Food Chemistry 303 (2020) 125394

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Vesicle based ultrasonic-assisted extraction of saponins in *Panax notoginseng*

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ARTICLE INFO

Keywords:
Panax notoginseng
Response surface methodology
Saponins
Ultra-high performance liquid chromatography
Ultrasonic-assisted extraction
Vesicle

ABSTRACT

A simple and effective vesicle based ultrasonic-assisted extraction (UAE) method was developed for extraction of active compounds in functional food. The target analytes were determined by ultra-high performance liquid chromatography with ultraviolet detector. Surfactant vesicle was adopted as extraction solvent. Different operating conditions including the type and concentration of vesicle, extraction time and solid to liquid ratio were investigated by single-factor experiments and response surface methodology. Optimized experimental conditions were 1% (w/v) of DTAB/SDS vesicle, 20 min of extraction time and 160 mg/mL of solid to liquid ratio. The proposed method provided good linearity in the linear range of 10–1000 μg/mL with regression coefficients larger than 0.999, low limits of detection of 27.64–55.67 ng/mL, good precision with relative standard deviations below 0.35%, and satisfactory recoveries of 83.84–90.92% for tested saponins. Consequently, the proposed vesicle based UAE method was well suited for the extraction of saponins in *Panax notoginseng*.

1. Introduction

Vesicles, the large aggregates of monomers, are like membrane-enclosed capsules and generally formed from natural and synthetic surfactants (Pascoe & Foley, 2003). The cationic/anionic (catanionic) surfactants readily form vesicle structures and have more merits than a single surfactant system because of the synergistic effects. The spherical structure of vesicle was consisted of a hydrophilic internal cavity and a bilayer hydrophobic shell composed of the tails of amphiphilic molecules. The behavior of self-aggregation is driven by multiple interactions including strong electrostatic interactions due to their counter charged head groups and the hydrophobic actions existed between their tails (Mala, Bagb, Ghosha, & Moulika, 2018). In the comparison of liposomes, an analogous closed bilayer vesicles made from phospholipid molecules was generally used as drug delivery carrier on the basis of a high biocompatibility (Li et al., 2019). However, vesicle also has potential application of drug delivery carrier in pharmaceutical industries. And the general conclusion is that most catanionic surfactant systems possess biodegradability and are gentle toxic to cells (Ghosh, Ray, Pramanik, & Ambade, 2016; Liang, Yeh, Liao, & Chou, 2015). Additionally, several advantages of vesicle are over liposomes. The formation of surfactant vesicles is spontaneous, easier for preparation, relatively low cost, more controllable and thermodynamic stable (Hong, Weekley, Grieb, & Foley, 1998; Jiang, Luan, Qin, Zhao, & Li, 2012; Wang et al., 2016). Kaler first reported the spontaneous formation of anionic and cationic surfactant vesicles in aqueous solution (Kaler, Murthy, Rodriguez, & Zasadzinski, 1989). The bilayer structure makes vesicles have many applications in drug delivery, biomimetic studies and food industry (Pascoe & Foley, 2003; Šegota & Težak, 2006). Moreover, compared to normal spherical micelle constructed by single surfactant solutions, vesicles have a larger number of solubilization sites, an alternative hydrophobic-hydrophilic selectivity and larger hydrodynamic diameter, which increased the interactions with various analytes (Fendler, 1987). Additionally, supramolecular solvents (SUPRAS) were employed for extracting different polarity substances into their ordered structures, which included vesicular SUPRASs and micellar SUPRASs, etc. However, preparation condition is extremely different between the vesicular SUPRAS and vesicles. There are two-steps for forming SUPRAS. After three-dimensional individual aggregates assembling, a new highly packed phase generates via the coacervation-inducing agent, pH or temperature stimulus (Ballesteros-Gomez, Caballero-Casero, García-Fonseca, Lunar, & Rubio, 2019; Torres-Valenzuela, Ballesteros-Gómeza, Saninb, & Rubioa, 2019), which is complicated. While the preparation of vesicles is one step and further aggregation is omitted. Therefore, vesicles are very possible to be employed for extracting target analytes in real samples. However, as far as we know, there are rare reports on the applications of vesicles in extraction fields.

Natural products derived from traditional Chinese medicines have attracted much attention in recent years. Herbal materials contain complex ingredients and the active compounds are usually present in low concentrations. For this reason, before introducing the samples into

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https://doi.org/10.1016/j.foodchem.2019.125394
Received 23 February 2019; Received in revised form 10 August 2019; Accepted 18 August 2019
Available online 23 August 2019
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analytical instruments, it is very significant to choose an appropriate extraction method. Conventional sample pretreatment techniques such as Soxhlet extraction, heat reflux extraction, distillation and liquid-liquid extraction have been used to extract desired compounds from various matrices (Farajzadeh, Khoshmaram, & Nabil, 2014; Yang, Wei, Huang, Lee, & Lin, 2013). But, they have many drawbacks, including the consumption of large volumes of organic solvents, tedious procedures, long extraction time, and the degradation of analytes due to the high temperature To overcome the above-mentioned drawbacks, various new extraction methods such as ultrasound-assisted extraction (UAE) (Briars & Paniwnyk, 2013), microwave-assisted extraction (Simsek, Sumnu, & Sahin, 2012) and supercritical fluid extraction (Lee, Charles, Kung, Ho, & Huang, 2010) have been proposed to extract target compounds from various samples. However, these methods still required a large amount of organic solvents and exhibited low extraction efficiency. Recently, a relatively green solvent, ionic liquids (ILs), have been applied to replace classical organic solvents in many extraction approaches such as ILs based UAE procedure (Sun & Armstrong, 2010; Zhang et al., 2011; Magiera and Sobik, 2017). Furthermore, it is necessary to develop more specific and environmental friendly methods because of the complexity of natural products and the particularity of active compounds. To the best of our knowledge, there have not yet been any reports on the application of vesicles as an extraction solvent for the extraction of active compounds from plants.

Panax notoginseng, the dried root and rhizome of *Panax notoginseng* (Burk.) F. H. Chen, is a traditional medicinal herb and functional food that has been cultivated for more than 400 years in China, especially in Yunnan Province (Guo, Cui, An, & Cai, 2010). Contemporary researches demonstrated that it was employed for treating many diseases, including cardiovascular diseases, trauma, body pains, internal and external bleeding (Lin, Wong, Wu, Huang, & Liu, 2003; Wang et al., 2016). The ingredients in *Panax notoginseng* are mainly saponins, amino acids, dencichine, flavonoids and polysaccharides, etc. (Wang, McEntee, Wicks, Wu, & Yuan, 2006). Among them, the saponins including ginsenosides and notoginsenosides are major bioactive constituents responsible for pharmacological activities (Du, Jerz, Waibel, & Winterhalter, 2003; Wang et al., 2006). Thus, the quality control of *Panax notoginseng* was generally focused on the determination of saponins. To date, several extraction methods including reflux extraction (Bai et al., 2009; Zhou, Razmovski-Naumovski, & Chan, 2015) and Soxhlet extraction (Sarvin, Stekolshchikova, Rodin, Stavrianidi, & Shpigun, 2018) have been developed for the extraction of saponins in *Panax notoginseng*. However, these methods usually required long extraction time and lots of organic solvents. Therefore, it is meaningful to develop a more efficient and environmental friendly method for extraction of saponins from *Panax notoginseng*.

In this work, a simple, effective, and environmental friendly UAE method which used surfactant vesicle as extraction solvent was established for extraction of saponins from *panax notoginseng*. The target compounds were determined by ultra-high performance liquid chromatography with ultraviolet detector (UHPLC-UV). Several experimental factors including the type and concentration of vesicle, extraction time and solid to liquid ratio were evaluated and optimized by single-factor experiments and response surface methodology (RSM). Additionally, validation experiments were carried out in the terms of linearity, precision, accuracy, and reproducibility.

2. Experimental

2.1. Reagents and materials

Dodecyltrimethylammonium bromide (DTAB), cetyltrimethylammonium bromide (CTAB), cetyltrimethylammonium chloride (CTAC), dihexadecyldimethylammonium bromide (DHAB), sodium dodecyl sulfate (SDS), and sodium octyl sulfate (SOS) were purchased from Sincopharm Chemical Reagent Co., Ltd. (Shanghai, China). Chromatography-grade methanol and acetonitrile were acquired from Merck Darmstadt Ltd. (Darmstadt, Germany). The purified water was provided by Hangzhou Wahaha Group Co., Ltd. (Hangzhou, China). All saponin standards (purity were ≥ 98%) including notoginsenoside R1, ginsenoside Rg1, ginsenoside Rb1, and ginsenoside Rd were obtained from Shanghai Winherb Medical Technology Co., Ltd. (Shanghai, China). Standard stock solutions were prepared in chromatographic grade methanol at a final concentration of 500 μg/mL, and then stored in a refrigerator at 4 °C. Working standard solutions were freshly prepared by dilution of the standard stock solutions in methanol. The *panax notoginseng* produced from Yunnan province were supplied by a local drugstore (Hangzhou, China).

2.2. Apparatus

Chromatographic analysis was performed on Agilent 1290 UHPLC system (Agilent Technologies, Santa Clara, CA) equipped with an UV detector. The Agilent SB-C_{18} column (1.8 μm, 4.6 × 50 mm i.d.) maintained at 40 °C was used for separation of target analytes. The mobile phase consisted of A (water) and B (acetonitrile). The elution gradient was: 0–1 min, 20% B; 1–2 min, 20–25% B; 2–3 min, 25–30% B; 3–4 min, 30–35% B; 4–5 min, 35% B; 5–6 min, 35–40% B; 6–7 min, 40–60% B; 7–8 min, 60–100% B; 8–9 min, 100% B; 9–10 min, 100–20% B. The flow rate was set at 0.4 mL/min during the whole process. The detection wavelength was set at 203 nm and the injection volume was set at 2 μL.

2.3. Vesicle preparation and UAE process

The formation of vesicle is achieved in two-step: dissolution and blend. The DTAB/SDS vesicle solution was prepared in a total surfactant concentration of 0.1–5% w/v with a weight ratio of 39.06/60.94 DTAB/SDS (Hong et al., 1998). Firstly, vesicle was prepared by dissolving accurately weighed DTAB in water, and then the fixed amount SDS was introduced into the solution. Finally, the catanionic surfactants mixture in aqueous media was vibrated at intermediate speed (150 r/min) for 30 min. CTAB/SOS or CTAC/SOS vesicles (14.8 mM/54.2 mM) in a mole ratio of 3/7 with a total surfactant weight percentage of 1.8% w/v (Pascoe & Foley, 2002; Schuster & Foley, 2005) were prepared through the same procedure. The DHAB vesicle was prepared by dissolving 50 mg of the double-chain surfactant in 10 mL water (Agbodjan & Khaledi, 2003), and then vibrated for 30 min. All the mixed solution was placed under ambient conditions for three days. No precipitate and change of color were observed. Physicochemical properties (mean diameter) of tested vesicles were shown in Table S1 (Supplementary materials).

A fixed amount of *panax notoginseng* treated by a crushing machine was added to the vesicle solution with the aim to obtain concentration at a ratio of solid/liquid 160 mg/mL, and then extracted by ultrasonication for 20 min.

2.4. Pharmacopeia method

As additional control, four saponins were extracted through pharmacopeia method (Chinese Pharmacopoeia, 2015 version): 0.5 g dried sample powder was precisely weighed and placed into a distilling flask contained 50 mL methanol. And then overnight immersion and 80°C water bath distillation was carried out.

Above extraction solution was treated by centrifuging (13,000 rpm, 5 min), the supernatant solution was filtered by a 0.22 μm nylon syringe filter prior to UHPLC analysis.

2.5. Experimental design

Response surface methodology (RSM) was utilized to determine the optimal combination of independent variables for the UAE of saponins

from *panax notoginseng*. The main experimental factors affecting extraction efficiency including the concentration of vesicle (A), extraction time (B) and solid to liquid ratio (C) were selected as independent variables, while the peak area of each saponin (notoginsenoside R1, ginsenoside Rg1, ginsenoside Rb1, and ginsenoside Rd) was chosen as the response (dependent) variables (Y). The optimization was carried out through a three-level, three-factor Box-Behnken design (BBD) project consisting of 17 experimental runs including five replicates at the central point. The choice of range and center point values of the three main variables was based on the results of single-factor test. The coded and actual values of the experimental factors for the BBD are shown in Table S2 (supplementary materials). All experiments were carried out according to above-mentioned UAE procedure. The average values of dependent parameters determined by UHPLC analysis were subjected to a second order polynomial model. The analysis of variance (ANOVA) was carried out to determine individual linear, quadratic and interaction regression coefficients using Design-Expert software version 8.0.6 (Stat-Ease, Inc.). The coefficient of determination (R^2) was used to assess the fitness of the quadratic polynomial equation to the experimental responses, and the significance of the model and independent variables was evaluated by computing the F value at p value < 0.05.

2.6. Statistical analysis

All optimization experiments were performed by three replicates and the data were expressed as the mean ± standard deviation (n = 3) (Fig. 1). Statistical calculation was performed by Excel software (2016 version). Design Expert 8.0.6 software (Stat-Ease, Inc., Minneapolis, MN, USA) was applied for BBD and analysis of variance (ANOVA).

3. Results and discussion

3.1. Optimization of UAE conditions with single-factor experiment

For the purpose to obtain the optimal extraction conditions, several factors including the type and concentration of vesicle, extraction time and solid to liquid ratio, which could influence the extraction efficiency of UAE procedure, were evaluated through the single factor experiment. Each parameter was performed in triplicate in the optimization process.

3.1.1. Type of vesicle

Vesicles are large self-assembly of monomers with a spherical bilayer structure, which contained an internal cavity of hydrophily. Thus, the special structure made vesicles have the ability to capture polar saponins in hydrophilic internal cavity. Additionally, the hydrophobic bilayer shell structure could provide hydrophilic hydrophobic discrimination power (Hong et al., 1998; Jiang et al., 2012). A detailed explanation was shown in Fig. 3. In this study, surfactant vesicle solutions were used as extraction solvents for extracting saponins from *panax notoginseng*. Four kinds of vesicles including DTAB/SDS, CTAB/SOS, CTAC/SOS and DHAB, which were prepared according to the Section 2.3, were evaluated to find the optimal vesicle extraction solvent, while the other extraction conditions (extraction time, 10 min; solid to liquid ratio, 10 mg/mL) were consistent. The experimental results are displayed in Fig. 1A. As can be seen from the histogram, different vesicles were acted as the X axis and Y axis showed the peak areas of four target analytes (notoginsenoside R1, ginsenoside Rg1, ginsenoside Rb1 and ginsenoside Rd) detected by UHPLC. The highest extraction efficiency of all the analytes was achieved when the DTAB/SDS vesicle was used as extraction solvent. The polar saponins were extracted by vesicle based on hydrophobic-hydrophilic interaction. The DTAB/SDS vesicle had the same alkyl chain length of anionic and

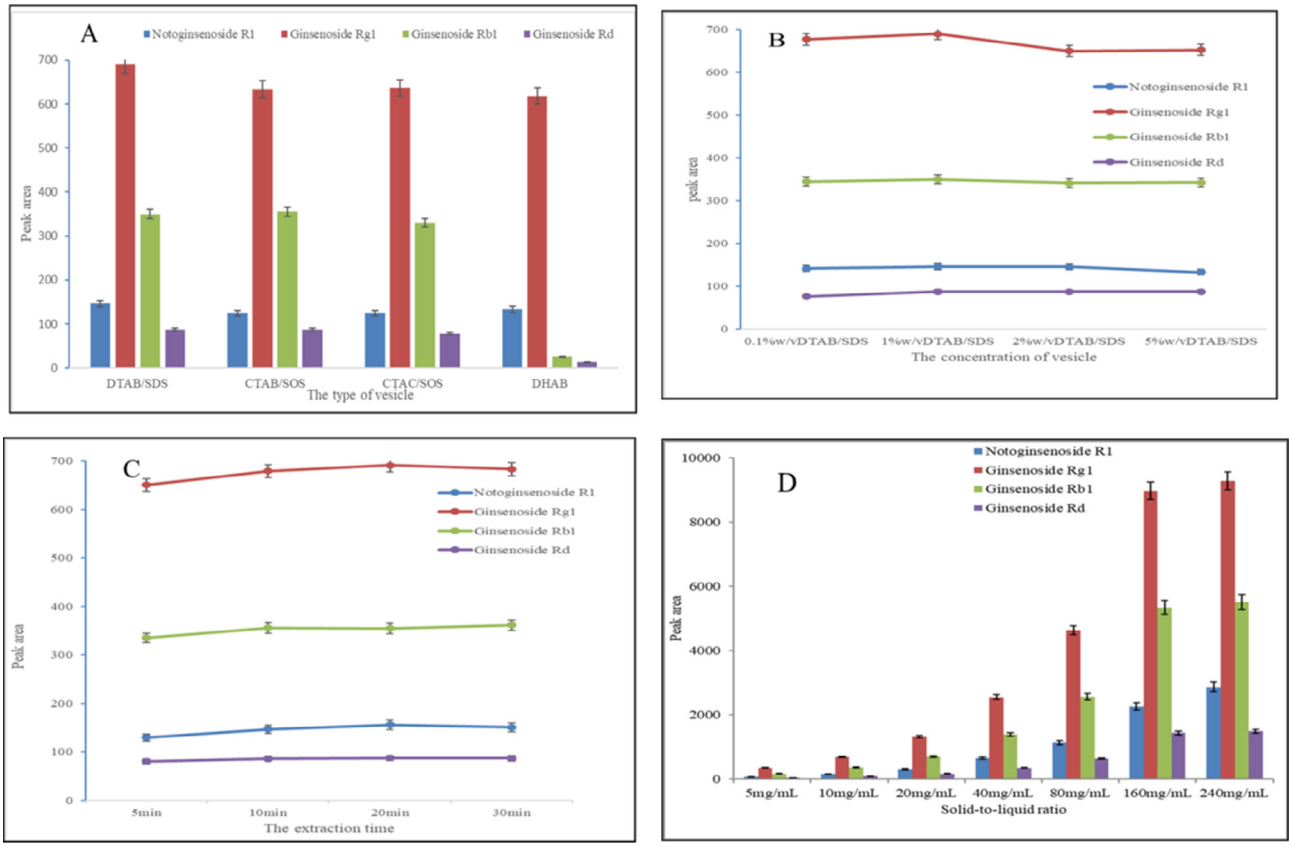

Fig. 1. The effect of the type of vesicle (A), the concentration of vesicle (B), extraction time (C) and solid to liquid ratio (D) on the extraction efficiency of four saponins.

cationic surfactants, which might result in a more compact and stable polar layer aggregates compared with other investigated vesicles. Further, there is a suitable value of molecular packing parameter ($P = v/(a_h \cdot L)$) for DTAB/SDS vesicle and a packing parameter theory was generally used to understand the self-assembly behavior (Chiappisi et al., 2019). In addition, the unique properties of DTAB/SDS vesicle and the special interaction with target analytes might facilitate the extraction. Conversely, the DHAB vesicle exhibited the lowest extraction efficiency. This might be due to the fact that the DHAB vesicle had a lower charge density and weak electrostatic interaction than other vesicles formed by oppositely charged surfactants. Therefore, the DTAB/SDS vesicle was chosen as the optimal extraction solvent.

3.1.2. Concentration of vesicle

The concentration of vesicle plays an important role in the vesicle based UAE procedure. For the purpose to obtain the highest extraction efficiency, the concentration of vesicle was investigated in the range of 0.1–5% (w/v) to find the appropriate vesicle concentration under the same experimental conditions (extraction solvent, DTAB/SDS vesicle; extraction time, 10 min; solid to liquid ratio, 10 mg/mL) in this work. The results were shown in Fig. 1B, which demonstrated that the peak areas of target analytes were increased with the concentration of DTAB/SDS vesicle increasing from 0.1 to 1% (w/v). It was likely attributed to the fact that the tested analytes could not be extracted completely by too low concentration of vesicle. Increasing the concentration of DTAB/SDS vesicle could increase the amount of vesicle aggregates, which could increase the extraction yields through enhanced hydrogen bond and hydrophobic-hydrophilic interactions with target analytes (Jiang et al., 2012). However, further increasing the concentration of vesicle from 1 to 5% (w/v) resulted in a decrease or invariableness in the extraction efficiency of target analytes. This phenomenon can be explained by the fact that the kinetic extraction equilibrium was achieved around the vesicle concentration of 1% (w/v). Further increasing the vesicle concentration might make the vesicle monomers start precipitation. Additionally, too much vesicles in the aqueous solution might cause some adverse effects on the mass transfer of target analytes into the vesicle solution. And the solubility of analytes in the aqueous phase may be affected. Owing to these reasons, 1% (w/v) was selected as the optimal concentration of DTAB/SDS vesicle for the extraction of saponins.

3.1.3. Effect of extraction time

The extraction time is a considerable factor that can significantly influence the extraction yields of target analytes in the UAE process. Since in the vesicle based UAE process, the cell wall of the *panax notoginseng* was destroyed by the ultrasonic wave and the vesicle solution could diffuse into the cell and then interact with the target analytes (Hong Ngoc, Quan Van, Bowyer, & Scarlett, 2018). Finally, the target compounds could dissolve into the extraction solvent. Definitely, the vesicle based UAE method was a time dependent process. Therefore, different extraction times (5, 10, 20 and 30 min) were evaluated in this study under the same extraction conditions (extraction solvent, DTAB/SDS vesicle; concentration of vesicle, 1% (w/v); solid to liquid ratio, 10 mg/mL) in order to acquire the highest extraction yields of tested analytes. Just as Fig. 1C revealed, the peak areas of four targeted compounds increased with the extraction time increasing from 5 to 20 min. The reason for this phenomenon might be that the target analytes could not completely transfer into the vesicle solution from the sample matrix due to the insufficient interaction between the extraction solvent and the analytes, when underwent too short extraction time. The highest extraction efficiency was achieved at extraction time of 20 min. And then corresponding peak areas remained an almost constant value or slightly decreased when further increasing the extraction time from 20 to 30 min. The possible reason was the fact that the extraction equilibrium was achieved when the extraction time was 20 min. Additionally, further increasing the extraction time, the extraction yields of target analytes might decrease due to the destruction of some vesicles caused by the ultrasonic wave. As a result, 20 min of extraction time was adopted for the following studies.

3.1.4. Effect of solid to liquid ratio

Solid to liquid ratio is a crucial variable that can significantly affect the extraction efficiency of analyzed compounds in the UAE process. The extraction solvent volume is related to the amount of the sample. In order to find the appropriate solid to liquid ratio, the effect of solid/liquid ratio was assessed by varying the solid to liquid ratio from 5 to 240 mg/mL, as other variables held constant (extraction solvent, DTAB/SDS vesicle; concentration of vesicle, 1% (w/v); extraction time, 20 min). The experimental results are displayed in Fig. 1D. It could be observed that the extraction yields of target analytes were increased with an increase of the solid to liquid ratio from 5 to 160 mg/mL. This phenomenon could be attributed to the fact that more target compounds could be extracted by the vesicle solution with the enhancement of solid to liquid ratio. However, further increasing the solid to liquid ratio in the range of 160–240 mg/mL, the peak areas of four targets increased slightly, which was insignificant gains being made with the price of larger solid to liquid ratio. The possible explanation was that the extraction equilibrium could be achieved at the solid to liquid ratio of 160 mg/mL. Meanwhile, too large solid to liquid ratio would lead to a difficulty in the process of the transfer of analytes from the sample matrix to the liquid phase. Further, too less volume of vesicle solution caused insufficient interaction between the sample and solvent. Besides, it is very important to reduce the consumption of sample and solvent while prioritizing the highest extraction efficiencies of tested analytes. To take the above situation into consideration, the solid to liquid ratio was set at 160 mg/mL in this study.

3.2. Optimization of UAE through BBD and RSM

3.2.1. Model fitting

Based on the results obtained from single-factor experiments, the optimum extraction conditions were further predicted by RSM through BBD project with the aim of maximum extraction yield of the studied responses. The experimental data showed in Table S2 (supplementary materials). The second-order model equations for four saponins provided in coded form were expressed as follows:

Notoginsenoside R1: Y_1

$$= +2354.86 + 21.84\,A - 8.95\,B + 689.56\,C - 1.98AB + 8.50AC - 8.57\,BC - 106.58A^2 - 51.41B^2 - 417.53C^2$$

Ginsenoside Rg1: Y_2

$$= +9082.08 + 13.37\,A - 0.10B + 2347.00\,C + 11.90AB - 4.95\,AC - 3.20BC - 45.34A^2 - 36.79B^2 - 2146.84C^2$$

Ginsenoside Rb1: Y_3

$$= +5374.64 + 23.80\,A - 33.41\,B + 1416.59\,C - 4.22AB + 32.23\,AC - 0.20BC - 183.44A^2 - 150.72B^2 - 1266.52C^2$$

Ginsenoside Rd: Y_4

$$= +1438.64 - 7.29\,A + 2.95\,B + 420.64\,C + 37.95AB - 6.67AC + 15.45\,BC - 105.13A^2 - 84.46B^2 - 288.68C^2$$

The four equations were used to construct the response surfaces and study the relationship of investigative variables and the responses of four saponins, which were carried out by investigating all levels of each independent variable at the same time. The results of ANOVA for all models are shown in Table 1. Some parameters were applied to estimate the chosen model. The p-values of the model and each coefficient less than 0.05 indicated that the model and each term were significant, while the p-values greater than 0.05 were considered insignificant. As can be seen from the Table 1, the p-values for four saponins models

Table 1
ANOVA of response surface model and predicted results for response of four analytes.

Source	Notoginsenoside R1		Ginsenoside Rg1		Ginsenoside Rb1		Ginsenoside Rd	
	F value	p-value	F value	p-value	F value	p-value	F value	p-value
Model	3657.14	< 0.0001	2696.65	< 0.0001	1674.07	< 0.0001	103.79	< 0.0001
A	27.06	0.0013	0.55	0.4842	2.93	0.1308	0.21	0.6601
B	4.55	0.0705	0.00003049	0.9957	5.77	0.0473	0.035	0.8578
C	26982.09	< 0.0001	16792.99	< 0.0001	10373.77	< 0.0001	702.26	< 0.0001
AB	0.11	0.7491	0.22	0.6563	0.046	0.836	2.86	0.1348
AC	2.05	0.1953	0.037	0.8522	2.68	0.1454	0.088	0.7748
BC	2.09	0.1919	0.016	0.9041	0.0001034	0.9922	0.47	0.5134
A^2	339.25	< 0.0001	3.3	0.1122	91.56	< 0.0001	23.09	0.002
B^2	78.92	< 0.0001	2.17	0.1841	61.81	0.0001	14.9	0.0062
C^2	5206.55	< 0.0001	7395.16	< 0.0001	4364.36	< 0.0001	174.09	< 0.0001
Lack of Fit	1.94	0.2648	1.3	0.3887	0.19	0.8993	1.76	0.2929
Adjusted R^2/R^2	0.9995	0.9998	0.9993	0.9997	0.9989	0.9995	0.9830	0.9926
Predicted value	2484.32		9486.43		5621.53		1514.68	
Experimental value	2517.00		9511.50		5874.30		1491.10	
Error in relation to predicted value (%)	1.32		0.26		4.5		−1.56	

were less than 0.0001, revealing that the regression models were remarkably significant. Moreover, the non-significant values of lack of fit with the p-values considerably larger than 0.05, indicated that the models fitted well with the response variables. The values of adjusted coefficients of multiple determination (adjusted R^2) and coefficients of multiple determination (R^2) were very close to 1, revealing that the models could predict the experimental data well (Karasu, Bayram, Ozkan, & Sagdic, 2019). Thus, the models were adequately reliable and accurate in this study.

3.2.2. Response surface analysis

As can be seen from Table 1, the individual terms of C, and the quadratic terms of A^2, B^2 and C^2 had remarkably significant influence ($p < 0.01$) on notoginsenoside R1 (Y_1), while the linear parameters A yielded significant influence ($p < 0.05$) as for notoginsenoside R1 (Y1) (Wang et al., 2019). However, the solid to liquid ratio (C) and its quadratic term (C^2) had significant effect ($p < 0.001$) on ginsenoside Rg1 (Y_2). As for ginsenoside Rb1 (Y_3) and ginsenoside Rd (Y_4), the factors with the greatest influence on extraction efficiency of the two response variables were B, C, A^2, B^2, C^2 and C, A^2, B^2, C^2, respectively. Three-dimensional (3D) response surface graphs demonstrated the interaction effects of the independent variables on four response variables (Y_1-Y_4), which were constructed by plotting two input variables as the X and Y axes, while the other independent variable was kept constant. Finally, the response surface 3D graphs corresponding to the response values affected by three independent variables were presented in Figs. S1–4 (see supplementary materials). As can be observed in these figures, the 3D plots showed the effect of three pairs of investigated factors on each response variables. The interaction of the concentration of vesicle and extraction time (AB) illustrated significant effect on all the responses. And their 3D response surface plots all reached a maximum point in the experimental range, which demonstrated that the ranges of variables were reasonable. However, the linear variables of A and B did not show much obvious influence on the construction of response surfaces when the terms of AC and BC were chosen as variables. In contrast, the variable of solid to liquid ratio (C) was remarkably significant. Based on the desirability of reducing the consumption of sample and reagents in the experiment, the optimal factors were obtained according to the analytical results of Design-Expert software while taking these requirements into consideration. The RSM model suggested that the maximum extraction yield values reached when the concentration of vesicle (A) was 1.03% w/v, extraction time (B) was 19.73 min and solid to liquid ratio (C) was 177.12 mg/mL, respectively. Predicted values of four dependent variables are shown in Table 1. In addition, for the purpose to validate the suggested optimal conditions, the experiments were performed in triplicate under the optimized conditions (the extraction time was set at 20 min for convenient). The experimental values are also summarized in Table 1. These data showed that predicted values were very close to experimental values with the error value lower than 4.5%, indicating the designed model was very accurate and reliable.

3.3. Method validation

The optimized vesicle based UAE procedure was validated in terms of linearity, inter- and intra-day precision, limits of detection (LOD) and limits of quantification (LOQ). The results are listed in Table 2. The calibration curves were generated by analyzing six levels of each standard solution in the linear range of 10–1000 μg/mL. Satisfactory linearities for the four curves were obtained with the correlation coefficients (r) in the range of 0.9991–0.9996. The intra- and inter-day precision was determined by injecting the mixed standard solution (50 μg/mL) six times a day and twice a day in three consecutive days, respectively. Satisfied precision was obtained with the relative standard deviations (RSDs) of peak area and retention time in the range of 0.006–0.486% and 0.114–0.351% for intra-day precision and inter-day

Table 2
Linear regression data, precision, limits of detection (LODs) and limits of quantification (LOQs) of the investigated compounds.

Analyte	Calibration curve				Precision (RSD%)				LOD	LOQ
	Calibration levels (n = 6)				Intra-day n = 6		Inter-day n = 6			
	r	Slopes	Intercepts	Linear ranges μg/mL	Retention time	Peak area	Retention time	Peak area	ng/mL	ng/mL
Notoginsenoside R1	0.9996	1.6045	1.1755	10–1000	0.015	0.348	0.312	1.230	31.86	105.13
Ginsenoside Rg1	0.9993	1.856	6.2594	10–1000	0.015	0.330	0.295	1.275	27.64	91.21
Ginsenoside Rb1	0.9991	1.1025	5.1552	10–1000	0.031	0.486	0.458	1.020	55.67	183.70
Ginsenoside Rd	0.9992	1.4345	2.6513	10–1000	0.006	0.075	0.114	3.351	34.94	115.30

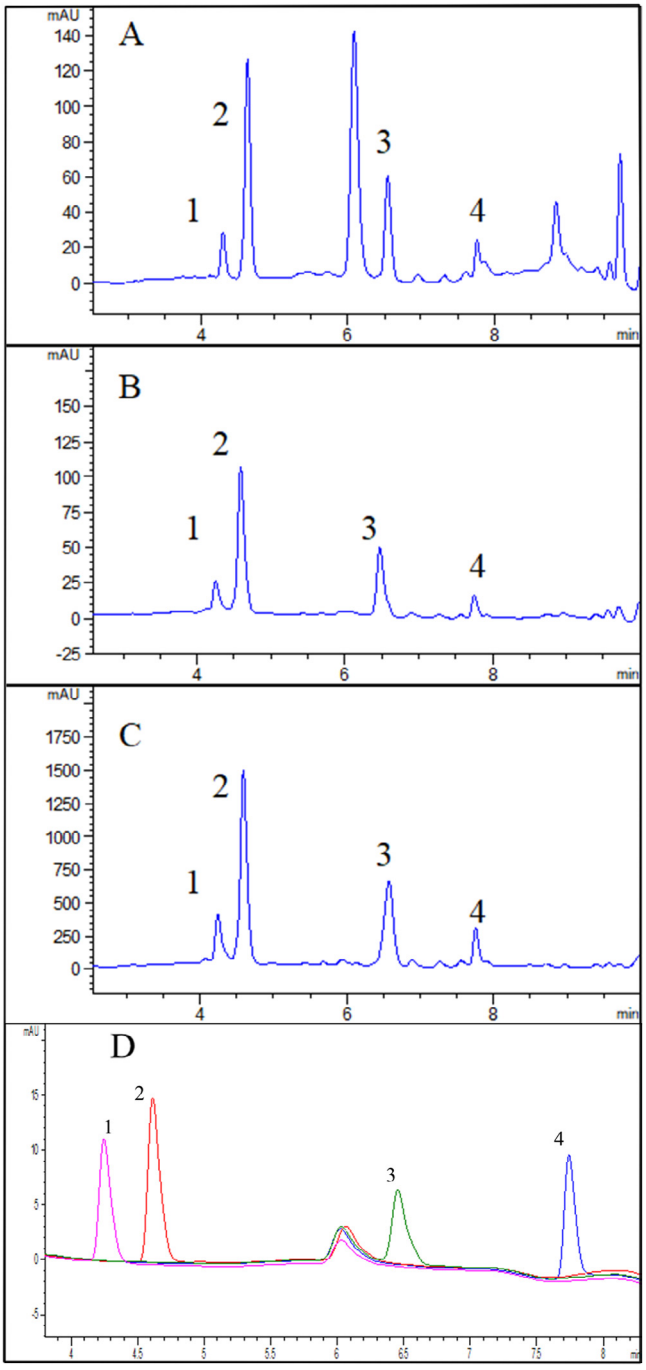

Fig. 2. The UHPLC-UV chromatograms of the Panax notoginseng extracts extracted under the following conditions (methanol as extraction solvent, 10 min of extraction time and 10 mg/mL of solid to liquid ratio.) (A), Panax notoginseng extracts extracted under the following conditions (1% (w/v) of DTAB/SDS vesicle as extraction solvent, 10 min of extraction time and 10 mg/mL of solid to liquid ratio.) (B) and Panax notoginseng extracts extracted under the optimal conditions (1% (w/v) of DTAB/SDS vesicle as extraction solvent, 20 min of extraction time and 160 mg/mL of solid to liquid ratio.) (C). Analytes: (1) notoginsenoside R1, (2) ginsenoside Rg1, (3) ginsenoside Rb1, (4) ginsenoside Rd (D). standard substances of saponins dissolved in vesicle solution.

precision, respectively. The LOD and LOQ were obtained according to signal-to-noise ratio of 3:1 and 10:1, respectively. It was observed that the LODs and LOQs of four analytes were in the range of 27.64–55.67 and 91.21–183.70 ng/mL, respectively. Consequently, the good sensitivity of the established method was confirmed.

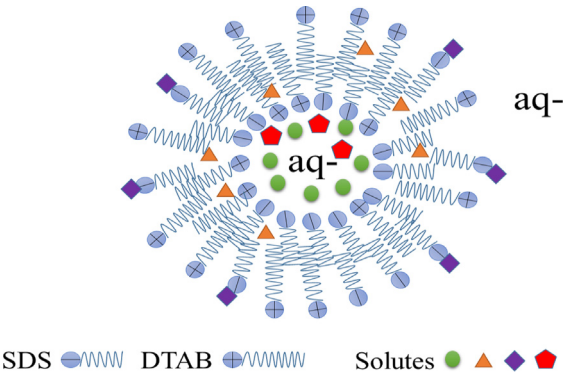

Fig. 3. The illustrate of interaction mechanism and formation vesicle.

3.4. Application of method

The proposed surfactant vesicle based UAE method was applied to extract and determine saponins in *panax notoginseng* under optimum conditions. The UHPLC-UV chromatograms of *panax notoginseng* extracted by methanol under UAE process (other conditions were as follows: extraction time, 10 min; solid to liquid ratio, 10 mg/mL) (A), *panax notoginseng* treated by DTAB/SDS vesicle (other conditions were as follows: concentration of vesicle, 1% (w/v); extraction time, 10 min; solid to liquid ratio, 10 mg/mL) (B) and *panax notoginseng* extracted by optimized vesicle based UAE process (other conditions were as follows: concentration of DTAB/SDS vesicle, 1% (w/v); extraction time, 20 min; solid to liquid ratio, 160 mg/mL) (C) are showed in Fig. 2. Obviously, the response of many non-interested compounds existed in Fig. 2(A), while the interference compounds in Fig. 2(B) became less and the response of targets was close to that in (A). However, chromatogram of (B) was not subjected to the optimal vesicle extraction condition. Finally, the chromatogram of Fig. 2(C) was obtained on the basis of the established method and peak areas of targets was much larger than (A). Additionally, no many non-targets were observed. Thus, the high extraction efficiency of developed method was confirmed. Finally, the contents of four target saponins extracted by various solvents was listed in Table S2. As can be seen, the contents of notoginsenoside R1, ginsenoside Rg1, ginsenoside Rb1 and ginsenoside Rd in *panax notoginseng* extracted by optimum condition were 9.539 ± 0.6880, 31.28 ± 1.068, 31.46 ± 1.567, 6.341 ± 0.1160 mg/g, respectively. The corresponding extraction yield (7.36%) was higher with the contrast of the extraction yield (3.14%) obtained from pharmacopeia method. The accuracy of the developed method was estimated by recovery experiment. Mixed standard solution was spiked in the *panax notoginseng* at two concentrations level (50 and 100 μg/mL), and each one was analyzed in triplicate. The average recoveries of four analytes were in the range of 83.84–90.92%. The reproducibility of the present extraction method was tested by analyzing three parallel samples extracted under the optimized method. The RSDs of retention times and peak areas were in the range of 0.081–0.484% and 0.168–2.184%, respectively. These results demonstrated that the established method was very accurate and reliable for extraction and determination of saponins in *panax notoginseng*.

3.5. Comparison of the analytical performance with other reported methods

The comparison with other established methods of saponins extraction was listed in Table 3. Compared with traditional ultrasound and pressurized liquid extraction method, the developed vesicle based UAE had potential advantages involving shorter extraction time, lower consumption of extraction solvent and energy. Additionally, other green extraction methods (ion liquid based UAE and microwave extraction) were evaluated and compared with the aim to highlight the advantages of the present approach. Obviously, higher extraction yield,

Table 3
The comparison with other reported methods.

Analytes	Samples	Extraction method	Extraction temperature (°C)	Extraction time (min)	Total extraction Yield[6] (wt%)	Solid to solvent ratio (g/ml)	Extraction solvent	References
Saponins	Catharanthus roseus stem	Ultrasound	55	35	–	1/100	Methanol	Hong Ngoc et al. (2018)
Nine Saponins[1]	Panax notoginseng	Pressurized liquid extraction	150	15	7.36	1/22	Methanol	Wan et al. (2006)
Five Saponins[2]	Panax notoginseng	Overnight immersion, ultrasonic	–	60 (ultrasonic)	–	1/40	70% methanol	Xia et al. (2017)
Saponins	Spent tea leaves	Microwave-assisted extraction	90	9	1.40	1/23.54	Water	Noor, Suzana, Armando, Khairiraihana, and Benjamin (2019)
Saponins	Fenugreek seed	Microwave-assisted extraction	70	3	–	1/10	60% ethanol	Akbari, Abdurahman, Yunus, and Fayaz (2019)
Four isoflavones[3]	Soy products	Ionic liquid-based ultrasound-assisted extraction	–	40	–	1/10	$[C_6MIM]Br$	Magiera and Sobik (2017)
Four Saponins[4]	Panax ginseng C. A. Mey	ILs–based ATPS extraction[7]	Room temperature	60	–	–	35 wt% $[C_4Tr]Br$ + 20 wt% NaH_2PO_4 + 42 wt% water	He, Dong, Feng, and Yao (2018)
Three Saponins[5]	Panax notoginseng	Overnight immersion, distillation	80	120 (distillation)	3.14	1/83.33	Methanol	Pharmacopeia method
Four Saponins[6]	Panax notoginseng	Vesicle based ultrasonic	Room temperature	20	7.36	1/6.25	1% (w/v) of DTAB/SDS vesicle	This work

[1] Notoginsenoside R1, ginsenoside Rg1, Re, Rf, Rb1, Rc, Rb2, Rb3 and Rd.
[2] Ginsenoside Rg1, Re, Rb1, Rd and notoginsenoside R1.
[3] Genistin, genistein, daidzin, and daidzein.
[4] Ginsenosides Rg1, Re, Rd and Rb1.
[5] Ginsenoside Rg1, Rb2, and notoginsenoside R1.
[6] Notoginsenoside R1, ginsenoside Rg1, ginsenoside Rb1, and ginsenoside Rd; 6: the computational formula of extraction yield: Extraction yield (wt%) = mass of total extracted/mass of feedstock × 100%.
[7] Ionic liquids based aqueous two-phase systems.

lower solvent consumption and extraction temperature, simpler extraction process were confirmed. Moreover, the results of pharmacopeia method were assessed as additional control. Above merits were further clarified. Therefore, this method was proved to be a reliable, efficient, simple, and environmental friendly technique.

4. Conclusions

A simple and new vesicle based UAE method in combination with UHPLC-UV was established and validated, and further it was used to extract and determine four saponins (notoginsenoside R1, ginsenoside Rg1, ginsenoside Rb1, and ginsenoside Rd) in *panax notoginseng*. Surfactant vesicle solution was used as extraction solvent. The extraction conditions were optimized with single-factor experiments and RSM. This was the first time that surfactant vesicle had been used as an extraction solvent for extracting active components in real samples. Compared with traditional extraction methods, the present method avoided using large amount of organic solvents, which met the principle of green chemistry. Good linearity, adequate reproducibility, acceptable recoveries, and low detection limits were obtained through validation experiment of the method. Overall, the developed vesicle based UAE coupled with UHPLC-UV was a simple, sensitive, reliable, low cost, and environmental friendly method for extraction and determination of saponins in *panax notoginseng*, and which also provided a reference for the extraction active compounds in other plants.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

This study was supported by the National Natural Science Foundation of China (81573552), Public Welfare Research Project of Zhejiang Province (LGF18H280006), Hangzhou Social Development of Scientific Research projects (20191203B13), Hangzhou 131 Middle-aged and Young Talent Training Plan (China, 2017).

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodchem.2019.125394.

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