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Development of an ultrasound-negative pressure cavitation fractional precipitation for the purification of (+)-dihydromyricetin from biomass

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Abstract−An ultrasound-negative pressure cavitation fractional precipitation method was developed to efficiently purify (+)-dihydromyricetin. The precipitation efficiency of the developed method was significantly higher than that of the ultrasound- and negative pressure-fractional precipitation methods. In particular, under an ultrasonic power of 180 to 250 W and a negative pressure intensity of −200 mmHg, highly pure (+)-dihydromyricetin could be obtained with a high yield of up to 97.56% in only one minute of precipitation. Kinetic analysis results showed that when both ultrasound and negative pressure were introduced during fractional precipitation, the rate constant increased by 1.4 to 7.1 times compared to the conventional fractional precipitation, and the activation energy decreased by −1,299 to −4,550 J/mol. In addition, the particle size of the precipitate was reduced by 1.9 to 4.0 times, and the diffusion coefficient increased by 2.3 to 5.0 times.

Keywords: (+)-Dihydromyricetin, Cavitation Fractional Precipitation, Ultrasound-negative Pressure, Diffusion Coefficient, Mass Transfer Coefficient

INTRODUCTION

(+)-Dihydromyricetin or (+)-ampelopsin (Fig. 1) is a major bioactive flavonoid isolated from the medicinal plants *Hovenia dulcis* and *Ampelopsis grossedentata* [1,2]. It has received great attention due to its health-benefiting activity, including antioxidant, antimicrobial, anti-inflammatory, anticancer, antidiabetic and neuroprotective activity [3,4]. Du et al. [5] reported the inhibitory effect of (+)-dihydromyricetin on portal hypertension. Yoshikawa and Murakami [6] also reported that this substance inhibited muscle relaxation, promoted metabolism in the body, and showed an anti-allergic effect by ethanol. The demand for (+)-dihydromyricetin is expected to continue to increase due to the ongoing research on the disease-therapeutic effect and its application in food and pharmaceutical industries [7].

To use the plant-derived bioactive substance (+)-dihydromyricetin as functional food or raw material for drugs, it is necessary to purify the active ingredient at scale. However, the economic feasibility of its mass production at an industrial scale is limited because expensive chromatography is involved during separation and purification, or the crude product without pretreatment is purified through high performance liquid chromatography (HPLC) [4].

Fractional precipitation is a simple and effective method to purify (+)-dihydromyricetin using solubility differences. A process was developed which could obtain high purity (>83.2%) (+)-dihydromyricetin through a fractional precipitation in 2008 [8]. However, it was difficult to adopt the process for mass production because of its long precipitation time (up to 32 hr). In 2014, a method to improve precipitation efficiency by increasing the surface area per precipitation volume using an ion exchange resin (Amberlite 200, Amberlite IR 120Na) was developed [9]. Furthermore, a more improved fractional precipitation process was developed using hydrophilic polymers, but the precipitation of (+)-dihydromyricetin still took a long time [3]. Recently, attempts have been made to improve precipitation efficiency using a cavitation effect. In general, the cavitation phenomenon generated by ultrasound or negative pressure increases the nucleation rate in the precipitation process. In 2021, the fractional precipitation of (+)-dihydromyricetin using ultrasound remarkably shortened the operating time [10]. In addition, a new green negative pressure cavitation precipitation for the purification of the anticancer substance paclitaxel has been reported [11]. This is a simple and effective method with efficient energy use and a small investment. Such improvements to the precipitation efficiency by ultrasound and negative pressure are based on the acoustic cavitation phenomenon, which includes the formation, growth, and collapse of cavitation bubbles [10,12]. Moreover, the

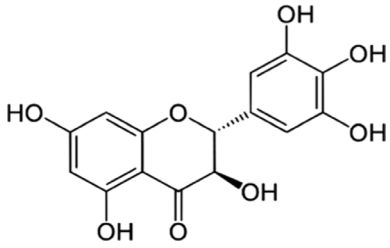

Fig. 1. The structure of (+)-dihydromyricetin ($C_{15}H_{12}O_8$, molecular weight: 320.25).

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collapse of the cavitation bubbles generates high-speed microjets of solution, intense localized heating, and high-pressure shock waves in the precipitation solution [13,14]. Based on this phenomenon, this study developed a new ultrasound-negative pressure cavitation fractional precipitation method by introducing both ultrasound and negative pressure simultaneously to effectively purify the biomass-derived (+)-dihydromyricetin. In addition, a kinetic analysis was conducted using the Johnson-Mehl-Avrami-Kolmogorov (JMAK) equation. The diffusion behavior of the molecules was also calculated quantitatively through the diffusion coefficient using the Stokes-Einstein equation. Additional fractional precipitation was performed introducing degassed solution and gas bubbles to investigate the mechanism of the ultrasound-negative pressure cavitation fractional precipitation in detail.

MATERIALS AND METHODS

1. (+)-Dihydromyricetin Sample

The crude extract of (+)-dihydromyricetin (purity: 54.84%) used in this study was procured from Guilin Natural Ingredient, Inc. (Guilin, China).

2. Fractional Precipitation

Conventional fractional precipitation, ultrasound-fractional precipitation, negative pressure-fractional precipitation, and ultrasound-

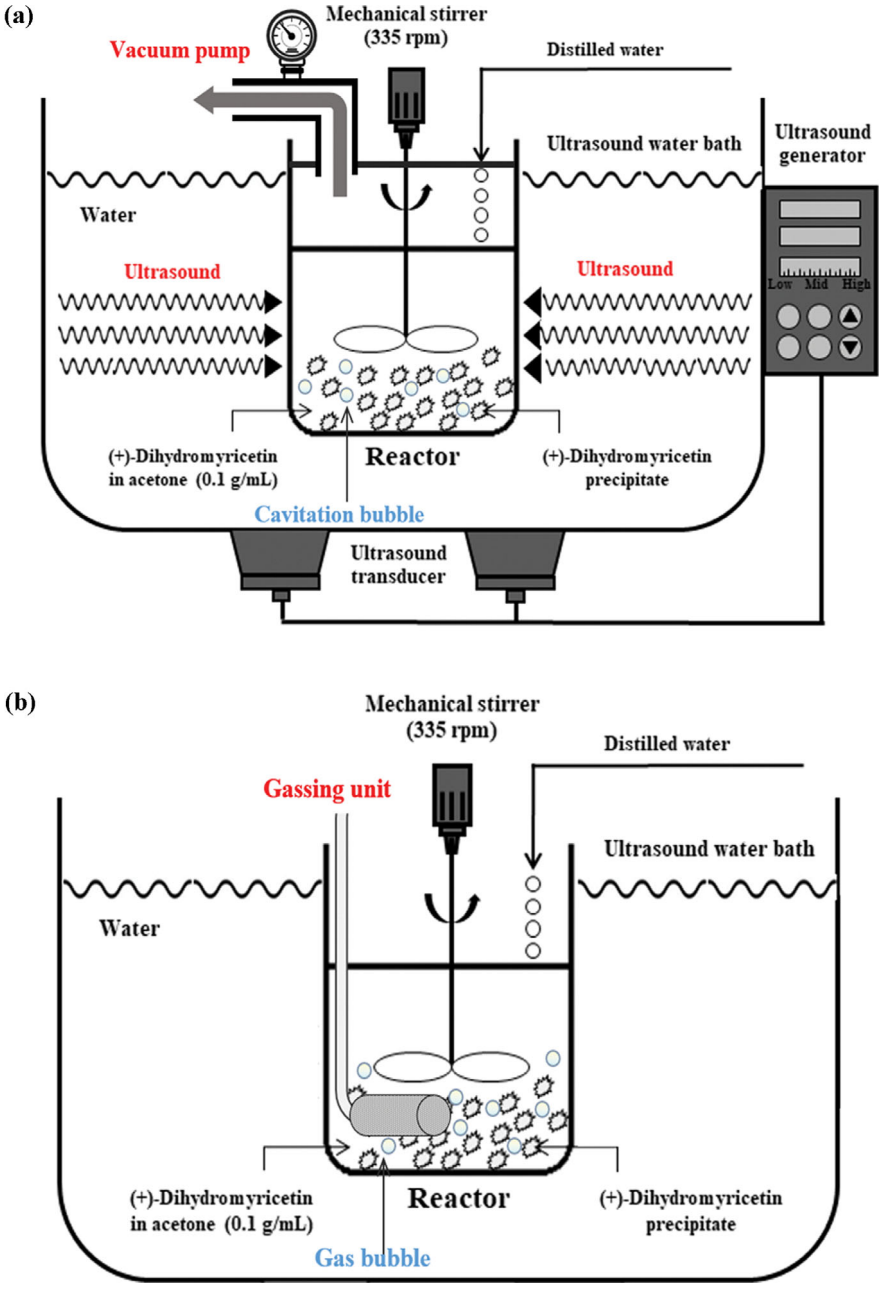

Fig. 2. Schematic diagram of different fractional precipitations for purification of (+)-dihydromyricetin from biomass. (a) Ultrasound-negative pressure cavitation fractional precipitation; (b) Fractional precipitation using gas bubbles.

negative pressure fractional precipitation were conducted to confirm the effect of ultrasound and negative pressure cavitation. The (+)-dihydromyricetin sample (purity: 58.84%) was dissolved in acetone (0.1 g/mL) and the solution was added drop by drop into distilled water (acetone/water ratio=1 : 5, v/v) while stirring (335 rpm) to induce precipitation [12]. All the fractional precipitation was conducted for different working times (1, 5, 10, 20, and 30 min) at 5 °C. Ultrasound-fractional precipitation was carried out under different ultrasonic power conditions (80, 180, and 250 W) in a 40 kHz ultrasonic bath (UC-10, Jeiotech, Korea). Meanwhile, negative pressure-fractional precipitation was carried out under varied negative pressure strengths (−100 and −200 mmHg). Negative pressure means a pressure lower than atmospheric pressure. The pressure was created with a vacuum controller unit (EYELA NVC-3000, Japan) and a diaphragm vacuum pump (EYELA NVP-1000, Japan). Ultrasound-negative pressure fractional precipitation involves varying both the ultrasonic power (80, 180, and 250 W) and the negative pressure strength (−100 and −200 mmHg). The schematics for the cavitation precipitation using ultrasound and negative pressure are presented in Fig. 2(a). Here, cases without ultrasound and negative pressure, without negative pressure, and without ultrasound represent conventional fractional precipitation, ultrasound-fractional precipitation, and negative pressure-fractional precipitation, respectively.

To investigate the mechanism of cavitation fractional precipitation, fractional precipitation using a degassed solution and gas bubbles was performed. First, degassing proceeded for the fractional precipitation solutions (acetone and distilled water) using an aspirator (A-3S, Tokyo Rikakikai, Japan) and an ultrasonic bath at an ultrasonic power of 540 W for 4 hr to investigate the effect of cavitation bubbles. Fractional precipitation was then performed with the degassed solutions at 250 W, −200 mmHg, and 250 W/−200 mmHg for 30 min each. The fractional precipitation using gas bubbles was also carried out by injecting gas bubbles into the precipitation solutions using a bubble generator (SH-A2, Amazonpet, Korea) with an orifice (diameter 0.5 mm) attached. The gas flow rate of the bubble generator was maintained at 1.75 mL/min and fractional precipitation was carried out under varied working times (1, 5, 10, 20, and 30 min schematics for the fractional precipitation using gas bubbles are presented in Fig. 2(b). All the experiments were performed in triplicate and the average values from the results were taken.

The precipitate obtained after filtering (150 mm, Whatman, Buckinghamshire, UK) was dried in a vacuum drying oven (UP-2000, EYELA, Japan) at 40 °C for 24 hr. The amount of (+)-dihydromyricetin from the dried sample was analyzed through HPLC.

3. (+)-Dihydromyricetin Analysis

The (+)-dihydromyricetin was quantitatively analyzed using an HPLC system (SCL-10 AVP, Shimadzu, Japan) with the C_{18} column (4.6×250 mm, 5 μm, Osaka Soda, Japan). A mobile phase of 20% acetonitrile was made to flow in the isocratic mode at a flow rate of 1.0 mL/min, and 30 μL of the sample was injected and analyzed at 274 nm through an ultraviolet (UV) detector. An authentic sample (purity: 98%) was purchased from Guilin Natural Ingredient, Inc. (Guilin, China). Each sample was analyzed in triplicate.

4. Morphology and Size of the Precipitate

An electron microscope (SV-35 Video Microscope System, Sometech, Korea) was used to examine the morphology and size of the precipitate in the fractional precipitation [12]. The (+)-dihydromyricetin precipitate was analyzed under high magnification (x200) and the size of the precipitate was measured from video images using IT-Plus software (Sometech, Korea).

5. Estimation of Diffusion Coefficient

The diffusion coefficient of (+)-dihydromyricetin molecules (B) diffused in the precipitation solvent (A) was calculated from Stokes-Einstein equation and can be expressed as Eq. (1) [11,15].

$$D_{AB} = \frac{kT}{6\pi r_0 \eta} \tag{1}$$

where, k is the Boltzmann constant (1.38×10^{-23} J/K), r_0 is the radius of the (+)-dihydromyricetin molecule, η is the dynamic viscosity of the solution, and T is the absolute temperature of the solution. The viscosity of the solution was measured with a viscometer (Viscolite 700, Hydromotion, UK).

6. Kinetic Studies

The Johnson-Mehl-Avrami-Kolmogorov (JMAK) equation is mainly used in the crystallization or precipitation process. It pertains to the kinetics of isothermal phase-transformation during nucleation and particle growth. It is expressed as Eq. (2) [11,12].

$$X(t) = 1 - e^{-kt^n} \tag{2}$$

Eq. (2) becomes Eq. (3).

$$\log\left(\ln\left(\frac{1}{1-X(t)}\right)\right) = n\log t + \log k \tag{3}$$

where, X(t) is the precipitation yield at any time t, k is the rate constant, and n is the Avrami index which describes the characteristics of the crystal structure and crystal nucleation. n and k were calculated from the slope and intercept of the straight line obtained by plotting $\log\left(\ln\left(\frac{1}{1-X(t)}\right)\right)$ versus logt using Eq. (3). The suitability of the JMAK model was judged by the coefficient of determination (r^2). For a good statistical model, r^2 should be close to 1, which means that the error between the analysis value and calculated value is small. In addition, $r^2>0.75$ represents a suitable model [16].

RESULTS AND DISCUSSION

1. Ultrasound-fractional Precipitation and Negative Pressure-fractional Precipitation

Though fractional precipitation is a simple method for purifying (+)-dihydromyricetin using solubility differences, it has limited uses in mass production due to its long precipitation time [10]. To solve this problem, fractional precipitation was first performed using the ultrasound cavitation effect. The results of conventional fractional precipitation (control) and fractional precipitation with different ultrasonic powers (80, 180, and 250 W) are presented in Fig. 3. In the case of the control, the (+)-dihydromyricetin yield was 73.86% with a precipitation time of 30 min. On the other hand, the maximum precipitation yield was as high as 82.37%, 92.33%, and 93.62% at the ultrasonic powers of 80, 180, and 250 W, respectively.

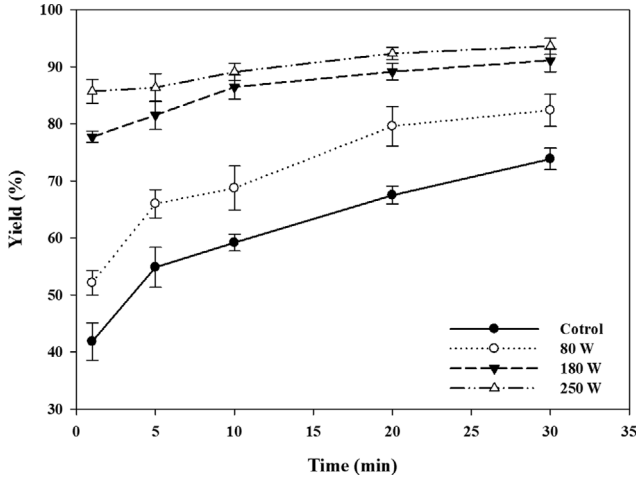

Fig. 3. Effect of ultrasound on the yield of (+)-dihydromyricetin from biomass.

As the precipitation time elapsed, the (+)-dihydromyricetin yields increased in both the control and ultrasound-fractional precipitation, and the yield increased as the ultrasonic power increased. Therefore, ultrasound-fractional precipitation could remarkably improve the precipitation efficiency of (+)-dihydromyricetin. The purity of (+)-dihydromyricetin was not much different, being between 83.13% and 90.57% regardless of ultrasound application (data not shown). These results are in good agreement with the results of the preceding studies [10,12].

A new green negative cavitation was then introduced into the fractional precipitation of (+)-dihydromyricetin. Negative pressure-fractional precipitation requires fewer additional devices and has a lower energy cost than ultrasound-fractional precipitation. In addition, negative pressure-fractional precipitation can be well utilized in the environmentally friendly commercial mass production process with a relatively simple process [11]. The yield of (+)-dihydromyricetin according to the precipitation at different negative pressures (−100 and −200 mmHg) is presented in Fig. 4. The maximum yield of (+)-dihydromyricetin was 91.02% and 100% at negative pressures of −100 mmHg and −200 mmHg at 30 min of precipitation time, respectively. As the negative pressure and precipitation time increased, the yield also increased. In particular, a negative pressure of −200 mmHg could recover most of the (+)-dihydromyricetin in a short working time (20 min). Like ultrasound-fractional precipitation, the precipitation efficiency of the negative pressure-fractional precipitation was significantly improved when compared to conventional fractional precipitation. Nonetheless, the purity of the precipitates was about 82.51 to 91.40%, not much different from that of the conventional fractional precipitation (data not shown). These efficiency improvements might have been attributed to not only the cavitation effect by the negative pressure but also by a mechanical effect by improving the fluidity of the precipitation solution [17]. The results are similar to those in the negative pressure cavitation fractional precipitation for the purification of paclitaxel from *Taxus chinensis* reported by Min and Kim [11].

2. Ultrasound-negative Pressure Fractional Precipitation

Ultrasound and negative pressure were simultaneously introduced to the factional precipitation of (+)-dihydromyricetin to investigate their synergistic effect. As shown in Fig. 5, after 10 min of precipitation, a high yield of more than 93.43% was observed at all conditions. At 30 min of precipitation the yield of (+)-dihydromyricetin was 96.30% (ultrasound/negative pressures: 80 W/−100 mmHg), 100% (80 W/−200 mmHg), 100% (180 W/−100 mmHg), 100% (180 W/−200 mmHg), 100% (250 W/−100 mmHg), and 100% (250 W/−200 mmHg), respectively. In all conditions, most of the (+)-dihydromyricetin could be recovered. As the ultrasonic power, negative pressure strength, and working time increased, the yield of (+)-dihydromyricetin also increased. Especially under 180 W/−200 mmHg and 250 W/−200 mmHg, most of the (+)-dihydromyricetin (>97.56%) could be recovered in a short working time (1 min). The purity of (+)-dihydromyricetin after the ultrasound-negative pressure fractional precipitation ranged from 84.63% to 91.45% (data not shown). When compared with the fractional precipitation introducing only ultrasound or negative pressure, there was a far higher yield given the same precipitation time when both

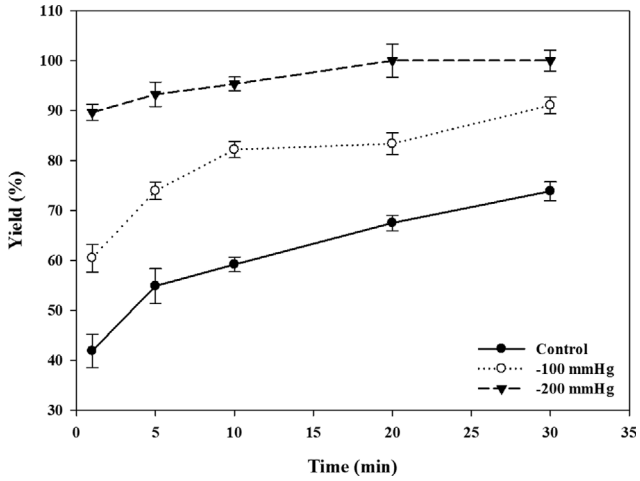

Fig. 4. Effect of negative pressure on the yield of (+)-dihydromyricetin from biomass.

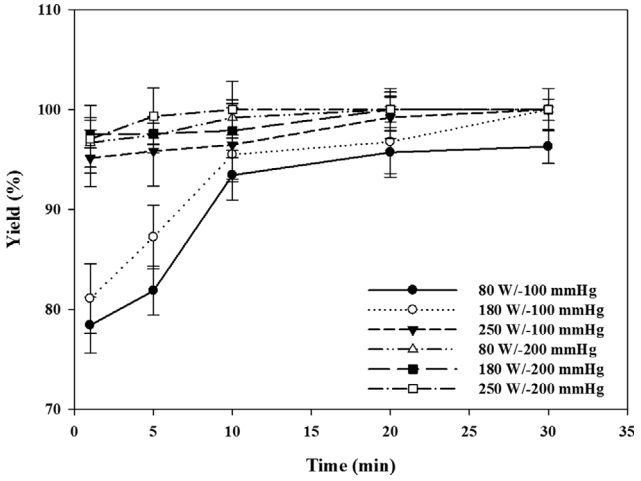

Fig. 5. Effect of ultrasound and negative pressure on the yield of (+)-dihydromyricetin biomass.

Table 1. Comparison of this study and previous studies for fractional precipitation of (+)-dihydromyricetin

Fractional precipitation method	Fractional precipitation time (hr)	Reference
Traditional method	~32	[8]
With ion exchange resin	~16	[9]
With gas bubble	0.5	[12]
With ultrasonic cavitation bubble	0.5	[10], This study
With negative pressure cavitation bubble	0.3-0.5	This study
With ultrasound-negative pressure cavitation bubble	0.02-0.17	This study

ultrasound and negative pressure were introduced. The comparison results of the precipitation times between conventional fractional precipitation and the method in this study for (+)-dihydromyricetin are presented in Table 1. In fractional precipitation using gas bubbles and ultrasound cavitation bubbles, which were developed recently, the precipitation time was shortened to 0.5 hr as compared to the longer precipitation times of the conventional fractional precipitation and fractional precipitation with ion exchange resins (surface area-increasing agent). The precipitation time of the negative pressure cavitation fractional precipitation developed in this study was 0.3 to 0.5 hr, shortening the precipitation time further compared to that of the preceding studies. In the case of ultrasound-negative pressure cavitation fractional precipitation, the precipitation time was approximately 0.02 to 0.17 hr, the shortest among the methods developed for the fractional precipitation of (+)-dihydromyricetin. That means that by introducing both ultrasound and negative pressure into the fractional precipitation process, the issues of long precipitation time in the conventional fractional precipitation could be significantly improved. Such results might have been attributed to the synergy effect of the ultrasound and negative pressure. Furthermore, the cavitation bubbles formed by the introduction of ultrasound and negative pressure acted as sites of heterogeneous nucleation, which lowered the free energy barrier for nucleation, promoting a higher precipitation rate [10,11,17]. In addition, the shock waves and microjets produced by the collapse of the cavitation bubbles might have affected homogeneous nucleation favorably.

3. Studies on the Mechanism of Fractional Precipitation

To investigate the effect of the bubbles on the improvement of precipitation efficiency in the cavitation fractional precipitation in detail, fractional precipitation was performed using degassed solutions (acetone and distilled water). Other conditions for precipitation were set the same except using degassed solutions, and fractional precipitation was performed under ultrasound and negative pressure conditions (250 W, −200 mmHg, and 250 W/−200 mmHg) for 30 min (Fig. 6). Cavitation bubbles were not generated in the precipitation solution made with the degassed solutions (i.e., no cavitation) and the yield of (+)-dihydromyricetin in the fractional precipitation without cavitation bubbles ranged from 65.19% to 73.00%, which was insignificant regardless of ultrasound and negative pressure. Such results were similar to the yields obtained through the conventional fractional precipitation. It showed a clear difference compared to the yield (93.62-100%) of fractional precipitation using cavitation bubbles. This indicated that the fractional precipitation efficiency was greatly improved by the cavitation bubbles [18].

When the ultrasound and negative cavitation bubbles are collapsed, it is accompanied with cavitation phenomena such as high-pressure shock waves, strong local heating, and high-speed microjets. On the other hand, gas bubbles only expand during their lifetime, unlike the cavitation bubbles; thus, there is no high temperature or high-pressure generation [12]. These characteristics were used in the fractional precipitation with gas bubbles in order to investigate the mechanism of the cavitation fractional precipitation in detail. The gas bubbles were injected into the precipitation solution while maintaining the gas flow rate at 1.75 mL/min. using a bubble generator. The fractional precipitation results with gas bubbles (gas flow rate: 1.75 mL/min) and those with ultrasound (250 W) and negative pressure (−200 mmHg) cavitation bubbles are presented in Fig. 7. In fractional precipitation where the gas bubbles were introduced, the yield reached 90.55% in 20 min of precipitation. The yield was remarkably improved with only gas bubbles in the fractional precipitation, giving similar results to the use of cavitation bubbles. In the case of fractional precipitation using gas bubbles instead of cavitation bubbles (yield: 82.01-91.65%), the precipitation efficiency was significantly improved compared to the control (41.83-73.86%). The results imply that the roles of the cavitation bubbles and gas bubbles in the fractional precipitation were similar. In summary, the bubbles themselves acted as nucleation sites rather than the high pressure shock waves, strong local heating, and high-speed microjets generated by the collapse of the cavitation bubbles, and attributed to the improved precipitation efficiency [12,18]. Based on this study, the mechanism of the cavitation bubble fractional precipitation was proposed in Fig. 8.

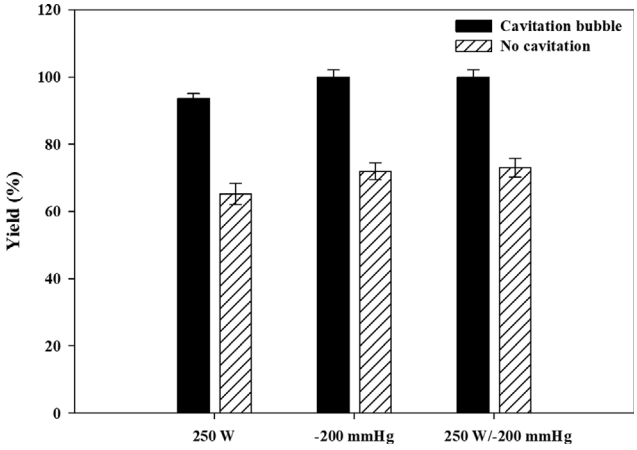

Fig. 6. Effect of the presence or absence of cavitation bubbles on the yield of (+)-dihydromyricetin.

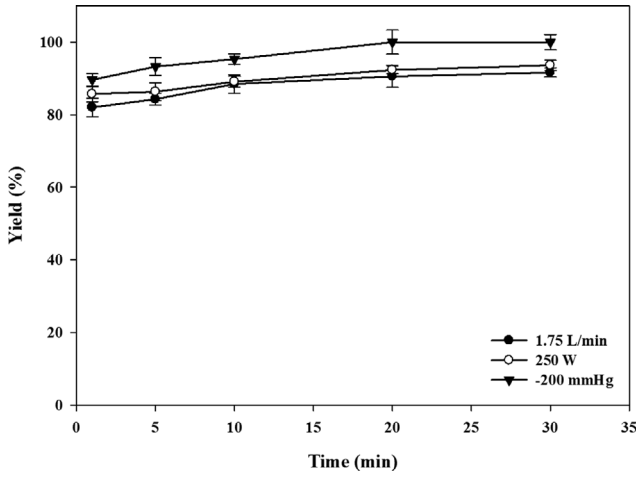

Fig. 7. Effect of cavitation bubbles (ultrasonic power 250 W, negative pressure −200 mmHg) and gas bubbles (gas flow rate 1.75 L/min) on the yield of (+)-dihydromyricetin.

4. Kinetic Study on the Fractional Precipitation Process

The experimental data were applied to the JMAK equation to plot $\log\left(\ln\left(\frac{1}{1-X(t)}\right)\right)$ versus logt. The JMAK index (n) and rate constant (k) were obtained from the slope and intercept, and these are presented along with the coefficient of determination (r^2) in Table 2. The obtained n values were 0.2738 (control), 0.0799 to 0.2490 (ultrasound-fractional precipitation), 0.1175 to 0.2614 (negative pressure-fractional precipitation), and 0.0799 to 0.3508 (ultrasound-negative pressure fractional precipitation). The values of k were 0.5196 in the control, and 0.7195 to 1.8789 under the ultrasound power of 80 to 250 W, which was an increase of 1.4 to 3.6 times compared to the control. In the case of negative pressure-fractional precipitation, the k values were 0.9114 to 2.097 under the negative pressures of −100 to −200 mmHg, thereby the k increased by 1.8 to 4.0 times compared to the control. The k values were 1.4214 to 3.7171 in the ultrasound-negative pressure fractional precipitation and it increased by 2.7 to 7.1 times compared to the control. Therefore, the precipitation rate increased the most with ultrasound-negative pressure fractional precipitation. In addition, the activation energy changes ($\Delta E_a = \Delta E_{a,\,given\ conditions} - \Delta E_{a,\,control}$) were calculated according to the ultrasonic power and negative pressure using the Arrhenius equation. By introducing ultrasound and negative pressure, ΔE_a decreased and, as the ultrasonic power and negative pressure intensity increased, ΔE_a was further decreased. These results indicate that the ultrasound and negative pressure had a synergy effect. The activation energy was decreased by the ultrasound and negative pressure introduced in the fractional precipitation, which

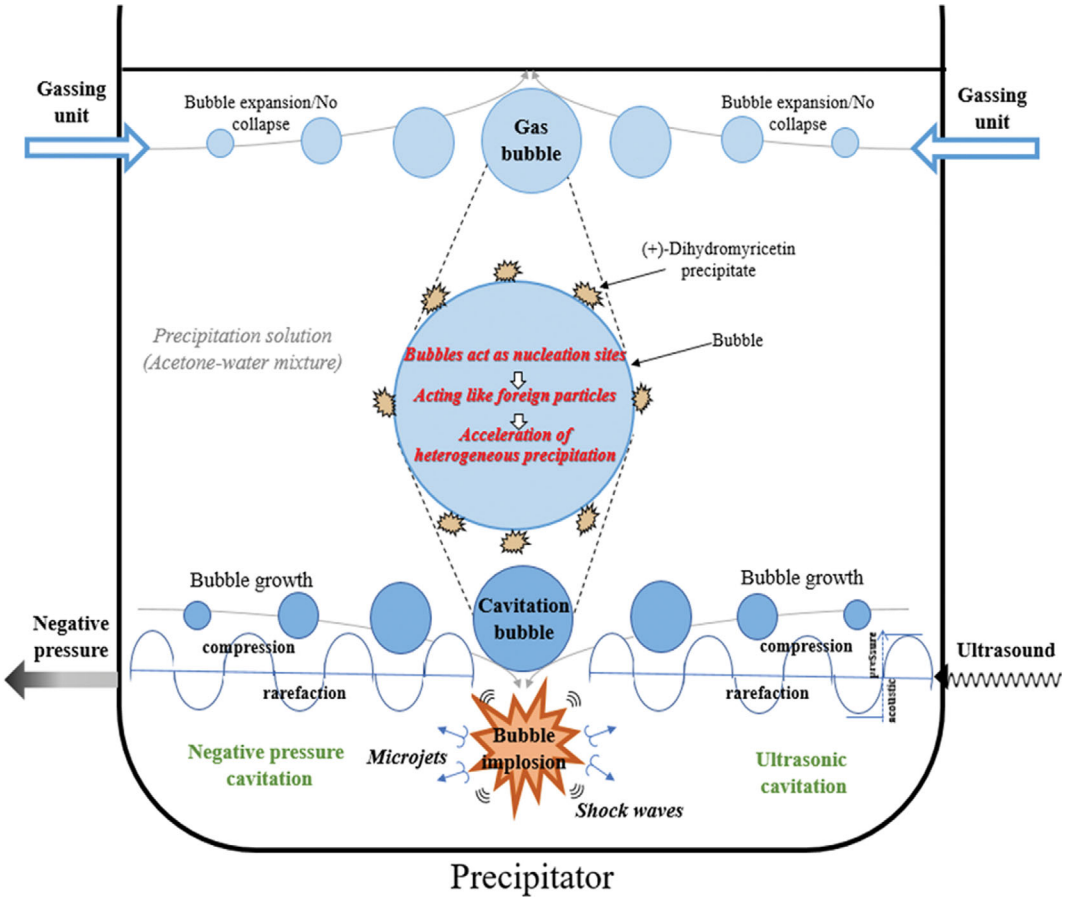

Fig. 8. Proposed mechanism for ultrasound/negative pressure cavitation bubble- and gas bubble-induced fractional precipitation of (+)-dihydromyricetin from biomass.

Table 2. Values of kinetic parameters for the fractional precipitation of (+)-dihydromyricetin at different ultrasonic powers and negative pressures

Negative pressure (mmHg)	Ultrasonic power (W)	n	k (min^{-1})	ΔE_a (J/mol)	r^2
0	0 (Control)	0.2738	0.5196	-	0.8820
	80	0.2490	0.7198	−754	0.9740
	180	0.1662	1.4103	−2,364	0.8861
	250	0.0799	1.8789	−2,918	0.7892
−100	0	0.2614	0.9114	−1,299	0.9592
	80	0.2447	1.4214	−2,327	0.8744
	180	0.2327	1.6070	−2,611	0.8852
	250	0.0418	3.0110	−4,063	0.9510
−200	0	0.1175	2.0970	−3,226	0.9803
	80	0.3217	2.8445	−3,932	0.7899
	180	0.1952	3.5157	−4,421	1.0000
	250	0.3146	3.7171	−4,550	0.8851

Table 3. Effect of ultrasound and negative pressure on viscosity, mean particle size, and diffusion coefficient

Negative pressure (mmHg)	Ultrasonic power (W)	Viscosity, η (cp)	Mean particle size, r_0 (μm)	Diffusion coefficient, D_{AB} ($\times 10^{-10}$ cm^2/s)
0	Control	1.0	14.802	1.375
	80	0.8	7.419	3.429
	180	0.8	5.692	4.470
	250	0.8	5.137	4.952
−100	0	0.8	7.995	3.182
	80	0.8	6.024	4.223
	180	0.8	5.286	5.383
	250	0.8	4.726	6.841
−200	0	0.8	4.807	5.293
	80	0.8	4.628	5.497
	180	0.8	3.996	6.367
	250	0.8	3.719	6.841

lowered the free energy barrier required for nucleation, thus accelerating precipitation [10-12]. The kinetic study suggested that the JMAK model was suitable for the fractional precipitation process of (+)-dihydromyricetin because $r^2>0.75$ in all the conditions.

5. Determination of Particle Size and Diffusion Coefficient

In general, for active pharmaceutical ingredients (API), smaller particle sizes have a better dissolution rate during formulation, better uniformity for drug dispersion, and better oral bioavailability, thereby increasing the utilization of the drug [19]. In addition, as the particle size is reduced, it becomes more effective in removing residual moisture and residual solvent after purification [20]. In this respect, the particle size reduction of (+)-dihydromyricetin is very important in terms of its utilization. The particle size r_0 of the precipitate in the control and cavitation fractional precipitation using the ultrasound and negative pressure was measured using an electron microscope. The results are arranged in Table 3. The particle size of the precipitate decreased in the cavitation fractional precipitation compared to that of the control, and this tendency was more pronounced as the ultrasonic power and negative pressure intensity increased. Quantitative comparison showed that the mean particle size until the precipitation reached equilibrium was 14.802 μm in the control, and the particle size was 5.137 to 7.419 μm (80-250 W), 4.807 to 7.995 μm (−100~−200 mmHg), and 3.719 to 6.024 (80 W/−100 mmHg~250 W/−200 mmHg), respectively, in the ultrasound and negative pressure fractional precipitations. Compared to the control, the particle size was 2.0 to 2.9 times smaller for ultrasound and 1.9 to 3.1 times smaller for negative pressure. On the other hand, when both ultrasound and negative pressure were introduced, the particle size decreased by 4.0 times. As the ultrasonic power and negative pressure intensity increased, the particle size was further decreased. In general, when ultrasound and negative pressure were introduced, the crystal size decreased due to abrasion between shock waves and particles [21].

To quantitatively investigate the diffusion behavior during fractional precipitation, the diffusion coefficient D_{AB} was calculated using the Stokes-Einstein equation (Table 3). The viscosity of the precipi-

tation solution was 1.0 cp in the control, and it was 0.8 cp in the fractional precipitation with the ultrasound and negative pressure. In the case of the control, D_{AB} was 1.375×10^{-10} cm^2/s and it was 3.429 to 4.952×10^{-10} cm^2/s (80-250 W) in the fractional precipitation with ultrasound, which was an increase of 2.5 to 3.6 times compared to the control. When negative pressure was introduced, the diffusion coefficient was 3.182 to 5.293×10^{-10} cm^2/s (−100, −200 mmHg), which was 2.3 to 3.8 times greater than that of the control. When both ultrasound and negative pressure were simultaneously introduced, the diffusion coefficient ranged from 4.223 to 6.841×10^{-10} cm^2/s (80 W/−100 mmHg~250 W/−200 mmHg), which was a 3.1 to 5.0 times increase. The increases of the diffusion coefficient in the fractional precipitation affected uniform nucleation. By introducing ultrasound and negative pressure, the diffusion coefficient increased, which also improved uniform nucleation [22,23]. Particularly, the diffusion coefficient in the ultrasound-negative pressure cavitation fractional precipitation showed a larger value than the fractional precipitation with either ultrasound or negative pressure alone, explaining the synergy effect of ultrasound and negative pressure.

CONCLUSIONS

An ultrasound-negative pressure cavitation fractional precipitation process was developed to efficiently purify (+)-dihydromyricetin, and the mechanism of the cavitation fractional precipitation was investigated. In the case of the conventional fractional precipitation, the yield of (+)-dihydromyricetin in 30 min of precipitation was 73.86%. However, the yield was 82.37-93.62% (80-250 W), 91.02-100% (−100~−200 mmHg), and 96.30-100% (80 W/−100 mmHg to 250 W/−200 mmHg) in ultrasound-fractional precipitation, negative pressure-fractional precipitation, and ultrasound-negative fractional precipitation, respectively. In particular, the ultrasound-negative pressure cavitation fractional precipitation could recover most of the (+)-dihydromyricetin (>97.56%) in a short operating time. The mechanisms of the cavitation fractional precipitation were investigated using a degassed solution and gas bubbles. The results showed that the bubbles themselves acted as nucleation sites during precipitation, increasing the nucleation rate and resulting in an improved precipitation efficiency. The kinetic study revealed that the rate constant increased by 3.6 times, 4.0 times, and 7.1 times in ultrasound-fractional precipitation, negative pressure-fractional precipitation, and ultrasound-negative pressure fractional precipitation, respectively, compared to the control. The changes of activation energy decreased in the ultrasound-negative pressure fractional precipitation. The size of the (+)-dihydromyricetin particles decreased by 4.0 times, and the diffusion coefficient increased by 5.0 times in ultrasound-negative pressure precipitation compared to the control. The results indicated that the precipitation rate became accelerated due to decreases in the activation energy by introducing ultrasound and negative pressure, and uniform nucleation was promoted due to decreases in the particle size and increases in the diffusion coefficient. Therefore, the synergy effect of ultrasound and negative pressure was confirmed through the quantitative comparison of the fractional precipitation efficiency.

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