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Solid–liquid extraction of andrographolide from plants—experimental study, kinetic reaction and model

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Abstract

Solid–liquid extraction is performed from leaves and stems of *Andrographis paniculata* in ethanol–water solvent, in order to obtain andrographolide. The first part of this work concerns the acquisition of the raw plant geometric and physicochemical characteristics. Then batch experiments are done in order to study the influences of the operating parameters (temperature, nature of the solvent and particles size). Furthermore, the destruction of the solute with high temperature is also studied. In the last part, a two-shape extraction model is proposed and compared with experimental data. This model includes the shape factor of the particles population composed of stems and leaves (cylinders and plates).

Keywords: Andrographolide; Solid–liquid extraction; Reaction; Batch experiment; Diffusion model

1. Introduction

Andrographis paniculata grows widely in the tropical area of Asia, and belongs to the acanthaceae family. It has an annual growth of 30–70 cm height. Its common name is King of Bitters. In Thailand, this plant has been extensively used for traditional medicine and helps against fever, dysentery, diarrhea, 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].

The objective of this work is to study the solid–liquid extraction of andrographolide. The first part deals with physicochemical properties acquisition from the dried raw plant. Then, in a second part, batch reactor experiments are performed in order to identify the influence of the operating parameters. In the third part, a mass transfer model is proposed, taking into account the differences in shapes of plant parts (leaves and stems) and initial concentrations of

the solute in the solid. The model is then compared with experimental data.

From a global point of view, this work will allow us to choose the good solvent and to identify the influence of the main operating parameters. This study will be integrated in a classical pharmaceutical industry methodology as the first step of the development and the implementation of a complete process.

2. Material and methods

2.1. Compositions and physicochemical properties

Three main diterpenoid lactones can be isolated from *A. paniculata*: andrographolide, neo-andrographolide and deoxyandrographolide. The molecular formula of andrographolide, which is an unsaturated trihydroxy lactone, is C₂₀H₃₀O₅. It can be easily dissolved in methanol, ethanol, pyridine, acetic acid and acetone but slightly dissolved in ether and water. Its physical properties were reported as follows [2]: m.p. is 228–230 °C, ultraviolet spectrum in ethanol: λ_{max} 223 nm. Andrographolide is the main component in mass in the plant. There are many general techniques to analyze andrographolide such as thin-layer chromatography

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Nomenclature

A	the specific area of the solid particle (m^2)
C	concentration in solid (kg/kg)
C_L	concentration in solution (kg/l)
C_S	concentration of solute in the solvent (kg/l)
C_1	concentration in leaves (kg/kg)
C_{10}	initial concentration in leaves (kg/kg)
C_2	concentration in stems (kg/kg)
C_{20}	initial concentration in stems (kg/kg)
D	diffusion coefficient of material (m^2/s)
e	diameter or thickness of particle solid (m)
E_a/R	activation energy/ R (K)
k	the pre-exponential factor (s^{-1})
K	constant value
l	length of solid (m)
m_0	total mass of solid (kg)
m_0^f	initial mass of solution in leaves (kg)
m_0^t	initial mass of solution in stems (kg)
R	the gas constant, 1.987 (cal/K mol)
T	temperature ($^{\circ}\text{C}$, K)
V_L	volume of solvent (m^3)
V_1	total volume in leaves (m^3)
V_2	total volume in stems (m^3)

Greek letters

ε	porosity (dimensionless)
η	viscosity (kg/m s)
ρ	density of solid (kg/m^3)
φ	geometric shape factor (dimensionless)

(TLC), column chromatography, and crystallization technique. In pharmaceutical chemistry, focus is made on extraction of the pure crystal of andrographolide [2].

2.2. Plant structure and geometric characteristics

A. paniculata samples were scanned with a Scanning Electronic Microscope in order to accomplish visual observations on its structure, especially the thickness and the shape of the plants parts (Fig. 1). As a result of the observations, the particles could be separated into two shape classes: cylinder for the stems and plate for the leaves. The average thickness of the leaves was $35\ \mu\text{m}$ and the average diameter of the stems was $700\ \mu\text{m}$. The density of the plant (ρ) was determined by helium pycnometry and its porosity ($\varepsilon = \text{volume of pores}/\text{total volume of the solid}$) equal to $837\ \text{kg m}^{-3}$ and 0.33, respectively.

2.3. HPLC analysis of andrographolide

The calibration curve was obtained using standard pure crystal coming from pharmaceutical department of Chulalongkorn University.

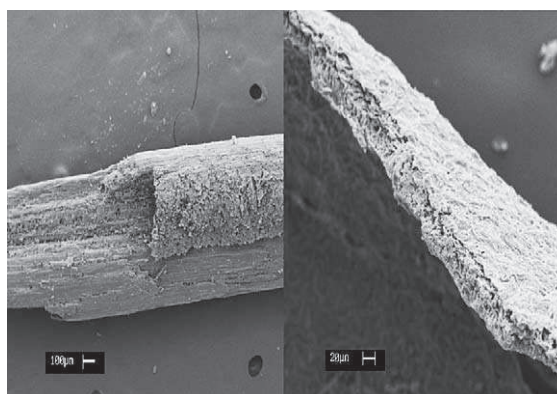


Fig. 1. Stem and leaf in SEM.

Analyses were performed by high-performance liquid chromatography (HPLC) with a system from Thermo Finnigan (Paris, France), Serie AS1000XR, with a pump Serie P1000XR and a Lichrosorb column (PR18, $123\ \text{mm} \times 4.6\ \text{mm}$) with a UV detector (Thermo Finnigan, France), Serie UV2000. The volume injection was $25\ \mu\text{l}$. The mobile phase was methanol/water (1/1, v/v) at constant $1\ \text{ml/min}$ flow rate. The UV detector was set to the wave length of $223\ \text{nm}$. In this study, all HPLC analysis have been done at least twice, with less than 1% difference.

2.4. Soxhlet experiments

The objective of the soxhlet experiments is to determine the initial concentration of the solute in the raw material.

The plant was separated by sieving into five size classes ($0.10\text{--}0.30$, $0.30\text{--}0.45$, $0.45\text{--}0.60$ and $0.60\text{--}0.80\ \text{mm}$). Then, a known mass of about $10\ \text{g}$ of *A. paniculata* was introduced in the soxhlet with $500\ \text{ml}$ of a 90% in volume of ethanol solution. The extraction was performed during $3.5\ \text{h}$ at normal pressure. Experimental conditions and measured concentrations of andrographolide are presented in Table 1. Each experiment has been done three times, with a variation of less than 3% in the results.

For experiments 3–7 in Table 1, the mass percentage of stems and leaves in each class can be calculated from the results of experiments 1 and 2, assuming that the matter is homogeneous. The percentage of leaves was then 80% in mass of the samples, except for the finest particle class (about 50% of leaves). But this class is not important in mass (less than 3%). The amount of andrographolide in leaves was then three times higher than in stems.

Furthermore, an experiment with pure water has been performed in the soxhlet, in the same operating conditions. After the soxhlet experiment was finished, less than $1\ \text{mg/g}$ of solid was extracted. A reaction of destruction of the solute should then be activated at high temperatures. This will be discussed in Section 3.5.

Table 1
Concentration of andrographolide and percentage of stems and leaves

No.	Diameter (mm)	[Andrographolide] (mg/ml solvent)	[Andrographolide] (mg/g solid)	Stems (%)	Leaves (%)	Nb of replication
1	Only leaves	0.888	44.48	0	100	3
2	Only stems	0.304	15.3	100	0	3
3	$d < 0.1$	0.598	29.91	49.68	50.32	3
4	$0.1 < d < 0.3$	0.751	37.23	22.12	77.88	3
5	$0.3 < d < 0.45$	0.795	39.11	19.97	80.03	3
6	$0.45 < d < 0.6$	0.765	39.02	21.18	78.82	3
7	$0.6 < d < 0.8$	0.766	38.23	20.13	79.87	3

Table 2
Experimental conditions of batch reactor

No.	Temperature (K)	Mass solid (g)	Diameter (mm)	% ethanol	Nb of replication
1	22	10	0.6–0.8	80	3
2	22	10	0.6–0.8	70	3
3	22	20	0.6–0.8	60	3
4	22	20	0.6–0.8	0	3
5	22	10	0.45–0.6	60	1
6	22	10	0.1–0.3	60	1
7	40	10	0.6–0.8	60	3
8	50	10	0.6–0.8	60	3
9	60	10	0.6–0.8	60	3
10	22	10	0.6–0.8	60	3
11	22	5	0.6–0.8	60	3

2.5. Batch reactor experiments

The objective of the batch experiments is to measure global kinetic of extraction and to analyze the influences of the operating parameters.

A volume of 500 ml of 0, 60, 70 and 80% in volume of ethanol solution was introduced in the 11 reactor, equipped with a mechanical agitation and a jacket. At initial time, a known mass of a particular size class of *A. paniculata* was introduced in the reactor. Liquid samples were taken at different times and the andrographolide concentration in the liquid phase was then measured by HPLC. As the analytic sample volume was 1 ml (compared with a total of 500 ml) the measurement was supposed to have no influence on the process. The operating conditions of the batch experiments are represented in Table 2.

3. Results and discussion

3.1. Effect of solid/liquid ratio

Fig. 2 shows the evolution of the concentration of andrographolide for different amount of solid particles. The andrographolide concentrations in the extracted solvent are nearly linear proportional to the initial mass of solid. These results show that in the experimental range, there is no solubilization limits in the solvent at 22 °C. Furthermore, this

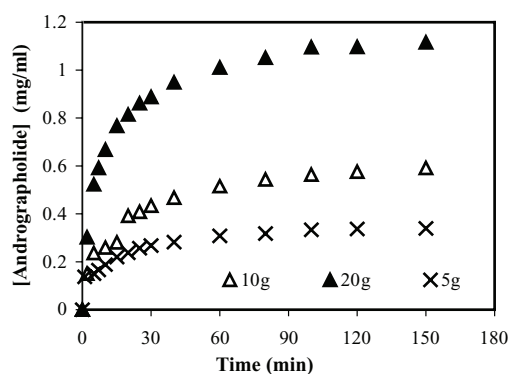


Fig. 2. Influence of the solid initial mass on the solute yield with 60% ethanol at 22 °C and diameter of solid 0.6–0.8 mm.

result allow us to compare the experiments with different initial solid mass (by using a reference mass).

3.2. Effect of solvent concentration

Fig. 3 shows the evolution of extracted andrographolide for experiments 1–4. The final concentration increased with the percentage of ethanol.

Ethanol and water have similar solubility properties because they contain hydroxyl group which is hydrophilic. Furthermore, the ethanol is an organic solvent whereas water is an inorganic solvent, andrographolide ($C_{20}H_{30}O_5$) which is a large hydrocarbon molecule can be better dissolved in

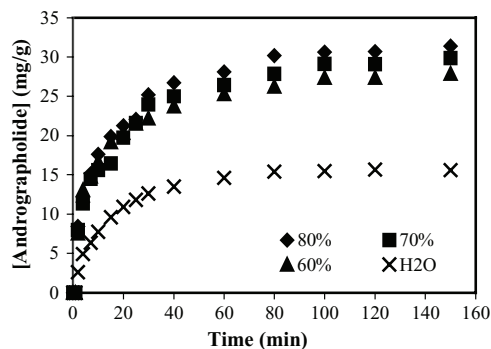


Fig. 3. Influence of the percentage of ethanol on the solute yield with 10g samples at 22 °C and diameter of solid 0.6–0.8 mm.

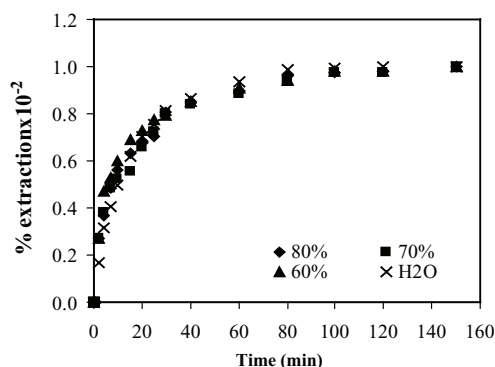


Fig. 4. Influence of the percentage of ethanol on the solute mass percentage with 10 g samples at 22 °C and diameter of solid 0.6–0.8 mm.

ethanol although andrographolide has more polar function [3].

Fig. 4 shows the evolution of the percentage of final extracted concentration. This percentage is determined by dividing each concentration by the final concentration. This representation allow us to study the dynamic of the phenomena, independently of the equilibrium. The curves show that the percentage of ethanol has no significant influence on the kinetic of extraction.

3.3. Effect of particle size

The graph in Fig. 5 shows the evolution of extracted andrographolide for experiments 3, 5, 6 and 10, drawn for the same amount of solid. There is no differences in the final concentrations in the experiments. This is in accordance with the soxhlet results.

Fig. 6 shows the evolution of the percentage of final extracted concentration. The curves show that the rate of extraction slightly increased with the decrease of the size of the particles. It is a classical result since the pore diffusion path increases with the particle size. But as the particles are sieved, the size classes obtained depend on the diameter for the stem (cylindrical shape) and on the width for the leaves

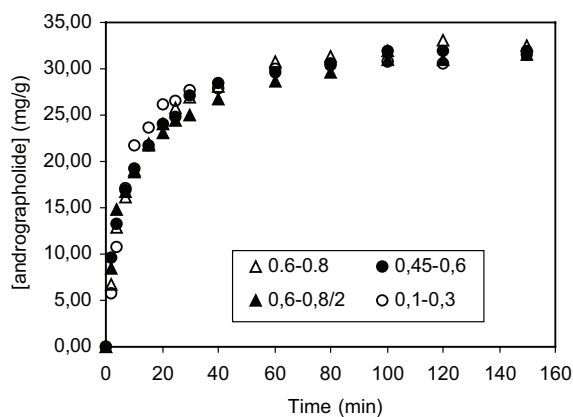


Fig. 5. Influence of the particle size on the solute yield with 10 g samples at 22 °C and 60% ethanol.

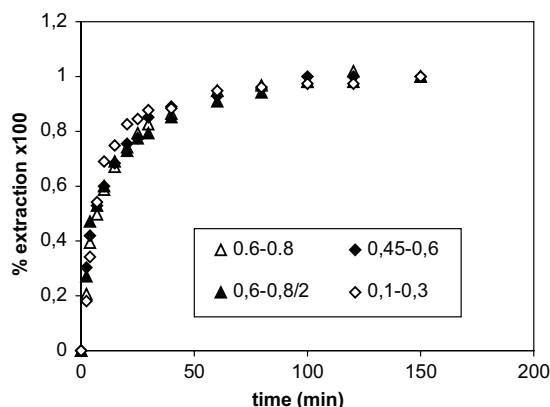


Fig. 6. Influence of the particle size on the solute mass percentage with 10 g samples at 22 °C and 60% ethanol.

(plate shape). Since, in the case of the leaves which contain the main amount of solute, the relevant dimension for the diffusion is the thickness, the effect of the size cannot be very important in the experimental results.

3.4. Effect of temperature

Fig. 7 shows the evolution of extracted andrographolide for experiments 7–9. (Experiment 3 does not appear in this figure because the used plant has a different location and then has not the same initial concentration.) The curves show that the final concentration increased with the temperature. This must be due to the classic thermodynamic effect of temperature on solubilization inside of the solid.

Fig. 8 shows the evolution of the percentage of final extracted concentration for experiments 3, 7, 8 and 9. The curve shows that the temperature increased the kinetic of extraction.

Since the diffusion coefficient can be written as a function of the temperature and the viscosity [4]: $D = f(K(T/\eta))$, the coefficient of diffusion D will increase with the temperature and so will do the apparent kinetic of extraction.

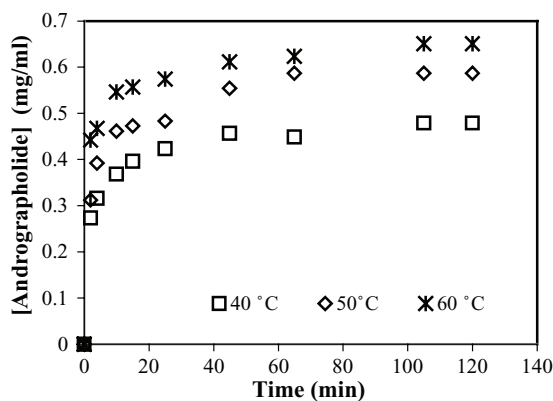


Fig. 7. Influence of the temperature on the solute yield with 10 g samples with 60% ethanol and diameter of solid 0.6–0.8 mm.

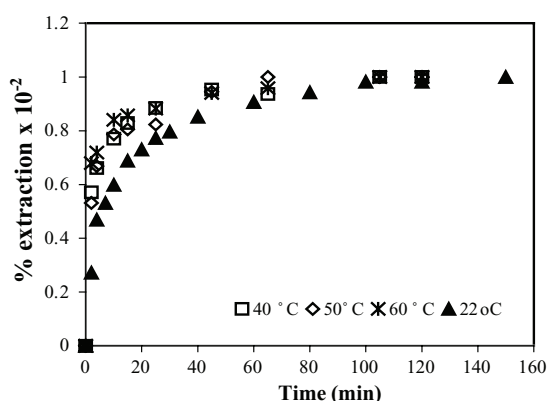


Fig. 8. Influence of the temperature on the solute mass with 10 g samples with 60% ethanol and diameter of solid 0.6–0.8 mm.

3.5. Kinetic of destruction of andrographolide

It might seem that raising the temperature would always be a good way to save time by making reaction go faster. However, the problem with raising the temperature is that all reactions are accelerated, including all the unwanted side reactions. Since andrographolide is a lactone, the opening of the lactone ring can be the most likely destruction mechanism. However, the lactone ring would react in different manner with water and ethanol. The former will open the ring by hydrolysis, whereas the latter will do it by trans-esterification. Hydrolysis is expected to be much faster reaction compared to trans-esterification. Then the rate of destruction should be depending both on the temperature and on the composition of the solvent.

The objective in this part was not to make a complete study of the rate of destruction but to explain the observed phenomena in soxhlet experiments (i.e. the very low amount of extracted andrographolide with low percentage of ethanol or pure water solvent) and help the temperature choice for the industrial implementation of the process. A simple first-order reaction was then be supposed in respect of andrographolide concentration to represent the global kinetic of destruction.

In a 1 l reactor, equipped with a mechanical agitation and a jacket, 500 ml of an aqueous ethanol solution was introduced. At initial time, a known mass of *A. paniculata* was introduced in the reactor and the extraction was performed at 22 °C during 3 h. Solid and liquid phase were then basically separated with a filter just to obtained most of the liquid phase. The solution was then heated to its boiling point and samples were taken and analyzed in order to obtain the kinetic of destruction of andrographolide. Three initial solutions were studied: 100% of water, 75 and 15% of ethanol so the destruction were done at three different temperatures: 97, 92 and 86 °C, measured in the reactor.

Fig. 9 shows the evolution of the reduction of andrographolide concentration for the different temperatures (initial kinetics) in logarithm scale.

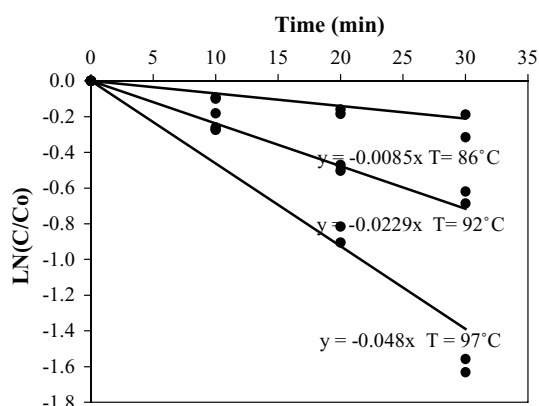


Fig. 9. Influence of the temperature on the kinetic of destruction of andrographolide (logarithm scale).

The Arrhenius equation implies that the rate of reaction depends on the fraction of molecules with kinetic energy of at least E_a . The kinetic can then be approached by a first order in respect of the andrographolide concentration. The apparent kinetic constant k increased with T and it can be expressed by the Arrhenius law:

$$k = k_0 e^{-E_a/RT} \quad (1)$$

k_0 and E_a/R are determined by drawing $\ln(k)$ versus $1/R$ (in Fig. 10):

$$k_0 = 3.89 \times 10^{25} \text{ min}^{-1} \quad (2)$$

$$\frac{E_a}{R} = 23\,200 \text{ K} \quad (3)$$

With these parameters it is possible to determine the percentage of andrographolide destroyed in the solution after 3 h as a function of the temperature. The temperature maximum to have a percentage inferior to 98% is 340 K.

Since the rate of destruction should be a function of both the temperature and the composition of the solvent, this first approach can certainly not reproduce all the experiments (as for the 370 K points). Then the obtained results in this chapter need more experiments in order to confirm the results but are enough for the main goal of this work.

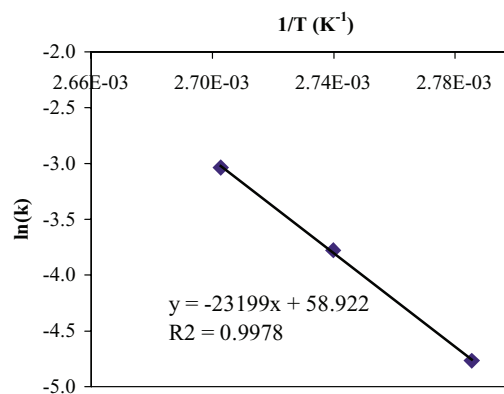


Fig. 10. Influence of the temperature on the constant of kinetic.

4. Mass transfer model

4.1. Model hypothesis

In order to describe the andrographolide transfer, the following hypotheses were used:

1. The solid particle has two shapes: plane shape corresponding to leaves and cylindrical shape corresponding to stems. The solute diffusion takes place in the transverse direction and is monodirectional.
2. Every particle is symmetrical and homogeneous.
3. The diffusion coefficient is constant in all experiment. The andrographolide concentration in a particle, C_1 , depends only on position and time.
4. The solvent in the batch reactor is perfectly mixed. The transfer resistance in the liquid phase is negligible and the andrographolide concentration in the solvent depends only on time.
5. The transport of the andrographolide particles is a diffusion phenomenon. It is described by a diffusion coefficient that relates to D_1 (or D) and independent of the time.
6. At the interface, the concentrations of every species in solution between the internal liquid (in pores) and external to particles are supposed to be equal.
7. Initial concentration in each experiment is calculated using the experiment at 150 min because the ability of extraction for each solvent is different.
8. The mass percentage of leaves in all samples is supposed to be about 80%.

4.2. General equations

The general diffusion model of solid–liquid extraction [5–7] is

$$\frac{\partial C(t, x)}{\partial t} = D \frac{1}{x^{\varphi-1}} \frac{\partial}{\partial x} \left(x^{\varphi-1} \frac{\partial C(t, x)}{\partial x} \right) \quad (4)$$

where t is the time, x the radial distance in the direction of material transfer (the thickness of plate is equal to $2e$). φ the geometric shape factor values for the shapes involved in the experiment.

For the leaves:

$$\frac{\partial C_1}{\partial t} = D \frac{\partial^2 C_1(t, x)}{\partial x^2} \quad (5)$$

For the stems:

$$\frac{\partial C_2(t, x)}{\partial t} = D \left(\frac{1}{x} \frac{\partial C_2(t, x)}{\partial x} + \frac{\partial^2 C_2(t, x)}{\partial x^2} \right) \quad (6)$$

The mass balance is

$$\begin{aligned} C_L(t) V_L + \int_0^e C_1(x, t) dV_1(x) + \int_0^e C_2(x, t) dV_2(x) \\ = C_{10} V_1 + C_{20} V_2 \quad \forall t \end{aligned} \quad (7)$$

4.3. Initial and boundary conditions

4.3.1. Initial conditions ($t = 0$)

- For the continuous phase: $C_s = 0$ (concentration of solution in the solvent),
- For the dispersed phase: $C_1 = C_{10}$ and $C_2 = C_{20} \forall x$.

4.3.2. Boundary conditions

- At the center of a particle ($x = 0$)

$$\left(x^{\varphi-1} \frac{\partial C(t, x)}{\partial x} \right)_{x=0} = 0 \quad \forall t \quad (8)$$

- At the interfacing ($x = e$), equality of flux of andrographolides (in mass):

The retiring flux of the solid (which has to be integrated for all the solid) is:

$$F = -DA \left(\frac{\partial C(t, x)}{\partial x} \right)_{x=e} \quad (9)$$

The incoming flux in the liquid is:

$$F = V_L \frac{dC_L(t)}{dt} \quad (10)$$

where V_1 is the solvent volume and A the specific area.

4.3.3. Numerical treatment

The particle diameter was discretized in space with second-order finite differences. For the boundary equations, a same order finite difference was used. The equations were treated with a Crank–Nicolson method [6]. The coefficient of diffusion was then modified to fit the experimental points using a simple dichotomy method based on a quadratic criterion.

5. Comparison between model and experiments

The diffusion coefficient D was adjusted in order to fit the experimental values for experiment 3, using the plate shape or the cylinder shape. Fig. 11 shows that the proposed model, with the identified value of $8.43 \times 10^{-14} \text{ m}^2/\text{s}$ for D , quite well described the extraction phenomena. However, it was not possible to completely fit the experimental points using only one-particle shapes (plate or cylinder) [8].

As in the batch experiments, 0.6–0.8 mm size class particles were used, soxhlet experiments gave that the percentage of leaves is 80%. The extraction was then simulated taking into account the two different shapes. Results are shown in Fig. 12.

A good fitting between experimental and numerical data was obtained. The identified values of the coefficient of diffusion for all the experiments are in Table 3, including the values of the identification criterion. The coefficient of diffusion varies from 8.43×10^{-14} to $11.05 \times 10^{-14} \text{ m}^2/\text{s}$ in ethanol solution at 22 °C. Temperature can increase this

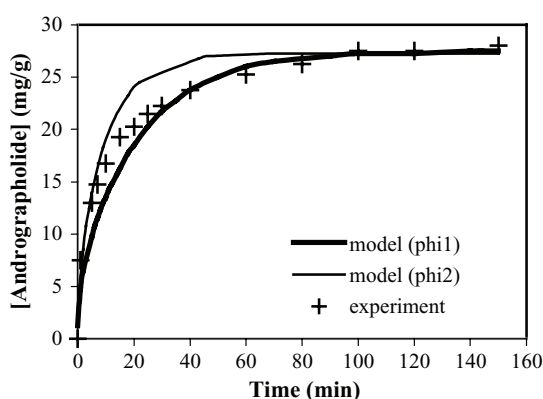


Fig. 11. Comparison between experimental data, plate model and cylinder model with 60% ethanol at 22 °C and diameter of solid 0.6–0.8 mm.

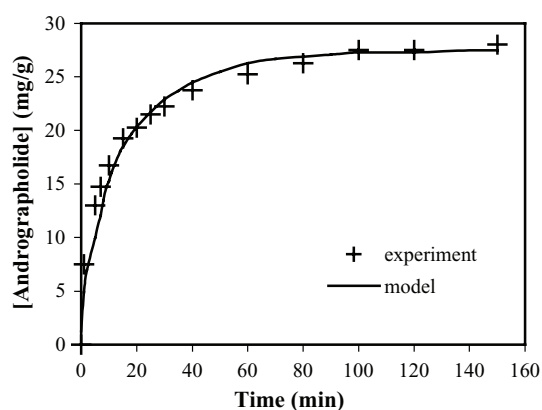


Fig. 12. Comparison between experimental data and the final model 60% ethanol at 22 °C and diameter of solid 0.6–0.8 mm.

Table 3
Identified coefficients of diffusion

Temperature (°C)	Diameter (mm)	% ethanol	$D \times 10^{14}$ ($\text{m}^2 \text{s}^{-1}$)
22	0.6–0.8	80	11.05
22	0.6–0.8	70	9.05
22	0.6–0.8	60	8.43
22	0.6–0.8	0	6.67
22	0.1–0.3	60	12.62
22	0.45–0.6	60	12.18
22	0.6–0.8	60	9.27
40	0.6–0.8	60	39.25
50	0.6–0.8	60	28.62
60	0.6–0.8	60	52.10

value until $52.1 \times 10^{-14} \text{ m}^2/\text{s}$ at 60 °C. The results confirm the experimental observation on the influences of the main parameters.

6. Conclusions

In this study, *A. paniculata* samples were scanned with a Scanning Electronic Microscope in order to accomplish

visual observations on its structure, especially the characteristic dimensions. As a result of the observations, the used solid can be separated into two groups with differences in structure, shape and characteristic dimension. For each group, soxhlet experiments were performed in order to obtain the ratio of leaves and stems contained in each experiment (the mean percentage of leaves is about 80%).

Then, batch extraction experiments were performed in order to study the influences of the operating parameters such as ethanol concentration, particle size and temperature. In this investigation, it has been found that the sizes of the particles have very little effect on both velocity of extraction and final concentration. The percentage of ethanol in the solvent has no effect on the kinetic of extraction but the final concentration increases with the percent of ethanol. The coefficient of diffusion D is higher when T increases. Finally, a reaction of destruction of the solute which is activated at high temperature (from about 67 °C) has been characterized. An extraction model is then proposed, taking into account the difference in shape of the particles. The experimental results are used to identify the diffusion coefficients for all experiments. The value of this coefficient varies from 8.43×10^{-14} to $52.1 \times 10^{-14} \text{ m}^2/\text{s}$ in ethanol solution depending on the temperature.

These results will now be used in the design of an industrial pilot. The planned apparatus will be a disc and doughnuts pulsed column. The influences of the operating parameters presented in this paper will allow us to choose the process conditions in term of solvent quality and temperature, based on economic or safety criteria.

Acknowledgements

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