP, CMP and GMP. And study for separation of the four nucleotides is of great significance [Ji et al., 1999]. Among the four nucleotides, the separation of CMP and UMP is the most difficult because they are the most similar in molecular structures, as well as some physical properties such as polarity and molecular mass. So the study on the kinetic modeling for chromatographic separation of CMP and UMP is significant for their separation optimization and scale-up.

This work gave a basic study for chromatographic simulation of separation of CMP and UMP. The chromatographic separation was simulated by the ED model. Then prediction performance of the model was validated in the different conditions, and the influence of adsorption isotherms on the precision of model prediction was discussed.

MODEL EQUATIONS

The ED model assumed that mass transfer across the column was infinitely fast and treated the finite rate of the mass transfer as a contribution to axial dispersion. The mass balance equation for each component was written:

$$\frac{\partial C_i}{\partial t} + (1/e - 1)\frac{\partial q_i}{\partial t} + u\frac{\partial C_i}{\partial x} = D_a\frac{\partial^2 C_i}{\partial x^2} \tag{1}$$

$$q_i = f(c_i) \tag{2}$$

The initial condition was:

$$C_i(0, x) = 0, \ 0 < x < L \qquad (\text{for } t = 0) \tag{3}$$

The Danckwerts boundary conditions [Rosen, 1952] were used for Eq. (1):

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$$u[Cf_i(t) - C_i(t, 0)] = -D_a \frac{\partial C_i(t, 0)}{\partial x} \qquad \text{(for x=0)} \qquad (4)$$

where $Cf_i(t) = \begin{cases} 0, & t < 0 \text{ and } t > t_p \\ C_i(t, 0), & 0 < t < t_p \end{cases}$

$$\frac{\partial C_i}{\partial x} = 0 \qquad \text{(for x=L)} \qquad (5)$$

When Eq. (2) was non-linear, the partial differential equations above mentioned had no analytical solution. Therefore, a finite difference method was applied for numerical solution [Czok and Guiochon, 1990; Ma and Guiochon, 1991]. In this method, Eq. (1) was rewritten as:

$$\frac{c_{i,z-1}^t - c_{i,z-1}^{t-1}}{\Delta t} + (1/e - 1)\frac{q_{i,z-1}^t - q_{i,z-1}^{t-1}}{\Delta t} + u\frac{c_{i,z}^t - c_{i,z-1}^t}{\Delta x} = 0 \qquad (6)$$

In Eq. (6), the apparent dispersion coefficient was set to zero, which was different from Eq. (1), and this error was eliminated by choosing appropriate values of the time and length increments as follows [Felinger et al., 2003]:

$$\Delta x = 2D_a e/u \qquad (7)$$

$$\Delta t = a_k e(1 + k')\Delta x/u \qquad (8)$$

a_k in Eq. (8) was a constant more than 1, usually 2 was reasonable [Guiochon et al., 1994].

EXPERIMENTAL

1. Instrumentation and Chemicals

The apparatus consisted of an East Chrom liquid chromatography system (Knauer, Germany), Well Chrom K2501 UV detector, manual injector with different sample loops (0.02 mL, 0.1 mL, and 1 mL); and a stainless steel chromatographic column (25 cm×0.46 cm) packed with SB C18. CMP and UMP (purity≥99%) were purchased from Shanghai QZU Bioscience & Biotechnology Co., LTD. 0.05 mol/L KH_2PO_4 (pH 3.3), CH_3OH (chromatographic grade).

2. Methods

2-1. Measurement of UV Absorbance-concentration Calibration Curve

The detector responses of CMP and UMP were calibrated at different concentrations in the range of 0-0.4 g/L, which were performed at 25 °C, 1 mL/min, 280 nm.

2-2. Measurement of UV Absorbance of Mixtures of CMP and UMP

Mixtures of CMP and UMP were injected in the amount of 0.02 mL and 0.1 mL, and the band profiles were recorded. The former injection was used for determination of parameters in the model, and the latter for validation of the model. Concentrations of CMP and UMP in mixtures were 0.5 mg/mL, respectively. Other experimental conditions were the same as in the first step.

2-3. Measurement of Adsorption Isotherms

The parameters of adsorption isotherms in this study were determined by the inverse method [Felinger et al., 2003], a method by minimizing the discrepancies between an experimental chromatogram and the model predictions. First, band profiles of the sample were measured experimentally. Then, an isotherm model was selected and its initial parameters were estimated, with which the band profiles were calculated and compared with the experimental con-
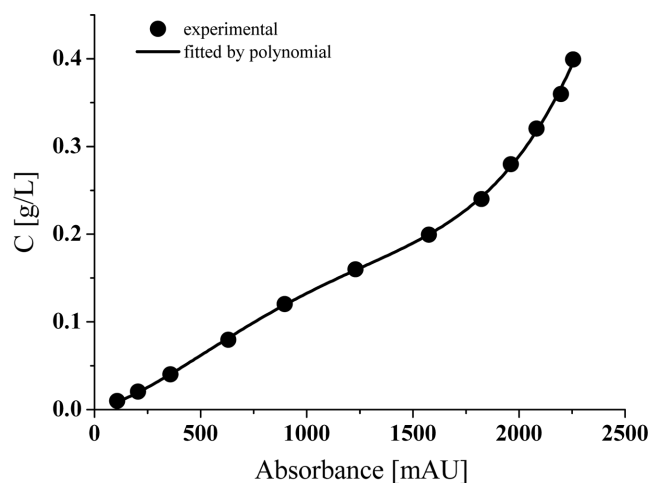
centrations as follows:

$$Fun = \sum_i (c_i^{exp} - c_i^{cal})^2 \qquad (9)$$

Parameters were changed and optimization method was used to minimize Fun. For the multi-dimensional optimization here, the super modified downhill simplex search method was used [Morgan and Burton, 1990]. Adsorption isotherms determined by this method are quite similar with that by frontal analysis [Felinger et al., 2003].
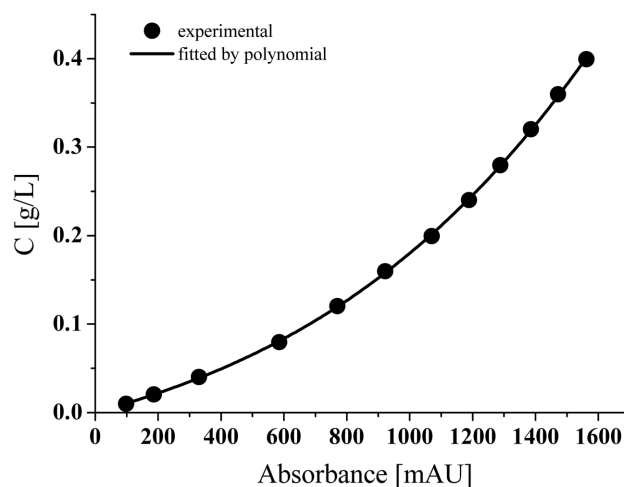
RESULTS AND DISCUSSION

1. UV Absorbance-concentration Calibration Curve

The result of model calculation was the values of concentration, while the result of experiment was the values of UV response. So we should, first of all, determine the UV-concentration relations of CMP and UMP. Fig. 1 illustrated the relationship between the concentrations and the corresponding UV response of CMP and UMP at the wavelength of 280 nm. And the UV absorbance-concentration calibration curves of CMP and UMP are written, respectively,

(a) CMP

(b) UMP

Fig. 1. UV-concentration relations of CMP and UMP at a wavelength of 280 nm.

as follows:

$$c_{CMP}=5.2\times10^{-14}\times A^4-1.9\times10^{-10}\times A^3+2.1\times10^{-7}\times A^2+6.1\times10^{-5}\times A \quad (10)$$

$$c_{UMP}=3.6\times10^{-11}\times A^3+4.3\times10^{-8}\times A^2+1.0\times10^{-4}\times A \quad (11)$$

where c_{CMP} and c_{UMP} were the concentrations of CMP and UMP (g/L), and A was the corresponding UV response (mAU).

Fig. 1 showed that the calibration curves of CMP and UMP were both severely non-linear, and obviously different from each other. So in the concentration range of 0-0.4 g/L, band profiles of CMP and UMP were determined not only by dispersion and resistance of interfacial film mass transfer, but also by the concentration. Then the velocity of each component in band profiles was affected by its corresponding concentration, which made the model calculation of CMP and UMP more complex.

2. Determination of Model Parameters
2-1. Determination of Total Porosity

Total porosity of the column was determined by methanol in this work. The retention time of methanol was 2.653 minutes at the flow rate of 1 mL/min. Total volume of the column was 4.153 mL. Total porosity can be determined by the following equation:

$$e=\frac{Qt_0}{V} \quad (12)$$

Here, the total porosity of the column, calculated from Eq. (12), is equal to 0.64.

2-2. Determination of Apparent Dispersion Coefficient

The apparent dispersion coefficient D_a could be determined by using the following equation:

$$D_a=\frac{uL}{2N} \quad (13)$$

The number of theoretical plates (N) is derived from the half peak width and the relative retention time by an analytical injection of CMP:

$$N=5.54\left(\frac{t_{RI}}{w_{1/2}}\right)^2 \quad (14)$$

At a flow rate of 1.0 mL/min, the number of theoretical plates N was 4374, and the apparent dispersion coefficient of the column, calculated from Eq. (13), was 0.0175 cm²/min.

3. Model Solution

The partial differential equation of ED model was numerically integrated by a modified Rouchon (finite difference) algorithm [Kaczmarski and Antos, 1996]. Main parts of the program were written in the C language, which were called through an interface file (MEX-file) in Matlab6.5.

4. Effects of Adsorption Isotherms on Model Prediction

Parameters in the ED model were determined when the injected sample amount was 0.02 mL. For the model validation, three kinds of competitive isotherms, i.e., the Langmuir, modified Langmuir and bi-Langmuir isotherms, are discussed at the sample amount of 0.1 mL.

4-1. Langmuir Isotherm

The classical competitive Langmuir isotherm model is written:

$$q_i=\frac{a_ic_i}{1+\sum_{j=1}^{n}b_jc_j} \quad (i=1, ..., n) \quad (15)$$

Table 1. Parameters of Langmuir isotherm obtained by inverse method

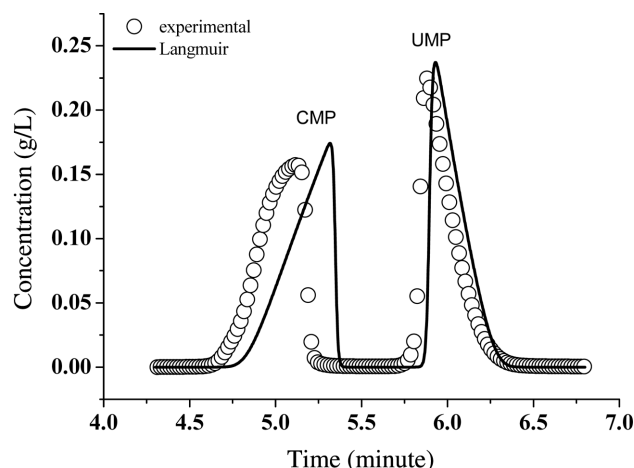| CMP		UMP	
a_1	b_1	a_2	b_2
1.5264	−0.4874	2.3756	0.1940

Fig. 2. Comparison of band profiles predicted by the ED model with experimental data $C_f=0.5$ g/L, Q=1 mL/min, λ=280 nm, V_{inj}=100 μL.

where a_i and b_j are numerical coefficients. A mixture of CMP and UMP was injected at a flow rate of 1.0 mL/min. The injected volume was 0.02 mL. The parameters of Langmuir isotherm obtained by the inverse method are listed in Table 1.

A second injection of the sample was carried out at the injected sample amount of 0.1 mL to validate the performance of the model with Langmuir isotherm. Model prediction was compared with experimental results in Fig. 2.

Fig. 2 shows that there was a deviation between the model calculation and experimental data, especially for CMP. Therefore, it could be concluded that the competitive Langmuir isotherm model was not satisfactory for the system of CMP and UMP. Then a modified Langmuir isotherm was used for better prediction performance of the model.

4-2. Modified Langmuir Isotherm

As had been shown, the parameter a in the competitive Langmuir isotherm was very sensitive [Felinger et al., 2003], and changed with concentration [Zhu et al., 1991]. So parameter a had very significant effect on the model prediction. Suppose that a is linearly changed with concentration, then Eq. (15) can be transformed as follows:
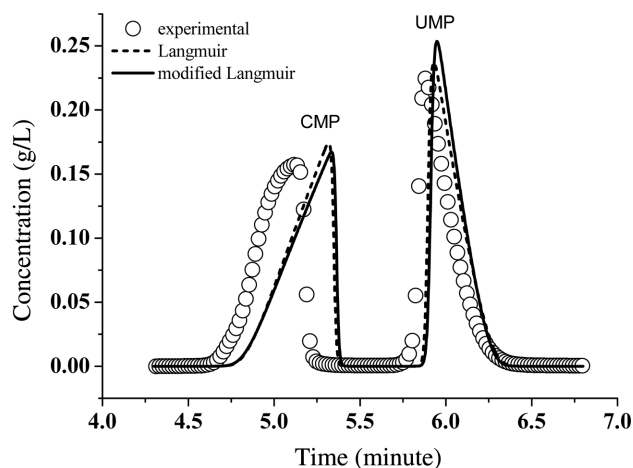
$$q_i=\frac{a_i(1+k_ic_i)c_i}{1+\sum_{j=1}^{n}b_jc_j} \quad (i=1, ..., n) \quad (16)$$

With the same experimental condition as in the section 4-1, parameters of modified Langmuir isotherm obtained by the inverse method are listed in Table 2.

For the second injection of the sample at the amount of 100 μL, model prediction with modified Langmuir isotherm is compared

Table 2. Parameters of modified Langmuir isotherm obtained by the inverse method

CMP			UMP		
a_1	b_1	k_1	a_2	b_2	k_2
1.5203	−0.4873	0.0569	2.3641	0.1837	0.0224

Fig. 3. Effect of Langmuir isotherm and modified Langmuir isotherm on the performance of model prediction C_f=0.5 g/L, Q=1 mL/min, λ=280 nm, V_{inj}=100 µL.

with experimental results in Fig. 3. The model with modified Langmuir isotherm was expected to have a better prediction than that with Langmuir isotherm because of more parameters. In this case shown in Fig. 3, however, the discrepancy between experimental data and model prediction is even larger. The main reason should be that equilibrium dispersive model with four parameters' Langmuir isotherm was already enough for the two-components-system here, and the ED model would overfit when the number of parameters in Langmuir isotherm was more than four, leading to the worse prediction. Here, overfitting is a phenomenon that the model is excessively trained and results in poor performance of prediction, which is actually a universal phenomenon in the modelling process for predictions such as artificial neural networks [Zupan and Gasteiger, 1993].

4-3. Bi-Langmuir Isotherm

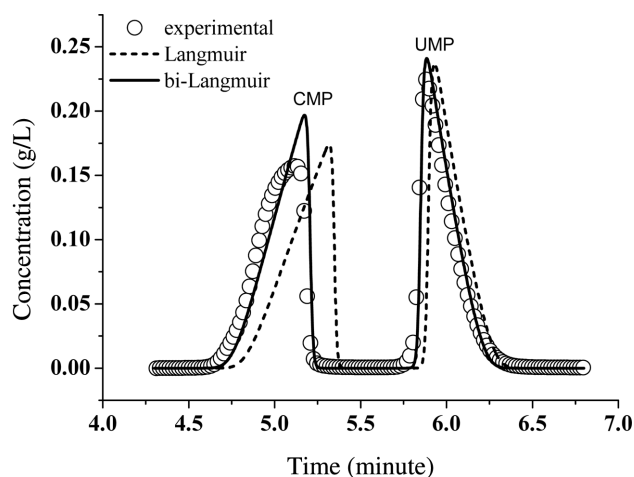The competitive bi-Langmuir isotherm model is written:

$$q_i = \frac{a_i^I c_i}{1+\sum_{j=1}^{n} b_j^I c_j} + \frac{a_i^{II} c_i}{1+\sum_{j=1}^{n} b_j^{II} c_j} \qquad (i=1, ..., n) \qquad (17)$$

The parameters of bi-Langmuir isotherm obtained by the inverse method are listed in Table 3.

For the second injection of the sample at the amount of 100 µL,

Fig. 4. Effect of Langmuir isotherm and bi-Langmuir isotherm on the performance of model prediction C_f=0.5 g/L, Q=1 mL/min, λ=280 nm, V_{inj}=100 µL.

Table 4. Prediction precision of the ED model with three different kinds of isotherms

Isotherms	Langmuir	Modified Langmuir	bi-Langmuir
ε	0.0075	0.0087	0.0008

model prediction with bi-Langmuir isotherm is compared with experimental results in Fig. 4. The precision of model prediction was particularly improved for both components when bi-Langmuir isotherm, instead of Langmuir isotherm, was used in the ED model, as shown in Fig. 4.

Prediction precision of the ED model with Langmuir, modified Langmuir and bi-Langmuir isotherm is quantitatively compared in Table 4. The prediction precision is defined as:

$$\varepsilon = \sum_{i=1}^{n} \int (c_i^{exp} - c_i^{cal})^2 dt \qquad (18)$$

From Table 4, ε of the model with bi-Langmuir isotherm is about 9.4 times smaller than that of Langmuir isotherm. It suggests that the bi-Langmuir isotherm described the system of CMP and UMP much better than the Langmuir isotherm. The number of parameters in the bi-Langmuir isotherm was eight, even more than that in modified Langmuir isotherm, but the performance of prediction was excellent without the phenomenon of overfitting. Hence, the conclusion could be drawn that the precision of ED model was very sensitive to the selection of adsorption isotherms.

CONCLUSION

The chromatographic separation of CMP and UMP was simu-

Table 3. Parameters of bi-Langmuir isotherm obtained by the inverse method

CMP				UMP			
a_1^I	a_1^{II}	b_1^I	b_1^{II}	a_2^I	a_2^{II}	b_2^I	b_2^{II}
1.3312	0.1452	−0.2911	0.3761	2.6095	−0.2695	−0.3899	0.2049

lated by the ED model, and adsorption isotherms in the ED model were determined by the inverse method. Excellent agreement was achieved with experimental band profiles and prediction of the ED model. Prediction performance of the ED model was discussed in detail under three different kinds of conditions. The results showed that prediction performance of the ED model was very sensitive to different selection of adsorption isotherm. Prediction of the ED model with modified Langmuir isotherm was not better than that with the competitive Langmuir isotherm, because it would overfit when the number of parameters in Langmuir isotherm was more than four, which led to the worse prediction. Prediction performance was particularly improved when bi-Langmuir isotherm was used in the ED model. The error of prediction by the ED model with bi-Langmuir isotherm was about 9.4 times smaller than that with Langmuir isotherm.

NOMENCLATURE

a	: coefficient of Langmuir isotherm [-]
b	: coefficient of Langmuir isotherm [L/g]
a^I, a^{II}	: coefficient of bi-Langmuir isotherm [-]
b^I, b^{II}	: coefficient of bi-Langmuir isotherm [L/g]
a_k	: constant in Eq. (8) [-]
c	: concentration in fluid phase [g/L]
c_i^{cal}	: calculated concentration [g/L]
c_i^{exp}	: experimental concentration [g/L]
C_f	: feed concentration [g/L]
D_a	: apparent dispersion coefficient [m²/s]
e	: total porosity [-]
i, j	: component index [-]
k	: coefficient of modified Langmuir isotherm [-]
k'	: retention factor [-]
L	: height of column [m]
n	: number of components [-]
N	: number of theoretical plates [-]
q	: concentration in stationary phase [g/L]
Q	: flow rate [m³/s]
t	: time [s]
t_0	: hold-up time [s]
t_p	: time during the constant concentration [s]
t_{Rl}	: retention time of CMP [s]
u	: superficial velocity [m/s]
V	: volume of the column [m³]
V_{inj}	: injected volume [μL]
$w_{1/2}$	: the half peak width [s]
x	: axial coordinate [m]
Δt	: time increment [s]
Δx	: space increment [m]
ε	: prediction precision [g²s/L²]

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