EFFECT OF SOLVENT FLOW RATE ON THE SUPERCRITICAL CARBON DIOXIDE EXTRACTION OF ANDROGRAPHOLIDE FROM ANDROGRAPHIS PANICULATA

A. C. Kumoro [1, 2] and **M. Hasan** [2]

[1] Dept. of Chemical Engineering, Faculty of Engineering, Diponegoro University. Prof. H. Soedarto, SH. Road, Tembalang Campus, Semarang - Indonesia.
Email: andrewkomoro@perdana.um.edu.my
[2] Dept. of Chemical Engineering, Faculty of Engineering, University of Malaya, Pantai Valley 50603 Kuala Lumpur, Malaysia

ABSTRACT

Malaysia is one of the major medicinal plants (*Andrographis paniculata*) producers in the world. Extraction of bioactive component (andrographolide) from ground dried leaves of this plant using supercritical carbon dioxide has been studied. The effect of solvent flow rates ranging from 7.95×10^{-6} to 6.36×10^{-5} kg/s on the extraction efficiency has been investigated. The extraction yield increased with the increase of solvent flow rate from 7.95×10^{-6} to 3.18×10^{-5} kg/s. However further increase of solvent flow rate reduced the extraction yield. Therefore, an optimum solvent flow rate at 3.18×10^{-5} kg/s was obtained for the system under study. The extraction process was found to be controlled by intraparticle diffusion of the andrographolide in the starting leaves particles.

Keywords: solvent, flow rate, supercritical carbon dioxide, extraction, andrographolide

1. INTRODUCTION

Andrographis paniculata NEES, locally known as Hempedu Bumi grows widely in the tropical area of South East Asia, India and China with plant height of 0.30 – 0.70 m. In these regions, this plant has been extensively used for traditional medicine and help against fever, dysentery, diarrhoea, inflammation, and sore throat (Wongkittipong *et al*., 2000). Furthermore, it is a promising new way for the treatment of many diseases, including HIV, AIDS, and numerous symptoms associated with immune disorders (Calíbrese *et al*., 2000).

Andrographolide, the main diterpenoid lactones identified in *A. paniculata* leaves, is grouped as an unsaturated trihydroxy lactone has molecular formula of $C_{20}H_{30}O_5$ (Choudhury *et al*., 1987; Rajani *et al*., 2000). 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. Its physical properties are; m.p. is 501- 503 K and ultraviolet spectrum in ethanol: λ_{max} is 223 nm (Rajani *et al*., 2000).

Conventional production methods such as solvent extraction and soxhlet, although effective for extraction, can lead to degradation of heat sensitive compounds as well as leave traces of toxic solvents in the solute. This is a concern for food and medicinal extracts (Tonthubthimthong *et al*., 2001). Supercritical fluid extraction (SFE) may be a viable alternative to solvent extraction methods. Carbon dioxide is generally the most desirable solvent for supercritical fluid extraction. Its low critical temperature (304.26 K) and pressure (7.38 MPa), make it effective for the extraction of heat sensitive (thermally labile) compounds. In addition, it is an inert, non-flammable, non-explosive, inexpensive, odourless, colourless clean solvent that leaves no solvent residue in the product; it is also non-toxic and is generally accepted as a harmless ingredient in pharmaceuticals and food (Tonthubthimthong *et al*., 2001). It also has low surface tension and viscosity and high diffusivity, which make it attractive as supercritical solvent (Roy *et al*., 1996).

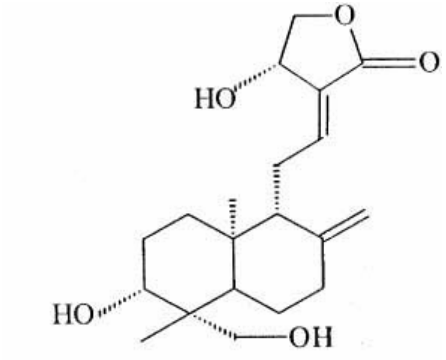

Fig. 1. Molecular structure of andrographolide

The diffusivity of supercritical carbon dioxide is one to two orders of magnitude higher than for other fluids, which permits rapid mass transfer, resulting in a larger extraction rate than that obtained by conventional liquid extraction (Tonthubthimthong *et al*., 2001).

The objective of this work is to investigate the effect of supercritical carbon dioxide flow rates on the extraction yield in the supercritical carbon dioxide extraction of andrographolide from *A. paniculata.*

2. METHODOLOGY

2.1 MATERIAL AND REAGENTS

Dried leaves of *A. paniculata* were collected from Malaysian Agricultural Research and Development Institute (MARDI) and stored in an air tight sealed container at ambient conditions. Without any pre-treatment the leaves were ground into powder and were sieved to obtain three different particle sizes. The andrographolide standard pure crystals (Purity 980 g/ kg) was supplied by Sigma - Aldrich (M) Sdn. Bhd. Carbon dioxide (999.70 g/ kg) was used as the solvent in this study and was purchased in the liquid form by Air Product (M) Berhad

2.2 SUPERCRITICAL CO_2 EXTRACTION

The own built supercritical fluid extraction apparatus used in this study is as described in Fig. 2. It consists of a 1.60×10^{-5} m^3 high-pressure stainless steel extraction vessel (10 mm inner diameter × 200 mm length). A reciprocating pump was used to compress and introduce CO_2 into the extraction system. The extraction process can be briefly described as follows: in a typical run, the extraction vessel was first filled with 3.0 g of dried - powdered A. *paniculata* leaves distributed in stainless steel balls in order to improve the leaves-solvent contact and to avoid channelling when CO_2 flowed through. The extraction vessel was then immersed in the water bath controlled by an electrical heater (Thermo Haake, model DC10) to within ± 0.1 K and the system was brought to the desired temperature. Liquid CO_2 was then charged into high-pressure pump (ISCO, model 260 DM) from the liquid CO_2 cylinder. The high-pressure pump further compressed the CO_2 to the desired pressure and continuously delivered the CO_2 to flow through the sample in the extraction vessel to extract the andrographolide at desired volumetric flow rate. The pressure of the system was determined by a pressure transducer (Druck, model PDCR 961). The extract was collected in a cold trap by depressurising the CO_2 at the backpressure regulator (Jasco, model 880-81). The cold trap was a glass vial immersed in a pack of diced ice that was maintained at 277 K. The samples were taken at one-hour interval and experiments were terminated after seven hours, and the total amount of extract collected was gravimetrically determined using a balance (Mettler Toledo, model AG 204) with an accuracy of ± 0.0001 g. The carbon

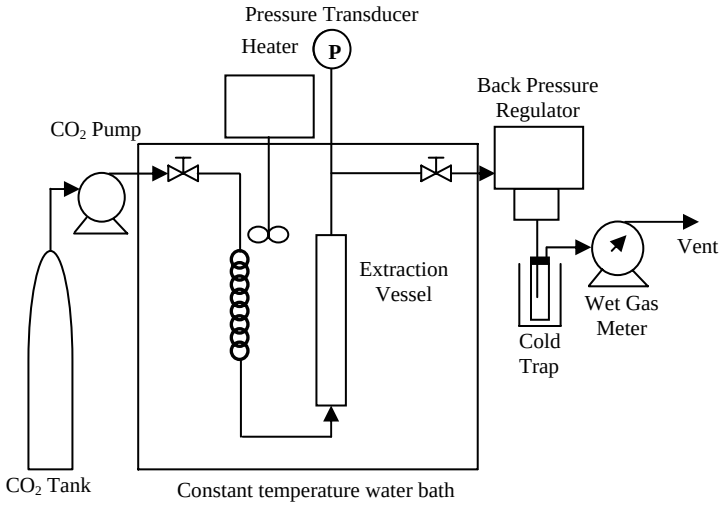

Fig. 2. The supercritical fluid extraction unit

dioxide amount consumed was determined using a wet gas meter (Alaxander Wright & Co., model DM 3A). The glass vials were wrapped with aluminium foil to prevent photo degradation of the andrographolide. For each trial, at least two experiments were carried out where the total amount of CO_2 that passed through the cell slightly varied, hence varying the total amount of extract collected. The extracts were then analysed their andrographolide content using high performance liquid chromatography. The relative error obtained between the duplicate experiments was less than 5 %.

3. RESULT AND DISCUSSIONS

In an attempt to investigate the effect of solvent flow rate on the extraction rate, a set of experiments was carried out at 313 K and 10 MPa, while the flow rate of CO_2 was varied from 7.95×10^{-6} to 6.36×10^{-5} kg/s. Figure 3 was constructed to show the effect of time with the flow rate as a parameter. It shows that the yield increases with increase in supercritical carbon dioxide flow rate, reaches a maximum value, and then decreases with further increase in the flow rate. The results obtained here can be explained as a trade-off between a mass transfer process and a thermodynamic equilibrium state. The interface gas phase concentration of the solute is a function of the mass transfer coefficient, or solvent flow rate, while equilibrium state is favoured by both high mass transfer rate and long residence time (Elkanzi and Singh, 2001). At low flow rates of the solvent, the mass transfer resistance limits the amount of solute transported into the bulk of the solvent and the supercritical carbon dioxide leaves the extractor unsaturated. As the flow rate is increased, mass transfer resistance continues to decrease until exiting solvent is saturated; and let equilibrium to be achieved and hence the maximum yield is attained. Further increase of the flow rate will reduce the

residence time causing the system to deviate from equilibrium and the solvent leaves the extractor unsaturated

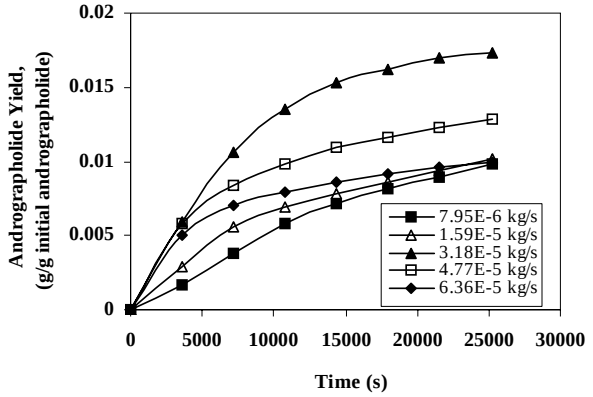

Fig. 3. Effect of solvent flow rate as on andrographolide yield as a function of time

despite the high mass transfer rate (Mira *et al*., 1999; Elkanzi and Singh, 2001). This is because the amount of solvent that is in excess to what is needed to penetrate the cellular structure of the leaves simply bypassed the extractable leaves (Saldana *et al*., 2002). This behaviour indicated the presence of a mass transfer resistance which is possibly intraparticle diffusion or an external film resistance (Machnauton and Foster, 1995). Thus in a semi batch supercritical fluid extraction, the optimum solvent flow rate has to be determined a priori. However, it should be noted that the condition of optimum solvent flow rate depends on the nature of the solvent – solute system, the geometry of the extractor, temperature and pressure (Elkanzi and Singh, 2001).

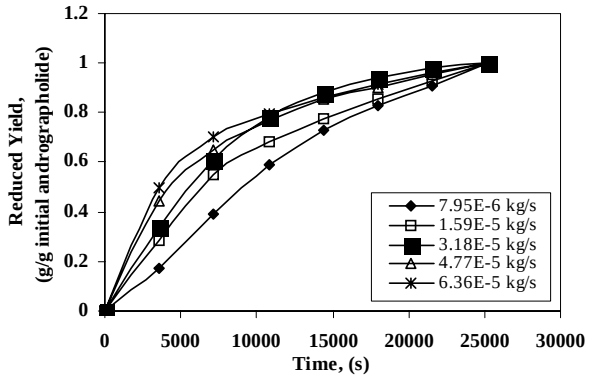

Fig. 4. Effect of solvent flow rate on reduced andrographolide yield as a function of time

Fig. 4 shows the effect of solvent flow rate on the reduced extract yield as a function of time. The reduced extract yields were then also plotted in Fig. 5 as a function of the amount of carbon dioxide consumed. The reduced yield was defined as the ratio of yield to final yield. Since the final yield does not accurately reflect the same value, reduced yield was used for the purpose of comparison as suggested by previous researchers (Goto *et al*., 1993; Roy *et al*., 1996). As can be seen in Fig. 4., the extraction rate is higher at larger carbon dioxide flow rate. On the other hand, the extraction rate in the plot of Fig. 5. was affected by supercritical carbon dioxide flow rate, even in the beginning of extraction run. This indicates that the exit concentration of extracts is dependent of flow rate especially at very high solvent flow rate, where equilibrium is not able to be considered to hold. The slopes of the extraction curve in the plot of reduced yield versus the amount of carbon dioxide consumed were smaller at higher flow rate. This figure indicated that intraparticle diffusion resistance is controlling the extraction process (Goto *et al*., 1993; Roy *et al*., 1996; Tonthubthimthong *et al*., 2001). If external mass transfer significantly affects the extraction process as in the case of desorption from activated carbon, the curve for lower flow rate becomes gentler in Fig. 5. All curves will lie in a single line if the extraction rate is not affected by supercritical carbon dioxide flow rate or solubility is controlling the extraction process (Goto *et al*., 1993).

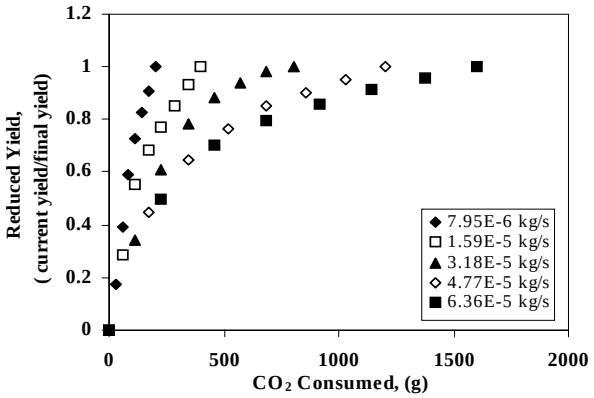

Fig.5. Effect of solvent flow rate on reduced andrographolide yield as a function of CO_2 consumed

4. CONCLUSIONS

In this study, the andrographolide extraction yield of approximately 0.0174 g andrographolide/g andrographolide present in the *A. paniculata* leaves using only supercritical CO_2 extraction was obtained. The extraction rate was found to be affected by intraparticle mass transfer of andrographolide. The best extraction condition was found to be at 313 K, 10 MPa and a flow rate of 3.18×10^{-5} kg/s for 3.0 g sample of *A. paniculata* leaves.

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