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Kinetics and thermodynamics of paclitaxel extraction from plant cell culture

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Abstract−We investigated the effect of temperature on the efficiency of paclitaxel extraction from biomass. In addition, kinetic and thermodynamic studies of this extraction process were performed. The concentration of extracted paclitaxel increased with increasing extraction temperature and extraction time. When the experimental data were applied to various kinetic models, the hyperbolic model (second-order model) was the most appropriate. The predictive model was developed to predict the concentration of extracted paclitaxel at different temperatures at a given time. The Gibbs free energy change was determined to be negative, while enthalpy change and entropy change were positive. These results indicate that this extraction process is spontaneous, endothermic, and irreversible.

Keywords: Paclitaxel, Extraction, Kinetics, Predictive Model, Thermodynamics

INTRODUCTION

Paclitaxel is a diterpenoid anticancer agent found in the bark of the yew tree [1]. Unlike other existing anticancer drugs, it inhibits cancer cell division in the mitotic phase with strong anticancer activity and low toxicity [2]. Approved by the U. S. Food and Drug Administration as a treatment for ovarian cancer, breast cancer, Kaposi's sarcoma, and non-small cell lung cancer, paclitaxel is currently the most widely used anticancer drug [3,4]. Its indications, which currently include acute rheumatic arthritis and Alzheimer's disease, are expanding continuously, and clinical tests for combined prescription with other treatment methods are being conducted. Thus, the demand for this drug is expected to increase steadily. The main methods of paclitaxel production include direct extraction from the yew tree [5], semi-synthesis by chemically bonding side chains after obtaining precursors (e.g., baccatin III, 13-dehydroxybaccatin III, 10-deacetylbaccatin III, 10-deacetylpaclitaxel) from the leaves of the yew tree [6], and plant cell culture from the bioreactor after inducing callus from the yew tree and then performing a seed culture [7]. Among these methods, plant cell culture enables stable mass production of paclitaxel of consistent quality in the bioreactor without being affected by external factors such as climate and environment [4].

Paclitaxel produced by plant cell culture is mostly contained in biomass (plant cells), and efficiently extracting paclitaxel from biomass plays an important role in increasing yield. The typical methods of paclitaxel extraction from biomass include conventional solvent extraction and microwave-assisted extraction [8,9]. The conventional solvent extraction is relatively inexpensive and efficient, but it involves the use of large amount of organic solvents and long operating time compared to the microwave-assisted extraction. Because of its feasibility and efficiency, the conventional solvent extraction is most commonly used for the commercial production of paclitaxel [4,8]. The efficiency of conventional solvent extraction was evaluated in a variety of organic solvents. Among these, the best result in terms of the extraction efficiency was obtained from methanol [10,11]. Most of the paclitaxel (>98%) could be extracted four times from biomass when the concentration of methanol was 90% or higher [10]. Furthermore, the yield of paclitaxel was not different at a biomass/methanol ratio of 1 : 1-1 : 6 (w/v) [12]. However, the extraction temperature, which is one of the main process parameters in the extraction of paclitaxel from biomass, was set only at room temperature; therefore, the effect of changes in extraction temperature has not been studied. In addition, most previous studies on extraction have been focused on the optimization of the experimental conditions or process factors and qualitative description of their effects [10-12], and very few on the quantitative analysis of extraction kinetics and thermodynamics. Kinetic characteristics are used to investigate and predict the pathway, rate, and extent of reaction [13-15]. In particular, the kinetic model is a useful engineering tool, considerably facilitating design, optimization, and simulation of the extraction process and contributing to utilization of energy, solvent, and time. Thermodynamic characteristics are used to investigate the spontaneity and reversibility of the reaction to understand the behavior of the extraction process [16-18]. Therefore, we investigated the effect of extraction temperature on the efficiency of paclitaxel extraction from biomass. Also, we applied the experimental data to various kinetic models and quantitatively analyzed the extraction process. Furthermore, Gibbs free energy change, enthalpy change, entropy change, and activation energy, which are thermodynamic parameters, were obtained to evaluate the energetic aspects of the extraction of paclitaxel.

MATERIALS AND METHODS

1. Plant Materials

A suspension of cells originating from *Taxus chinensis* was maintained in darkness at 24 °C with shaking at 150 rpm. The suspen-

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sion cells were cultured in modified Gamborg's B5 medium [19] supplemented with 30 g/L sucrose, 10 mM naphthalene acetic acid, 0.2 μM 6-benzylamino purine, 1 g/L casein hydrolysate, and 1 g/L 2-(*N*-morpholino)ethanesulfonic acid. Cell cultures were transferred to fresh medium every two weeks. During prolonged culture for production purposes, 4 μM $AgNO_3$ was added at the initiation of culture as an elicitor, and 1 and 2% (w/v) maltose were added to the medium on days 7 and 21, respectively. Following cultivation, biomass (plant cells) was recovered using a decanter (CA150 Clarifying Decanter; Westfalia, Germany) and a high-speed centrifuge (BTPX 205GD-35CDEFP; Alfa-Laval, Sweden). The biomass was provided by Samyang Genex Company, South Korea.

2. Paclitaxel Analysis

Dried residue was redissolved in methanol for quantitative analysis by using an HPLC system (SCL-10AVP, Shimadzu, Japan) with a Capcell Pak C18 column (250×4.6 mm, Shiseido, Japan). Elution was performed in a gradient using a distilled water-acetonitrile mixture varying from 65 : 35 to 35 : 65 within 40 min (flow rate= 1.0 mL/min). The injection volume was 20 μL, and the effluent was monitored at 227 nm with a UV detector. Authentic paclitaxel (purity: 97%) was purchased from Sigma-Aldrich and used as a standard [8]. Each sample was analyzed in triplicate.

3. Biomass Extraction

According to previous studies [8-12], methanol is the most effective organic solvent to extract paclitaxel from biomass in the conventional solvent extraction method. In addition, the extraction efficiency is not different at a biomass/methanol ratio of 1 : 1-1 : 6 (w/v). The possibility of paclitaxel degradation at high temperatures (>50 °C) was considered [8]. Thus, the batch extraction was conducted at a fixed biomass/methanol ratio of 1 : 2 (w/v) and different extraction temperatures (25, 30, 35, 40, and 45°C)) and extraction times (1, 2, 4, 6, 8, 10, 20, and 30 min) with stirring at 570 rpm in order to investigate the effect of temperature on the extraction efficiency of paclitaxel. The extraction temperature and stirring speed were controlled by using a thermostat (PS-1000; EYELA, Japan). After extraction, the mixture was filtered under vacuum in a Buchner funnel through filter paper (150 mm, Whatman). Methanol extract was concentrated and dried at 40 °C) under vacuum for HPLC analysis. The paclitaxel yield was calculated as follows:

$$\text{Yield (\%)} = \frac{\text{Quantity of pure paclitaxel in crude extract}}{\text{Quantity of pure paclitaxel in biomass}} \times 100 \quad (1)$$

4. Kinetic Model

Kinetic models can be divided into physical and empirical ones [15]. Physical models are based on physical phenomena of mass transfer through biomass and from external solid surfaces into the bulk of the solvent phase. They can be very complex and are usually simplified to make data processing easier. Empirical models describe mathematically variations of extractive substance amount in either biomass or liquid extract with time. These models are usually simpler than physical ones but are still suitable for engineering purposes. In this study, we compared five empirical models to each other for the extraction of paclitaxel from biomass.

Weibull's model applied to the process of nuclide release from low-level radioactive paraffin waste [20] can be used for most dissolution reactions and is expressed as Eq. (2), which can be rearranged to obtain the linear Eq. (3).

$$q = 1 - e^{-(t/\delta)^m} \quad (2)$$

$$\ln(-\ln(1-q)) = m\ln t - m\ln\delta \quad (3)$$

where δ is the scale parameter correlated with the extraction rate constant and means the time needed to accomplish approximately 63% of the extraction; is the shape parameter. If m is less than 1, in the case of the extraction process, the parabolic extraction behavior shows a high slope at initial extraction and then a gentle one [15].

Elovich's model used in the extraction of polycyclic aromatic hydrocarbon from coal tar-contaminated soil [21] and the adsorption of dye-chitosan [22] can be applied to most chemical adsorption processes and is expressed as Eq. (4).

$$q = E_0 + E_1 \ln t \quad (4)$$

When the extraction rate exponentially decreases with increasing extraction yield, Eq. (5) is established.

$$\frac{dq}{dt} = \beta e^{-\alpha t} \quad (5)$$

where α and β are constants ($\beta = E_1 e^{E_0/E_1}$ and $\alpha = 1/E_1$). In addition, β means the initial extraction rate because of $(dq/dt) \rightarrow \beta$ at $q \rightarrow 0$ [14,22].

The phenomenological model suggested by Patricelli et al., working on the extraction of uninterrupted oil from sunflower seeds, is based on the extraction mechanism with two steps. The extract is rapidly washed by solvent in the first step and then slowly diffused in the suspension [23]. It can be expressed as Eq. (6).

$$C_t = C_e^w(1 - e^{-k_w t}) + C_e^d(1 - e^{-k_d t}) \quad (6)$$

where C_e^w is the concentration of extracted paclitaxel at equilibrium during the washing step (mg/mL), C_e^d is the concentration of extracted paclitaxel at equilibrium during the diffusion step (mg/mL), k_w is the mass transfer coefficient for the washing step (min^{-1}), and k_d is the mass transfer coefficient for the diffusion step (min^{-1}). In addition, the relation between C_e^w and C_e^d can be expressed as Eq. (7).

$$C_e = C_e^w + C_e^d \quad (7)$$

where C_e is the concentration of extracted paclitaxel at equilibrium (mg/mL). So and Macdonald used this model for the extraction of oil from canola. They also suggested two distinct steps of diffusion: slow, unhindered diffusion and very slow, hindered diffusion. It is expressed as Eq. (8) [24].

$$C_t = C_e^w(1 - e^{-k_w t}) + C_e^{d1}(1 - e^{-k_{d1} t}) + C_e^{d2}(1 - e^{-k_{d2} t}) \quad (8)$$

where C_e^{d1} is the concentration of extracted paclitaxel at equilibrium during the unhindered diffusion step (mg/mL), C_e^{d2} is the concentration of extracted paclitaxel at equilibrium during the hindered diffusion step (mg/mL), k_{d1} is the mass transfer coefficient for the unhindered diffusion step (min^{-1}), and k_{d2} is the mass transfer coefficient for the hindered diffusion step (min^{-1}).

In addition, the relation among C_e^w, C_e^{d1}, and C_e^{d2} can be expressed as Eq. (9).

$$C_e = C_e^w + C_e^{d1} + C_e^{d2} \quad (9)$$

These models were applied to the extraction of oil from olive cake [25,26]. Furthermore, Eq. (6) compared to Eq. (8) has fewer parameters and therefore it was easier and more adequate to determine parameters [25].

The hyperbolic model, known as the Peleg's model [27], was applied to the extraction of medicinal herbs from sorrel calyces [28], the extraction of polyphenols from soybeans [29], and the extraction of polyphenols from grape seeds [30]. It can be used in most solid-liquid extraction processes and is expressed as Eq. (10), which can be rearranged to obtain the linear Eq. (11). It mathematically and easily explains the moisture sorption curve.

$$q = \frac{K_1 t}{1 + K_2 t} \quad (10)$$

$$\frac{t}{q} = \frac{1}{K_1} + \frac{K_2}{K_1} t \quad (11)$$

Eq. (10) is the same as a second-order extraction model applied to the extraction of antioxidants from pomegranate mare [31]. As shown in Eq. (12) and Eq. (13), the extraction order is first-order at the very beginning and then becomes zero-order at equilibrium. K_1 is the initial extraction rate (min^{-1}) and K_1/K_2 is the Peleg capacity constant to represent the maximum extraction yield [15].

$$q_{t\to 0} = K_1 t \quad (12)$$

$$q_{t\to \infty} = \frac{K_1}{K_2} \quad (13)$$

The second-order model is similar to the hyperbolic model. This model can be used when it is assumed that the extract is dissolved rapidly by solvent in the initial extraction and then diffused slowly in the suspension [32]. It has been applied to many solid-liquid extraction processes such as the extraction of protopine from *Fumaria officinalis* [32] and the extraction of water-soluble compounds from *Tilia* sapwood [33]. According to the second-order rate law, the rate of dissolution is expressed as Eq. (14).

$$\frac{dC_t}{dt} = k_2 (C_e - C_t)^2 \quad (14)$$

where k_2 is the extraction rate constant for the second-order model (mL/mg·min).

The initial and boundary conditions to solve Eq. (14) are as follows:

$$C_t = 0 \text{ at } t = 0 \quad (15)$$

$$C_t = C_t \text{ at } t = t \quad (16)$$

When Eq. (14) is integrated on the basis of that, Eq. (17) is obtained:

$$C_t = \frac{k_2 C_e^2 t}{1 + k_2 C_e t} \quad (17)$$

By rearranging Eq. (16), the linear Eq. (18) can be obtained and the extraction rate can be written as Eq. (19).

$$\frac{t}{C_t} = \frac{1}{k_2 C_e^2} + \frac{t}{C_e} \quad (18)$$

$$\frac{C_t}{t} = \frac{1}{\left(\frac{1}{k_2 C_e^2}\right) + \left(\frac{t}{C_e}\right)} \quad (19)$$

where h is the initial extraction rate (mg/mL·min) and shown in Eq. (20).

$$h = \left(\frac{C_t}{t}\right)_{t\to 0} = k_2 C_e^2 \quad (20)$$

It is applied to Eq. (18) and then the concentration of extracted paclitaxel depending on extraction temperature and extraction time can be expressed as Eq. (21).

$$C_t = \frac{t}{\left(\frac{1}{h}\right) + \left(\frac{t}{C_e}\right)} \quad (21)$$

5. Temperature Extraction Coefficient

The temperature extraction coefficient indicates increasing the extraction of paclitaxel from biomass for every 10 °C increase in extraction temperature. It is defined as Eq. (22), which can be rearranged to linear Eq. (23).

$$q_T = q_{T0\gamma}^{T_d/10} \quad (22)$$

$$\ln q_T = \ln q_{T_0} + \frac{T_d}{10} \ln \gamma \quad (23)$$

where γ is the temperature extraction coefficient, q_T is the yield of paclitaxel in the extract at equilibrium at T_d (°C), and q_{T_0} is the yield of paclitaxel in the extract at equilibrium at 0 °C [16,26].

6. Thermodynamic Analysis

It is important to conduct thermodynamic analysis to investigate spontaneity, heat of reaction, and reversibility of the extraction process. The relation between activation energy (E_a) (kJ/mol) and the second-order extraction rate constant (k_2) is expressed as Eq. (24) according to the Arrhenius equation. It can be rearranged to linear Eq. (25).

$$k_2 = k_0 e^{-E_a/RT} \quad (24)$$

$$\ln k_2 = \ln k_0 + \left(\frac{-E_a}{R}\right)\frac{1}{T} \quad (25)$$

The Gibbs free energy change (ΔG^0) (kJ/mol) is an indication of spontaneity of a reaction. A negative value means a spontaneous reaction and a positive one means a non-spontaneous reaction. Enthalpy change (ΔH^0) (kJ/mol) is an indication of heat of a reaction. A negative value means an exothermic reaction and a positive one means an endothermic reaction. Entropy change (ΔS^0) (J/mol·K) is an indication of the disorder of a reaction. Values higher than zero mean an irreversible reaction [17]. The relation between ΔG^0 and an equilibrium constant (K_e) shown in Eq. (26) is expressed as Eq. (27). In addition, according to the van't Hoff equation shown in Eq. (28), ΔH^0 and ΔS^0 can be determined by using K_e and extraction temperature.

$$K_e = \frac{C_e}{C_{se}} \quad (26)$$

$$\Delta G^0 = -RT \ln K_e \quad (27)$$

$$\ln K_e = -\frac{\Delta H^0}{RT} + \frac{\Delta S^0}{R} \quad (28)$$

where C_{se} is the concentration of paclitaxel at equilibrium in the biomass (mg/mL) and R is the universal gas constant (8.314 J/mol·K).

7. Statistical Methods

In the kinetic models, except the phenomenological model, parameters were calculated from their linearized forms by linear regression using Sigmaplot 10.0 (Systat Software Inc., USA). The parameters of the phenomenological model were determined by the non-linear least squares fitting method of Origin 6.0 (Microcal Software Inc., USA). The concordance between experimental data and calculated values was established by the coefficient of determination (r^2) and the root mean square deviation (RMSD). The was computed using the following [29,30]:

$$\mathrm{RMSD} = \sqrt{\frac{1}{n_T}\sum_{i=1}^{n_T}(\text{experimental})^2 - (\text{calculated})^2} \quad (29)$$

where n_T is the number of experimental runs.

RESULTS AND DISCUSSION

1. Effect of Extraction Temperature and Time

The concentration of extracted paclitaxel depending on extraction temperature (25-45 °C) and extraction time (1-30 min) is shown in Fig. 1. The extraction temperatures had remarkable effects on the paclitaxel concentrations in the extract. The concentration of extracted paclitaxel rapidly increased within the first 1 min, and then displayed a slow extraction until reaching equilibrium. Two periods of extraction can easily be observed: washing, characterized by a rapid increase in the concentration of paclitaxel in the early beginning of the process, and slow extraction, characterized by a slow increase in the concentration with the progress of extraction until equilibrium. This phenomenon has been shown in various solid-liquid extraction processes [17,32,33]. The concentrations of extracted paclitaxel were significantly increased with the increased extraction temperature. This result can be explained by the fact that the rise in temperature increases the solubility and the facility

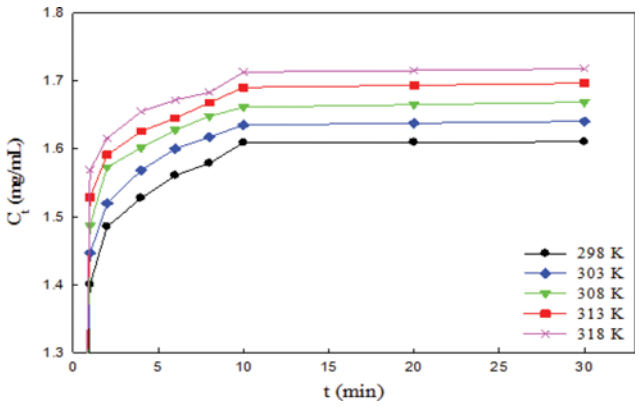

Fig. 1. Effect of extraction temperature on the concentration of extracted paclitaxel from biomass. The biomass amount, biomass/methanol ratio, and stirring speed were 5 g, 1 : 2 (w/v), and 570 rpm, respectively.

Table 1. Model parameters and statistical correlation values of various kinetic models for extraction of paclitaxel from biomass

Model		Temperature (°C)				
		25	30	35	40	45
Weibull's	m	0.0950	0.0896	0.0797	0.0742	0.0661
	δ (min)	4.158	2.600	1.499	0.955	0.511
	r^2	0.983	0.993	0.965	0.986	0.986
	RMSD	0.01529	0.01484	0.01337	0.01047	0.00867
Elovich's	E_0	0.582	0.601	0.620	0.633	0.649
	E_1	0.0349	0.0329	0.0292	0.0272	0.0241
	β	$6.25*10^5$	$2.79*10^6$	$4.84*10^7$	$3.68*10^8$	$1.16*10^{10}$
	r^2	0.984	0.992	0.965	0.986	0.986
	RMSD	0.01539	0.01505	0.01362	0.01073	0.00892
Phenomenological	C_e^w (mg/mL)	1.423	1.451	1.478	1.495	1.508
	C_e^d (mg/mL)	0.187	0.193	0.203	0.211	0.219
	C_e (mg/mL)	1.610	1.644	1.681	1.706	1.727
	k_w (min^{-1})	3.427	3.651	3.804	4.045	4.233
	k_d (min^{-1})	0.191	0.207	0.217	0.228	0.248
	r^2	0.991	0.998	0.995	0.984	0.985
	RMSD	0.01133	0.01057	0.01192	0.01155	0.01636
Hyperbolic	K_1 (min^{-1})	3.644	4.296	4.812	5.118	5.479
	K_2 (min^{-1})	5.442	6.307	6.951	7.270	7.687
	K_1/K_2	0.670	0.681	0.692	0.704	0.713
	r^2	0.999	0.999	0.999	0.999	0.999
	RMSD	0.00646	0.00491	0.00464	0.00657	0.00747

of diffusion of the paclitaxel while decreasing viscosity [16,26]. It was also confirmed by thermodynamic effects depending on the extraction temperature. The extraction process was endothermic. The paclitaxel concentrations in the extract at all investigated temperatures reached equilibrium at extraction time of 10 min. The corresponding concentrations at 25, 30, 35, 40, and 45 °C were 1.61, 1.63, 1.66, 1.69, and 1.71 mg/mL and equilibrium yields were 66.4, 67.5, 68.6, 69.8, and 70.7%, respectively.

2. Kinetic Analysis

The experimental data shown in Fig. 1 were applied to the empirical models (Weibull's model, Elovich's model, phenomenological model, hyperbolic model, and second-order model) to investigate the characteristics of extraction of paclitaxel from biomass. r^2 and RMSD values of each model were considered for the assessment of model adequacies.

The experimental data were applied to the linear form of Weibull's model shown in Eq. (3), and results of analyses are summarized in Table 1. The calculated values of δ, which is the scale parameter to represent the time needed to accomplish approximately 63% of the extraction, were 4.158, 2.600, 1.499, 0.995, and 0.511 min at 25, 30, 35, 40, and 45 °C, respectively, indicating that the δ values decreased as the extraction temperature increased. Thus, it was found that the extraction rate was elevated as the extraction temperature increased. The calculated values of m, which is the shape parameter, were 0.0950, 0.0896, 0.0797, 0.0742, and 0.0661 at 25, 30, 35, 40, and 45 °C, respectively, indicating that the values decreased as the extraction temperature increased. A similar tendency was found in a previous study [15]. In addition, the shape parameter (0.0661-0.0950) was lower than 1, which represents that the curve is parabolic with a high initial slope followed by an exponential shape. These results can be found in Fig. 1. Weibull's model had relatively high values of r^2 (0.965-0.993) and low values of RMSD (0.00867-0.01529), as shown in Table 1. The yield calculated slightly increased to the certain value after 10 min of extraction time required to reach equilibrium. This result was because external diffusion continues without equilibrium (saturation) according to this model equation.

The experimental data were applied to Eq. (4), which is Elovich's model, and results of analyses are summarized in Table 1. The calculated values of β, which is a constant that represents the extraction rate at $t \rightarrow 0$, were 6.25×10^5, 2.79×10^6, 4.84×10^7, 3.68×10^8, and 1.16×10^{10} at 25, 30, 35, 40, and 45 °C, respectively, indicating that the β values increased as the extraction temperature increased. Elovich's model had relatively high values of r^2 (0.965-0.992) and low values of RMSD (0.00892-0.01539). The yield calculated in this model also slightly increased to the certain value after 10 min of extraction time required to reach equilibrium. However, the increase was comparable to that in Weibull's model and therefore the value of was low.

The experimental data were applied to Eq. (6), which is the phenomenological model; results of analyses are summarized in Table 1. Non-linear least squares fitting was performed to determine the parameters. The calculated values of C_e^w, which is the concentration of extracted paclitaxel at equilibrium due to the washing step, were 1.423, 1.451, 1.478, 1.495, and 1.508 mg/mL at 25, 30, 35, 40, and 45 °C, respectively. The calculated values of C_e^d, which is the concentration of extracted paclitaxel at equilibrium due to the diffusion step, were 0.187, 0.193, 0.203, 0.211, and 0.219 mg/mL at 25, 30, 35, 40, and 45 °C, respectively. The C_e^w and C_e^d values increased with increasing extraction temperature. The calculated values of k_w, which is the mass transfer coefficient for the washing step, were 3.427, 3.651, 3.804, 4.045, and 4.233 min^{-1} at 25, 30, 35, 40, and 45 °C, respectively. The calculated values of k_d, which is the mass transfer coefficient for the diffusion step, were 0191, 0.207, 0.217, 0.228, and 0.248 min^{-1} at 25, 30, 35, 40, and 45 °C, respectively. The k_w and k_d values increased with increasing extraction temperature because of increasing the mass transfer coefficient of the paclitaxel while decreasing viscosity [16,26]. Based on these results, it was determined that the extraction rate was elevated as the extraction temperature increased. The calculated values of C_e, which is the concentration of extracted paclitaxel at equilibrium, were 1.610, 1.644, 1.681, 1.706, and 1.727 mg/mL at 25, 30, 35, 40, and 45 °C, respectively, indicating that the C_e values increased as the extraction temperature increased. The concentration of extracted paclitaxel at equilibrium due to the washing step was much higher than that due to the diffusion step. The mass transfer coefficient for the washing step was higher than that for the diffusion step. It was confirmed that paclitaxel was rapidly washed initially in the extraction process and a large amount of paclitaxel was extracted to the solvent in the washing step, which was followed by slow diffusion and a small quantity of paclitaxel was extracted to the solvent in diffusion step. The paclitaxel extraction from biomass was mainly controlled by diffusion. The phenomenological model had relatively high values of r^2 (0.984-0.998) and low values of RMSD (0.01057-0.01636). When the activation energy in each step was determined by applying k_w and k_d to the Arrhenius equation shown in Eq. (24), it was 8.281 kJ/mol in the washing step and 9.861 kJ/mol in the diffusion step. The value of the activation energy was greater for the diffusion step than for the washing step, meaning that the former step was more strongly influenced by extraction temperature than the latter step. Therefore, we decided that the rate-limiting step in this extraction process was the diffusion step. These results are comparable to those of the extraction of oil from olive cake (washing: 8.56 kJ/mol, diffusion: 9.88 kJ/mol) [26] and higher than those of the extraction of oil from hempseed (washing: 4.31 kJ/mol, diffusion: 6.99 kJ/mol) [16].

The hyperbolic model (Peleg's model) shown in Eq. (11) was fitted to the experimental data by linear regression; results of analyses are summarized in Table 1. The calculated values of K_1, which is the initial extraction rate, were 3.644, 4.296, 4.812, 5.118, and 5.479 min^{-1} at 25, 30, 35, 40, and 45 °C, respectively, indicating that the K_1 values increased as the extraction temperature increased. The calculated values of K_1/K_2, which is the Peleg capacity constant to represent the maximum extraction yield, were 0.670, 0.681, 0.692, 0.704, and 0.713 at 25, 30, 35, 40, and 45 °C, respectively, indicating that the K_1/K_2 values increased as the extraction temperature increased. The hyperbolic model had the highest values of r^2 (0.999) and the lowest values of RMSD (0.00464-0.00747). It was confirmed that the hyperbolic model was the most appropriate for this extraction process. The calculated value of yield in this model reached equilibrium at 10 min of extraction. These results indicate that the reaction approaches zero-order kinetics with the progress of extraction in the diffusion step. In addition, the experimental data were comparable to the calculated values based on the

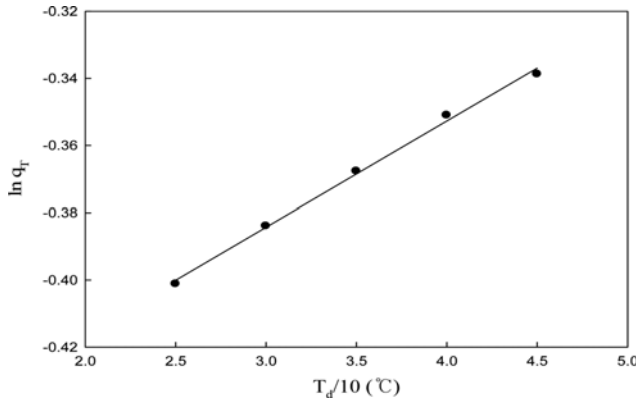

Fig. 2. Plot of $\ln q_T$ versus T_d/10 for extraction of paclitaxel from biomass.

lowest RMSD in this model.

3. Temperature Extraction Coefficient

According to the hyperbolic model, also called Peleg's model, the yield of paclitaxel in the extract could be determined when equilibrium was reached at each temperature with the Peleg capacity constant. Based on this result, the temperature extraction coefficient (γ) could be decided. As shown in Fig. 2, the plot of $\ln q_T$ against T_d/10 of Eq. (23) was found to be linear (r^2=0.997). The value of γ, obtained from the slope of the plot, was about 1.032. It indicates that the yield increased by a factor of about 1.032 for every 10 °C rise in temperature. The yield of paclitaxel in the extract at equilibrium at 0 °C, calculated from the intercept of the plot, was 61.9%. The obtained value of γ was higher than that in the extraction of oil from olive cake (1.02) [26] and in the extraction of oil from hempseed (1.012-1.027) [16]. It was because the extraction of paclitaxel from biomass was more sensitive to temperature than the extraction of oil from olive or hempseed.

4. Predictive Model

When the extract was rapidly dissolved by solvent and then slowly diffused to the solvent, the second-order model, which is similar to the hyperbolic model that was the most suitable for this extraction process, could be used. The parameters of the second-order model

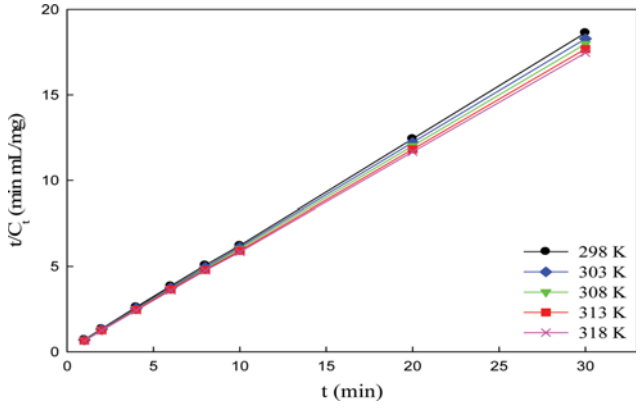

Fig. 3. Second-order model linear regression for extraction of paclitaxel from biomass at various extraction temperatures. The biomass amount, biomass/methanol ratio, and stirring speed were 5 g, 1 : 2 (w/v), and 570 rpm, respectively.

Table 2. Parameters of second-order model for extraction of paclitaxel from biomass

Temperature (°C)	Model parameter			r^2
	C_e (mg/mL)	h (mg/mL min)	k_2 (mL/mg min)	
25	1.621	9.138	3.476	0.999
30	1.650	10.150	3.727	0.999
35	1.677	11.501	4.089	0.999
40	1.705	12.395	4.264	0.999
45	1.726	14.052	4.719	0.999

can be expressed as a function of extraction temperature and extraction time, which are the main process parameters in the extraction process. Thus, the concentration of extracted paclitaxel can be predicted depending on changes in extraction temperature and extraction time.

According to linear Eq. (18) in the second-order model, linear regression analysis of t/C_t over extraction time was performed, and the result is shown in Fig. 3. The parameters and the value of r^2 at all investigated temperatures are presented in Table 2. The values of k_2, which is the extraction rate constant for the second-order model, were 3.476, 3.727, 4.089, 4.264, and 4.719 mL/mg·min at 25, 30, 35, 40, and 45 °C, respectively, indicating that the k_2 values increased with increasing extraction temperature. The values of C_e, which is the concentration of extracted paclitaxel at equilibrium, were 1.621, 1.650, 1.677, 1.705, and 1.726 mg/mL at 25, 30, 35, 40, and 45 °C, respectively, indicating that the C_e values also increased with increasing extraction temperature because of increased paclitaxel solubility at higher temperatures. Temperature strongly affects the solubility of extracts in the extraction process [17,26,29]. The calculated values of h, which is the initial extraction rate, were 9.138, 10.150, 11.501, 12.395, and 14.052 mg/mL·min at 25, 30, 35, 40, and 45 °C, respectively, indicating that the h values increased as the extraction temperature increased. From these results, it was determined that the initial extraction rate and the total extraction rate increased with increasing extraction temperature, and that the concentration of extracted paclitaxel at equilibrium increased. This result was also shown in other acceptable models and a similar trend was observed in other solid-liquid extraction processes [17, 31-33]. The calculated value of h was much higher than that in the extraction of protopine from *Fumaria officinalis* (0.0087-0.034 mg/mL·min at 15-75 °C) [32] and in the extraction of water-soluble compounds from *Tilia* sapwood (0.0193-0.103 mg/mL·min at 40-90 °C) [33], comparable to that in the extraction of antioxidant from pomegranate mare (10.512-20.217 mg/mL·min at 25-40 °C) [31] at low temperature and lower at high temperature. In addition, this extraction process was less sensitive to temperature because the variation in depending on extraction temperature was smaller than that in other extraction processes.

Regression analysis of h and C_e depending on extraction temperature was performed to express the concentration of extracted paclitaxel as a function of extraction temperature and extraction time. Here h includes the extraction rate constant for the second-order model, and therefore it can be expressed as the Arrhenius

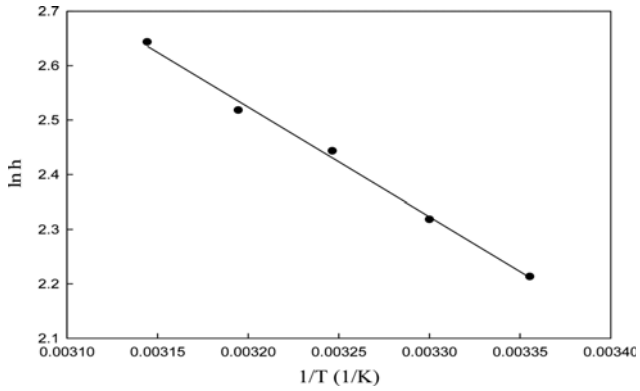

Fig. 4. Plot of lnh versus 1/T for extraction of paclitaxel from biomass.

equation shown in Eq. (24). As shown in Fig. 4, when h depending on extraction temperature was used in linear regression analysis as the linear form of the Arrhenius equation shown in Eq. (25), the value of r^2 was 0.995; h was expressed as a function of extraction temperature in Eq. (30).

$$h = 7742\exp\left(\frac{-2010}{T}\right) \tag{30}$$

When C_e depending on extraction temperature was used in first-order and second-order regression analysis, the value of r^2 was 0.997 and 0.999, respectively (data not shown). These values of r^2 were not significantly different. Therefore, first-order regression analysis containing fewer parameters was more appropriate and less sensitive to temperature. Thus, C_e was expressed as a function of extraction temperature as shown in Eq. (31).

$$C_e = 0.00527T + 0.05402 \tag{31}$$

Eq. (30) and (31) were substituted in Eq. (21) and the concentration of extracted paclitaxel at any time was expressed as a function of extraction temperature and extraction time as shown in Eq. (32).

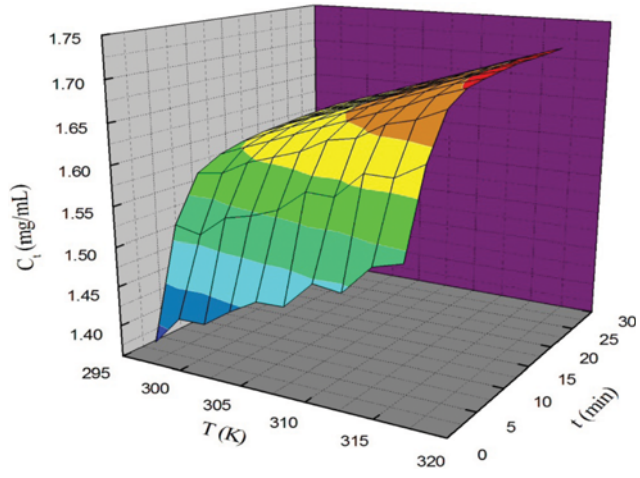

Fig. 5. Predictive model for extraction of paclitaxel from biomass at any extraction time and extraction temperature.

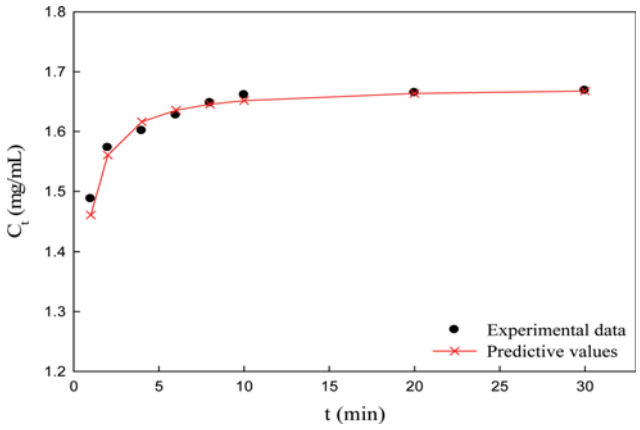

Fig. 6. Comparison between experimental data and predictive values on the concentration of extracted paclitaxel from biomass. The biomass amount, biomass/methanol ratio, stirring speed, and extraction temperature were 5 g, 1 : 2 (w/v), 570 rpm, and 30 °C, respectively.

$$C_t = \frac{t}{1.292\times10^{-4}\exp\left(\frac{2010}{T}\right) + \left(\frac{t}{0.00527T + 0.05402}\right)} \tag{32}$$

Based on Eq. (32), a three-dimensional graph of the concentration of extracted paclitaxel depending on extraction temperature and extraction time was plotted and shown in Fig. 5. As extraction temperature and extraction time increased, the concentration of extracted paclitaxel increased. At 10 min of extraction, equilibrium was reached and then the concentration of extracted paclitaxel hardly changed. The concentration of extracted paclitaxel determined by the predictive model and the experimental data was compared and the result shown in Fig. 6. A good fit between experimental data and calculated values was obtained.

5. Thermodynamic Analysis

When k_2, which is the extraction rate constant for the second-order model, was used in first-order and second-order regression analysis depending on extraction temperature, the value of r^2 was 0.984 and 0.988, respectively (data not shown). These values of r^2 were not significantly different. Therefore, first-order regression analysis containing fewer parameters was more appropriate and less

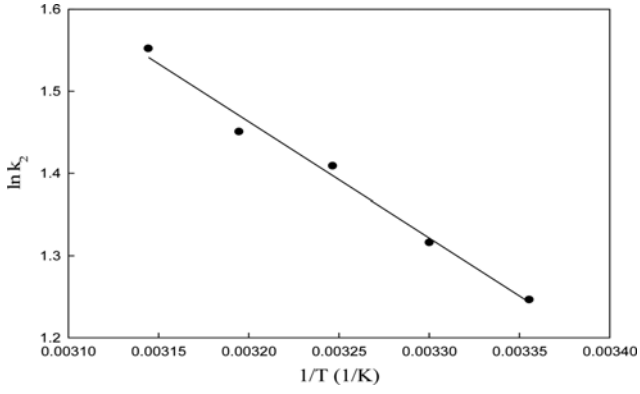

Fig. 7. Plot of $\ln k_2$ versus 1/T for extraction of paclitaxel from biomass.

Table 3. Thermodynamic parameters for extraction of paclitaxel from biomass

Temperature (°C)	K_e	ΔG^0 (kJ/mol)	E_a (kJ/mol)	ΔH^0 (kJ/mol)	ΔS^0 (J/mol K)
25	2.025	−1.749			
30	2.138	−1.915			
35	2.251	−2.078	11.747	8.040	32.85
40	2.378	−2.254			
45	2.478	−2.399			

sensitive to temperature. As shown in Fig. 7, when k_2 and extraction temperature were used in linear regression analysis based on the linear form of the Arrhenius equation shown in Eq. (25), the value of E_a was 11.747 kJ/mol (r^2=0.988). This value is characteristic of an extraction process of a physical nature [26]. This activation energy was much higher than that in the extraction of protopine from *Fumaria officinalis* (5.182 kJ/mol) [32] and the extraction of oil from sunflower seeds (4.2 kJ/mol) [34], comparable to that in the extraction of antioxidants from pomegranate mare (14.541 kJ/mol) [31], and much lower than that in the extraction of water-soluble compounds from *Tilia* sapwood (21.9 kJ/mol) [33] and the extraction of oil from *Jatropha curcas* (26.676 kJ/mol) [35].

K_e and ΔG^0 at all investigated temperatures were determined by Eq. (26) and Eq. (27). ΔH^0 and ΔS^0 were calculated from the slope and intercept of the plot of $\ln K_e$ versus 1/T of Eq. (28). The results are summarized in Table 3. The values of K_e were 2.025, 2.138, 2.251, 2.378, and 2.478 at 25, 30, 35, 40, and 45 °C, respectively, indicating that the K_e values increased as the extraction temperature increased. This result is because C_e increased as the extraction temperature increased. The values of ΔG^0 were −1.749, −1.915, −2.078, −2.254, and −2.399 kJ/mol at 25, 30, 35, 40, and 45 °C, respectively. The negative value of ΔG^0 for paclitaxel extraction from biomass confirms that the process is feasible and spontaneous and that the degree of spontaneity of the extraction increases with increasing the extraction temperature as ΔG^0 becomes more negative. The decrease in the negative value of ΔG^0 with an increase in temperature indicates that the paclitaxel extraction from biomass becomes more favorable at higher temperatures. The value of ΔH^0 was 8.040 kJ/mol. The value of ΔH^0 was positive, indicating that the extraction is endothermic requires energy during the process. This value was lower than that in the extraction of gossypol from defatted cottonseed meal (27.749-28.093 kJ/mol) [17], the extraction of oil from olive cake (12.910 kJ/mol) [26], the extraction of oil from sunflower seeds (11.200 kJ/mol) [34], and the extraction of oil from castor cake (12.270 kJ/mol) [36] and much higher than that in the extraction of polyphenols from barley (2.326 kJ/mol) [18]. Furthermore, ΔH^0 should be less than E_a in an endothermic reaction and this condition was satisfied. The positive value of ΔS^0 (+32.85 J/mol·K) indicates that the process is irreversible.

CONCLUSIONS

We investigated the effect of temperature on the efficiency of paclitaxel extraction from biomass using methanol. In addition, kinetic and thermodynamic studies of this extraction process were performed. The concentration of extracted paclitaxel increased as the extraction temperature increased. At all investigated temperatures, the concentration of extracted paclitaxel rapidly increased in the initial stage of extraction (~1 min) and then slowly increased and reached equilibrium at extraction time of 10 min. When the experimental data were applied to various kinetic models, the hyperbolic model (second-order model) showed the highest value of r^2 and the lowest value of RMSD, and therefore it was the most suitable. From acceptable models, the quantity of extracted paclitaxel at initial and equilibrium and the rate of extraction increased as the extraction temperature increased. The extraction process consists of washing and diffusion steps: the washing step was faster than the diffusion step and had more paclitaxel extracted in the solvent, indicating that the extraction of paclitaxel from biomass was mainly controlled by diffusion. The value of temperature extraction coefficient was about 1.032, indicating that the yield of paclitaxel increased by a factor of about 1.032 for every 10 °C rise in extraction temperature. The extraction rate constant, the concentration of extracted paclitaxel at equilibrium, and the initial extraction rate, which are parameters of the second-order model, were expressed as a function of extraction temperature. It was applied to the second-order model to obtain a predictive model for the concentration of extracted paclitaxel depending on extraction temperature and extraction time. A good fit between experimental data and calculated values was obtained. From thermodynamic analysis of the extraction process, the Gibbs free energy change was negative and the absolute value increased with increasing extraction temperature. The extraction process was spontaneous, and spontaneity increased as the extraction temperature increased. Both enthalpy change and entropy change were positive, indicating that the extraction is endothermic and irreversible.

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NOMENCLATURE

C_e : concentration of extracted paclitaxel at equilibrium [mg/mL]
C_e^d : concentration of extracted paclitaxel at equilibrium during the diffusion step [mg/mL]
C_e^{d1} : concentration of extracted paclitaxel at equilibrium during the unhindered diffusion step [mg/mL]
C_e^{d2} : concentration of extracted paclitaxel at equilibrium during the hindered diffusion step [mg/mL]
C_e^w : concentration of extracted paclitaxel at equilibrium during the washing step [mg/mL]
C_{se} : concentration of paclitaxel at equilibrium in the biomass [mg/mL]
C_t : concentration of extracted paclitaxel at any time [mg/mL]
E_0 : constant of Elovich's model
E_1 : constant of Elovich's model
E_a : activation energy [kJ/mol]

ΔG^0 : Gibbs free energy change [kJ/mol]
h : initial extraction rate [mg/mL·min]
ΔH^0 : enthalpy change [kJ/mol]
k_0 : pre-exponential factor [mL/mg·min]
k_2 : extraction rate constant for the second-order model [mL/mg·min]
k_d : mass transfer coefficient for the diffusion step [min^{-1}]
k_{d1} : mass transfer coefficient for the unhindered diffusion step [min^{-1}]
k_{d2} : mass transfer coefficient for the hindered diffusion step [min^{-1}]
k_w : mass transfer coefficient for the washing step [min^{-1}]
K_1 : initial extraction rate [min^{-1}]
K_2 : constant related to maximum extraction yield [min^{-1}]
K_e : equilibrium constant
m : shape parameter of Weibull's model
n_T : number of experimental runs
q : yield of paclitaxel in the extract at any time
q_T : yield of paclitaxel in the extract at equilibrium at any temperature [°C]
q_{T0} : yield of paclitaxel in the extract at equilibrium at 0 °C
r^2 : coefficient of determination
R : universal gas constant (8.314 J/mol·K)
ΔS^0 : entropy change [kJ/mol·K]
t : time [min]
T : absolute temperature [K]
T_d : Celsius temperature [°C]

Greek Letters

α : parameter of Elovich's model
β : parameter of Elovich's model
γ : temperature extraction coefficient
δ : scale parameter of Weibull's model [min]

Subscript

RMSD : root mean square deviation

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