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## Estimation of partition, free and specific diffusion coefficients of paclitaxel and taxanes in a fixed bed by moment analysis: experimental, modeling and simulation studies

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**ABSTRACT.** Paclitaxel, as known Taxol®, is an important agent in cancer treatment, founded in mixture with many structural analogs, or taxanes, present in natural source or plant tissue culture broth. The adsorption techniques are used in the purification of paclitaxel from that complex mixture, but despite of the strategy it is important to know the basic parameters associated with any process, such as isotherms and mass transfer parameters. In this paper is presented a simple model to estimate these parameters by moment analysis. After to consider linear isotherm for adsorption, the partition coefficient, free and effective diffusion coefficients of paclitaxel and four major components, in a plant tissue culture broth, were estimated from the first and second moments of peaks in pulse-elution chromatograms. The experimental chromatograms at two flow rates are compared with those ones from model, also proposed in this work. The experimental results of free diffusion coefficient are compared with that ones from the Literature.

**Keywords:** cancer, taxol, mass transfer.

## Estimativa dos coeficientes de partição, difusividades livre e específica do paclitaxel e outras taxanas em um leito fixo pela técnica da análise de momento: estudos experimental, de modelagem e simulação

**RESUMO.** O paclitaxel, também conhecido como Taxol®, é um importante agente anti cancerígeno, encontrado em mistura com vários compostos análogos, reconhecidos como taxanas, em fontes naturais ou em culturas de caldo cultivado. Técnicas de adsorção são utilizadas para extrair o paclitaxel dessa mistura complexa. Todavia, independentemente da estratégia de separação do paclitaxel, é fundamental o conhecimento de parâmetros básicos associados a qualquer processo, tais como isotermas de equilíbrio e coeficientes de transferência de massa. Apresenta-se neste artigo um modelo simples para estimar tais grandezas utilizando-se a técnica de análise de momentos de curvas cromatográficas. Após admitir que a adsorção obedeça isoterma linear, os valores dos coeficientes de partição, de difusão livre e de difusão efetiva para o paclitaxel e outros quatro componentes presentes no caldo cultivado, são estimados a partir dos primeiros e segundos momentos de pulsos cromatográficos. Os cromatogramas experimentais são comparados com aqueles advindos de um modelo também utilizado neste artigo, bem como o valor experimental do coeficiente de difusão do paclitaxel é comparado com valores advindos do uso de correlações encontradas na Literatura.

**Palavras-chave:** câncer, taxol, transferência de massa.

### Introduction

Cancer is a health public worldwide important problem that must be considered in various area of the knowledge, including engineering process. In USA, appear a million cases by year, and in the South East of the Brazil, cancer is the second cause of death, just losing for the cardiovascular diseases. The introduction of chemotherapy to cancer combat results in significant taxes of tumors cure, that didn't controlled with success by exclusive use of surgery

and/or radiotherapy. Researchers around the world have particular attention in the study of natural products as possible source of antineoplastic agents. Due to diversity of the chemical structures founded in theses products, there are big chances to identify news molecules with anti-tumor activities. The paclitaxel discovery offers good points for this reasoning way.

Paclitaxel is an diterpene compound and has the molecular formula  $C_{47}H_{51}NO_{14}$ , with molecular weight 853.92. There are many structural analogs, or

taxanes, such as cephalomannine and baccatin, present in the natural source of paclitaxel or plant tissue culture broth (PTC broth). One of the challenge in the paclitaxel processing is its separation, and the major portion of the purification cost is due to the separation of paclitaxel from a large number of taxanes with similar molecular structures (CARDELINA II, 1991). Conventional batch chromatography had been used for paclitaxel separation from PTC broth (WU et al., 1996). This technique, however, is expensive and has low yield and low productivity. A simulated moving bed (SMB), which saves solvent and increases adsorbent utilization, could result in a more economical separation process. Despite of the separation techniques, it is fundamental to know the basic parameters associated with any process, such as isotherms and mass transfer parameters.

The association of chromatographic technique and moment analysis of the response peaks is a powerful approach for adsorption studies. The moment analysis in chromatography and, as extension, the pulse analysis have been applied to measure the transport rate, adsorption parameters and bed characteristics for single and multicomponent systems (CHIAHARA et al., 2005; HARLICK; TEZEL, 2000, 2003; SILVA JR. et al., 2005; YAMAMOTO et al., 2001), and to evaluate effective diffusion coefficients in a macroreticular resin catalyst (DOGAN; DOGU, 2003). The pulse chromatographic method can be used to evaluate the influence of packing (WU; CHING, 2002), and connecting devices, such as frits, on the performance of a chromatographic column (WU; CHING, 2003). The behavior of a chromatographic column where a single solute is fed in the presence of a suitable modifier can be analyzed in the context of the pulse propagation of the solute, whose retention depends on the modifier concentration, as studied by Ströhlein et al. (2006).

Moment analysis is a useful tool for determining partition coefficient, and mass transfer parameters from pulse experiments, as verified since Schneider and Smith (1968). In this paper the moment analysis method is applied to determine adsorption constants, free and effective diffusion coefficients of taxane mixture from PTC broth, in a fixed bed of polystyrene divinyl-benzene copolymer adsorbent resin. The experimental chromatograms at two flow rates are compared with those ones from numerical solutions of a detailed rate model.

## Theory

In order to apply moment analysis to a pulse response curve to estimate linear adsorption constants or partition coefficients, and free and

effective diffusion coefficients, the following assumptions are made: i) the mobile phase is a dilute solution; ii) velocity is constant throughout column cross-section; iii) no chemical reactions occur; iv) Henry's law describes solute uptake – linear isotherm; v) intraparticle diffusion is described by pore diffusion; vi) external mass transfer from the bulk liquid to the pores is described by film mass transfer; vii) axial dispersion effects are considered. Based on these hypotheses the following equations are obtained (SCHNEIDER; SMITH, 1968):

Solute mass balance in the mobile phase for each solute presents in the PTC broth, as showed in Equation 1,

$$\frac{\partial c}{\partial t} = E_b \frac{\partial^2 c}{\partial z^2} - v \frac{\partial c}{\partial z} - \left( \frac{1-\epsilon}{\epsilon} \right) \frac{6k_f}{d_p} (c - c|_{r=R_p}) = 0 \quad (1)$$

where:

$c$  is solute concentration in the mobile phase ( $\text{ML}^{-3}$ );  $t$ , time;  $z$ , axial coordinate;  $E_b$ , axial dispersion coefficient ( $\text{L}^2\text{T}^{-1}$ );  $v$ , liquid interstitial velocity ( $\text{LT}^{-1}$ );  $\epsilon$ , bed porosity;  $k_f$ , film mass transfer coefficient ( $\text{L}^2\text{T}^{-1}$ );  $d_p$ , average particle diameter ( $\text{L}$ );  $R_p$ , average radius particle ( $\text{L}$ ).

Solute mass balance in the porous phase for each solute presents in the PTC broth, as presented in Equation 2,

$$[\epsilon_p + (1-\epsilon_p)k_p] \frac{\partial q}{\partial t} = \epsilon_p D_p \frac{1}{r^2} \frac{\partial}{\partial r} \left( r^2 \frac{\partial q}{\partial r} \right) \quad (2)$$

where:

$\epsilon_p$ , particle porosity;  $k_p$ , equilibrium partition constant;  $q$ , solute concentration in the porous phase ( $\text{ML}^{-3}$ );  $D_p$ , effective pore-phase diffusion coefficient ( $\text{L}^2\text{T}^{-1}$ );  $r$ , radial coordinate.

The initial and boundary conditions that describe a pulse injection into a chromatographic column are from Equation 3 up to Equation 9,

$$z = 0; 0 \leq t < t_0; c = c_0 \quad (3)$$

$$z = 0; t > t_0; c = 0 \quad (4)$$

$$z > 0; t = 0; c = 0 \quad (5)$$

$$z = Lc; t > t_0; c = 0 \quad (6)$$

$$r \geq 0; t = 0; q = 0 \quad (7)$$

$$\frac{\partial q}{\partial r} \Big|_{r=0} = 0 \quad (8)$$

$$\varepsilon_p D_p \frac{\partial q}{\partial r} \Big|_{r=R_p} = \frac{6k_f}{d_p} (c - c \Big|_{r=R_p}) \quad (9)$$

the subscripts in these equations represent the initial conditions;  $L_c$  is the packed bed height (L). The first,  $\mu$ , and the second,  $\sigma$ , absolute moments are defined as follows

$$\mu = \int_0^\infty c(t) t dt \Big/ \int_0^\infty c(t) dt \quad (10)$$

$$\sigma^2 = \int_0^\infty c(t) (t - \mu)^2 dt \Big/ \int_0^\infty c(t) dt \quad (11)$$

It's possible to apply the Laplace-Carson transform to the solute continuity equations, Equations 1 and 2, and obtained analytical solutions for pulse elution curves. By substituting the analytical solution into Equations 10 and 11, one can obtain the explicit expressions for the first and second moments, respectively,

$$\mu = \frac{L_c}{v} \left[ 1 + \left( \frac{1-\varepsilon}{\varepsilon} \right) K \right] + \frac{t_0}{2} \quad (12)$$

$$\sigma^2 = \left( \frac{2L_c}{v} \right) \left( \frac{E_b}{v^2} \right) \left[ 1 + \left( \frac{1-\varepsilon}{\varepsilon} \right) K \right]^2 + \left( \frac{2L_c}{v} \right) \left( \frac{1-\varepsilon}{\varepsilon} \right) K^2 \left( \frac{d_p}{6k_f} + \frac{d_p^2}{60\varepsilon_p D_p} \right) + \frac{t_0^2}{12} \quad (13)$$

with  $K = \varepsilon_p + (1 - \varepsilon_p) k_p$

For a system with linear adsorption isotherms, the efficiency of a chromatographic column can be evaluated by a height equivalent to that of a theoretical plate (HETP), which is defined according to the Van Deemter equation as follows:

$$\text{HETP} \equiv L_c \left( \frac{\sigma'^2}{t_R^2} \right) \quad (14)$$

where:

$$\sigma'^2 = \sigma^2 - \sigma_0^2 \quad (15)$$

$$t_R = \mu - \mu_0 \quad (16)$$

where:

$t_R$  represents the retention time (T), and  $\mu_0$  and  $\sigma_0$  include, respectively, the delay in retention and dispersion due to extra column dead volume.

By substituting Equations 12 and 13 into definition 14, one obtains

$$\text{HETP} = 2 \left( \frac{E_b}{v} \right) + 2\chi^2 \left( \frac{1-\varepsilon}{\varepsilon} \right) \left( \frac{d_p}{6k_f} + \frac{d_p^2}{60\varepsilon_p D_p} \right) v \quad (17)$$

in which:

$$\chi^2 = \left\{ K \left[ 1 + \left( \frac{1-\varepsilon}{\varepsilon} \right) K \right]^{-1} \right\}^2 \quad (18)$$

The axial dispersion coefficient  $E_b$  can be estimated from a correlation by Athalye et al. (1992):

$$E_b = d_p \varepsilon v \left[ \frac{\varepsilon v d_p}{(1-\varepsilon) D_{AB}} \right]^{1/6} \quad (19)$$

$D_{AB}$  é the free diffusion coefficient ( $L^2 T^{-1}$ ). The film mass-transfer coefficient,  $k_f$ , presents in Equation 17 is calculated from a correlation by Wilson and Geankoplis (1966):

$$\frac{d_p k_f}{D_{AB}} = \frac{1.09}{\varepsilon} \left( \frac{d_p \varepsilon v}{D_{AB}} \right)^{1/3} \quad (20)$$

#### Parameter estimation by moment analysis

The operating parameters depend on the bed and particle characteristics ( $D$ ,  $L_c$ ,  $d_p$ ,  $\varepsilon$ ,  $\varepsilon_p$ ) such as the partition coefficient  $k_p$  (in the case of linear isotherm), and mass-transfer parameters ( $D_{AB}$ ,  $D_p$ ,  $E_b$ ,  $k_f$ ). In this paper, we know ( $D$ ,  $L_c$ ,  $d_p$ ,  $\varepsilon$ ,  $\varepsilon_p$ );  $E_b$  and  $k_f$  are calculated from Equations 19 and 20, respectively. The unknown parameters ( $D_{AB}$  and  $D_p$ ) can be determined from a moment analysis. This technique consists of analyzing the solute concentration as a function of time at the outlet of a fixed bed in response to a concentration pulse at the entrance of the bed.

The partition coefficient for each component, under the assumption of dilute solutions and negligible adsorption interference, is obtained from Equations 12 and 16, or

$$\frac{L_c}{t_R} = \frac{\varepsilon v}{\varepsilon + (1-\varepsilon)\varepsilon_p + (1-\varepsilon)(1-\varepsilon_p)k_p} \quad (21)$$

For systems with linear adsorption isotherms, the efficiency of a chromatographic column can be quantified as the height equivalent to a theoretical plate (HETP), which can be related to the liquid-phase linear velocity and the key mass-transfer

parameters of the system through Equation 17. If one substitutes Equations 19 and 20 into Equation 17, one obtains,

$$y = a_1 x_1^{1/6} + a_2 x_1^{2/3} + a_3 x_2 \quad (22)$$

in which:

$$y = \frac{L_c}{2v} \left( \frac{\sigma'^2}{t_R^2} \right) \quad (23)$$

$$x_1 = \frac{1}{D_{AB}} \quad (24)$$

$$x_2 = \frac{1}{D_p} \quad (25)$$

$$a_1 = d_p \left( \frac{d_p \epsilon v}{1 - \epsilon} \right)^{1/6} \quad (26)$$

$$a_2 = \frac{(\epsilon d_p)^{2/3}}{6.54 v^{1/3}} \left( \frac{1 - \epsilon}{\epsilon} \right) \chi^2 \quad (27)$$

$$a_3 = \frac{d_p^2}{60 \epsilon_p} \left( \frac{1 - \epsilon}{\epsilon} \right) \chi^2 \quad (28)$$

It is possible to estimate the free and effective diffusion coefficients by Equation 22, associated with  $x_1$  and  $x_2$ , Equations 24 and 25, respectively. Equation 22 produces two new equations:

$$y_1 = \alpha_1 x_1^{1/6} + \alpha_2 x_1^{2/3} + a_3 x_2 \quad (29)$$

$$y_2 = \alpha_4 x_1^{1/6} + \alpha_5 x_1^{2/3} + a_3 x_2 \quad (30)$$

where:

$$\alpha_1 = d_p \left( \frac{d_p \epsilon v_1}{1 - \epsilon} \right)^{1/6} \quad (31)$$

$$\alpha_2 = \frac{(\epsilon d_p)^{2/3}}{6.54 v_1^{1/3}} \left( \frac{1 - \epsilon}{\epsilon} \right) \chi^2 \quad (32)$$

$$\alpha_4 = d_p \left( \frac{d_p \epsilon v_2}{1 - \epsilon} \right)^{1/6} \quad (33)$$

$$\alpha_5 = \frac{(\epsilon d_p)^{2/3}}{6.54 v_2^{1/3}} \left( \frac{1 - \epsilon}{\epsilon} \right) \chi^2 \quad (34)$$

The free diffusion coefficient in Equation 24 is obtained from:

$$(y_1 - y_2) = (\alpha_1 - \alpha_4) x_1^{1/6} + (\alpha_2 - \alpha_5) x_1^{2/3} \quad (35)$$

After  $x_1$  is calculated, this value is substituted into Equation 31 to estimate the effective diffusion coefficient.

## Material and methods

HPLC grade acetonitrile was purchased from Fisher Scientific (Fairlawn, NJ). HPLC grade tetrahydrofuran (THF) was obtained from Sigma Chemical Co. (St. Louis, MO). Pure ethanol was purchased from McCormick Distilling Co. (Weston, MA). The ethanol was degassed prior to use by sonicating in an ultrasonic bath (Fairlawn, NJ). Distilled deionized water (DDW) was obtained from a Milli-Q™ system by Millipore (Bedford, MA). All solvents used were filtered through 0.2-μm Nylon 66 filter from Alltech (Deerfield, IL). The polystyrene divinyl-benzene copolymer adsorbent (Dow XUS 43493) used in all low-pressure liquid chromatography (LPLC). This adsorbent has essentially two types of pores: large pores to allow for effective mass transfer and smaller pores, taking up about 20 to 30% of the total pore volume, to provide sufficient capacity for relatively small molecules (MW < 10000).

## Instrumentation

A Pharmacia (Piscataway, NJ) Fast Protein Liquid Chromatography (FPLC) system was used for the batch elution experiments. This system consists of two pumps (Pharmacia P-500), a liquid chromatography controller (Pharmacia LCC-500), an injection valve (Pharmacia MV-7), and a fraction collector (Pharmacia Frac-100). Omnifit low-pressure glass columns (each 1.5 cm I.D. × 15 cm, non-adjustable) used in the batch elution were purchased from Alltech Associates Inc. (Deerfield, IL).

HPLC was used to analyze the collected fractions and construct (off-line) the effluent concentration history. The HPLC system consisted of two pumps (Waters 510), a tunable single-wavelength detector (Waters 486) and an injector (Waters U6K). Waters Millennium 2010 software was used for data collection. A Waters Nova-Pak C18 column and a premixed mobile phase of water:acetonitrile:tetrahydrofuran (60:30:10 v v<sup>-1</sup> v<sup>-1</sup>), at a flow rate of 1.0 mL min.<sup>-1</sup> were used. The sample injection volume was 10 μL. The chromatograms were monitored at a wavelength of 227 nm with a single-wavelength detector (Waters 486). All solvents were degassed for approximately 20 minutes prior to analysis, and the

column was washed with acetonitrile after analysis. The simple isocratic assay requires a very short analysis time and can detect taxanes with very low concentrations (0.2 ppm). A single column can be used for more than 500 injections with this method.

To quantify the concentrations of the eluted components, a series of standard solutions in pure ethanol needed to be analyzed with the HPLC assay. Because the only available standard solution was paclitaxel, the calibration curve (peak area vs. concentration at 227 nm) for paclitaxel was also used for the other components.

The polystyrene divinyl-benzene copolymer adsorbent particles as received are spherical and have a large average diameter ( $> 600 \mu\text{m}$ ). The sorbent was chopped and sieved to a particle diameter range of 150 to  $300 \mu\text{m}$ . The particles became irregular in shape after the chopping. The irregular particles resulted in a higher pressure-drop than spherical particle of the same size. A slurry technique was used to pack the particles into the columns.

Two pulse elution experiments were carried out on the column described in Table 1. In each experiment, the column was pre-equilibrated with 60:40 v v<sup>-1</sup> ethanol:water. The same feed is used for the two batch-elution experiments. After the injection of the feed the column was immediately eluted with 60:40 v v<sup>-1</sup> ethanol:water. Fractions were collected at regular time intervals at the outlet of the column. The elution profiles of the eluted components were constructed according to the HPLC analysis of the fractions collected in the experiments.

**Table 1.** Column and particle characteristics.

Parameter	Value
Packed bed height, $L_c$	12.3 cm
Column inner diameter, $D$	1.50 cm
Average bed void fraction, $\varepsilon$	0.32
Particle void fraction, $\varepsilon_p$	0.46
Average particle diameter, $d_p$	$240 \mu\text{m}$

## Results and discussion

The feed used for the single-column experiments was a crude mixture from Bristol-Myers Squibb Co., and was dissolved in 60:40 ethanol:water v v<sup>-1</sup>. When passed through a C18 HPLC column according to the method described above, the resulting chromatogram showed four major impurities: Tr21, Tr18, Tr10, and Tr9 (the impurities were named on the basis of their retention times in the HPLC chromatogram). Paclitaxel had a retention time of 12 min. in the

HPLC chromatogram and it was also named Tr12. The paclitaxel concentration was 155.6 ppm. In the batch elution experiments, the impurities, which have HPLC retention times of 5, 9.5 and 16 min. (denoted as Tr5, Tr9.5, and Tr16), were found to have very similar elution behavior in the Dow columns as Tr21. Therefore, in the subsequent designs and simulations, these four components were treated as a single pseudo-component with the same properties as Tr21. These data are presented in Table 2. For paclitaxel, Tr9, Tr18, and Tr21, the mass balance is closed.

**Table 2.** PTC broth crude concentrations.

Component	Feed concentration (ppm)
Tr21	302.8
Tr18	28.5
Paclitaxel	155.6
Tr9	14.7
Tr10	49.8

Two pulses of crude mixture dissolved in 60% ethanol were run at  $1 \text{ mL min}^{-1}$  and  $3 \text{ mL min}^{-1}$ . The chromatograms are shown in Figure 1 (points). The partition coefficients were obtained from the first-moment of the eluted pulse of each component, according to Equation (21). The results are presented in Table 3. This table shows that the paclitaxel partition coefficient lies between two light components (Tr18 and Tr21) and two heavy components (Tr9 and Tr10).

**Table 3.** Partition coefficients of taxanes.

Component	$k_p$ (mL mL <sup>-1</sup> solid volume)
Tr21	15.09
Tr18	38.67
Paclitaxel	40.03
Tr9	58.28
Tr10	82.52

The diffusion coefficients values estimated from moment analysis are presented in Table 4, which shows that each component has almost the same diffusivity. For paclitaxel, its value is  $2.56 \times 10^{-4} \text{ cm}^2 \text{ min}^{-1}$ .

**Table 4.** Diffusivities value of taxanes.

Component	$D_{AB}$ ( $10^4 \text{ cm}^2 \text{ min}^{-1}$ )	$D_p$ ( $10^4 \text{ cm}^2 \text{ min}^{-1}$ )
Tr21	2.53	0.38
Tr18	2.56	0.92
Paclitaxel	2.56	0.59
Tr9	2.56	0.73
Tr10	2.58	1.31

If one uses the effective diffusion coefficient from Mackie and Meares (1955),

$$D_p = \frac{\epsilon_p}{(2 - \epsilon_p)^2} D_{AB} \quad (36)$$

and it uses the experimental value for paclitaxel  $\epsilon_p = 0.46$ , and  $D_{AB} = 2.56 \times 10^{-4} \text{ cm}^2 \text{ min}^{-1}$ , the value for  $D_p$  is  $D_{AB} = 0.50 \times 10^{-4} \text{ cm}^2 \text{ min}^{-1}$ , which it is close to experimental value estimated in this work,  $D_p = 0.59 \times 10^{-4} \text{ cm}^2 \text{ min}^{-1}$ .

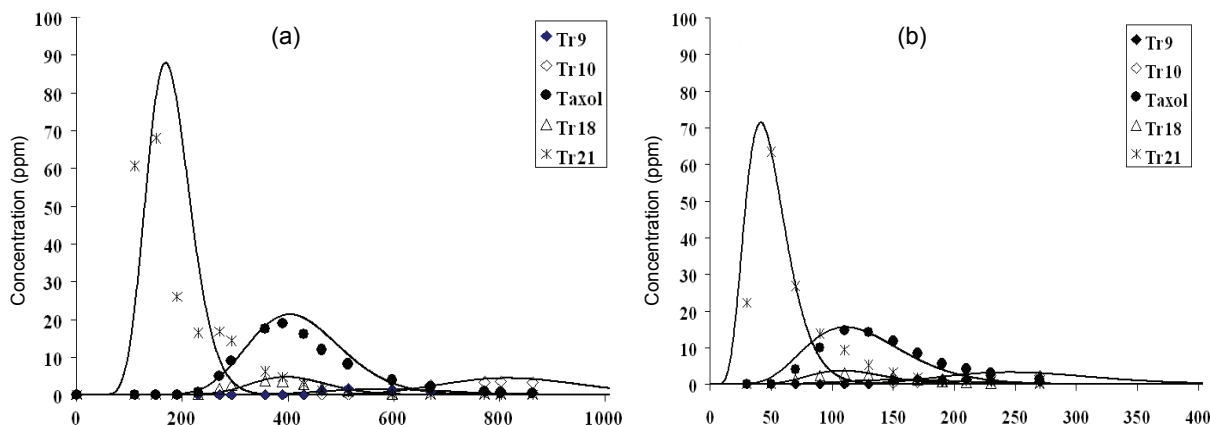
Table 5 shows a comparison between the experimental value of  $D_p$  for paclitaxel estimated in this study compared to those estimated from equation founded in Literature. It is possible to check that relative error calculated by,

$$d = \frac{D_{AB\text{cal}} - D_{AB\text{exp}}}{D_{AB\text{exp}}} \times 100\% \quad (37)$$

The numerical solution of the mass-balance equations, Equations 1 and 2, are obtained from VERSE-LC code (VERSatile Reaction Separation simulator for adsorption and Liquid Chromatography processes), developed at Bioseparation Laboratory, School of Chemical

Engineering, Purdue University. The simulations are based on a detailed model and numerical solution of the model equations. These equations are formulated based on mass conservation principles and fundamental constitutive relations, and are discretized using orthogonal collocation on finite elements, and the DASPK solver is used in the time domain (BERNINGER et al., 1991). This numerical method has been used in the studies of many adsorption systems (MA et al., 1996; ERNEST JR. et al., 1997; KOH et al., 1998; CREMASCO et al., 2001).

The intrinsic parameters (isotherms and diffusivities coefficients) are listed in Tables 3 and 4. Figure 1 shows the comparison between experimental (points) and simulated results (continuous curves). For all of the components except Tr21, the simulations based on the estimated isotherms and mass-transfer parameters are in close agreement with the experimental data. For component Tr21, there is strong tailing in each of the batch elution profiles, which is a good indication of isotherm non-linearity.



**Figure 1.** Comparison of experimental chromatogram with simulation results. (a)  $Q = 1 \text{ mL min}^{-1}$ ; (b)  $Q = 3 \text{ mL min}^{-1}$ .

**Table 5.** Comparison of paclitaxel diffusion coefficients from various correlations (CREMASCO, 1998).

Author(s)	Equation	$D_{AB}$ ( $10^4 \text{ cm}^2 \text{ min}^{-1}$ )	Error (%)
Scheibel (1954) apud Cremasco (1998)	$\frac{D_{AB}\mu_B}{T} = \frac{K}{V_{b_A}^{1/3}}$ , where: $K = 8.2 \times 10^{-8} \left[ 1 + \left( \frac{3V_{b_B}}{V_{b_A}} \right)^{1/3} \right]$	2.40	-6.25
Wilke and Chang (1955) apud Cremasco (1998)	$\frac{D_{AB}\mu_B}{T} = \frac{7.4 \times 10^{-8} (\phi M_B)^{1/2}}{V_{b_A}^{0.6}}$	1.75	-31.64
Lusis and Ratchiff (1968) apud Cremasco (1998)	$\frac{D_{AB}\mu_B}{T} = \frac{8.52 \times 10^{-8}}{V_{b_A}^{1/3}} \left[ 1.40 \left( \frac{V_{b_B}}{V_{b_A}} \right)^{1/3} + \left( \frac{V_{b_B}}{V_{b_A}} \right) \right]$	2.55	-0.39
Hayduk and Minhas (1982) apud Cremasco (1998)	$\frac{D_{AB}\mu_B}{T} = 1.33 \times 10^{-7} \frac{T^{0.47} \mu_B^{1+\xi}}{V_{b_A}^{0.71}}$ , where: $\xi = \frac{10.2}{V_{b_A}} - 0.71$	2.85	11.33
Siddiqi and Lucas (1986) apud Cremasco (1998)	$\frac{D_{AB}\mu_B}{T} = 9.89 \times 10^{-8} \mu_B^{0.093} \left( \frac{V_{b_B}^{0.265}}{V_{b_A}^{0.45}} \right)$	2.20	-14.06

## Conclusion

A simple method was proposed in this paper to estimate diffusion coefficients and linear isotherm parameters for paclitaxel and other similar taxanes present in a plant tissue culture broth. The crucial hypothesis of this method is about the linear isotherm assumption, which it shows reasonable for four species. However, Tr21 compound is the most concentrated, and could be present a non-linear isotherm. Then, would be influence in the adsorption of the others species and to influence in their partitions and diffusivities parameters. But, it was verified the good approximation for paclitaxel diffusion coefficient, that presents a good agreement with that ones founded in the literature. The batch chromatograms at two different flow rates were in good agreement with the predictions from a detailed rate model. The partition coefficient of paclitaxel lies between two light components and two heavy components. This result is important for future paclitaxel purification, and it shows the necessary two steps of separation, at least.

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## References

- ATHALYE, A. M.; GIBBS, S. J.; LIGHTFOOT, E. N. Predictability of chromatographic protein separations: study of size-exclusion media with narrow particle size distribution. **Journal of Chromatography**, v. 589, n. 1-2, p. 71-85, 1992.
- BERNINGER, J. A.; WHITLEY, R. D.; ZHANG, X.; WANG, N. H. L. A versatile model for simulation of reaction and nonequilibrium dynamics in multicomponent fixed-bed adsorption processes. **Computers and Chemical Engineering**, v. 15, n. 2, p. 749-768, 1991.
- CARDELINA II, J. H. HPLC Separation of Taxol and Cephalomannine. **Journal of Liquid Chromatography**, v. 14, n. 4, p. 659-665, 1991.
- CHIAHARA, K.; KANEKO, Y.; TERAOKA, T.; MIZUOCHI, H. Study of multicomponent gas adsorption into MSC5A by chromatograph. **Adsorption**, v. 11, n. 1, p. 249-253, 2005.
- CREMASCO, M. A. **Fundamentos de transferencia de massa**. Campinas: Unicamp Press, 1998.
- CREMASCO, M. A.; HRITZKO, B. J.; XIE, Y.; WANG, N. H. L. Parameters estimation for amino acids adsorption in a fixed bed by moment analysis. **Brazilian Journal of Chemical Engineering**, v. 18, n. 2, p. 181-194, 2001.
- DOGAN, M.; DOGU, G. Dynamics of flow and diffusion of adsorbing gases in Al<sub>2</sub>O<sub>3</sub> and Pd-Al<sub>2</sub>O<sub>3</sub> pellets. **AIChE Journal**, v. 49, n. 12, p. 3188-3198, 2003.
- ERNEST JR., M. V.; WHITLEY, R. D.; MA, Z.; WANG, L. N. H. Effect of mass action equilibria on fixed bed multicomponent ion exchange dynamics. **Industrial and Engineering Chemistry Research**, v. 36, n. 1, p. 212-226, 1997.
- HARLICK, P. J. E.; TEZEL, F. H. A novel solution method for interpreting binary adsorption isotherms from concentration pulse chromatography data. **Adsorption**, v. 6, n. 4, p. 293-309, 2000.
- HARLICK, P. J. E.; TEZEL, F. H. Use of concentration pulse chromatography for determining binary isotherms: comparison with statically determined binary isotherms. **Adsorption**, v. 9, n. 3, p. 275-286, 2003.
- KOH, J.-H.; WANKAT, P. C.; WANG, L. N.-H. Pore and surface diffusion and bulk-phase mass transfer in packed and fluidized beds. **Industrial and Engineering Chemistry Research**, v. 37, n. 1, p. 228-239, 1998.
- MA, Z.; WHITLEY, R. D.; WANG, L. N.-H. Pore and surface diffusion in multicomponent adsorption and liquid chromatography systems. **AIChE Journal**, v. 42, n. 5, p. 1244-1262, 1996.
- MACKIE, J. S.; MEARES, P. The Diffusion of electrolytes in a cation-exchange resin membrane. **Proceedings of Royal Society of London, Series A, Mathematical and Physical Sciences**, v. 232, n. 1191, p. 498-509, 1955.
- SCHNEIDER, P.; SMITH, J. Adsorption rate constant from chromatography. **AIChE Journal**, v. 14, n. 5, p. 762-771, 1968.
- SILVA JR., I. J.; VEREDAS, V.; CARPES, M. J. S.; SANTANA, C. C. Chromatographic separation of bupivacaine enantiomers by HPLC: parameters estimation of equilibrium and mass transfer under linear conditions. **Adsorption**, v. 11, n. 2, p. 123-129, 2005.
- STRÖHLEIN, G.; MORBIDELLI, M.; RHEE, H.-K.; MAZZOTTI, M. Modeling of modifier-solute peak interactions in chromatography. **AIChE Journal**, v. 52, n. 2, p. 565-573, 2006.
- YAMAMOTO, S.; OKAMOTO, A.; WATLER, P. Effects of adsorbent properties on zone spreading in expanded bed chromatography. **Bioseparation**, v. 10, n. 1-3, p. 1-6, 2001.
- WILSON, E. J.; GEANKOPLIS, C. J. Liquid mass transfer at very low Reynolds numbers in packed beds. **Industrial Engineering Chemical Fundamentals**, v. 5, n. 1, p. 9-14, 1966.
- WU, D.-J.; MA, Z.; AU, B. W.; WANG, N.-H. L. Recovery and purification of paclitaxel using low-pressure liquid chromatography. **AIChE Journal**, v. 43, n. 1, p. 232-242, 1996.
- WU, Y. X. C. B.; CHING, C. B. The theoretical study of the effect of packing heterogeneity on HPLC by



computational fluid dynamics. **Chromatographia**, v. 56, n. 11, p. 679-686, 2002.

WU, Y. X. C. B.; CHING, C. B. Theoretical study of the effect of frit quality on chromatography using computational fluid dynamics. **Chromatographia**, v. 57, n. 5, p. 239-337, 2003.

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