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MODELLING OF SUPERCRITICAL CARBON DIOXIDE EXTRACTION OF ANDROGRAPHOLIDE FROM *ANDROGRAPHIS PANICULATA* LEAVES BY EMPLOYING INTEGRAL DESORPTION CONCEPT

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ABSTRACT

Mathematical formulation is necessary for the optimisation and scale-up of a separation unit such as the extraction of biomolecules from their natural resources. The objective of this work is to obtain a quantitative description of the supercritical carbon dioxide extraction (SCDE) of andrographolide from Andrographis paniculata leaves. This work presents the development of a mathematical model based on first order desorption rate of solute into supercritical fluid to describe the SCDE of andrographolide from Andrographis paniculata leaves. Numerical calculation was carried out using a commercial algebraic optimisation code, namely the MATLAB software. In this work, calculation was accomplished to obtain the adjustable parameter of the model using experimental data obtained from the SCDE of andrographolide at different operating temperature and pressure. The calculation results show that this model agrees very well with the experimental data. Therefore, this model is capable for the modelling of SCDE of andrographolide from Andrographis paniculata leaves.

Keywords: *modelling, supercritical carbon dioxide extraction, andrographolide, Andrographis paniculata, MATLAB*

INTRODUCTION

Andrographis paniculata NEES, locally known as Hempedu Bumi and commonly called as King of Bitter grows widely in the tropical area of South East Asia, India and China with annual growth having 30 – 70 cm height. In Malaysia, this plant has been extensively used for traditional medicine and help against fever, dysentery, diarrhoea, inflammation, and sore throat. Furthermore, it is a promising new way for the treatment of many diseases, including HIV, AIDS, and numerous symptoms associated with immune disorders [1].

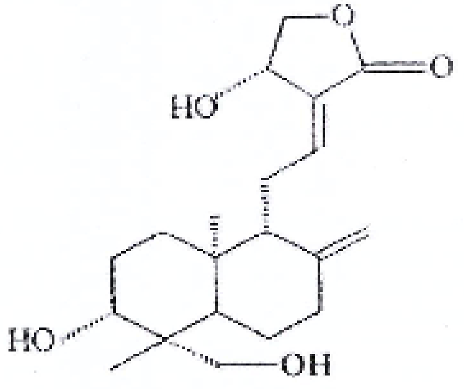

Fig. 1: Molecular structure of andrographolide [3]

Three main diterpenoid lactones identified in the *Andrographis paniculata* leaves were andrographolide, neoandrographolide and deoxyandrographolide [2, 3, 4]. Andrographolide, which is grouped as an unsaturated trihydroxy lactone has molecular formula of $C_{20}H_{30}O_5$. The molecular structure of andrographolide is shown in Figure 1. Andrographolide can be easily dissolved in methanol, ethanol, pyridine, acetic acid and acetone, but slightly dissolved in ether and water. The melting point of this substance is 228° – 230 °C, while its ultraviolet spectrum in ethanol, λ_{max} is 223 nm [3]. Hitherto, there are some techniques can be used for the analysis of andrographolide, such as thin layer chromatography (TLC) [4, 5], high - performance liquid chromatography (HPLC) [2, 6, 7] and crystallisation techniques [3].

Extraction using organic solvent is the most common method of separating bioactive components from their natural hosts. However, since the solvents are not able to be completely removed by the current processing techniques and their traces may remain in the final product, the extraction using only organic solvent is no longer attractive from clinical, environmental, energy and extraction time points of view [8, 9]. Therefore, supercritical fluid extraction is a better alternative method to extract andrographolide from *Andrographis paniculata* leaves, since this method offers shorter extraction times, cheaper operating cost, higher extraction selectivity, safer condition (non toxic, non flammable, non hazardous) and adjustable solvating power [10].

MATHEMATICAL MODEL DEVELOPMENT

Consider a packed bed of leaf particles with an initial concentration of solute $S = S_0$. Fresh supercritical fluid is introduced into the bed, which is operated isothermally. The global mass balance of this condition can be represented by the following equation:

$$\alpha \frac{\partial C}{\partial t} + u \frac{\partial C}{\partial z} - \alpha \left(D_z \frac{\partial^2 C}{\partial z^2} + D_x \frac{\partial^2 C}{\partial x^2} \right) = -\left(1-\alpha\right) \frac{\partial S}{\partial t} \tag{1}$$

where C and S are the concentration of solute in the supercritical solvent and concentration of solute in the leaf particles in g/cm^3, respectively. While, α, t and u are the bed void fraction, time in minutes and supercritical fluid velocity in cm/s, and D_z and D_r are the radial and axial diffusion coefficients in cm^2/s.

Although several components exist in the sample matrix, the fitting parameter has been made by taking into account just a single one, called solute. This is a pseudo component, which represents the global behaviour of the analytes. The extraction system is considered as a fixed bed comprising of two phases:

a. solid (static): matrix, which holds the solute.
b. fluid (mobile): supercritical solvent (solute concentration in the fluid phase is negligible due to solute's low solubility).

The solvent flow rate and physical properties are constant during the extraction course. Pressure losses, temperature gradients and heat of dissolution are neglected in the bed. Superficial velocity is constant and it is calculated from supercritical fluid flow rate, by neglecting extracted solute flow rate. The bed void fraction is constant. The time required by the supercritical solvent containing solute to flow from the exit of bed to the exit of separator is negligible. Axial dispersion can be ignored due to dominant axial mass transfer caused by bulk flow of supercritical solvent. In spite of extraction cell geometry ratio (L/D) = 10 <<100, radial dispersion is also plausible to be neglected. The mass balance in the bulk supercritical fluid phase in the column may now be written as:

$$\alpha \frac{\partial C}{\partial t} + u \frac{\partial C}{\partial z} = -\left(1-\alpha\right) \frac{\partial S}{\partial t} \tag{2}$$

The initial and boundary conditions are:

$$t = 0, \forall_z \rightarrow C = 0 \tag{3.a}$$

$$z = 0, \forall_t \rightarrow C = 0 \tag{3.b}$$

Because of the lack of information on supercritical fluid – solid mass transfer coefficient, effective diffusivity of andrographolide in supercritical carbon dioxide, and isotherms, the mass balance in the dried leaves is expressed in terms of linear or first order desorption kinetics, which may be written by:

$$\frac{\partial S}{\partial t} = -k_d S \tag{4}$$

where k_d is the desorption rate constant in 1/min.
The initial condition is:

$$t = 0, \forall_z \rightarrow S = S_0 \tag{5}$$

The concentration at the exit of the extraction column (C_e) then can be obtained by solving equations (2) to (5) and is expressed by:

$$C_e = \left(\frac{1-\alpha}{\alpha}\right) S_0 \left[\exp\left\{-k_d\left(t - \frac{\alpha L}{u}\right)\right\} - \exp\{-k_d t\}\right] \tag{6}$$

The total amount of extracted andrographolide can be then calculated by integrating C_e with respect with time. Therefore the fraction of extracted andrographolide ($F(t)$) can be calculated using the following equation:

$$F(t) = \left(\frac{1-\alpha}{k_d \alpha}\right) S_0 (1 - \exp\{-k_d t\}) + \exp\left\{-k_d\left(t - \frac{\alpha L}{u}\right)\right\} - \exp\left\{k_d\left(\frac{\alpha L}{u}\right)\right\} \tag{7}$$

The optimum values of k_d at each experiment were obtained by choosing the best or optimum value of k_d to minimise the sum of the squares of the difference between the experimental extraction yield (F_{exp}), and the estimated extraction yield (F_{calc}) or SSE, using the developed model as shown in equation (7):

$$Min(SSE) = \sum_{1}^{n} \left(F_{calc} - F_{exp}\right)^2 \tag{8}$$

The inputs of the calculation are α (0.8455), S_0 (4.35×10^{-4} g/cm^3), t (varied from 0 to 420 minutes), L (21.3 cm) and u (2 cm/s) into the minimisation function of one variable using the MATLAB software to obtain minimum value of SSE in equation (8).

Effect Of Temperature And Supercritical Fluid Density On Desorption Rate Constant

In previous studies there have been found different desorption rate constant dependences on temperature. Tan and Liou [11] reported that desorption efficiency of ethyl acetate from activated carbon using supercritical carbon dioxide decreased with temperature when pressure was fixed at 8.81 MPa, and an optimum temperature of about 40 °C was found when pressures were fixed and higher than 10.13 MPa. However, the desorption efficiency of toluene from activated carbon using supercritical carbon dioxide was found to increase with temperature by other researcher [12]. If the desorption rate constant in this system follows the Arrhenius law, the following linear relation should exist:

$$\ln k_d = -\left(\frac{E}{RT}\right) + \ln k_{d0} \tag{9}$$

Where E, R and T are the apparent desorption activation energy in J/mol, universal gas constant in J/mol K and temperature in Kelvin, respectively. The k_{d0} is the standard desorption rate constant in 1/min.

In supercritical fluid technology, temperature and pressure have significant roles in adjusting supercritical fluid density, which is strongly related to its solvating power. If it is assumed that k_{d0} is function of supercritical fluid density, the following relationship is proposed in this study:

$$k_{d0} = k_1 \rho^n \tag{10}$$

where ρ is the supercritical fluid density (g/cm^3), and k_1 and n are the constants of this equation.

RESULTS AND DISCUSSIONS

For the calculation in this work, the initial andrographolide content in the *Andrographis paniculata* leaf particles was 1.2×10^{-1} g/100 g ground - dried leaves. It was determined by exhaustive standard soxhlet extraction of 5 grams of ground - dried leaves using 1.50×10^{-4} m^3 of pure methanol for 24 hours. Five random samples were analysed and an average value of initial andrographolide content was then taken. The data of supercritical carbon dioxide extraction of andrographolide from *Andrographis paniculata* leaves were obtained from the literature [13]. Figure 2 shows the increase of extraction rate with increasing temperature from 30 ° to 40 °C at 10 MPa although solubility of andrographolide at 40 °C is lower than that at 30 °C. This phenomenon is due to the increase of desorption kinetic constant with temperature according to Arrhenius dependence [14]. In addition, analytes can be strongly bound to the real-world samples; therefore a certain amount of energy is required to the extraction system. Increasing the temperature would be expected to increase the kinetics of the desorption process of analytes, which are strongly bound to the sample matrix [15, 16, 17]. Since extraction of solute from plant matrices is basically a desorption process, increasing the desorption kinetic constant would lead to increase in the extraction rate and extract yield. The integral desorption model fitted the experimental data very well as represented by the dashed lines in Figure 2. Almost all the data and calculation results collapsed at the same point showing a good agreement between the calculated results and the experimental data.

However, further increase of temperature reduced the extract yield. This is because the carbon dioxide density reduced significantly when temperature increases from 40° to 60 °C resulted in the reduction of the solubility of andrographolide. The density of carbon dioxide is the predominant factor for the dissolution of organic molecules in supercritical carbon dioxide. The density of carbon dioxide strongly determined the number of interactions between carbon dioxide and solute molecules. If enough interactions occurred, the cohesive forces between individual solute molecules were broken down rapidly and solubilisation would occur. Therefore, solubilisation of solute in carbon dioxide will be a function of the molecular weight of the solute and the level of interaction between carbon dioxide and solute molecules [18]. At constant pressure, the density of the solvent decreased with an increase in temperature, but vapour pressure of the solute increased with an increase in temperature [19]. The solvent density change will become more effective and can easily overcome the effect of solute vapour pressure change on the extraction rate [20]. This finding agrees well with supercritical carbon dioxide extraction of poly-cyclic aromatic hydrocarbons from environmental samples reported by Yang *et al.* [17].

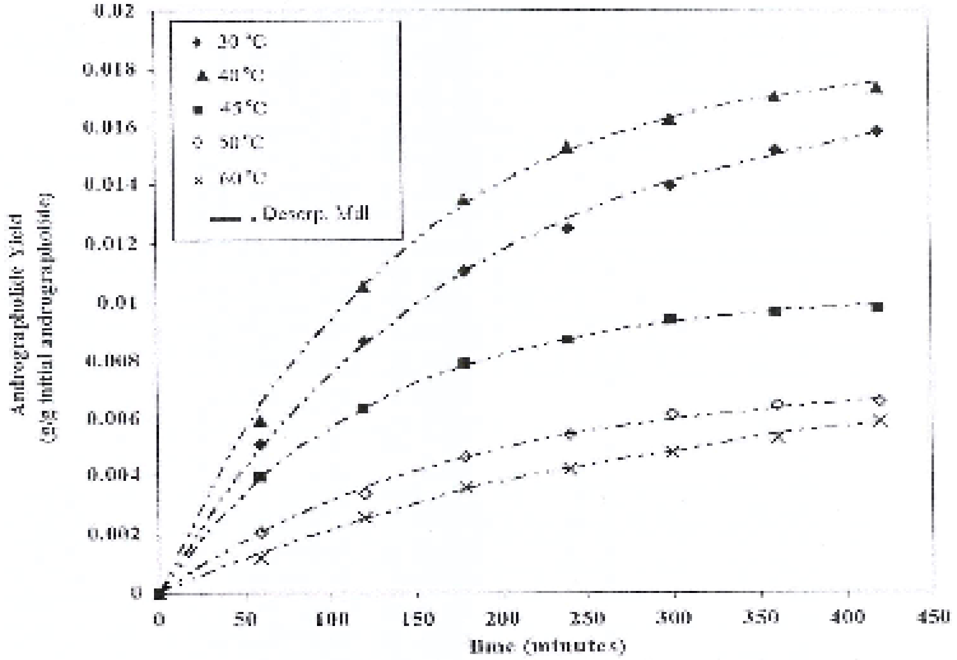

Fig. 2: Effect of temperature on extraction yield as a function of time at 10 MPa using 0.375 mm particle size and solvent flow rate of 3.18×10^{-5} kg/s.

Table 1: *Desorption rate constant as function of temperature at 10.0 MPa (k_{d0} = 0.58484, E = 11491760.0426 J/mol and the value of SSE = 1.7873E-7)*

T, (°C)	$k_d \times 1000$, (1/min)	$k_{d_{calc}} \times 1000$, (1/min)	ARD^1, (%)
30	5.90	6.10	2.32
40	7.10	7.10	0.08
45	7.80	7.60	2.49
50	8.30	8.10	2.19
60	9.00	9.20	2.38
		$AARD^2$ =	1.89

where $^1 ARD = ABS\left(\frac{k_d - k_{d_{calc}}}{k_d}\right)$, $^2 AARD = \frac{1}{N}\sum_1^n ABS\left(\frac{k_d - k_{d_{calc}}}{k_d}\right)$, and N is the number of data.

The results of this work show that the desorption rate decreased with the increase of temperature due to endothermicity of adsorption process and lower yields were obtained at higher temperatures. Table 1 shows the optimised desorption rate constant as a function of temperature. The value of desorption rate constants increased with the increase of temperature. With the present observation, the Arrhenius expression $\ln k_d = -\left(\frac{E}{RT}\right) + \ln k_{do}$ was obtained, where $k_{do} = 0.5848$ (1/min), $E = 11491700.04$ J/mol and 1.89 % *AARD* was generated.

Tan and Liou [11] obtained the desorption activation energies (*E*) of toluene from activated carbon at different temperature were in the range of 1.45 to 3.95 × 10[illegible] J/mol, which are in the same order with the *E* value obtained in this study. Correlation of desorption rate constant with supercritical solvent density is presented in Table 2. The desorption rate constants were found to increase with the decrease of supercritical solvent density. This fact indicated that desorption of andrographolide from ground – dried leaves of *Andrographis paniculata* is more favourable at lower supercritical solvent density. The following correlation between desorption rate constant and supercritical solvent density is then proposed: $k_{dc} = k_r \rho^n$, with $k_r = 5.41\times10$[illegible] (1/min) and n = -0.43 and gives an *AARD* of 2.05 %.

Table 2: Desorption rate constant as function of supercritical CO₂ density ($k_r = 0.0054131$, $n = -0.42797$ and the value of SSE = 2.0154E-7)

Density × 100, (g/cm3)	$k_d \times 1000$, (1/min)	$k_{d,calc} \times 1000$, (1/min)	*ARD*, (%)
72.91	5.90	6.20	3.28
56.13	7.10	6.90	1.92
45.61	7.80	7.60	2.56
37.37	8.30	8.20	0.59
29.11	9.00	9.20	1.88
		AARD =	2.05

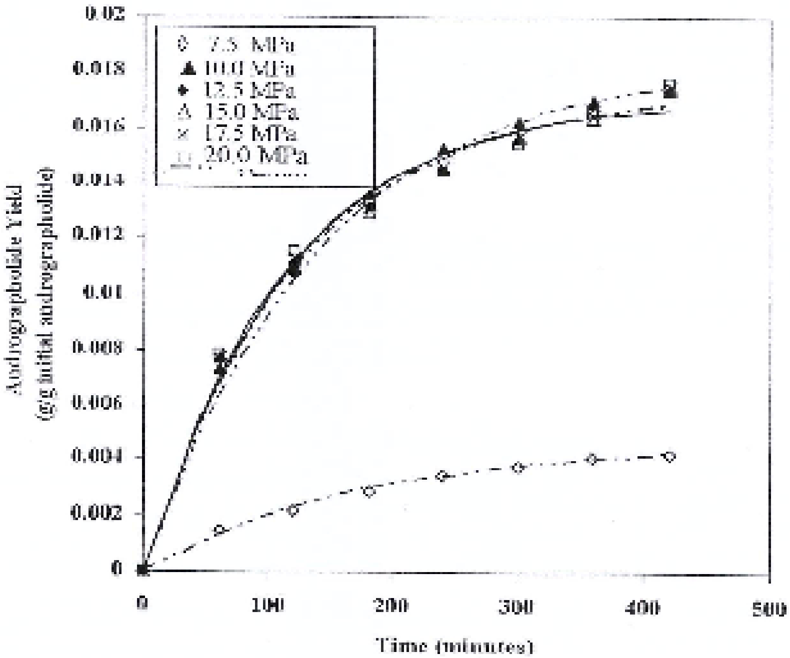

Fig. 3: Effect of pressure on extraction yield as a function of time at 40 °C using 0.375 mm particle size and solvent flow rate of 3.18×10^{-5} kg/s.

Figure 3 shows that integral desorption model fitted the experimental data fairly well, while Table 3 shows the optimum desorption rate constants obtained at different pressures. The desorption rate constants increased sharply when pressure increased from 7.5 to 10.0 MPa. However, their values increased slightly when pressures increased from 12.5 to 20.0 MPa. This pressure effect may be due to the increase of supercritical solvent density with the increase of operating pressure. For practical purpose, a pressure of 10.0 MPa may be sufficient. Numerous studies have indicated that the solubility of a solute in supercritical solvent increases with density [21]. Tan and Liou [11] reported that desorption activation energy was less at higher pressure and was the evidence that operations at higher pressures are more favourable for regeneration of activated carbon using supercritical carbon dioxide. Therefore, the desorption of solute by supercritical fluid from a solid matrix may be easier at higher fluid density or at higher operating pressure. This fact agrees well with the finding in this work.

Table 3: Optimum desorption rate constanst for integral desorption model at various pressures studied at 40 °C

Pressure (MPa)	$k_d \times 1000$, (1/min)
7.5	5.73
10.0	7.10
12.5	8.15
15.0	8.27
17.5	8.72
20.0	8.87

CONCLUSIONS

A mathematical model based on unsteady state first order solute desorption has been developed and tested for the calculation of SCDE profile of andrographolide from *Andrographis paniculata* leaves at different temperatures and pressures. The extraction profiles obtained from the calculation using this model were in a good agreement with the experimental data obtained from the literature. The desorption rate constants were also well correlated with temperature and supercritical solvent density. Thus, the modelling presented in this work can be used in the optimisation and scale - up of a supercritical fluid extraction unit. This model is capable for the modelling of SCDE of andrographolide from *Andrographis paniculata* leaves.

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ABBREVIATIONS AND NOTATIONS

Parameters	Meaning	Unit
AARD	= Average Absolute Relative Deviation	-
ARD	= Absolute Relative Deviation	-
C	= Solute concentration in the fluid phase in bed void volume	g/cm^3
C_e	= Solute concentration in exit stream of fluid	g/cm^3
D	= Diameter of extractor	cm
D_r	= Radial dispersion coefficient	cm^2/s
D_z	= Axial dispersion coefficient	cm^2/s
E	= Apparent desorption activation energy	J/mol
F_{calc}	= Calculated fractional extraction yield as a function of extraction time	-

Parameters	**Meaning**	**Unit**
F_{exp}	= Experimental fractional extraction yield as a function of extraction time	-
$F(t)$	= Fractional extraction yield as a function of extraction time	-
k_d	= Desorption rate constant	1/min
k_{dcalc}	= Calculated desorption rate constant	1/min
k_{ds}	= Standard desorption rate constant	1/min
k_ρ	= Density based desorption rate constant in equation (10)	1/min
L	= Length of extractor	cm
n	= Constant in equation (10)	-
N	= Number of data	-
R	= Universal gas constant	J/(mol.K)
S	= Concentration of solute in leaf particles	g/cm^3
S_0	= Initial concentration of solute in leaf particles	g/cm^3
SSE	= Sum of squares of errors	-
t	= Time	minutes
T	= Temperature	°C, K
u	= Supercritical fluid velocity	cm/s
x	= Radial coordinate in the extractor	cm
z	= Axial coordinate in the extractor	cm
α	= Void bed fraction	-
ρ	= Supercritical fluid density	g/cm^3