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# Analysis of Isoflavone Daidzein in *Puerariae radix* with Micelle-Mediated Extraction and Preconcentration

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Nonionic surfactant oligo(ethylene glycol) monoalkyl ether (Genapol X-080) was employed as an alternative and effective solvent for the extraction of daidzein from *Puerariae radix* for the first time. Optimum experimental conditions were established. With 5% Genapol X-080 (w/v), liquid/solid ratio of 25:1 (mL/g), and ultrasonic-assisted extraction for 45 min, the extraction percentage of daidzein reached the highest value. For the preconcentration of daidzein by cloud-point extraction (CPE), sodium chloride was added to the solution to facilitate the phase separation and increase the preconcentration factor by reducing the volume of the surfactant-rich phase. The preconcentration factor for daidzein was about 13. Satisfactory results were obtained for the analysis of daidzein from *P. radix* with this established method.

**KEYWORDS: Micelle-mediated extraction; daidzein; *Puerariae radix*; cloud-point extraction; HPLC**

## INTRODUCTION

Isoflavones are a widespread group of natural products, and their biochemical and pharmacological properties have been studied and reported recently (*1*, *2*). In particular, daidzein (**Figure 1**), as a major isoflavone, has shown antigiardial activity (*3*), antioxidant action, and potential antidiabetic properties (*4*, *5*). Daidzein exists widely in soy foods, traditional Chinese medicines such as *Puerariae radix*, and human fluids. Therefore, the analysis of this compound is of prime importance. *Puerariae radix*, referred to as "Ge-Gen" in Chinese, is a commonly used Chinese herb, which exerts sedative and antipyretic actions and is often used to treat influenza, wrist stiffness, and headache (*6*). *P. radix* and its medical preparations are also used as clinical medicine to treat coronary heart disease, myocardial infarction, and hypertension (*7*). Daidzein is an important isoflavone in *P. radix*. The determination of daidzein in *P. radix* was not easy because of the complexity of components in the derived extract.

For the analysis of daidzein in *P. radix*, extraction procedures with organic solvents have been reported by several researchers with subsequent analysis by high-performance liquid chromatography (HPLC) coupled to mass spectroscopy (*8*) and capillary electrophoresis (*9*−*11*). The detection limits of these reported methods were 1.77 mg/mL with UV detection (*9*) and 0.28 mg /mL with electrochemical detection (*11*). But conventional extraction methods had some disadvantages, such as the use of large amounts of toxic and flammable organic solvents and time-consuming preconcentration procedure.

To minimize the use of organic solvents and simplify the operating procedure, many other extraction methods have been developed in recent years. Among them, the micelle-mediated extraction method offers a good and convenient alternative. It generally involves two parts: solubilize and purify daidzein from solid herbal materials into the aqueous surfactant solution and then preconcentrate the daidzein on the basis of phase separation by the cloud point methodology. Compared with the conventional extraction systems, the micelle-mediated extraction has the following advantages: very small amounts of the relatively nonflammable and nonvolatile surfactants required, good capacity to solubilize solutes of different types and nature, ability to concentrate solutes with high recoveries; safety and cost benefits, and easy disposal of the waste surfactant (*12*).

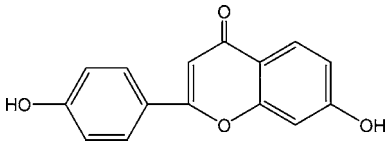


**Figure 1.** Molecular structure of daidzein.

Cloud-point extraction (CPE) has been reported in many studies concerning the extraction and preconcentration of solutes from water (*13*−*16*), human serum (*17*, *18*), urine (*19*, *20*), and soil (*21*, *22*). However, little was known about the use of surfactant solution as a solvent for the extraction of chemical constituents from herbal products (*23*, *24*). In this study, nonionic surfactant solution (Genapol X-080) was employed as an alternative and effective solvent for the extraction of daidzein from *P. radix*. The surfactant-rich phase obtained from CPE of daidzein can be analyzed by HPLC without further cleanup or evaporation steps. Compared with other reported methods (*9*, *11*), the sensitivity of this method was satisfactory.

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## MATERIALS AND METHODS

**Chemicals and Reagents.** *Puerariae radix* in the form of small pieces was purchased from Jiashitang Pharmaceutical Group Co. (Beijing, China). It was pulverized and sieved to generate samples with particle sizes up to 100 mesh. Daidzein was purchased from Lancaster Chemical Co. Nonionic surfactant oligo(ethylene glycol) monoalkyl ether (Genapol X-080) was obtained from Fluka. Aqueous surfactant solutions of different concentration levels were prepared by weighing an appropriate amount of the surfactant and directly dissolving it in deionized water. The standard solutions of daidzein were prepared by dissolving daidzein in 5% Genapol X-080. All other reagents were of analytical grade. Deionized water was used throughout.

**Apparatus.** HPLC was performed on a HP G1311A Quat Pump System equipped with a HP G1315 diode-array detector and HP G1328A manual injector (Hewlett-Packard). The analytical column was a Diamonsil C18 (150 × 4.6 mm i.d, 5 $\mu$m) column connected with an Agilent Zorbax extend-C18 guard column (12.5 × 2.1 mm i.d., 5 $\mu$m). The column temperature was controlled at 25 °C. Ultrasonic-assisted extractions (UAE) were performed with a Transsonic SB3200 apparatus (50 kHz, 120 W, Branson, Shanghai, China). Avanti J-25 high-speed refrigerated centrifuge (Beckman) was used for centrifugation. A HZQ incubator shaker (Harbin, China) was used to perform the shake flask extraction. A versatile plant pulverizer (Tianjin, China) was used to make the plant materials into powder. Cloud-point extraction was carried out in a thermostatic water bath.

**Extraction and Preconcentration of Daidzein from *Puerariae radix*.** *P. radix* was ground into powder and dried at 50 °C for 6 h. One gram of *P. radix* was accurately weighed and placed in a 50 mL centrifuge tube, and 25 mL of Genapol X-080 solution of various concentrations was added. The extraction was performed in an ultrasonic bath for 45 min. The *P. radix* extracts were centrifuged at 10 000 rpm for 10 min, and the supernatant was filtered through 0.45 $\mu$m membrane and analyzed by HPLC.

Different types of extractants [5% Genapol X-080, methanol, acetic ether, ethanol, 50% methanol, dichloromethane−methanol (v/v 1:4), cyclohexane, and *n*-hexane] were employed to compare the extraction efficiency under the following experimental conditions: 1 g of sample, 25 mL of extractants, and 45 min extraction time. The shake flask extraction mode was compared with UAE mode. The shake flask extraction mode was performed in an incubator shaker at 160 rpm for 4 h at 40 °C. Genapol X-080 (5%) was used as the extractant.

The experiment of preconcentration of the extracted daidzein by phase separation of the aqueous surfactant solution was carried out by extracting 1 g of *P. radix* with 25 mL of 5% Genapol X-080 in an ultrasonic bath for 45 min. After centrifugation, the supernatant was transferred into a 50 mL centrifuge tube. An appropriate amount of sodium chloride was added to the sample solution and vortex-dissolved for 2 min. The sample solution was then kept in a thermostatic water bath at 50 °C until the solution completely separated into two distinct phases—the upper phase was the small volume of surfactant-rich phase. The aqueous phase was sucked out with a long needle, and the sticky surfactant-rich phase was left in the tube. Methanol was added into the tube to lower the viscosity of the surfactant-rich phase. An aliquot (10 $\mu$L) of the solution was injected into the HPLC system for analysis after filtration through a 0.45 $\mu$m nylon membrane.

**Determination of Daidzein in *Puerariae radix* by HPLC.** The HPLC mobile phase consisted of methanol−1% aqueous solution of acetic acid (55:45 v/v). The flow rate and detection wavelength were set at 1 mL/min and 250 nm, respectively. Peaks in the chromatograms were identified by comparison with retention times and UV spectra of the daidzein standard. Quantification of daidzein was based on calibration graphs, and the extraction percentage (w/w) of daidzein was defined as the amount of daidzein extracted per unit mass of plant tissues.

## RESULTS AND DISCUSSION

**Analysis of Daidzein by HPLC.** Calibration graphs were plotted on the basis of linear regression analysis of the integrated peak areas (integration units, *y*) versus concentrations (micrograms per milliliter, *x*) of daidzein in the standard solution. Each standard solution was analyzed three times. The detector response was linear from 0.78 to 62.4 $\mu$g/mL for daidzein ($y = 64.54x + 37.95$, $R = 0.9998$, $n = 5$).

**Table 1.** Intra- and Interday RSD of Daidzein ($n = 5$)

| content of daidzein ($\mu$g/mL) | intraday RSD (%) | interday RSD (%) |
|---|---|---|
| 62.4 | 0.71 | 0.70 |
| 15.6 | 1.1 | 1.3 |
| 0.78 | 3.6 | 4.8 |

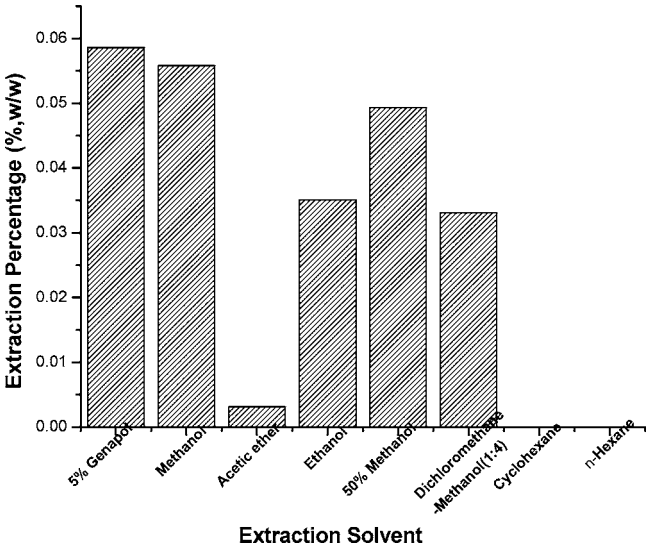


**Figure 2.** Comparison of the results of extraction with different solvents. Solvent volume, 25 mL; *P. radix*, 1 g; ultrasonic-assisted extraction time, 45 min; temperature of extraction, 40 °C.

It showed good linear relationships between the peak areas and the concentrations. The detection limit of dadizein is 0.07 $\mu$g/mL (based on a signal-to-noise ratio of 3).

To assess the precision of this method, standard solutions of daidzein were injected five times on the same day and in a 5-day period. The coefficient variations of intraday and interday studies were less than 4% and 5%, respectively. The precision as well as accuracy of this assay was satisfactory (**Table 1**).

**Comparison of Different Extractants for the Extraction of Daidzein.** To investigate the extraction effect of Genapol X-080, the extraction percentage results were compared between 5% Genapol X-080 and various commonly used extractants. **Figure 2** shows the extraction percentage for daidzein. It could be seen that the extraction efficiency of 5% Genapol X-080 was the highest, and *n*-hexane and cyclohexane had almost no extraction efficiency. The extraction efficiency of 5% Genapol X-080 was a bit higher than that of methanol. It was probably due to the more complete diffusion of surfactant solution into the particles of the herbal material and the solubility-enhancing effect of the surfactant micelles.

**Optimization of the Micelle-Mediated Extraction by Experimental Design.** In our preliminary study, several types of aqueous surfactant solution including Triton X-100, Triton X-114, Triton X-45, and Genapol X-080 were all tried as the extraction solvents. It was found that the Triton X series interfered severely with determination of the daidzein because of their strong UV absorbance. Genapol X-080 is a poly(oxyethylene glycol) monoether-type surfactant that has eight oxyethylene units and tridecyl alkyl moieties. It does not absorb wavelengths above 210 nm; thus it will not interfere with the

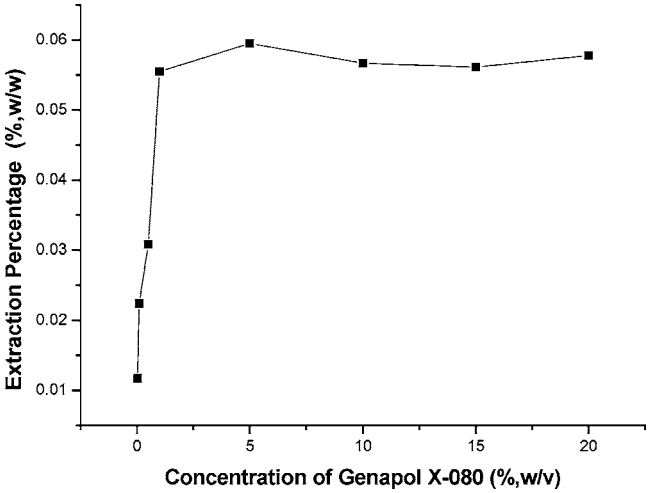


**Figure 3.** Effect of concentration of Genapol X-080 (percentage, w/v) on extraction percentage of daidzein. *P. radix*, 1 g; solvent volume, 25 mL; ultrasonic-assisted extraction time, 45 min; temperature of extraction, 40 °C.

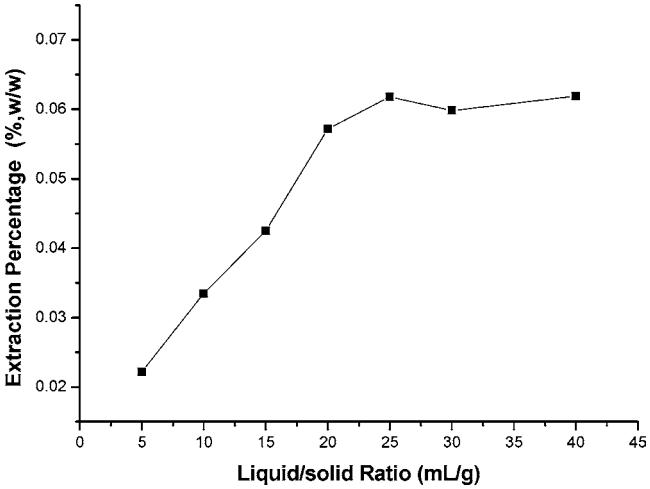


**Figure 4.** Effect of liquid/solid ratio on extraction percentage of daidzein. Solvent volume, 10 mL; concentration of Genapol X-080, 5% (w/v); ultrasonic-assisted extraction time, 45 min; temperature of extraction, 40 °C.

determination of the daidzein. In this study, Genapol X-080 was chosen as the CPE surfactant. Its critical micellar concentration (cmc) is 0.05 mmol/L (0.028% w/v), and its cloud point is 42 °C in pure water.

The effect of surfactant concentration on the extraction percentage of daidzein from *P. radix* is shown in **Figure 3**. It can be seen from **Figure 3** that the extraction percentage of daidzein increased sharply with the concentration of surfactant between 0.03% and 5%. While in the concentration range of 5−20%, the extraction percentage of daidzein remained fairly constant. Daidzein, as a kind of hydrophobic compound, could not be extracted by pure water; whereas it was demonstrated that it could be extracted by surfactant solution at certain concentrations by the results of this experiment. The ability of the aqueous nonionic Genapol X-080 solution in extracting daidzein may be related to the solubility-enhancement effect of the surfactant micelles. It was reported that certain surfactants were known to increase the mass-transfer coefficient during the desorption of pollutants from soil to water, presumably due to better swelling of the soil organic matters and more complete diffusion of the solvent into the solid matrix (*25*). Since maximum extraction percentage could be obtained at surfactant concentrations above 5%, 5% Genapol X-080 was used in the following study.

The effect of liquid/solid ratios on the extraction percentage of daidzein is shown in **Figure 4**. It was found that the extraction percentage of daidzein increased with the increase of liquid/solid ratios and reached the maximum range above the ratio value of 25. So the liquid/solid ratio of 25:1 (mL/g) was chosen for complete extraction of daidzein from *P. radix*.

The effect of extraction time on the extraction percentage of daidzein from *P. radix* was also studied and is shown in **Figure 5**. On the basis of the extraction efficiency and for economic reasons, the extraction time was set as 45 min in the following experiments.

To investigate the effect of extraction mode on the extraction percentage of daidzein, the shake flask extraction method (40 °C, 160 rpm, 4 h) was compared with the UAE method. The results indicated that the UAE method had higher extraction efficiency. The extraction percentage by UAE was 0.060%, while the extraction percentage with the shake flask extraction method was only 0.055%. Because the cavitation effect of ultrasonic wave facilitated the interaction between the surfactant

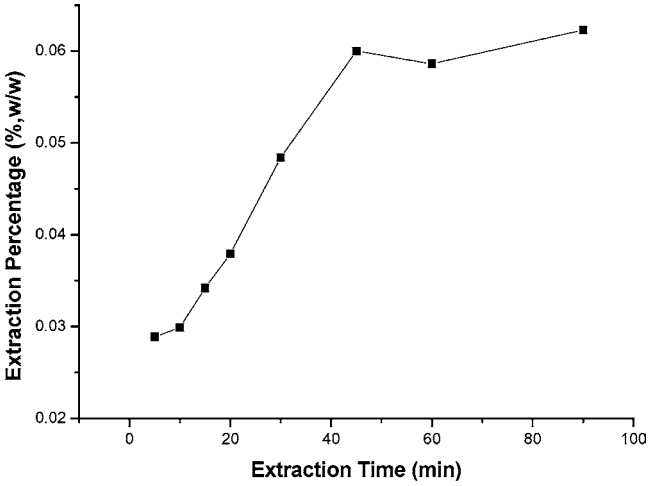


**Figure 5.** Effect of extraction time on extraction percentage of daidzein. *P. radix*, 1 g; solvent volume, 25 mL; concentration of Genapol X-080, 5% (w/v).

solution and daidzein in herbal material, a higher extraction percentage could be obtained with the UAE method in a shorter extraction time.

**Preconcentration of Daidzein by Cloud-Point Extraction.** Daidzein was preconcentrated by cloud-point extraction after micelle-mediated extraction. There were two commonly used methods to induce CPE: one method was by raising the temperature of the sample solution above the cloud-point temperature of the surfactant, and the other was by adding electrolyte to facilitate the separation of the two phases since it may decrease the cloud point temperature (*26*, *27*) and at the same time increase the density of the bulk aqueous phase (*28*). First we tried the temperature-induced CPE at 50 °C, but the time for complete separation of the two phases was longer than 2 h. So electrolytes were added to facilitate the separation of the two phases. Electrolytes that could be used in CPE include urea, sodium chloride, sodium azide, and potassium chloride (*29*). In this study, sodium chloride was the modifier of choice because it was both cost-effective and environmentally friendly. The influence of the content of sodium chloride was studied by adding different amounts of sodium chloride into the solution, and heating the solution in thermostatic water bath at 50, 55, and 60 °C.

**Table 2.** Effects of NaCl Amount and Water Bath Temperature on Concentration of Daidzein in *P. radix* with 5% Genapol X-080

| | 50 °C | | 55 °C | | 60 °C | |
|---|---|---|---|---|---|---|
| amount of NaCl added (g/mL) | preconcentration factor | recovery[a] (%) | preconcentration factor | recovery[a] (%) | preconcentration factor | recovery[a] (%) |
| 0.04 | 3.9 | 104.2 | 4.1 | 91.2 | 4.0 | 104.6 |
| 0.07 | 3.9 | 106.2 | 4.0 | 98.7 | 4.0 | 85.1 |
| 0.10 | 3.6 | 90.9 | 4.0 | 88.2 | 4.1 | 85.7 |
| 0.20 | 13.3 | 100.6 | 12.7 | 91.3 | *b* | *b* |
| 0.30 | 13.4 | 99.1 | 13.0 | 89.7 | *b* | *b* |

[a] The recovery was obtained by comparing the value after CPE with that prior to CPE. [b] Undivided.

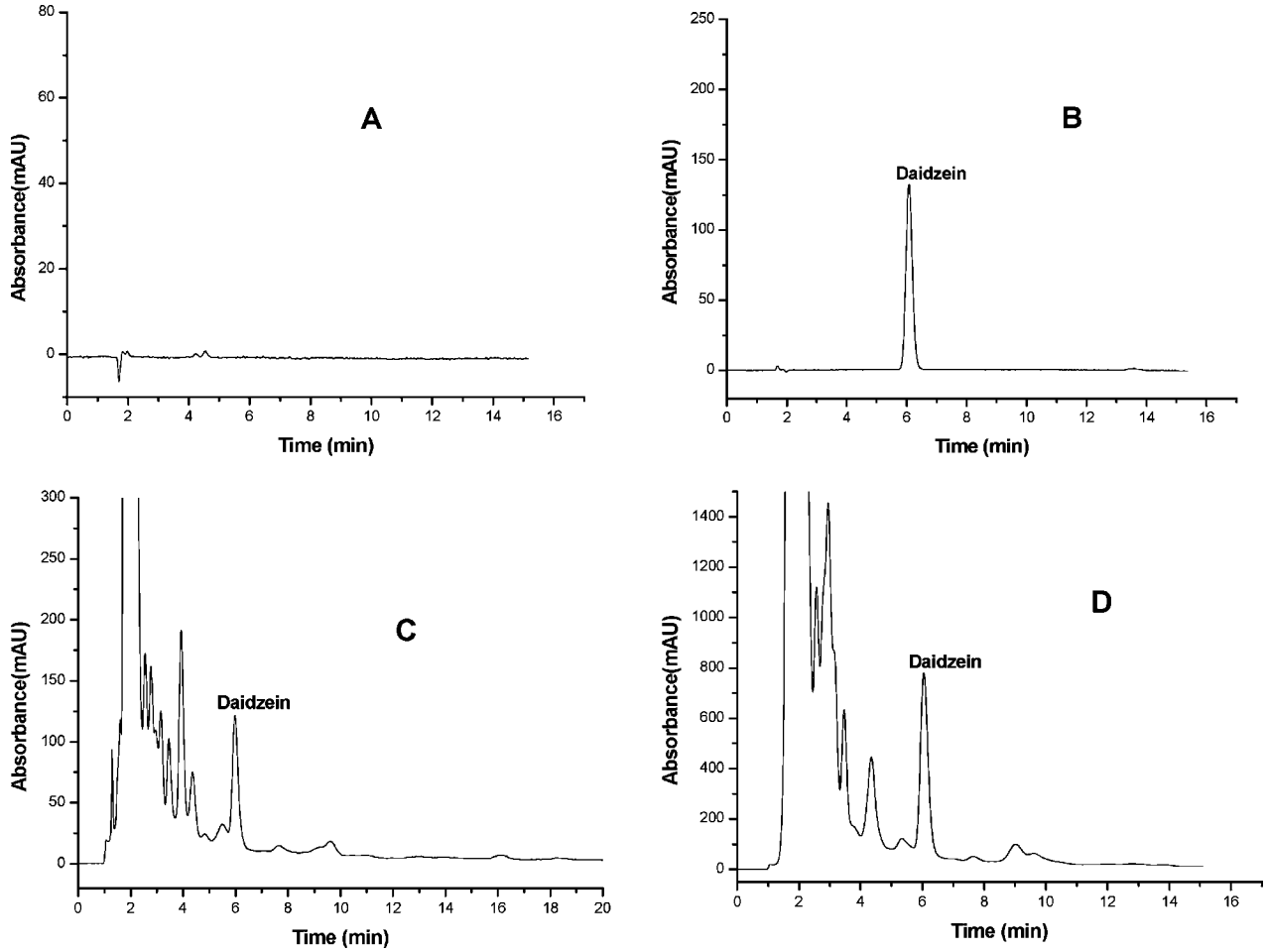


**Figure 6.** HPLC chromatograms of daidzein extracted from *P. radix*. HPLC conditions: Diamonsil C18 column (150 × 4.6 mm i.d., 5 $\mu$m) connected with an Agilent Zorbax extend-C18 guard column (12.5 × 2.1 mm i.d., 5 $\mu$m) controlled at 25 °C; mixture of methanol–1% aqueous acetic acid (55:45 v/v) as mobile phase at a flow rate of 1.0 mL/min; detection wavelength set at 250 nm. (A) Chromatogram of Genapol X-080 (5% w/v); (B) chromatogram of authentic daidzein; (C) chromatogram of the extract prior to cloud-point extraction (non-preconcentration sample); (D) chromatogram of the surfactant-rich phase after cloud-point extraction. *P. radix*, 1 g; original sample solution, 25 mL; concentration of Genapol X-080, 5%; equilibration temperature, 50 °C; equilibration time, 10 min; amount of salt added, 5 g.

**Table 2** shows the effects of the amount of salt on the volume of the whole solution and the temperature of the water bath. It was clearly shown that the preconcentration factor increased significantly as a result of increasing the amount of salt added. When the amount of salt added exceeded 0.2 g/mL, the preconcentration factor reached a maximum value. The complete separation of the two phases could be finished in 10 min. The preconcentration factor was about 13. With the increase of temperature, the recovery declined. When the temperature was above 60 °C, the solution could not be divided into two separate phases. So, in the following experiments, the added amount of sodium chloride was selected to be 0.2 g/mL and the temperature of the water bath was set at 50 °C. To reduce the viscosity of the surfactant-rich phase before injection into the HPLC system, methanol was added to the surfactant-rich phase and the final concentration multiple was fixed to 10.

The preconcentration effect of the CPE with 5% aqueous Genapol X-080 solution as the extractant is clearly demonstrated in **Figure 6**. **Figure 6D** shows a chromatogram of preconcentrated daidzein present in the surfactant-rich phase. For comparison, **Figure 6C** shows a chromatogram of the aqueous Genapol X-080 solution containing daidzein prior to CPE. The content of daidzein in *P. radix* was found to be 0.061%, and RSD was 1.1% ($n = 3$). The recovery of the cloud-point extraction for daidzein in *P. radix* was close to 99.6% (0.294

mg of daidzein was added and 0.295 mg was found), and RSD was 0.7% ($n = 3$).

**Conclusion.** In this work, satisfactory results for the extraction and preconcentration of daidzein from *P. radix* were obtained without use of flammable and potentially toxic organic solvents. Micelle-mediated extraction/CPE was demonstrated to be a potentially powerful tool for the solubilization, purification, and preconcentration of active ingredients from herbal medicines. This capability should be highly valuable in the large-scale extraction and purification of active ingredients from herbal materials. It should be noted that a key step in the purification process would likely be surfactant removal, which can be carried out by various methods based on exploiting the differences in size, charge, and hydrophobicity between the surfactant and extracted compounds (*30*). A popular method of removing nonionic surfactants is via hydrophobic adsorption of the surfactants with polystyrene resins (*31*, *32*). The resins are usually added batchwise to the preparation and removed, together with the bound surfactants, simply by centrifugation or filtration. It has been reported recently that polar active ingredients in herbal medicine could possess significant pharmaceutical properties (*33*). But the polar active ingredients could not be extracted efficiently with conventional organic solvents. Thus micelle-mediated extraction/CPE provided a perfect choice for extraction and preconcentration of both polar and nonpolar compounds from herbal products prior to determination by chromatography or other techniques for research or quality control purposes.

## LITERATURE CITED


(1) Faraj, A.; Vasanthan, T. Soybean isoflavones: Effects of processing and health benefits. *Food Rev. Int.* **2004**, *20*, 51−75.

(2) Davis, S. R.; Dalais, F. S.; Simpson, E. R.; Murkies, A. L. Phytoestrogens in health and disease. *Recent Prog. Horm. Res.* **1999**, *54*, 185−211.

(3) Khan, I. A.; Avery, M. A.; Burandt, C. L.; Goins, D. K.; Mikell, J. R.; Nash, T. E.; Azadegan, A.; Walker, L. A. Antigiardial activity of isoflavones from *Dalbergia frutescens* bark. *J. Nat. Prod.* **2000**, *63*, 1414−1416.

(4) Arora, A.; Nair, M. G.; Strasburg, G. M. Antioxidant activities of isoflavones and their biological metabolites in a liposomal system. *Arch. Biochem. Biophys.* **1998**, *356*, 133−141.

(5) Vedavanam, K.; Srijayanta, S.; O'Reilly, J.; Raman, A.; Wiseman, H. Antioxidant action and potential antidiabetic properties of an isoflavonoid-containing soyabean phytochemical extract (SPE). *Phytother. Res.* **1999**, *13*, 601−608.

(6) Editor committee of Jiangsu New Medical College.*Encyclopedia of traditional Chinese medicine*; Shanghai Science and Technology Press: Shanghai, China, 1995; p 2307.

(7) Yue, H. W.; Hu, X. Q. The medicinal value of *Pueraiae radix* and puerarin for heart vas. *Chin. J. Comb. Chin. Med. West. Med.* **1996**, *16*, 382−384.

(8) Rong, H. J.; Stevens, J. F.; Deinzer, M. L.; De Cooman, L.; De Keukeleire, D. Identification of isoflavones in the roots of *Pueraria lobata*. *Planta Med.* **1998**, *64*, 620−627.

(9) Wang, C. Y.; Huang, H. Y.; Kuo, K. L.; Hsieh, Y. Z. Analysis of *Puerariae radix* and its medicinal preparations by capillary electrophoresis. *J. Chromatogr. A* **1998**, *802*, 225−231.

(10) Chen, G.; Zhang, J, X,; Ye, J, N. Determination of puerarin, daidzein and rutin in *Pueraria lobata* (Wild.) Ohwi by capillary electrophoresis with electrochemical detection. *J. Chromatogr. A* **2001**, *923*, 255−262.

(11) Cao, Y. H.; Lou, C. G.; Zhang, X.; Chu, Q. C.; Fang, Y. Z.; Ye, J. N. Determination of puerarin and daidzein in *Puerariae radix* and its medicinal preparations by micellar electrokinetic capillary chromatography with electrochemical detection. *Anal. Chim. Acta* **2002**, *452*, 123−128.

(12) Saitoh, T.; Hinze, W. Concentration of hydrophobic organic compounds and extraction of protein using alkylammoniosulfate zwitterionic surfactant mediated phase separations (cloud point extractions). *Anal. Chem.* **1991**, *63*, 2520−2525.

(13) Casero, I.; Sicilia, D.; Rubio, S.; Perez-Bendito, D. An Acid-Induced Phase Cloud Point Separation Approach Using Anionic Surfactants for the Extraction and Preconcentration of Organic Compounds. *Anal. Chem.* **1999**, *71*, 4519−4526.

(14) Martínez, R. C.; Gonzalo, E. R.; Jiménez, M. G. G.; Pinto, C. G.; Pavón, J. L. P.; Méndez, J. H. Determination of the fungicides folpet, captan and captafol by cloud-point preconcentration and high-performance liquid chromatography with electrochemical detection**.** *J. Chromatogr. A* **1996**, *754*, 85−96.

(15) Froschl, B.; Stangl, G.; Niessner, R. Combination of micellar extraction and GC-ECD for the determination of polychlorinated biphenyls (PCBs) in water. *Fresenius' J. Anal. Chem.* **1997**, *357*, 743−746.

(16) Yu, H. X.; Man, B. K. W.; Chan, L. L. N.; Lam, M. H. W.; Lam, P. K. S.; Wang, L. S.; Jin, H. J.; Wu, R. S. Cloud-point extraction of nodularin-R from natural waters. *Anal. Chim. Acta* **2004**, *509*, 63−70.

(17) Sirimanne, S. R.; Barr, J. R.; Patterson, D. G. Quantification of polycyclic aromatic hydrocarbons and polychlorinated dibenzo-*p*-dioxins in human serum by combined micelle-mediated extraction (cloud-point extraction) and HPLC. *Anal. Chem.* **1996**, *68*, 1556−1560.

(18) Sirimanne, S. R.; Patterson, D. G.; Ma, L.; Justice, J. B. Application of cloud-point extraction-reversed-phase high-performance liquid chromatography: A preliminary study of the extraction and quantification of vitamins A and E in human serum and whole blood. *J. Chromatogr. B* **1998**, *716*, 129−137.

(19) Fang, Q.; Du, M.; Huie, C. W. On-line incorporation of cloud point extraction to flow injection analysis. *Anal. Chem.* **2001**, *73*, 3502−3505.

(20) Du, M.; Wu, W.; Ercal, N.; Ma, Y. F. Simultaneous determination of 3-nitro tyrosine, *o*-, *m*-, and *p*-tyrosine in urine samples by liquid chromatography−ultraviolet absorbance detection with pre-column cloud point extraction. *J. Chromatogr. B* **2004**, *803*, 321−329.

(21) Merino, F.; Rubio, S.; Perez-Bendito, D. Acid-induced cloud point extraction and preconcentration of polycyclic aromatic hydrocarbons from environmental solid samples. *J. Chromatogr. A* **2002**, *962*, 1−8.

(22) Evdokimov, E.; Von Wandruszka, R. Decontamination of DDT-polluted soil by soil washing cloud point extraction. *Anal. Lett.* **1998**, *31*, 2289−2298.

(23) Choi, M. P. K.; Chan, K. K. C.; Leung, H. W.; Huie, C. W. Pressurized liquid extraction of active ingredients (ginsenosides) from medicinal plants using nonionic surfactant solutions. *J. Chromatogr. A* **2003**, *983*, 153−162.

(24) Fang, Q.; Yeung, H. W.; Leung, H. W.; Huie, C. W. Micelle-mediated extraction and preconcentration of ginsenosides from Chinese herbal medicine. *J. Chromatogr. A* **2000**, *904*, 47−55.

(25) Sahoo, D.; Smith, J. A. Enhanced trichloroethene desorption from long-term contaminated soil using Triton X-100 and pH increases. *Environ. Sci. Technol.* **1997**, *31*, 1910−1915.

(26) Materna, K.; Milosz, I.; Miesiac, I.; Cote, G.; Szymanowski, J. Removal of phenols from aqueous streams by the cloud point extraction technique with oxyethylated methyl dodecanoates as surfactants. *Environ. Sci. Technol.* **2001**, *35*, 2341−2346.

(27) Materna, K.; Cote, G.; Szymanowski, J. Cloud point of aqueous solutions containing oxyethylated methyl dodecanoates: effects of surfactant hydrophilicity, nature of added electrolyte, and water activity. *J. Colloid Interface Sci.* **2004**, 269, 466−471.

(28) Akita, S.; Takeuchi, H. Cloud-point extraction of organic-compounds from aqueous-solutions with nonionic surfactant. *Sep. Sci. Technol.* **1995**, *30*, 833−846.
(29) Hinze, W. L.; Pramauro, E. A critical review of surfactant-mediated phase separations (cloud-point extractions): theory and applications. *Crit. Rev. Anal. Chem.* **1993**, *24*, 133−177.
(30) Furth, A. J. Removing unbound detergent from hydrophobic proteins. *Anal. Biochem.* **1980**, *109*, 207−215.
(31) Holloway, P. W. A simple procedure for removal of Triton X-100 from protein samples. *Anal. Biochem.* **1973**, *53*, 304−308.
(32) Cheetam, P. S. J. Removal of Triton X-100 from aqueous solution using Amberlite XAD-2. *Anal. Biochem.* **1979**, *92*, 447−452.
(33) Kajiwara, H. Motif 2 in adenosine kinase homologous ginseng polypeptide showed affinity to D-ribose by capillary zone electrophoresis and surface plasmon resonance. *J. Chromatogr. A* **1998**, *817*, 173−179.

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