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Studies on the interactions between ginsenosides and liposome by equilibrium dialysis combined with ultrahigh performance liquid chromatography-tandem mass spectrometry

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ABSTRACT

To study the interactions between components of *Panax* Ginseng and liposome biomembrane, we applied the equilibrium dialysis system combined with ultrahigh performance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS) approach to analyze and identify the bioactive components of ginseng. Moreover, the effect of pH value has also been investigated on their interactions between the ginsenosides of ginseng extract and biomembrane. The result shows that seven kinds of ginsenosides have obvious interactions with biomembrane in comparison with the standards in terms of tandem mass spectrometry (MS/MS) data along with retention time, including four panaxadiol ginsenosides (Rb_1, Rb_2, Rc, Rd) and three panaxatriol ginsenosides (Re, Rf, Rg_2). The value of binding degree decreased with the increase of molecular weight. The sugar moieties which are attached to C-20 were the main factor affecting the binding degree of panaxadiol ginsenosides. The interactions between panaxadiol ginsenosides and biomembrane correlate to the type and number of sugar moieties in ginsenosides. The sugar moieties which are at C-6 and C-20 have been shown to influence the value of binding degree for panaxatriol ginsenosides. In addition, the pH value has been shown to have an impact on the interactions. Overall, ginsenoside Rd has a better absorption character among the seven ginsenosides. In the study, we have screened the potential bioactive components of ginseng in vitro using the equilibrium dialysis-UPLC-MS/MS method, and then predicted the potential bioactivities of ginseng, which contribute to the investigation of the efficacy of ginseng.

1. Introduction

Panax ginseng C.A. Meyer has been widely used in traditional Chinese medicine (TCM) for over 2000 years. Numerous studies have shown that *Panax* ginseng possess many pharmacological properties, which are demonstrated in the central nervous system [1,2], prevention of the aging process [3], cardiovascular systems [4,5], antitumor and immunomodulatory effects [6,7], antioxidant activity [8] and so on. While the pharmacological properties of ginseng are mainly attributed to ginsenosides [1–9], which have in common the attachment of one or more sugar chains to the aglycone. According to the structures of the aglycones, ginsenosides mainly include dammarane triterpenes with (20S)-protopanaxadiol or (20S)-protopanaxatriol, oleanolic acid type saponins [10]. The multiple components of ginseng play the therapeutic role by the synergistic reaction among them. Thus a rapid and reliable screening method was necessary at the aspect of separation and identification of the potential bioactive ingredients simultaneously in early drug discovery.

The different parts of organism, which is a complex system, are in different physiological environments. There are two vital steps in terms of the absorption of oral drug, intestinal absorption and target cell interaction. Therefore, one of the important steps in screening and identifying the active ingredient of compounds is prediction of drug-membrane penetration. In recent years, several methods have been applied in the characterization of the interaction properties of captured drugs with biological systems (DNA, protein, cell, etc.) in vitro, such as Caco-2 model [11–13], equilibrium dialysis method [14–16] and parallel artificial membrane permeation assay (PAMPA) [17,18]. Since the structures of liposomes formed by self-assembly in water of phospholipids are similar to cell membranes and suitable for experiments under physiological conditions, the interaction analysis of drugs with liposomes should be superior in predicting of drug transport across membranes. Therefore, liposomes are extensively used in investigating the interaction properties of drugs with biological systems. Though the

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ingredients of TCMs can freely pass through the dialysis membrane in equilibrium dialysis experiment, after combining with liposome they could not pass through the dialysis membrane with a certain molecular weight cutoff. Beigi and collaborator first proposed that bonding drugs to the immobilized-liposome chromatographic (ILC) columns and then they studied the partitioning of drugs into liposomes in vitro [19]. Many studies illustrated that a good correlation between liposome biomembranes and an intestinal mucosa model holds for a series of drugs [16,19–21].

The ultrahigh performance liquid chromatography (UPLC) approach has possessed higher chromatographic performance, and a new generation of short column packed with 1.7 μm particles was used in the technique. Thus several parameters have been improved, such as speed, resolution, sensitivity, and peak capacity compared with common high performance liquid chromatography (HPLC). In recent years, UPLC combining with mass spectrometry (MS) or tandem mass spectrometry (MS/MS) has become a technique of choice in many laboratories. The analytical method fulfills key requirements in terms of rapidity, sensitivity, selectivity, and peak-assignment certainty for the analysis at low concentrations of complex matrices (e.g., biological fluids, plant extracts, and food and environmental samples) [22–27].

In the present study, the egg phosphatidylcholine was used for the production of liposome biomembranes. The drug-membrane permeability of potential bioactive constituents of ginseng was studied by using the liposome equilibrium dialysis method. The dialysis samples were analyzed by using UPLC and UPLC-MS/MS, respectively. The multiple reaction monitoring (MRM) function, which is a sensitive and specific quantitative method of MS/MS analysis, was used to monitor all the target ions simultaneously from each analyte. By comparison of each chromatographic peak area of ginseng extract dialysate before and after interaction with liposme biomembranes at different pH values, the interaction properties of ginsenosides with biomembranes could be predicted. The approach in this paper appears to simulate the physiological environment preferably and can provide a basis for the prediction of the absorption of ginseng in human body.

2. Experimental

2.1. Reagents and chemicals

Egg phosphatidylcholine was obtained from Shanghai Chemical Reagents (Shanghai, China). A dialysis bag with a molecular weight cutoff of 8000–14,000 Da and a diameter of 36 mm was purchased from Dingguo Biotec. Co. (Beijing, China). Acetonitrile of HPLC grade was obtained from Fisher Scientific (Fair Lawn, NJ, USA). Deionized water was prepared using the Milli-Q plus water purification system (Bedford, MA, USA). All the other chemicals were at least of analytical grade. All samples were filtered through a 0.22 μm membrane filter before analysis.

The ginsenosides Rb_1, Rb_2, Rc, Rd, Re, Rf and Rg_2 were provided by the Chinese authenticating institute of material and biological products (Beijing, China) and the chemical structures of the reference compounds are shown in Fig. 1.

2.2. Sample preparation

Freshly harvested 5 year growth *Panax* Ginseng was purchased from Fusong (Jilin, China). One hundred grams of powdered root of ginseng which were selected by 80 mesh sieve were refluxed thrice with ethanol: water (80:20, v/v) at a sample-to-solvent ratio 1:10 (w/v) for 1.5 h. The upper layer of the extract was combined, filtered, concentrated decompression until without alcohol odor. Then, the concentrated solution was degreased with twice volume of diethyl ether for three times. The aqueous layer was extracted thrice with equal volume of water-saturated *n*-butanol and the extract liquid was combined. After the solvent was evaporated via decompression approach, the extract was dissolved in water and lyophilized to obtain total ginsenoside extract powder which was stored in plastic bags at room temperature. Afterward, 10 mg/mL of ginseng extract under different buffer conditions (pH 5.4, pH 7.0, and pH 7.4) were obtained by dissolving the right amount of extract powder in corresponding ammonium acetate buffer respectively.

2.3. Preparation of liposome membranes

There are two major methods which are solvent evaporation and solvent injection for preparing liposomes [16,28,29]. Liposomes which were produced by the solvent evaporation method indicated sedimentation when stored at 4 °C, while they were stabilized at 4 °C by the ethanol injection method. The latter method was employed in our experiment. Succinctly, 0.6 g of phosphatidylcholine was weighed into a beaker and soaked with 6 mL of ethanol, and then poured into an injector with 10 mL capacity. The concentration of ammonium acetate buffer used in the experiment was 10 mmol/L. Meanwhile, another beaker containing 10 mL of pH 5.4 ammonium acetate buffer was placed in a water-bath with magnetic stirrer, in addition, the buffer solution was heated in the water-bath at 60 °C. Then the phosphatidylcholine solution was added dropwise to the ammonium acetate buffer slowly, furthermore, in the whole experimental process the buffer was stirred continuously till the remaining ethanol was removed. Thus, 60 g/L of liposome dispersion solution was obtained by the ethanol injection method, and the other two liposome dispersion solutions (pH 7.0 and pH 7.4) were also produced by exactly the same procedure.

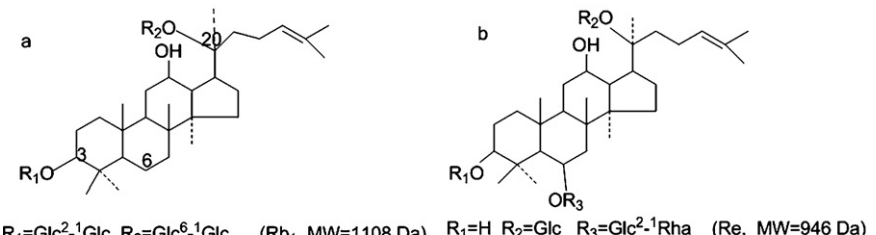

Fig. 1. Chemical structures of panaxadiols (a) and panaxatriols (b).

2.4. Equilibrium dialysis experiment

A 7-cm length of dialysis bag containing one milliliter of ginseng extract solution and 1 mL of liposome solution was placed into a centrifuge tube including 10 mL of buffer solution for dialysis at 4 °C. Meanwhile, a blank sample was running and the liposome solution was substituted by 1 mL of buffer solution, while other steps followed exactly the same procedure of the experimental sample under the same buffer conditions. After equilibration for 24 h, the dialysate of ginseng extract outside the bag was obtained for analysis by UPLC-PDA (photo-diode array) and UPLC-MS/MS method. The "blank dialysate" and "interaction dialysate" represent the ginseng extract before and after interaction with liposome, respectively.

2.5. UPLC-PDA and UPLC-MS/MS analyses

UPLC-PDA and UPLC-MS/MS analyses were performed utilizing ACQUITY™ UPLC H-Class system equipped with an autosampler, a PDA detector and a Xevo TQ MS spectrometer (Waters Corp., Milford, MA, USA). An Acquity UPLC® BEH C18 column (50 mm × 2.1 mm i.d., 1.7 μm; Waters Corp., Milford, MA, USA) was used for chromatographic separation in the UPLC-PDA assay section, while the Acquity UPLC® BEH Shield RP 18 column (50 mm × 2.1 mm i.d., 1.7 μm; Waters Corp., Milford, MA, USA) was applied in the UPLC-MS/MS analysis. The binary mobile phase consisted of acetonitrile (solvent A) and water (solvent B), the flow rate was set at 0.3 mL/min, and 10 μL of sample was injected. The gradient elution program was started with 25% A (75% B) and increased linearly to 50% A (50% B) in 5 min, then to 90% A (10% B) over 3 min, further to 100% A in the next 2 min and maintained this gradient for 2 min. The detection wavelength was selected at 195 nm. The column temperature was kept constant at 35 °C. The Empower Chromatography Software was applied in data acquisition and data processing.

The electrospray ionization (ESI) source of the mass spectrometer was connected to the UPLC system via a capillary to the PDA cell outlet. Additionally, the injection volume was 3 μL considering the sensitivity of the instrument. The source temperature and desolvation temperature were set at 150 and 350 °C, respectively. The spray voltage was set at 3.0 kV. The flow of collision gas (argon) was 0.16 mL/min, while the flow rates of the cone gas (nitrogen) and desolvation gas (nitrogen) were set to 60 and 800 L/h, respectively. The tandem mass analyses were operated in the multiple reaction monitoring mode for the accurate quantitative analysis of analytes in positive and negative ion modes. We optimized the cone voltages and the collision energies in direct flow-injection mode. The quantitative ion transitions with appropriate instrumental parameters for the MRM detection are shown in Table 1. Additionally, the intra-day and inter-day precision and accuracy were investigated at three levels (five parallels of each concentration). The accuracy of the method was estimated as relative error (RE), while the precision was expressed by the relative standard deviation (RSD). The data were acquired via MassLynx4.1 software (Waters), and the MassLynx4.1 with TargetLynx was applied to data processing.

3. Results and discussion

3.1. Optimization of experimental conditions

To develop a suitable and robust liquid chromatography (LC) approach, in the preliminary experiments several experimental parameters were investigated, including the column temperature, the mobile phase and the gradient elution program. In order to obtain the stable chromatographic resolution conditions, other parameters such as injection volume and column temperature were also investigated. The optimal separation of ginsenosides was achieved using a gradient elution with acetonitrile and water within 12 min. The PDA detection was performed in the range of 190–400 nm at 1 nm/step. Based on the maximum absorption of the ginsenosides in the ultraviolet (UV) spectra of the three-dimensional chromatograms obtained by PDA detection, the UV detection wavelength was set at 195 nm, where all of the ginsenosides could be detected and had adequate absorption. In the above conditions, more peaks with better peak shape were clearly shown in the chromatogram of the extract solution of ginseng. The UPLC-MS/MS analysis for the interactions between saponins of ginseng and liposome biomembrane was performed in MRM mode. In order to obtain the most specific and sensitive detection parameters of different kinds of ginsenosides, the IntelliStart function was applied to optimize the cone voltages and the collision energies. In this process, the ginsenoside standard solutions were infused into the mass spectrometer via a syringe pump, respectively. By the combined mode, the appropriate instrumental parameters were obtained at a flow rate of 20 μL/min. The validation studies were determined including intra-day precision and accuracy, and inter-day precision and accuracy. The precision and accuracy results met the requirements of an analytical assay (Table S1). Two precursor/product ion pairs of each ginsenoside were selected for creating MS/MS method (Figs. 2 and 3), so we can collect sufficient data for the integrative research. The highest sensitive ion pair was chosen for the quantitative analysis (Figs. S1and S2), meanwhile another one was elected as qualitative ion pairs for the confirmatory analysis.

3.2. Investigating the active components of Ginseng by UPLC-PDA

The "interaction dialysate" and "blank dialysate" of the ginseng extracts were collected according to the procedure described in the experimental section and analyzed by UPLC-PDA under the identical conditions. Based on the comparison of the obtained chromatograms of "interaction dialysate" and "blank dialysate", the permeable property of compounds from the ginseng extract could be deduced. The permeable components in the ginseng extract which interacted with the liposome membranes would be partly confined in the semi-permeable membrane of the dialysis bag, so that they could not be collected in the equilibrium dialysate. Consequently, obvious decrease of the chromatographic peak area indicated the stronger binding of compounds to liposome membranes, whereas no apparent change of the UPLC peak area indicated that there were weak or no interactions between those components and liposome membranes through comparing the

Table 1
The quantitative ion pairs and parameters for MRM of ginsenosides.

Ginsenoside	t_R (min)	Precursor ion → product ion (*m/z*)	Cone voltage (V)	Collision energy
Rb_1	2.40	1131.76 → 365.21	82	58
Rb_2	2.72	1101.76 → 335.21	82	58
Rc	2.58	1101.82 → 335.21	82	58
Rd	3.11	969.69 → 789.67	76	48
Re	1.04	969.69 → 789.66	72	42
Rf	2.30	823.60 → 365.22	80	48
Rg_2	2.79	807.60 → 349.21	76	42

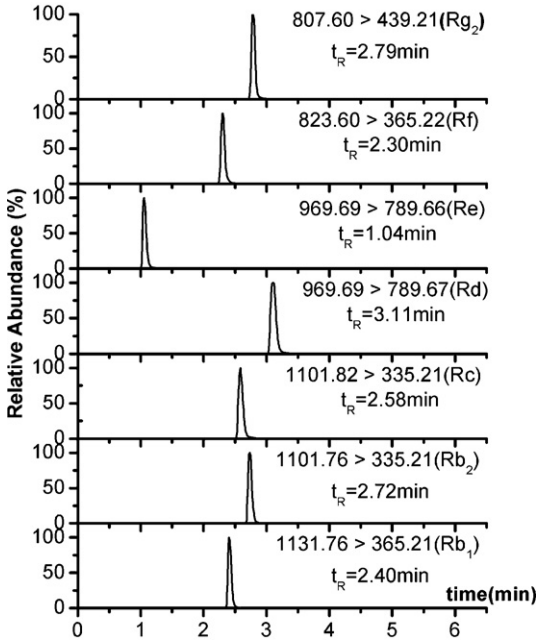

Fig. 2. The MRM chromatograms of the quantitative ion pairs of ginsenosides.

"interaction dialysate" and "blank dialysate" of the ginseng extract. Accordingly, the permeable components and non-permeable components of the ginseng extract could be easily distinguished from the obtained chromatograms of different dialysate. Finally, the bioactive ingredients of the ginseng extract were speculated.

Fig. 4 illustrates the UPLC chromatograms of the ginseng extract before and after binding with liposome membranes at different pH values (pH 5.4, pH 7.0, and pH 7.4). Comparing with the chromatograms of reference compounds, the area of six chromatographic peaks from the ginseng extract dialysate decreased obviously via the analysis of UPLC-PDA, while the decrease of these peak areas reflected the interaction with liposome. The interacting strength between permeable component and liposome membrane

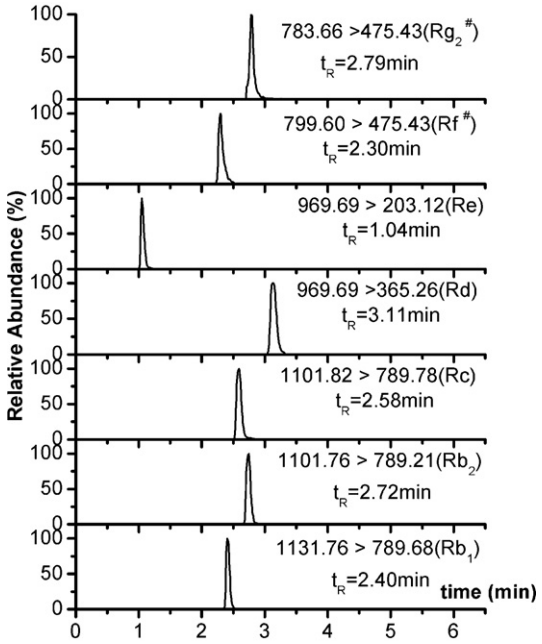

Fig. 3. The MRM chromatograms of the qualitative ion pairs of ginsenosides (# negative ionization mode).

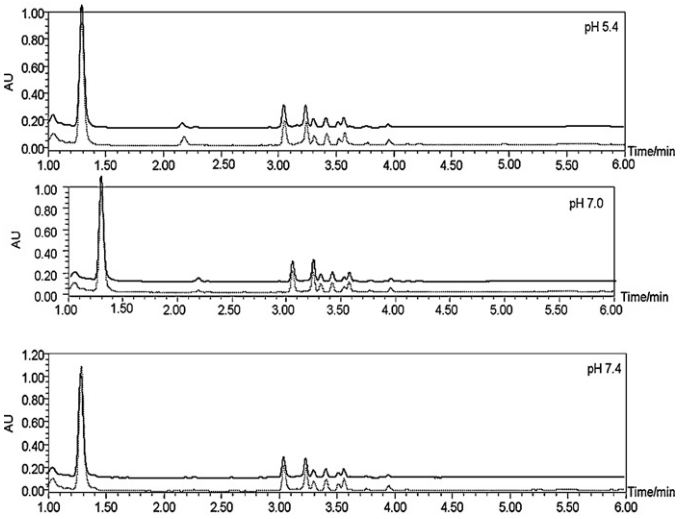

Fig. 4. The UPLC-UV chromatograms of the dialysate of ginseng extract at different pH values (dash line: experiment without biomembranes; solid line: experiment with liposome biomembranes).

could be characterized by the decreased percentage of peak area, which was called binding degree. The binding degree was calculated as follows:

$$\text{Binding degree} = \frac{A_a - A_b}{A_a} \times 100\% \quad (1)$$

Here, A_a and A_b denote the peak areas of a compound before and after interaction with liposome membranes of the chromatograms, respectively. The binding abilities between compounds and liposome membranes in the environment were shown by the binding degrees. It is worth noting that the binding degrees are obtained under a competitive situation. The binding degree was greater when the interaction of the permeable component with liposome membrane is stronger. The binding degree was calculated using Eq. (1). It was found that the interacting strengths of these compounds with liposome membranes were different from each other. The result showed that ginsenoside Rd was the most active ingredient among the seven ingredients of ginseng in our experimental conditions by comparing the chromatograms of ginsenoside references with the chromatograms of dialysate.

The ginsenoside standards were used to the essential confirmation of the active compounds from the ginseng extract in UPLC-PDA approach, but the complex matrix of ginseng extract would interfere in the analyses of its bioactive compounds. So we further applied the liquid chromatography-tandem mass spectrometry (LC-MS/MS) method to investigate the bioactive components from the ginseng extract.

3.3. UPLC-ESI-MS/MS assay

The MRM mode, via monitoring the mass-to-charge ratio (m/z) of parent ions and diagnostic fragment ions for quantification, is the most selective and sensitive approach. It is the main quantitative method of triple stage quadrupole mass spectrometer. The MS parameters were optimized via injecting the solutions of ginsenoside standards, and there were two ion pairs selected for the identification and quantification of the target compound. In this study, two most intense ion transitions were chosen to develop a sensitive MRM method. The highest ion transition was selected as a quantifier for quantification while another one was selected as a qualifier for confirmatory analysis. The precursor/product ion pairs for the quantitative and qualitative analyses of all the studied

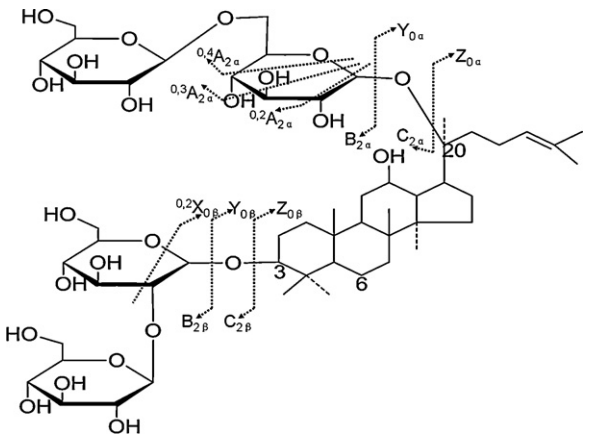

Fig. 5. The fragmentation pathways of ginsenosides in tandem mass spectrometry (taking the structure of ginsenoside Rb_1 as the example).

ginsenosides, as well as the appropriate instrumental parameters for MS/MS detection in MRM mode are listed in Table 1.

In the present study, the fragment ions of ginsenosides were labeled according to the nomenclature described by Costello and coworkers [30,31]. The saccharide chain at C-3 and C-6 positions are defined as β-chain sugar moieties, while that at C-20 position of the aglycone is named as the α-chain while under most MS-MS conditions the bond cleavage occurred at this position. The fragment ions retaining the charge at the reducing terminus containing the aglycone are termed Y and Z (glycosidic bond cleavages) and X (cross-ring cleavages), whereas B, C (glycoside cleavages), and A (cross-ring cleavages) ions retaining the charge at the sugar moieties correspond to the complementary ions of Y, Z and X, respectively. Cross-ring cleavage ions are also designated by superscript numbers indicating the two-bond cleavage. Fig. 5 shows the fragmentation pathways of ginsenoside Rb_1 on the basis of the nomenclature proposed by Costello and collaborators.

In electrospray ionization-mass spectrometry (ESI-MS) spectra, the sodiated molecular ion was observed as the predominant ion in positive mode while in negative mode the $[M-H]^-$ ion was the most abundant ion. The sodium-cationized ginsenoside molecule was probably derived from glass containers and as an impurity picked up during the preparation of the sample while the sample was not doped with metal salts [32–34]. In this research the most abundant $[M+Na]^+$ ions of ginsenosides obtained in positive mode had higher sensitivity and intensity to permit quantitative measurement than the $[M-H]^-$ ions in negative mode. In this case we chose the quantitative ion pairs of ginsenosides in positive mode. $C_{2\alpha}$ fragment ions formed by glycosidic cleavages were selected as the quantitative ions of ginsenosides Rb_1, Rb_2 and Rc. The ions at *m/z* 365 (Rb_1) and 335 (Rb_2 and Rc) correspond to two hexose residues (162+180+23) and one pentose residue and one hexose residue (162+150+23), respectively. The ion at *m/z* 789 (ginsenosides Rd and Re) was generated by loss of α-chain sugar residue from $[M+Na]^+$ ion and selected as a quantifier for quantification. $[M+Na]^+$ ion produced $C_{2\beta}$ fragment ions at *m/z* 365 and 349, corresponding to the product ion for the MRM analysis of ginsenosides Rf and Rg_2, respectively. In addition, another ion pair was selected for qualitative analysis. The $Z_{0\alpha}$ ions, which were formed by glycosidic cleavages as well, were selected as the qualitative ion pairs of ginsenosides Rb_1, Rb_2 and Rc. The qualitative ion pairs of ginsenosides Rd and Re were $C_{2\beta}$ ion at *m/z* 365 and $C_{2\alpha}$ ion at *m/z* 203, respectively. It showed that their qualitative ions corresponded to the complementary ions of their quantitative ions, respectively. However, the qualitative ions at *m/z* 475 of ginsenosides Rf and Rg_2 were selected in negative mode, which is [aglycon-H]$^-$ ion formed by losing β-chain sugar residue of deprotonated molecule $[M-H]^-$ ion via glycosidic cleavage. Fig. 6 shows the chromatograms of the ginseng extracts before and after interaction with liposome membranes at different buffer conditions (pH 5.4, pH 7.0, and pH 7.4). The binding degree of ginsenosides from *Panax* ginseng extract was calculated using Eq. (1), and the results are summarized in Table 2. It indicated that seven kinds of ginsenosides had obvious

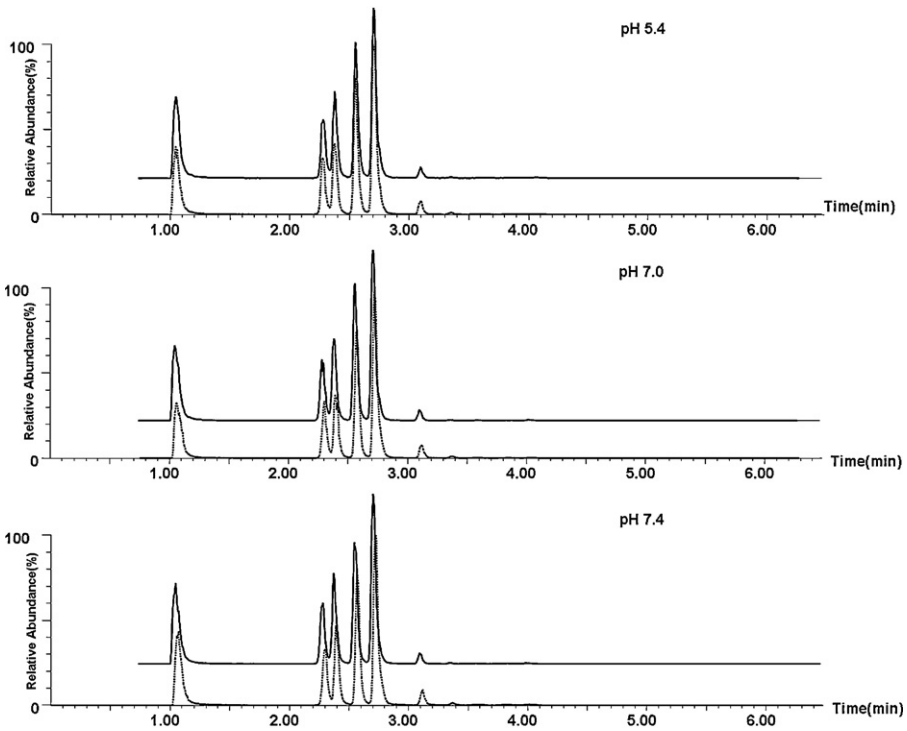

Fig. 6. The total ion chromatograms of the dialysate of ginseng extract at different pH values (dash line: experiment without biomembranes; solid line: experiment with liposome biomembranes).

Table 2
The binding degree of ginsenosides interacting with liposome at different pH values analyzed by UPLC-MS/MS method.

Ginsenosides	Binding degree (%, ±SD, $n = 3$)		
	pH 5.4	pH 7.0	pH 7.4
Re	0.26 ± 0.03	3.87 ± 0.07	5.65 ± 0.81
Rf	1.60 ± 0.17	1.50 ± 0.04	4.23 ± 0.75
Rb_1	2.14 ± 0.11	3.05 ± 0.11	0.19 ± 0.04
Rg_2	12.77 ± 0.42	8.85 ± 0.56	8.82 ± 0.27
Rc	15.20 ± 0.28	5.39 ± 0.08	2.89 ± 0.10
Rb_2	16.67 ± 1.23	12.26 ± 0.71	9.13 ± 0.38
Rd	36.83 ± 0.70	34.31 ± 1.22	29.43 ± 1.07

Values are presented as means ± SD (standard deviation).

interactions with biomembrane in comparison with the standards in terms of MRM data along with retention time (t_R), including four panaxadiol ginsenosides (Rb_1, Rb_2, Rc and Rd) and three panaxatriol ginsenosides (Re, Rf and Rg_2).

The order of binding degree of panaxadiol ginsenosides was Rd > Rb_2 > Rc > Rb_1, moreover, ginsenoside Rd was the most active component of the extract of ginseng in different buffer environment. The results were analyzed further from the structures of four panaxadiol ginsenosides (Fig. 1). The group of ginsenoside Rd at the C-20 position was monosaccharide residue, while it was disaccharide moiety for the other three panaxadiol ginsenosides. The more sugar residues, the greater polarity, thus the interaction between ginsenosides and biomembranes was weaker. Ginsenosides Rb_2 and Rc are isomers, their difference is the type of terminus sugar residues. The difference of the structure of ginsenosides would affect the interactions between ginsenosides and lipsomes. The group of panaxatriol ginsenosides Re, Rf and Rg_2 at position C-20 is glucose residue, H and H, respectively. There is a disaccharide moiety attached to C-6 of the three panaxatriol ginsenosides, and yet the terminus sugar residues vary from one panaxatriol ginsenoside to another. The disaccharide chain is Glc^2-^{1}Rha, Glc^2-^{1}Glc and Glc^2-^{1}Rha corresponding to ginsenosides Rg_2, Rf and Re, respectively.

The different parts of organism, which is a complex system, are in different physiological environments. There are two vital steps in terms of the absorption of oral drug, they are intestinal absorption and target cell interaction. The intestinal absorption of oral drug is a very complex physiological process. Passing through all the buffer environment of intestinal epithelial cell surface (pH 5.4), the external cell (pH 7.0) and internal cell (pH 7.4) is necessary for the drug through the epithelial cell of the intestine into the blood circulation. Consequently, the interactions between components of *Panax* Ginseng and liposome biomembrane were studied under different buffer conditions (pH 5.4, pH 7.0, and pH 7.4) to fit the absorption of ginsenoside in the intestine of the human body. The result shows that the binding degree value of ginsenosides Rb_1, Rb_2, Rc, Rd and Rg_2 decreased with the increase of pH value, while the binding degrees value of ginsenosides Re and Rf have a slight increase with the increase of pH value. To infer, the conformation of ginsenosides changed in different buffer conditions, thereby which influenced the interaction between ginsenosides and liposome biomembranes. The interaction of panaxadiol ginsenosides with liposome membranes was stronger than panaxatriol ginsenosides at pH 5.4, in addition, the binding degrees of ginsenosides decreased to a certain extent at pH 7.0 and pH 7.4.

The binding degrees of ginsenosides to the liposome membranes were greater when the molecular weight decreased. Moreover, the binding capability of panaxadiol ginsenosides to the liposome was stronger than that of panaxatriol ginsenosides. The saccharide chain at C-20 was the major cause affecting the binding degree of panaxadiol ginsenosides, in which, monosaccharide residue > disaccharide sugar moiety, pyranose > furanose, low molecular weight glycosyl > high molecular weight glycosyl. The saccharide chains attached at C-6 and C-20 showed the influence for the binding degree of panaxatriol ginsenosides. Furthermore, the binding degree of panaxatriol ginsenosides declined when the saccharide chain was at the C-20 position. Meanwhile, the saccharide chain linked at C-6 affected the binding degree of panaxatriol ginsenosides to the liposome membranes, the influence order of the saccharide chain was as follows: monosaccharide residue > disaccharide sugar moiety, low molecular weight glycosyl > high molecular weight glycosyl. The binding degree of ginsenoside Rd to liposome was stronger than the other ginsensides in our experiment, which indicate the strong interaction between ginsenoside Rd and biomembrane. From the results, we speculate that ginsenoside Rd has a better absorption character among the seven ginsenosides.

4. Conclusions

In the present work, the equilibrium dialysis method has been applied to study the interactions of ginsenosides with liposome biomembranes, and then the potential bioactive components of *Panax* ginseng extract were analyzed and identified by UPLC-PDA and UPLC-ESI-MS/MS approach. The mass spectrometry data further confirmed these results. In this paper, the pH value of buffer solution was changed for fitting the physiological environment of the human body, so that the absorption of ginsenoside in the intestine and the interaction with the target cell could be simulated well. The result demonstrates that this assay can be a preliminary process for screening potential active components from ginseng extract, and thus provides a reference for in vivo absorption of ginseng.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.jchromb.2013.01.035.

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