

A Simple Method to Determine the Adsorption Isotherm of Taxol from a Yew Tree

Young Kwang Park, Sung Taik Chung and Kyung Ho Row*

Department of Chemical Engineering, Inha University, Incheon 402-751, Korea

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Abstract—Taxol, a powerful anticancer agent, was extracted from yew extract and separated by NP-HPLC (Normal-Phase High Performance Liquid Chromatography). At the mobile phase composition of hexane/IPA/MeOH, 90/4/6 vol%, taxol was preparatively separated in a chromatographic column with large packings (15 μ m). The purified taxol was used to determine the adsorption isotherm of taxol. From the experimental results, the adsorption isotherm was obtained as the following Langmuir type,

$$C_s = \frac{59.5C_M}{1 + 2.0C_M}$$

To obtain the adsorption isotherm, only two injections of a small and large amount of taxol were required, and the Langmuir adsorption parameters were calculated from the assumption of monolayer coverage on the solid surface.

Key words : Taxol, Yew Tree, Langmuir Adsorption Isotherm, Chromatography

INTRODUCTION

Taxol (Paclitaxel), an antitumor drug isolated from the bark of the Pacific yew (*Taxus brevifolia*) has demonstrated clinical effectiveness in ovarian and breast carcinomas [Rao et al., 1996]. Discovered by Wall's group in 1967, taxol is a chemically and pharmacologically unique diterpene. The highly functionalized taxane diterpenes are known as various species of *Taxus* and a few members of the *Taxodiaceae*.

Chromatographic methods have been developed to detect and isolate taxanes from the above sources on analytical and semi-preparative base. A number of analytical HPLC methods exist, including the use of silica or C_{18} phase and reversed-phase separations on phenyl, and cyano-bonded silica [Cardellina II, 1991]. In normal-phase, a preparative separation of taxol under overload condition was conducted where high alcohol concentrations were added in mobile phase to increase sample solubility [Wu et al., 1995].

Recently, experimental work on the separation of taxol in both analytical and preparative scales has been performed at the High-Purity Separation Laboratory in Inha Univ. [Chang et al., 1997, 1998]. To purify taxol from the yew tree, a Nova-Pak column and a pentafluoro phenyl (PFP) bonded silica column were utilized with the binary or ternary system of the mobile phase [Jung et al., 1997]. The adsorption isotherm is the fundamental thermodynamic property of importance in theoretical and experimental studies of the separation processes based on the distribution of solutes between two phases. It describes the distribution between the mobile phase and the stationary phase in a functional form usually derived on the

basis of a theoretical model.

Based on previous experimental results, in the optimum condition of mobile phase to separate taxol from the extracts of yew, the taxol was purified. The goal of this paper is to determine the adsorption isotherm of taxol by a simple methodology using a few injections. When a large sample of taxol was injected, the peak characterized by a sharp fronting and a right-angled triangle shows the Langmuir isotherm. The experimental data and the calculated profiles of taxol with the resulting parameters of the Langmuir isotherm were compared.

METHODOLOGY

The simplest model for adsorption chromatography assumes that the solute, S, confined in the aqueous phase (aq) is adsorbed to the ligand, L, of a solid (s) chromatography surface. The process in chromatography is expressed by Eq. (1).



The adsorption process is characterized by an association constant, k_A

$$k_A = \frac{k_1}{k_2} \quad (2)$$

where k_1 and k_2 are the forward and backward rate constants, respectively. A dissociation constant, k_D , is a reciprocal of the association constant, k_A . The association constant is given by

$$k_A = \frac{[S-L]}{[S][L]} = \frac{C_s}{C_M(q_m - C_s)} \quad (3)$$

C_M is the concentration of solute in the mobile phase and C_s is the concentration of adsorbed solute at equilibrium, and q_m is the maximum (monolayer) capacity of the chromatogra-

*To whom correspondence should be addressed.

E-mail : rowkho@dragon.inha.ac.kr

phic medium for the solute. When $C_s \ll q_m$, Eq. (3) may, with the aid of the relationship $K_D = C_s/C_M$, be used to express the relationship between the distribution coefficient and the association and dissociation constant,

$$k_s = \frac{1}{K_D} = \frac{K_D}{q_m} \quad (4)$$

The retention factor, k' , is defined by the amount of solute in the stationary phase to that in the mobile phase. The retention factor is related to the distribution coefficient, K_D , expressing the concentration of solute in the stationary phase, C_s , over that in the mobile phase, C_M , by

$$K_D = k' \frac{V_M}{V_s} \quad (5)$$

From the following, Eq. (6), it may be noted that the concentration of adsorbed solute is asymptotically approaching the maximum capacity of the gel medium, and that this will be reached at lower mobile phase concentration for solutes with higher association constants [Sofer and Hagel, 1997].

$$C_s = \frac{C_M q_m}{C_M + k_A} = \frac{C_M q_m k_A}{C_M k_A + 1} \quad (6)$$

In case of the Langmuir isotherm, Eq. (6), q_m (monolayer capacity) may be derived from column experiments [Snyder et al., 1987].

$$q_m = \sqrt{2N} \frac{k'}{1+k'} \frac{k'_{low}}{k'_{low}-k'} \quad (7)$$

$$N = 16 \left(\frac{t_r}{W} \right)^2 \quad (8)$$

where q , N and k' are measured at high load of sample and k'_{low} at low load.

EXPERIMENTAL

1. Extraction and Pretreatment

The yew powder was provided by Forest Genetics Research Institute (Suwon). The extraction was carried out using methanol (1 l) from 10 g dried materials of the powder for a day. The concentrated methanol extract (125 ml) was partitioned with chloroform (CHCl_3 , 100 ml) and water (100 ml) for about 30 minutes and 2-14 hours to allow for any emulsion to clear. The solvent layer was drained off from the bottom into glass containers. The CHCl_3 -extract was evaporated by a rotary evaporator (LABO-THERM SW 200, Resona Technics Co.), stripped of its solvent to a thick syrup. The syrup was dissolved in methanol and a concentration of 5.0 mg/ml was prepared for the subsequent separation step.

The methanol solution of the extract was slowly passed through a silica open column with the mobile phase of methylene chloride/acetone, 97/3 vol%. The eluent was changed to 30% acetone in methylenechloride and we obtained the band containing taxol [Chang et al., 1998].

2. Chromatography

The HPLC system consisted of a pump (600E, Waters), a U6K injector (2 ml sample loop), a pressure gauge (maximum pressure : 3,000 psi, Span instrument Co.), and a 486 UV-vis-

ible tunable wavelength absorbance. The semi-preparative column (3.9×300 mm) was packed with Lichrospher Si 60 (15 μm , Merck Co.). Eluents were monitored at 228 nm wavelength. The mobile phase was hexane, IPA and methanol in ACS grade purchased from J. T. Baker (Phillipsburg NJ, U. S. A.).

The standards of cephalomannine, 10-deacetyl taxol and taxol were generously donated by NCI (the National Cancer Institute, Bethesda, MD, USA). The injection volume and the flow rate of mobile phase were 25 μl -2 ml and 1.5 ml/min, respectively. The columns were maintained at ambient temperature. Back pressure problems were avoided by the rinsing column frequently with the corresponding mobile phase.

RESULTS AND DISCUSSION

The analysis of the breakthrough curve yields a point on adsorption isotherm. By frontal analysis, the parameters of the distribution equation can be determined by fitting the model to experimental data [Jandera et al., 1996]. Contrary to the frontal analysis, using experimental data at different sample sizes, we estimated a non-linear isotherm from the experimental peak. The parameters of the non-linear adsorption isotherm were calculated by a simple method.

In the column packed with silica of 15 μm , the composition of the mobile phase was Hexane/IPA/MeOH, 90/4/6 vol % in an isocratic mode. Fig. 1 shows that in this condition, taxol was resolved from the yew tree. As sample size of taxol increased, the retention time was decreased (Fig. 2). Taxol was collected and concentrated for measuring the adsorption isotherm of taxol. By a simple methodology, first K_D and q_m were calculated by Eqs. (5) and (7), respectively. Also k_A was obtained by Eq. (4). Finally the parameters, $q_m k_A$ and k_A of adsorption isotherm of taxol were determined. At the larger sample size of 0.16 mg, the peak shape was a right-angled triangle, while at the lower amount, 0.03 mg the shape of the peak showed a gaussian elution profile. Therefore, the adsorption isotherm of taxol was well expressed as Langmuir isotherm [Hadden et al., 1971]. Table 1 shows the results of two experimental runs with the different sample sizes. At a concentration of 0.146 mg/ml, the injection volumes were 0.2 ml

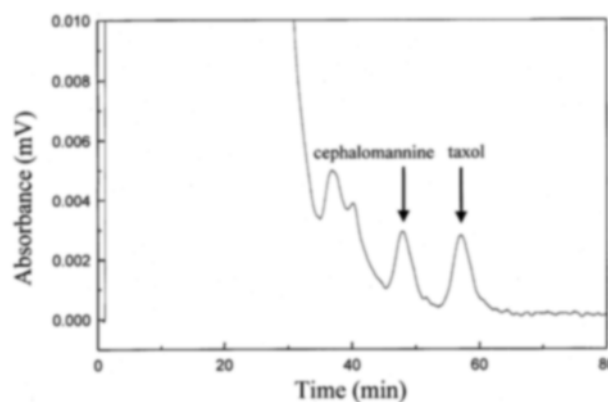


Fig. 1. Purification of taxol from extract of Yew tree. (Hexane/IPA/MeOH, 90/4/6 vol%)

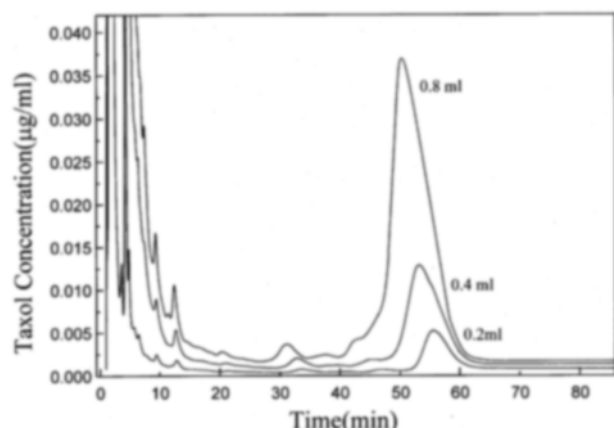


Fig. 2. Increase in sample size of taxol by NP-HPLC.
(same mobile phase compositions as in Fig. 1)

Table 1. Parameters of Langmuir adsorption isotherm of taxol

Injection volume (ml)	Sample size (mg)	Retention time (min)	N	k'	K _D	k _A (ml solid/mg)
0.2	0.03	55.6	538	36.5	59.5	2.0
0.8	0.16	50.2	334	32.9	53.6	1.8

and 0.8 ml, respectively. This indicates that the sample size was larger, so the K_D value by Eq. (5) and k_A were decreased.

The calculated q_m was 29.5 [mg/ml solid]. From the experimental result, k' was calculated and the value of V_M/V_s was 0.61 [-]; therefore, by Eq. (5) we also found K_D. Finally, the following Langmuir isotherm of taxol was obtained by Eq. (6),

$$C_s = \frac{59.5C_M}{1 + 2.0C_M} \quad (9)$$

The calculated parameters k_Aq_m and k_A were 59.5 [-] and 2.0 [ml solid/mg], respectively. The adsorption isotherm of taxol is shown in Fig. 3.

Neglecting the axial dispersion term, the mass balance equation is expressed as follows,

$$u \frac{\partial C_M}{\partial x} + \varepsilon \frac{\partial C_M}{\partial t} + (\bullet - \varepsilon) \frac{\partial C_s}{\partial t} = 0 \quad (10)$$

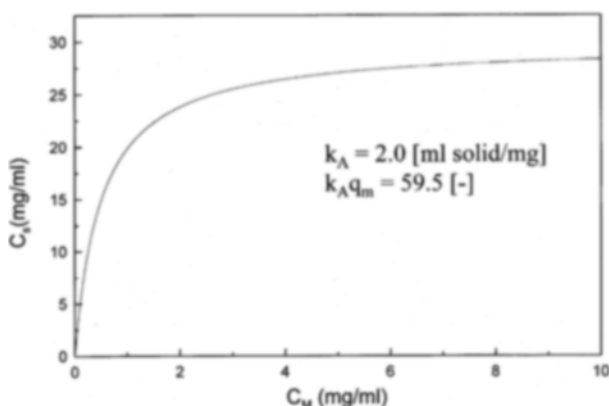


Fig. 3. Langmuir adsorption isotherm of taxol.

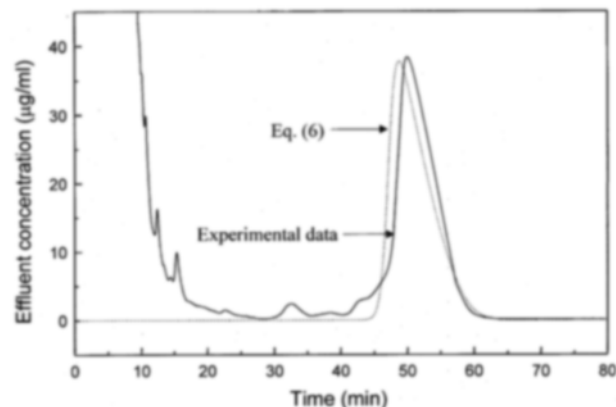


Fig. 4. Comparison of experimental data and calculated profiles by Eq. (6) of taxol.
(concentration : 0.16 mg/ml, injection vol : 0.8 ml)

where u is the linear velocity of the mobile phase, ε the total porosity of a chromatographic bed. As C_s is related a function of C_M in Eq. (9), a numerical scheme of the Finite Difference Method (FDM) was used, and Eq. (10) was discretized as

$$u \Delta t (C_{Mx+\Delta x,t} - C_{Mx,t}) + \varepsilon (C_{Mx+\Delta x,t+\Delta t} - C_{Mx,t}) + (\bullet - \varepsilon) (C_{sx,t+\Delta t} - C_{sx,t}) = 0 \quad (11)$$

As shown in Fig. 4, the dotted profile was numerically calculated and was compared with the experimental data. The agreement between the two elution profiles was relatively good. This means that the Langmuir adsorption isotherm obtained by the simple method describes well the adsorption process of taxol in the chromatographic column.

CONCLUSION

Taxol was extracted from a yew tree and separated by semi-preparative scale normal phase HPLC in isocratic mode. The mobile phase composition was hexane/IPA/MeOH, 90/4/6 vol %. Under the experimental conditions, the purified taxol was used for measuring the adsorption isotherm by a simple methodology. From the two experimental profiles, the parameters k_A and k_Aq_m of the Langmuir adsorption isotherm of taxol were calculated as 2.0 [ml solid/mg] and 59.5 [-], respectively. Because of the good coincidence to the experimental data, the resulting adsorption isotherm could be used.

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NOMENCLATURE

- k_A : association constant [ml solid/mg]
- k_D : dissociation constant [mg/ml solid]
- C_M : solute in the mobile phase [mg/ml]
- C_s : concentration of adsorbed solute [mg/ml]
- q_m : maximum (monolayer) capacity [mg/ml solid]

K_D : distribution coefficient [-]
 q : injection amount in high load [mg/ml solid]
 N : number of theoretical plate [-]
 k' : retention factor in high load [-]
 k'_{low} : retention factor in low load [-]
 t_r : retention time of component [min]
 w : band width of component [min]

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