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Modelling of Andrographolide Extraction from *Andrographis Paniculata* Leaves in a Soxhlet Extractor

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Abstract

Andrographolide is the main diterpenoid lactone contained in the leaves of *Andrographis paniculata*. This bioactive component has multifunctional medicinal properties such as activity against fever, dysentery, diarrhoea, inflammation, and sore throat as well as immune disorder. To date, extraction of andrographolide from *Andrographis paniculata* is usually carried out using liquid organic solvent.The extraction was carried out by employing methanol as solvent using standard soxhlet method. Five grams of ground-dried *Andrographis paniculata* leaves was extracted using 1.50×10^{-4} m^3 of methanol at different extraction times. The crude methanolic extracts were then analysed their andrographolide content using high performance liquid chromatography. A mathematical model based on rapid mass transfer at the interphase of the solid-liquid surface and introduction of volumetric mass transfer coefficient has been developed to describe the extraction phenomena. The final form of the model is $E_s = 0.12 \times (1 - e^{-1.69E-04\, t})$, where E_s = total extract, (g) and t = extraction time, (second). The model showed good agreement with the experimental data by generating *AARD* of about 0.46 %.

Keywords: modelling, extraction, andrographolide, Andrographis paniculata, soxhlet

1.0 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 of 0.30 - 0.70 m 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].

Three main diterpenoid lactones identified in the *Andrographis paniculata* leaves were andrographolide, neo-andrographolide 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 and deoxyandrographolide are shown in Figure 1. Andrographolide can be easily dissolved in methanol, ethanol, pyridine, acetic acid and acetone, but slightly dissolved in ether and water. Its physical properties were summarised as follows [3]: m.p. is 228^o – 230^oC and ultraviolet spectrum in ethanol: λ_{max} is 223 nm. Andrographolide is the main component in the leaves of *Andrographis paniculata*. Hitherto, there are some techniques that can be used for the analysis of andrographolide such as thin

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layer chromatography (TLC) [4, 5], high - performance liquid chromatography (HPLC) [2, 6, 7] and crystallisation techniques [3].

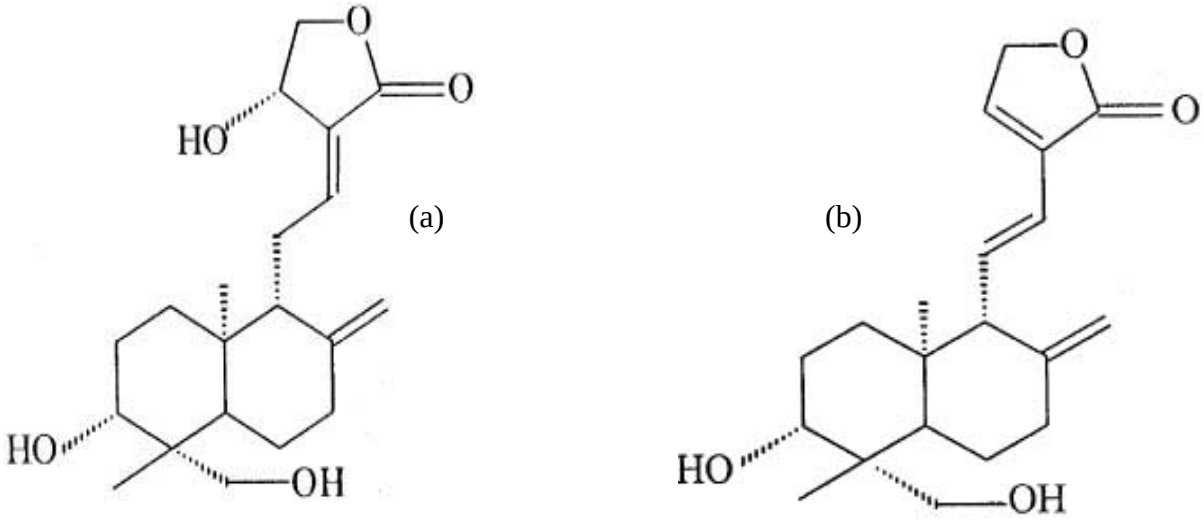

Figure 1. Molecular structure of (a) andrographolide and (b) deoxyandrographolide [3]

Conventional soxhlet extraction is one of the most common methods of separating bioactive components from natural resources. The most outstanding advantages of conventional soxhlet extraction are as follows [8]:

(1) The sample is repeatedly brought into contact with the fresh portions of the solvent, thereby helping to displace the transfer equilibrium.
(2) The temperature of the system remains relatively high since the heat applied to the distillation flask reaches the extraction cavity to some extent.
(3) No filtration is required after the leaching step.
(4) Sample throughput can be increased by simultaneous extraction in parallel.
(5) It has the ability to extract more sample mass than other extraction methods and non-matrix dependent.

However, for toxicological reasons, drug and medicine manufacturers are increasingly required to minimise the number of solvents employed in pharmaceutical process. Certain types of solvents of known toxicity and environmental hazard (e.g. benzene, chlorocarbons) are no longer permitted to be used in the manufacture of pharmaceuticals. At the same time, the maximum content of individual solvents in drugs is regulated. The presence of a solvent in the extract may also affect the kinetics of crystallisation and the shape of the product's crystals (morphology), which is an important factor that determines the product's quality [9].

In order to optimise the utilisation of solvent in the solid-liquid extraction of bioactive components from natural resources using their suitable solvent, estimation of the extract yield obtained is necessary. The objective of this work is to develop a simple mass transfer model for the estimation of extract yield in a soxhlet extraction system.

2.0 Modelling of Solid-Liquid Extraction in a Soxhlet Extractor

The phenomenon of solid-liquid extraction in the soxhlet extractor is schematically shown in Figure 2.

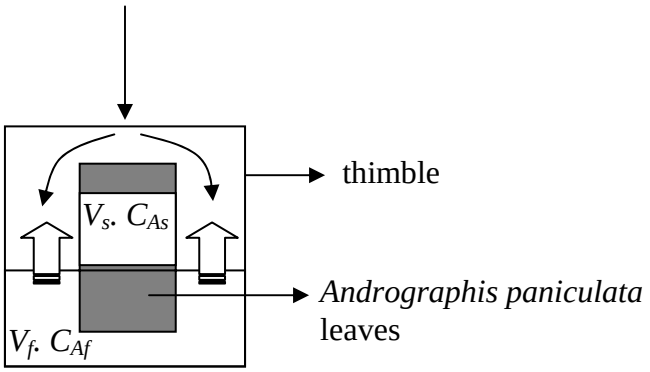

Figure 2 Mass balance in the cellulosic extraction thimble

In order to describe the andrographolide transfer from the ground-dried leaf particles to the bulk of liquid solvent, the following hypotheses were used:

1. Every leaf particle is symmetrical and homogeneous.
2. The mass transfer coefficient is constant in all experiments.
3. The solvent in the extractor is perfectly mixed. The transfer resistance in the liquid phase is negligible and the andrographolide concentration in the solvent depends only on time.
4. The transfer of the andrographolide is a diffusion phenomenon and independent of time.
5. At the interface, the concentration of andrographolide in the solution between the internal liquid (in pores) and external to particles are equal.

The mathematical model for mass transfer in the soxhlet extraction can be then developed as follows:
The total mass balance of solute in the leaf particles can be represented by:

$$-r_A = -\frac{d\left(V_s.C_{As}\right)}{dt} \tag{1}$$

Based on the assumption that the solute is uniformly distributed in the solid phase, the mass balance for the solute in the solid phase can be written as follows:

$$\frac{d(V_s.C_{As})}{dt} = Vs.\frac{dC_{As}}{dt} + C_{As}\frac{dV_s}{dt} \tag{2}$$

Since the solute content in the leaf particles is very little, the leaf particles do not shrink after the solute is released into the liquid solvent. Therefore, equation (2) can be simplified into:

$$\frac{d(V_s.C_{As})}{dt} = V_s.\frac{dC_{As}}{dt} \tag{3}$$

When a solute material is transferred from one phase to another across an interface that separates the two, the resistance to mass transfer in each phase causes a gradient concentration in each phase [10]. However, a single film of interphase mass transfer is adequate to represent a system involving liquid – solid or gas- solid mass transfer [11]. No experiment in this work was devoted to measure the total mass transfer surface area of the leaf particles. Therefore, the concept of flux or solute mass transfer per unit surface area is

not applicable. As suggested in the literature [11], the volumetric mass transfer coefficient (k_{sa}) is therefore introduced to solve this problem.

The volumetric mass transfer of solute from the solid particle surface into the bulk liquid is given by:

$$r_A = k_{sa}.V_s.(C_{As} - C_{Af}^{*}) \quad (4)$$

where k_{sa} and V_s are the total volumetric solid - liquid mass transfer coefficient at the solid phase and the total volume of solid particles, respectively. The C_{Af}^{*} is the saturation concentration of solute in the liquid phase, which is equal to the equilibrium concentration of solute at the solid surface.

The interphase mass transfer between the solid surface and liquid is assumed to be very fast causing no accumulation of solute in the solid - liquid interface and therefore the concentration of solute in the solid surface is always in equilibrium with the concentration of solute in the bulk liquid. Linear correlation was taken to represent this assumption [12]:

$$C_{Af}^{*} = K.\, C_{As}. \quad (5)$$

where K is the equilibrium adsorption coefficient, while C_{As} is the concentration of andrographolide in the leaf particles.

Substitution of equations (4) and (5) into equation (3) and rearranging it, the following equation is generated:

$$\frac{dC_{As}}{C_{As}} = -\left[k_{sa}\left(1-K\right)\right]dt \quad (6)$$

Integration of equation (6) from $t = 0$ to $t = t$ and $C_{As} = C_{AS0}$ to $C_{As} = C_{As}$, resulted in the following equation:

$$C_{As} = C_{AS0}.e^{-k_{sa}(1-K)t} \quad (7)$$

Since the value of $k_{sa.}(1- K)$ is always constant, therefore this value was taken as D. The amount of solute collected in the liquid phase is then calculated using the following equation:

$$E_s = C_{Af}.V_f = V_s.\left(C_{As0} - C_s\right) = V_s.C_{As0}\left(1-e^{-Dt}\right) \quad (8)$$

while $V_s.\, C_{As0}$ is the initial solute content in the leaf particles. The final form of the equation obtained is:

$$E_s = B.(1-e^{-Dt}) \quad (9)$$

where E_s = total extract, g.
t = extraction time, seconds.
B & D = equation constants.

3.0 Materials and Methods

3.1 Materials

Dried - ground leaves of *Andrographis paniculata* were collected from Malaysian Agricultural Research and Development Institute (MARDI). Andrographolide standard compound having 98 % of purity was supplied by Sigma - Aldrich (M) Sdn. Bhd. and deoxyandrographolide standard compound with 99 % of purity was purchased from LKT Laboratories, Inc. (USA). Methanol (*Merck*, HPLC grade, 99.8%) was purchased from Bibi

Saintifik Sdn. Bhd., while deionised water was generated in the Analytical Laboratory, Department of Chemical Engineering, University of Malaya

3.2 Solvent Extraction

Prior to solvent extraction study, 5 grams of dried - ground leaves of *A. paniculata* was placed in a cellulose thimble (25 mm × 100 mm). An amount of 1.50×10^{-4} m^3 of solvent was used for the extraction using a standard Soxhlet method at various extraction times in a Soxhlet extraction system (BÜCHI Extraction System Model B-811, Switzerland). The solvent extracts were then concentrated using vacuum rotary evaporator (BÜCHI Rotavapor Model R-144, Switzerland) and completely dried in an atmospheric oven.

3.3. HPLC Analysis of Andrographolides

Analyses were performed using high-performance liquid chromatography (HPLC) with a system from Shimadzu (Japan). Prior to HPLC analysis, the dried extract samples were dissolved in methanol/water (54/46, v/v) solution to form 1.00×10^{-5} m^3 solution. They were then sonicated for 30 minutes and filtered using N-25-4 Nylon Iso-Disc Filter having 0.45 μm pore diameter. The column used for analysis was reverse phase C18-Thermo Hypersil ODS 250 mm × 4.6 mm, 5 μm particle diameters, while a UV detector was used. The volume injection was 2.00×10^{-8} m^3. The mobile phase was methanol/water mixture (54/46, v/v) and it was delivered into the column at constant 1.67×10^{-8} m^3/s flow rate. During the analysis the wavelength of the UV detector was programmed as 250 nm from 0 to 4.5 minutes followed by 223 nm from 4.5 to 10 minutes. Chromatographic peaks were identified by comparison with the retention time of the standards. Linear calibrations of standards at accuracy of more than 99 % were carried out for the quantification of the A. *paniculata* extracts. Single injection of solvent (blank) was also made to determine the retention time of the solvent. The calibration graphs for andrographolide and deoxyandrographolide were linear from 1.1-24 and 1.05-24 $\times 10^{-3}$ kg/m^3, respectively. Recoveries were obtained as 99.1-99.2% with Relative Standard Deviation (RSD) of 1.1-1.8%.

4.0 Results and Discussions

The extracts obtained from the extraction of dried – ground leaves of *Andrographis paniculata* at different extraction time are tabulated in Table 1. As expected, the extracts obtained in this work increased with the increase of extraction time. This is because more solute can be extracted when extraction time is increased, due to longer solid – liquid contact time.

The model was then verified by the experimental data obtained from extraction of five grams of dried – ground leaves of *Andrographis paniculata* using pure methanol as solvent, while extraction time was varied from 3600 to 43200 seconds. The result of this work is presented in Figure 3. It was found that extraction rate was very rapid in the beginning of the extraction process due to the large mass transfer driving force, namely the difference of solute concentration at the solid and liquid phase. However, prolong of extraction time resulted in the reduction of extraction rate due to the reduction of mass transfer driving force. There was no significant increase of extract collected after 25200 seconds of extraction time. The model agreed well with the experimental data and the constant B and D were found to be 0.12 and 1.69×10^{-4}, respectively. The comparison of the experimental and calculated extract weight is

also tabulated in Table 1. The absolute average relative deviation (*AARD*) of the model was found to be 0.46 %. As can be seen in Figure 3, the solid line represents the proposed model and almost all the experimental data fall into the solid line showing good agreement between the model and the experimental data.

Table 1. Comparison of experimental and calculated extract weight

Time (second)	**Extract (g)**		**ARD** [1)]
	Experimental	**Calculated**	**(%)**
0	0	0	0
3600	0.0556	0.0542	1.18
7200	0.0826	0.0837	0.94
10800	0.0993	0.0998	0.44
18000	0.1140	0.1134	0.53
25200	0.1178	0.1174	0.34
32400	0.1185	0.1186	0.08
43200	0.1188	0.1190	0.19
		AARD [2)]**=**	0.46

1) $ARD = ABS\left|\frac{Calculated - Experiment}{Calculated}\right| \times 100\%$

2) $AARD = \frac{\sum_{1}^{N} ARD}{N}$, where N = number of experiments

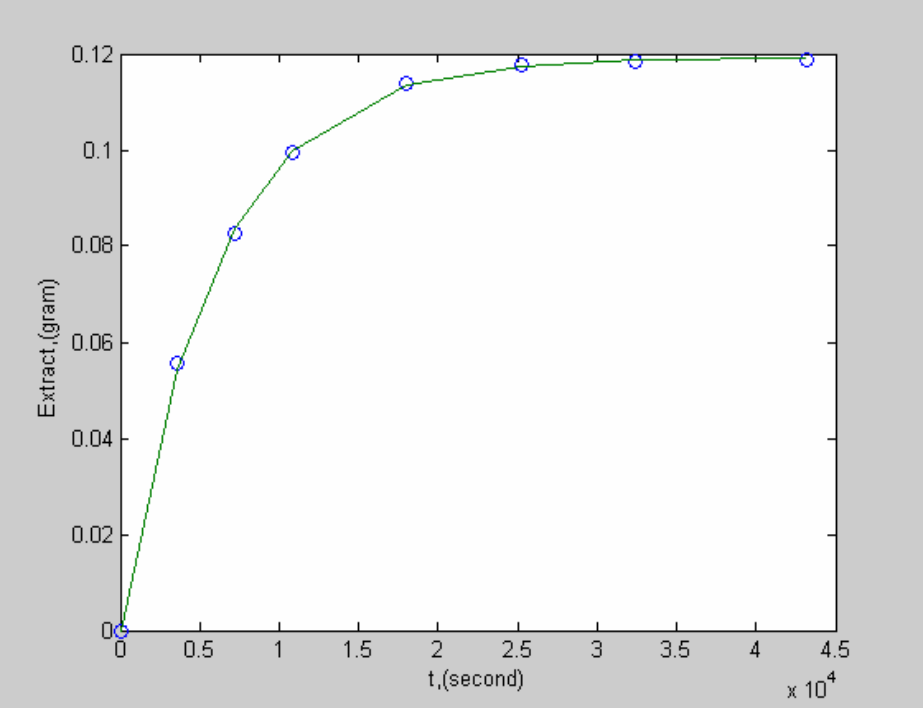

Figure 3 Comparison of extract weight calculated from the model and experimental data

The introduction of volumetric mass transfer coefficient and rapid mass transfer approach in the interface of solid – liquid were found to be adequate for the system under study. This finding is useful especially when the measurement of the total surface area of the solid particles is not possible. This model also reduces the number of experiments to obtain the equilibrium adsorption constant. However, the constants of the proposed equation will be dependent on the solvent and material used in the extraction system.

5.0 Conclusions

A mathematical model based on rapid mass transfer at the interphase of solid - liquid surface and introduction of volumetric mass transfer coefficient has been developed to describe the extraction phenomena. The final form of the model is $E_s = 0.12 \times (1 - e^{-1.69E-04\ t})$. The model showed good agreement with the experimental data by generating *AARD* of about 0.46 %.

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References

[1] Calabrese, C., S. H. Berman, and J. G. Babish. 2000. A phase I Trial of Andrographolide in HIV Positive Patients and Normal Volunteers., *Phytother. Res*. 14: 333 - 338.
[2] Wongkittipong, R., L. Prat, S. Damronglerd, and C. Gourdon. 2000. Solid-Liquid Extraction of Andrographolide from Plants-Experimental Study, Kinetic Reaction and Model. *Sepn. Purifn. Technol*. 40: 147-154.
[3] Rajani, M., N. Shrivastava, and M. N. Ravishankara. 2000. A Rapid Method for Isolation of Andrographolide from *Andrographis Paniculata* Nees (Kalmegh). *Pharmaceut. Biol.* 38: 204 -209.
[4] Choudhury, B. R., S. J. Haque, and M. K. Poddar. 1987. *In vivo* and *in vitro* Effects of Kalmegh (*Andrographis paniculata*) Extract and Andrographolide on Hepatic Microsomal Drug Metabolising Enzymes. *Planta Med*. 53: 135-140.
[5] Puri, A., R. P. Saxena, and K. C. Saxena. 1993. Immunostimulant Agents from *Andrographis paniculata*. *J. Nat. Prod.* 56: 995-999.
[6] Tang, F., D. X. Xiang, and H. D. Li. 2000. Reversed-Phase HPLC Determination Of Andrographolide and 14-Deoxy-11, 12-Didehydroandrographolide In Andrographis *Paniculata* And Its Preparations. *Yaowu-Fenxi-Zazhi*. 20 (6): 420-422.
[7] Li, W. and J. F. Fitzloff. 2004. HPLC - Photodiode-Array Determination of Bioactive Diterpenoids from Plant Materials and Commercial Products of *Andrographis paniculata*, *J. Liq. Chromatogr. Relat.Technol*. 27(15): 2407-2420.
[8] Luque de Castro, M. D. and L. E. Garcia-Ayuso. 1998. Soxhlet Extraction of Solid Materials: An Outdated Technique with a Promising Innovative Future. *Anal. Chim. Acta*. 369: 1-10.
[9] Kolar, P., J. W. Shen, A. Tsuboi, T. Ishikawa. 2002. Solvent Selection for Pharmaceuticals. *Fluid Phase Equil*. 194 -197: 771-782.
[10] Perry, R H., D. W. Green, and J. O. Maloney. 1988. *Perry's Chemical Engineer's Hand Book*. New York: McGraw-Hill Book Company.
[11] Sediawan, B. S. and A. Prasetyo. 1997. *Pemodelan Matematis dan Penyelesaian Numeris dalam Teknik Kimia dengan Pemrograman Bahasa BASICc dan FORTRAN*. Yogyakarta: Andi
[12] Goto, M., M. Sato., and T. Hirose. 1993. Extraction of Peppermint Oil by Supercritical Carbon Dioxide. *J. Chem. Eng. Japan*. 29 (4): 401-407.