

Application of gas anti-solvent process to the recovery of andrographolide from *Andrographis paniculata*

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Abstract—The gas anti-solvent (GAS) process was employed to extract andrographolide, which is the active ingredient found in *Andrographis Paniculata*, using carbon dioxide as an anti-solvent. The effects of temperature, flow rate and solvent type on the extraction recovery, particle size and morphology were investigated in this study. The experiments were conducted at the temperature ranging from 25–45 °C, carbon dioxide flow rate of 1–15 mL/min, and various types of organic solvents (methanol, ethanol, acetone and N,N-dimethylformamide). The extracted product was analyzed using high performance liquid chromatography (HPLC). The highest extraction yield was found to be 1.24 g andrographolide per 100 g of *A. paniculata* when using acetone as a solvent, carbon dioxide flow rate of 5 mL/min and the temperature of 35 °C. It was also found that no significant change in size or morphology of the precipitates was observed when changing temperature, carbon dioxide flow rate and solvents.

Key words: Dense Gas, GAS Process, Andrographolide, *Andrographis paniculata*, Carbon Dioxide

INTRODUCTION

The use of dense gases in the area of extraction has been well documented over the last few decades. The adjustable solvent strength and gas-like transport properties make dense gases efficient solvents for the extraction process. In addition, the increased scrutiny of industrial solvents by governments and awareness of pollution control have motivated the use of dense gases as extracting agents for food, beverages and spices [1–3]. Recently, the application of dense gas has also been extended to the area of the gas-anti-solvent (GAS) process. In dense gas anti-solvent processes, the solid of interest, which should have a relatively low solubility in the dense gas, is dissolved in an organic solvent. The solution is then expanded by contacting with the dense gas. The solvating power of the solvent phase decreases upon expansion since the dense gas is partially or completely miscible with the solvent. At a critical expanded volume, the solution becomes saturated. Further expansions or higher anti-solvent concentrations make the liquid phase become super-saturated; therefore the solute precipitates out of the solution, often as fine and uniform particles. The product can be separated from the solvent/anti-solvent mixture by further washing with fresh anti-solvent across a collection device such as a filter. The washing step is not only required for preventing re-dissolution of the solute into the solvent, but also for removing the residual organic solvent from the product [4–10].

Andrographis paniculata is a famous Chinese medical plant containing a variety of bioactive ingredients such as andrographolide, neo-andrographolide and deoxyandrographolide. The amount of andrographolide in the *Andrographis paniculata* was reported to be approximately 1.7% by weight [11]. Andrographolide can be used for anti-inflammatory, anti-microbial, anti-platelet aggregation

and anti-HIV activities [12]. The molecular structure of andrographolide is shown in Fig. 1. To account for the effect of andrographolide, it is required to take a large amount of *A. paniculata* daily. For example, for the treatment of fever, sore throat and diarrhea it is necessary to take 5–10 capsules of ground *A. paniculata* per dose and 3–4 times a day. Therefore, it would be beneficial to extract the active bioactive ingredient and use it as a concentrated medicine.

Kumoro and Hasan [13] reported that the best extraction condition of andrographolide from the ground dried leaves of *A. paniculata* was obtained when using supercritical carbon dioxide at 10 MPa, 40 °C and a flow rate of 2 mL/min. The extraction yield was found to be 0.0174 gram andrographolide per gram of andrographolide present in the *A. paniculata* leaves or equivalent to 2.01×10^{-3} gram andrographolide per gram of dried leaves.

Ge and co-workers [14] found that in the supercritical carbon

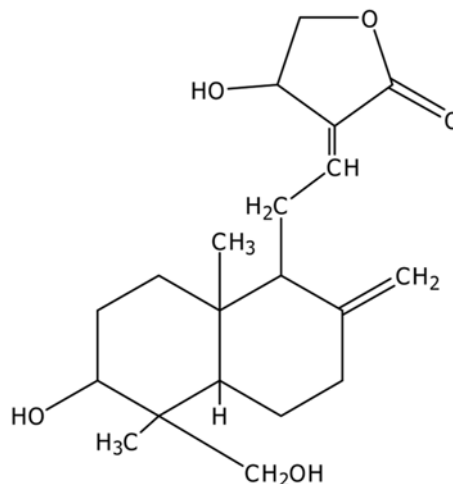


Fig. 1. Molecular structure of andrographolide.

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dioxide extraction of andrographolide from *A. paniculata* leaves the extraction rate and yield increased as the operating temperature and pressure increased. However, the andrographolide content in the extracts was very low. The extraction yield was found to be 3.81×10^{-3} gram of andrographolide per gram of dried leaves. They also reported that the use of ethanol as co-solvent can enhance the extraction yield and the andrographolide content.

Due to the low solubility of andrographolide in supercritical carbon dioxide, it is not feasible to extract this active ingredient on a large scale. However, andrographolide can be easily dissolved in various organic solvents such as methanol, ethanol, acetone and dimethylformamide (DMF). Therefore, it is worthwhile to investigate the feasibility of the gas anti-solvent (GAS) process for extraction of andrographolide from organic solvents. In this study, the effects of temperature, flow rate and solvent type on the extraction yield, particle size and morphology of andrographolide were investigated.

MATERIALS AND METHODS

1. Materials

Ground dried leaves of *A. paniculata* from Chao Krom Per, Thailand were used as received. Andrographolide (Aldrich, 98% purity) was used as a standard in the HPLC analysis. Methanol, ethanol, acetone and dimethylformamide (99.8% purity) were purchased from Italmar (Thailand) Co., Ltd. and used as organic solvents. Carbon dioxide (high purity grade, TIG) was used as an anti-solvent. All chemicals and reagents were used without further purification.

2. Extraction of Andrographolide using Organic Solvents

The extraction of andrographolide using organic solvents (ethanol, methanol, acetone and DMF) was carried out by dissolving 50 grams of ground dried leaves of *A. paniculata* into 100 mL of an organic solvent. The solution was then stirred by a magnetic stirrer at 600 rpm for 2 hour at room temperature. After that the extracted solution was filtered and analyzed for the andrographolide content using HPLC. The column used for analysis was Inertsil® ODS-3 (4.6×150 mm, 5 µm). The volume of injection was 20 µL. Methanol and water in the volume ratio of 60 : 40 was used as a mobile phase with the flow rate of 1.0 mL/min. The sample was detected using UV vis at the wave length of 254 nm. In the case of using ethanol as a solvent, extractions were repeated five times with the use of fresh solvent.

3. Extraction of Andrographolide using the GAS Process

The schematic diagram of the GAS process is shown in Fig. 2.

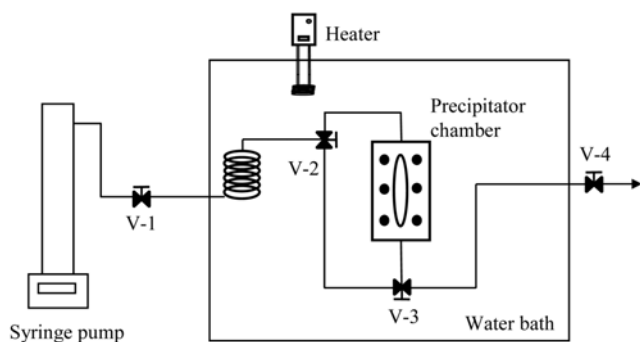


Fig. 2. Schematic diagram of the experimental GAS apparatus.

Precipitation of andrographolide was conducted by charging the vessel (Jerguson sight gauge series no. 32) with 4 mL of the solution extracted from part 2.2. The system temperature was controlled to within 0.1 °C using a recirculation heater (Thermoline Unistat 130). Liquid CO₂ was fed to a syringe pump (ISCO model 260D) and delivered through a preheating coil, which was immersed in the water bath. The precipitation chamber was then brought to the desired pressure by passing CO₂ from the pump through a 0.5 µm filter from the bottom. The rate of pressurization was set at the desired flow rates (1, 3, 5, 10 and 15 mL/min). The pressure of the system was increased up to 90 bar in order to ensure a complete precipitation. Precipitated samples were then washed with CO₂ at 90 bar for approximately 100 mL of CO₂ to remove residual solvent. After washing, the system was depressurized and a sample was taken for analysis. The percentage recovery of andrographolide relative to andrographolide content in the organic solvents can be calculated using the following equation:

$$\% \text{Recovery} =$$

$$\frac{\text{weight of andrographolide from the GAS process}}{\text{weight of andrographolide from organic solvent extraction}} \times 100$$

The morphology of the original andrographolide and the precipitates obtained by the GAS process was analyzed by scanning electron microscopy (SEM) (Hitachi S4500). Samples were coated with gold using a sputter coater (Edwards) prior to analysis.

RESULTS AND DISCUSSION

In the study of andrographolide extraction using ethanol, the amount of extracted andrographolide is shown in Table 1. The total amount of andrographolide in the sample was found to be 1.15 g per 100 g of ground dried leaves. It was found that using an extraction time of 2 hours was sufficient to extract this active ingredient from the sample since approximately 93.9% of the total andrographolide was extracted within the first 2 hours. Therefore, in the study of andrographolide extraction using other organic solvents, the extraction time

Table 1. Amount of andrographolide extracted using ethanol

No. of extractions	Amount of ethanol (mL)	Weight of andrographolide (g) per 100 g of ground dried leaves
1	100	1.08
2	100	0.03
3	80	0.02
4	80	0.01
5	80	0.01

Table 2. Amount of andrographolide extracted after 2 hours using different organic solvents

Solvent	Weight of andrographolide (g) per 100 g of ground dried leaves
DMF	1.48
Acetone	1.28
Methanol	1.27
Ethanol	1.08

of 2 hours was then applied. The amounts of extracted andrographolide using different organic solvents are shown in Table 2. It was found that DMF is the most efficient solvent to extract andrographolide. This could be due to the fact that the dipole moment and dielectric constant of DMF are higher compared to those of other organic solvents. As a result, DMF is a better solvent to dissolve the polar andrographolide. However, the maximum amount of andrographolide extracted in this study was lower than the reported literature (1.7 g per 100 g of ground dried leaves). This could be attributed to the differences in the variety of the plant and extraction techniques [11].

The solutions of andrographolide in different organic solvents obtained from the above section were then used as starting materials in the GAS process. The effects of CO₂ flow rate, temperature and type of organic solvents on the percentage recovery were investigated.

1. Effect of Carbon Dioxide Flow Rate

The effect of carbon dioxide flow rate on the % recovery of andrographolide was studied using the flow rates of 1, 3, 5, 10 and 15 mL/min, respectively. The system temperature was maintained at 35 °C. Fig. 3 shows the % recovery of andrographolide obtained from various organic solvents. It was found that as the flow rate of CO₂ decreased from 15 to 5 mL/min, the % recovery was increased. This could be due to the fact that a decrease in CO₂ flow rate resulted in a longer time for the system to reach equilibrium. As a result, more andrographolide can be precipitated out of the organic solvents. However, when decreasing the CO₂ flow rates from 5 to 3 and 1 mL/min, respectively, a reduction in the % recovery was observed. This may be due to the fact that using low CO₂ flow rate resulted in a slow solution expansion rate. Therefore, low level of supersaturation was obtained and thus lower amounts of andrographolide were precipitated out of the solution.

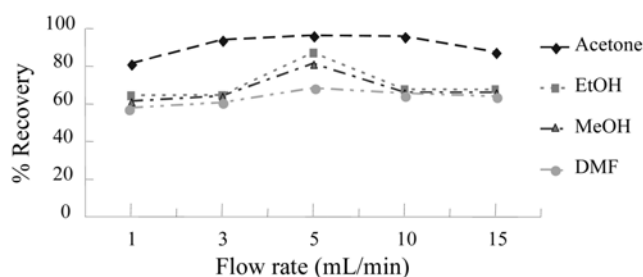


Fig. 3. Effect of CO₂ flow rate on the % recovery at 35 °C.

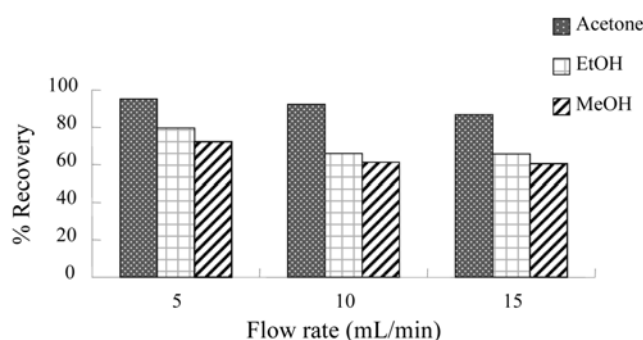


Fig. 4. Effect of CO₂ flow rate on the % recovery at 40 °C.

Similar results were obtained when the GAS experiments were carried out at 40 °C and 45 °C. As shown in Figs. 4 and 5, the highest % recovery was achieved when using the CO₂ flow rate of 5 mL/min.

An increase in the expansion rate is expected to have an effect on the particle size. Due to the high levels of supersaturation generated by high expansion rates, rapid rates of nucleation and little growth rate will result in small particles [15]. Chen and co-workers [16] reported that the size of andrographolide crystal precipitated from the supercritical anti-solvent (SAS) process was decreased when the solution flow rate increased. However, it should be noted that in Chen *et al.*'s research andrographolide solution was prepared by dissolving andrographolide (99% purity) in ethanol. As a result, the change in particle size they observed was not influenced by other materials present in the ground dried leaves of *A. paniculata*. In this study, the effect of carbon dioxide flow rate on the shape and morphology of precipitate was studied. The particle size of the original andrographolide as received from Aldrich was as high as 130 μm, as shown in Fig. 6(a). The unprocessed particles are irregular in shape with a broad size distribution. Andrographolide particles precipitated by the GAS process at 35 °C using acetone as a solvent with various CO₂ flow rates are shown in Figs. 6(b)-(d). The SEM images

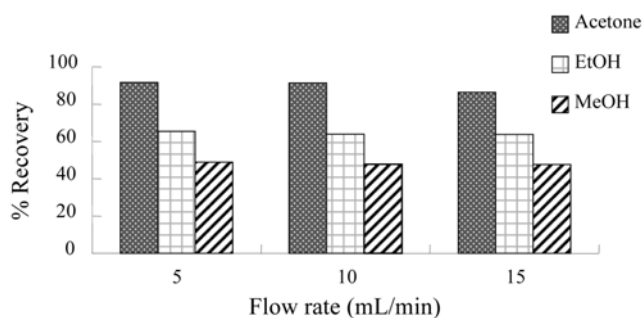


Fig. 5. Effect of CO₂ flow rate on the % recovery at 45 °C.

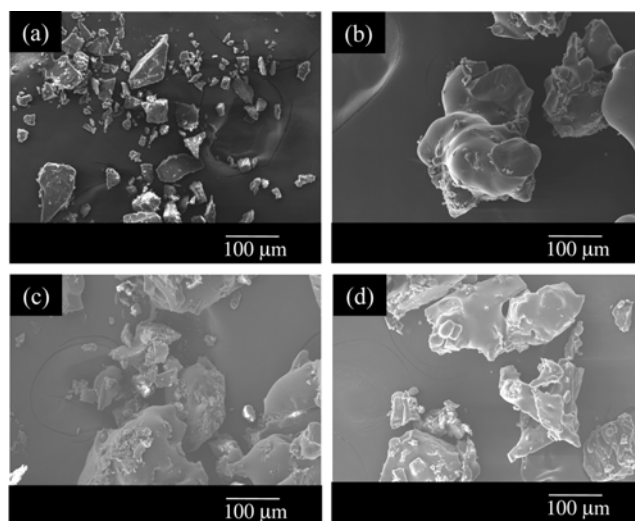


Fig. 6. SEM micrographs of andrographolide (a) original material and the precipitates obtained by GAS at 35 °C from andrographolide solution in acetone with different CO₂ flow rates (b) 5 mL/min (c) 10 mL/min (d) 15 mL/min.

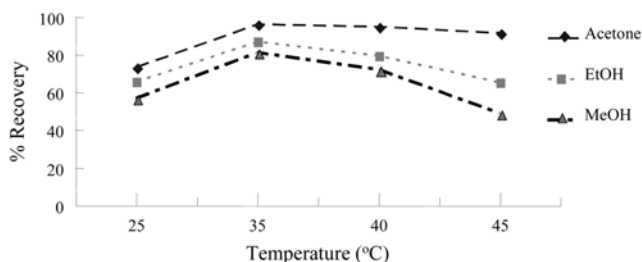


Fig. 7. Effect of temperature on % recovery when using CO₂ flow rate of 5 mL/min.

of processed particles reveal that particles were irregular in shape, aggregated and bigger than the original materials. In addition, it was found that andrographolide precipitated from the GAS process were not in a dry powder form, but they were sticky-like materials. This could be because other materials present in the ground dried leaves of *A. paniculata* were precipitated with the andrographolide at the same time.

2. Effect of Temperature

The effect of temperature on the % recovery was studied for the precipitation of andrographolide from organic solutions using the flow rate of CO₂ at 5 mL/min. The temperature of the system was varied from 25 °C to 45 °C. The % recovery of andrographolide at various temperatures is shown in Fig. 7. It was found that as the temperature was increased from 25 °C to 35 °C, the % recovery of andrographolide precipitated from all of the organic solvents increased. As the temperature increased, the diffusivity of CO₂ in the organic solvents increased, hence a higher degree of supersaturation in the solution was obtained. As a result, higher amount of andrographolide was precipitated at 35 °C. However, when increasing the temperature from 35 °C to 45 °C, the % recovery of andrographolide was decreased. This could be due to the fact that at a specific pressure the density of CO₂ decreased as the temperature increased; therefore, the CO₂ solubility in the organic solvents decreased. In addition, the solubility of andrographolide in the organic solvents increased as the temperature increased. Combining with these two effects, lower degree of supersaturation in the solution was obtained. As a result, lower % recovery of andrographolide was achieved at temperature higher than 35 °C.

The highest % recovery was also observed when the GAS experiments were carried out at 35 °C using CO₂ flow rate of 10 and 15 mL/min.

3. Effect of Solvent

The effect of solvent on the % recovery was investigated. The

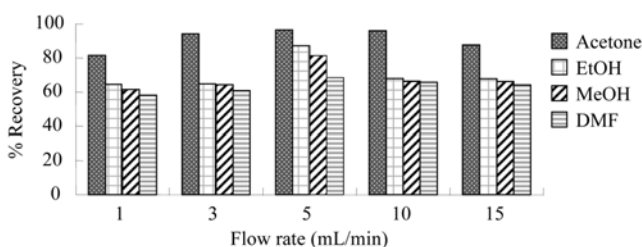


Fig. 8. Effect of solvent on % recovery at 35 °C using different CO₂ flow rates.

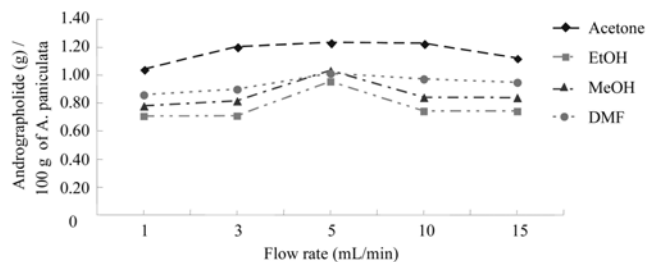


Fig. 9. Amount of andrographolide precipitated using various solvents at 35 °C.

solvents used in the study were methanol, ethanol, acetone and DMF. The precipitation process was performed in the temperature ranging from 25–45 °C and using the CO₂ flow rate in the range of 1–15 mL/min. It was found that at the same precipitation conditions, the obtained % recovery was highest when andrographolide was precipitated from acetone. As shown in Fig. 8, the % recovery was in the order of acetone > ethanol > methanol > DMF. Since CO₂ can dissolve in acetone better than in other organic solvents, the supersaturation could be obtained more easily as CO₂ was slowly added to the acetone system. Fig. 9 shows the amount of andrographolide precipitated from various organic solvents at different CO₂ flow rates. The highest amount of andrographolide precipitated was found to be 1.24 g andrographolide per 100 g of *A. paniculata* when using acetone as a solvent. In addition, it was found that the amount of andrographolide precipitated from DMF was higher than that precipitated from ethanol and methanol. This was because the solubility of andrographolide in DMF was greater than that in ethanol and methanol (as shown in Table 2).

It was reported that solvent type has a dramatic effect on the morphology of precipitates produced from the GAS process. Various morphologies can be obtained as a result of the solvent-solute interaction [4,15,17]. In this study, the effect of solvent type on the morphology of andrographolide was also examined. The SEM images of particles precipitated by GAS from various organic solvents are illus-

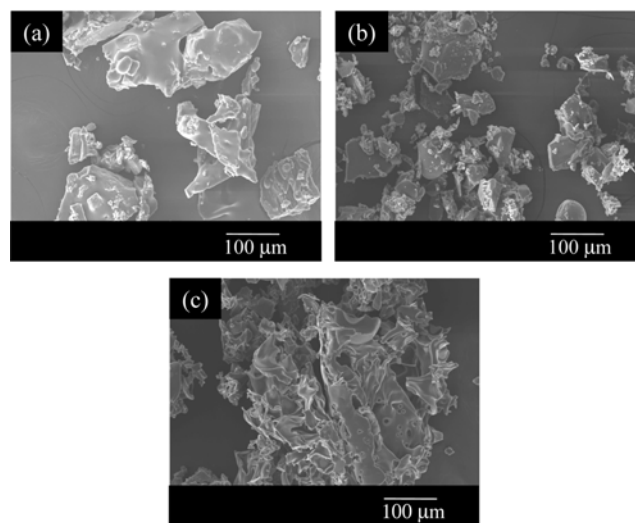


Fig. 10. SEM micrographs of particles precipitated by GAS at 35 °C using CO₂ flow rate of 15 mL/min from andrographolide solution in (a) acetone (b) ethanol (c) methanol.

trated in Fig. 10. The precipitates obtained from all conditions were found to be larger than the unprocessed andrographolide. This could be attributed to the fact that more than one compound was precipitated with andrographolide.

CONCLUSION

In this paper, it is demonstrated that it is practical to extract andrographolide from the ground dried leaves of *A. paniculata* with various organic solvents, followed by the precipitation by the GAS process. The highest andrographolide extraction was found to be 1.48 g andrographolide per 100 g of ground dried leaves of *A. paniculata* when using DMF as a solvent with the extraction time of 2 hours. Precipitation of andrographolide from the organic solvents was then successfully performed by the GAS process using CO₂ as an anti-solvent. The highest extraction yield was found to be 1.24 g andrographolide per 100 g of *A. paniculata* when using acetone as a solvent, carbon dioxide flow rate of 5 mL/min and the operating temperature of 35 °C.

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