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Separation of Soybean Isoflavone Aglycone Homologues by Ionic Liquid-Based Extraction

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Supporting Information

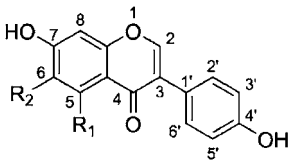
ABSTRACT: The separation of a compound of interest from its structurally similar homologues is an important and challenging problem in producing high-purity natural products, such as the separation of genistein from other soybean isoflavone aglycone (SIA) homologues. The present work provided a novel method for separating genistein from its structurally similar homologues by ionic liquid (IL)-based liquid−liquid extraction using hydrophobic IL−water or hydrophilic IL/water−ethyl acetate biphasic systems. Factors that influence the distribution equilibrium of SIAs, including the structure and concentration of IL, pH value of the aqueous phase, and temperature, were investigated. Adequate distribution coefficients and selectivities over 7.0 were achieved with hydrophilic IL/water−ethyl acetate biphasic system. Through a laboratory-scale simulation of fractional extraction process containing four extraction stages and four scrubbing stages, genistein was separated from the SIA homologues with a purity of 95.3% and a recovery >90%.

KEYWORDS: *bioactive constituents, flavonoid, soybean isoflavone aglycones, ionic liquid, liquid−liquid extraction, hydrogen bonding*

INTRODUCTION

Over the past few decades, natural bioactive constituents such as flavonoids have received increased attention for their health-promoting benefits and are considered to be major resources of drugs and food additives all over the world.[1−3] More than 5000 kinds of flavonoids are known.[4] As the bioactive flavonoids always exist in plant tissues together with several structural related homologues and because each homologue may possess physiological activities specific to or contrary from the others, it is necessary to separate the compound of interest from the mixture. However, the separation is very challenging because of the high structural similarity of the flavonoid homologues.

A good sample for the separation of flavonoid homologues is the production of soybean isoflavones, namely, soybean isoflavone aglycones (SIAs). Soybean is one of the most widely cultivated crops around the world and is rich in proteins, lipids, carbohydrates, and secondary metabolites such as isoflavones and saponins.[5] Of all the bioactive constituents, SIAs are gathering intense interest because they are a group of phytoestrogens and serve a variety of estrogen-related functions,[6,7] such as prevention and treatment of cancers, arteriosclerosis, and osteoporosis. As the content of SIAs in soybeans is rather low, SIAs are prepared by hydrolyzing soybean isoflavone glucosides separated from soybean meal, the byproduct of soybean processing for oil. The three types of SIA in soybean are daidzein, glycitein, and genistein (Figure 1). Their chemical structures, differing only in the type and position of the substituted groups, determine the biological activities of the SIAs. Genistein, bearing an additional phenolic hydroxyl group at C_5 position, has been proved to possess the highest antioxidant activity and bioavailability in human beings and therefore is the focus of much research.[8,9] Thus, there is a strong demand to obtain genistein with high purity. Because of the high structural similarity of the SIAs, isolating the most antioxidative genistein is really difficult.



compound	R_1	R_2
daidzein	H	H
glycitein	H	OCH_3
genistein	OH	H

Figure 1. Chemical structure of SIAs.

Several methods have been developed for the separation of SIA homologues, involving preparative chromatography,[10,11] high-speed counter-current chromatography (HSCCC),[12] crystallization,[10,13] etc. Although separation could be achieved, these methods still have limitations. Preparative chromatography is limited to low absorption capacity as well as high solvent and absorbent consumption. The HSCCC method has the disadvantages of large solvent and energy consumption. The crystallization method bears problems of low recovery and purity. The above-mentioned problems and drawbacks are encountered not just in SIA homologue separation; in fact, they are common problems for separating flavonoid homologues or even producing other natural bioactive compounds. Therefore,

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economical and effective separation methods should be developed.

Ionic liquids (ILs) are gathering considerable attention as potential substituents of conventional organic solvents due to their unique properties, such as negligible vapor pressures, high specific solvent abilities, and tunable structures.[14,15] Moreover, the dipolarity/polarizability and hydrophobicity as well as hydrogen-bonding acidity and basicity of the ILs can also be tuned finely by different cation and anion combinations.[16,17] Therefore, the unique properties of ILs are combined with the advantages of liquid extraction to improve the efficiency of the separation processes, such as the extraction of metal ions,[18] phenolic compounds,[19] dyes,[20] and organic acids;[21] aromatics/aliphatics separations;[22,23] removal of sulfur- and nitrogen- containing compounds from diesel and gasoline fuels;[24] and extraction of biomolecules (amino acids,[25] proteins,[26] antibiotics,[27] and alkaloids[28]) from various media. In addition, ILs were also used for extracting flavonoid mixtures from raw materials.[29] The application of ILs for extractive separation of structural similar compounds and homologues is also promising because of such advantages. The liquid−liquid extractive separation of limonene and linalool was performed by Arce et al. with IL as extractant, and the studied ILs (1-ethyl-3-methylimidazolium ethylsulfate and 1-ethyl-3-methylimidazolium methanesulfonate) showed higher selectivities than common extractants.[30,31] In our previous study, the natural phenolic compounds, tocopherol homologues, were separated by IL-based liquid−liquid extraction with a selectivity of δ-tocopherol to α-tocopherol up to 21.3, through a separation mechanism mainly based on the hydrogen-bonding interaction between the IL's anion and the hydroxyl group on the tocopherols.[32,33]

Therefore, in this paper we aim for the selective separation of the compound of interest from flavonoid homologues by IL-based liquid−liquid extraction, and the separation of genistein from SIA homologues was performed. Common hydrophobic IL−water biphasic system and a novel liquid−liquid biphasic system consisting of hydrophilic IL, water, and ethyl acetate were employed for separating genistein from daidzein and glycitein. Factors that influence the extraction equilibrium, including the chemical structure of IL, pH values, and composition of IL in the extraction solvent as well as temperature, were investigated. In addition, continuous multistage extractions were performed by both calculation and experimental verification.

MATERIALS AND METHODS

Materials. All of the ionic liquids (Figure 2) used in this work, including hydrophobic ILs ([BMIm][PF_6], [HMIm][PF_6], [OMIm][PF_6], and [BMIm][NTf_2]) and hydrophilic ILs ([EMIm][BF_4], [EPy][BF_4], [EMIm]Br, [EPy]Br, [EMIm]Cl, and [HOEtMIm]Cl) were purchased from Lanzhou Greenchem. ILS (LICP. CAS, Lanzhou, China) with mass fraction purities of >99%. The water mass fraction of ILs were determined by Karl Fischer titration, and values lower than 0.2% for hydrophobic ILs and around 0.7% for hydrophilic ILs were found. The raw materials containing soybean isoflavone glycosides were supplied by Heilongjiang Jiusan Oil and Fat Co., Ltd. (China). The SIAs were prepared according to the method described in our previous work,[34] with mass fractions of daidzein, glycitein, and genistein of 48.1, 20.6, and 27.2%, respectively.

Extraction Equilibrium Experiments. The extraction equilibrium experiments with hydrophobic IL−water biphasic system were performed as described below. First, 30.0 mg of SIA mixture was well-mixed with 100 mL of water or aqueous solution. The aqueous solution was prepared by adding hydrochloric acid or sodium hydroxide to water to adjust the pH values at 4, 6, 8, and 10. Second, aliquots of the solution were contacted with an equal volume of pure IL in a conical flask under vigorous shaking using a thermostatic rotary shaker at 323 K and 200 rpm until the SIAs were totally dissolved. Third, the flask was cooled and vigorously shaken in the same thermostatic rotary shaker at 313 K and 200 rpm for several hours to reach phase equilibrium and then allowed to settle for at least 5 h at the same temperature. Finally, samples were taken from both phases and diluted with methanol for HPLC analysis.

In extraction experiments with the hydrophilic IL/water−ethyl acetate biphasic system, a certain amount of the SIA mixture was dissolved in ethyl acetate to prepare a solution at 0.3 mg mL^{-1}, and an aqueous extraction solvent with definite IL mole fraction (5−80%) was prepared by gravimetric method (±0.0001 g). Then equal volumes of both solutions were mixed in a conical flask under vigorous shaking for 2 h using a thermostatic rotary shaker at a set temperature and 200 rpm. The next settling and sampling procedures were performed according to the same method as described for the hydrophobic IL−water biphasic system. The temperature was in the range from 303 to 328 K, from the lowest temperature that could be maintained in summer when the experiments were performed to a temperature below the boiling point of ethyl acetate (350 K).

The distribution coefficient (D_i) of solute i and selectivity of solute i to solute j ($S_{i/j}$) were calculated according to eqs 1 and 2

$$D_i = C_i^{e}/C_i^{r} \quad (1)$$

$$S_{i/j} = D_i/D_j \quad (2)$$

where C_i^{e} and C_i^{r} refer to the concentrations (mg mL^{-1}) of solute in the extraction phase and in the raffinate phase, respectively.

The extraction equilibrium experiments were performed repeatedly at least three times, and the relative uncertainties of the distribution coefficients were <3% for the hydrophilic IL/water−ethyl acetate biphasic system. When the hydrophobic IL−water biphasic system was used, as the concentration of SIAs in water-rich phase is rather low, the HPLC peak areas of the SIAs in the water-rich phase were too small, and it is difficult to measure the data accurately. In such cases, the relative uncertainties of the distribution coefficients were <8.5%.

Multistage Countercurrent Extraction and Fractional Extraction. Calculations were performed under ideal conditions: assuming the distribution coefficients were constant under the studied SIA mixture's total concentration and homologues' composition in feed solvent, and the mutual solubility of the biphasic system was ignored. The distribution coefficients and selectivity of the SIAs in [EMIm]Br/water (10:90, molar ratio)−ethyl acetate at 313 K were employed. As the distribution coefficients of daidzain and glycitein were close (1.09 and 1.08), the distribution coefficient 1.08 was used for daidzain and glycitein during calculation. The scrubbing solvent employed in fractional extraction was ethyl acetate.

The experimental continuous multistage countercurrent extraction and fractional extraction were performed with the "funnel method",[35] and the operating patterns are shown in Figure S1 in the Supporting Information (SI). The [EMIm]Br/water (10:90, molar ratio)−ethyl acetate biphasic system was employed. The operation temperature was maintained at 313 K. The countercurrent extraction was carried out with five extraction stages with the solvent-to-feed ratio (S/F) of 1:1. In every other line, 20 mL of ethyl acetate with an SIA concentration of 0.3 mg mL^{-1} and 20 mL aqueous IL solution were added. The fractional extraction was carried out under the following conditions: the number of equilibrium stages for extraction section and scrubbing section were both 4; the initial concentration of SIAs in the feed was 0.3 mg mL^{-1}; the flow ratio of extraction solvent, feed, and scrubbing solvent was deduced to be 10:1:2.5. Ethyl acetate was employed as scrubbing solvent. In every other line, 10.5 mL of aqueous IL solution and 30 mL of ethyl acetate dissolving SIAs were added in the extraction section, and 7.5 mL of aqueous IL solution dissolving SIAs and 30 mL of ethyl acetate were added in the scrubbing section. The SIA concentrations and compositions were determined according to the calculation results. Samples were taken and diluted by methanol for HPLC analysis.

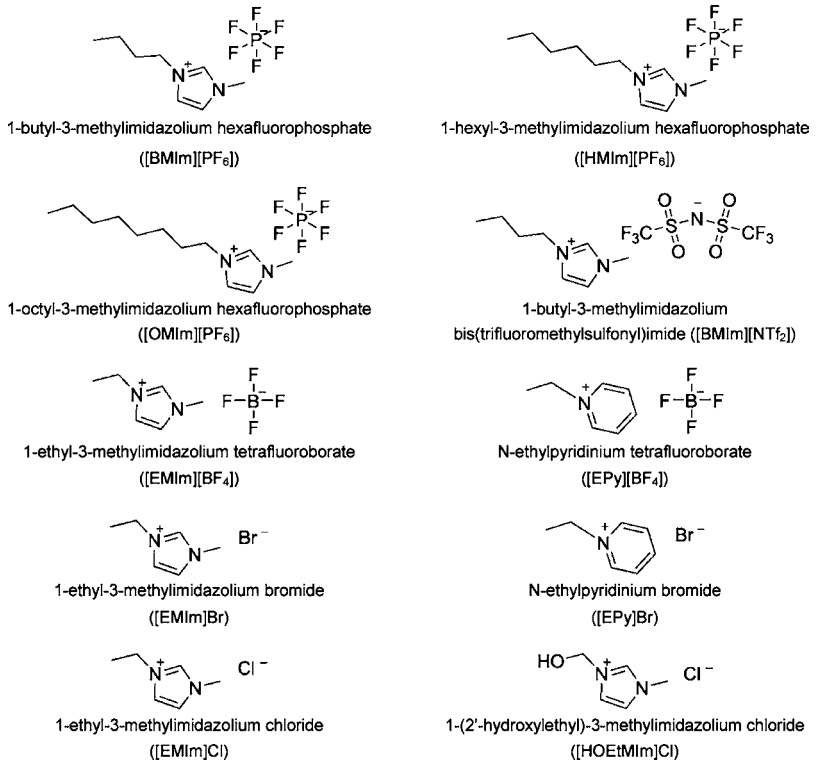

Figure 2. Chemical structures of the ILs in this study and their abbreviated names.

Table 1. Distribution Coefficients and Selectivities of the SIAs in Hydrophobic IL−Water Biphasic Systems at 313 K[a]

	distribution coefficient			selectivity	
IL	Dai	Gly	Gen	Gen/Dai	Gen/Gly
$[BMIm][PF_6]$	51.4 ± 2.2	41.9 ± 1.9	182.6 ± 8.2	3.6	4.4
$[HMIm][PF_6]$	47.1 ± 3.2	34.7 ± 2.1	131.9 ± 8.7	2.8	3.8
$[OMIm][PF_6]$	46.0 ± 3.9	42.5 ± 1.1	177.8 ± 10.7	3.9	4.2
$[BMIm][NTf_2]$	45.8 ± 2.9	36.4 ± 2.8	120.4 ± 6.6	2.6	3.3

[a]The initial concentrations of daidzein (Dai), glycitein (Gly), and genistein (Gen) in the aqueous solution were 0.14, 0.06, and 0.08 mg mL^{-1}, respectively. The volume ratio was 1:1.

HPLC Analysis. HPLC analysis of the SIAs was performed according to the literature.[36] A Waters series chromatograph (including a 1525 binary pump, a thermostat, a 2487 dual λ absorbance detector, and an autosampler) and a Waters Novapak C_{18} column (4 μm, 3.9 mm × 150 mm) were used. The column temperature was maintained at 313 K. The mobile phase consisted of solution A (acetic acid/water = 1:1000, v/v) and solution B (acetic acid/acetonitrile = 1:1000, v/v) with gradient elution as follows: 0 → 12.5 min, 13 → 30% B (v/v, linear gradient); 12.5 → 15.5 min, 30 → 36% B (v/v, linear gradient); 15.5 → 16.5 min, 36 → 100% B (v/v, linear gradient); 16.5 → 19.0 min, 100% B (v/v, isocratic); 19.0 → 20.0 min, 100 → 13% B (v/v, linear gradient); 20.0 → 30.0 min, 13% B (v/v, isocratic). The flow rate was 1 mL·min^{-1}. The wavelength was set at 254 nm. The retention times for daidzein, glycitein, and genistein were around 9.8, 10.7, and 13.2 min, respectively.

RESULTS AND DISCUSSION

Extractive Separation of SIA Homologues with Hydrophobic IL−Water Biphasic System. The distribution coefficients of SIA homologues with commonly used hydrophobic imidazolium-based ILs with $[PF_6]^-$ and $[NTf_2]^-$ anions as extractant listed in Table 1 were >30.0, and the selectivities of genistein to other SIAs were >3.0, indicating that the SIAs have higher affinity to ILs than to water and that genistein could be selectively separated from its homologues with hydrophobic IL as extractant.

The decreasing order of distribution coefficients of SIAs genistein > daidzein > glycitein can be attributed to two factors as suggested by the finding that the phenolic compounds could interact with IL through hydrophobic and hydrogen-bonding interactions.[37] On the one hand, the hydrogen-bonding interaction strength order between the acidic phenolic hydroxyl groups of SIAs and the IL's anion is in agreement with the hydrogen-bonding acidity order of SIAs: the additional phenolic hydroxyl group in genistein rendering genistein shows the highest hydrogen-bonding acidity, and the electron-denoting C_6 methoxyl group around the phenolic hydroxyl group in glycitein reduces its hydrogen-bonding acidity. On the other hand, the hydrophobicity of SIAs influences their solubility in water and then influences the distribution behavior. In aqueous environment, the intramolecular interaction between the adjacent C_5 hydroxyl group and the C_4 ketone group in genistein minimizes the hydrophilic contribution of both groups relative to the contribution of the single ketone group in daidzein, and the additional methoxyl group at C_6 in glycitein enhances the aqueous solubility;[38] the hydrophobicity of the SIAs follows the order genistein > daidzein > glycitein.[39] The differences in both

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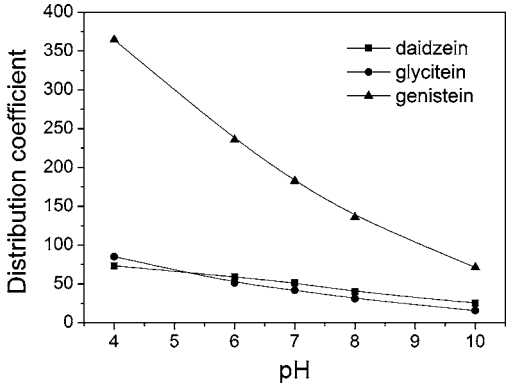

Figure 3. Effect of pH value of the aqueous phase on distribution coefficient of daidzein, glycitein, and genistein. The IL used was [BMIm][PF_6]. The initial concentrations of daidzein, glycitein, and genistein in the aqueous solution were 0.14, 0.06, and 0.08 mg mL^{-1}, respectively. The volume ratio was 1:1. The temperature was 313 K.

hydrogen-bonding interaction and hydrophobicity could result in the strongest interaction between IL and genistein and the weakest interaction between IL and glycitein, which further determined the order of distribution coefficients.

Effect of the Chemical Structure of IL. The distribution coefficient of the SIAs obtained by ILs with different anions generally followed the order [BMIm][NTf_2] < [BMIm][PF_6] under the same experimental condition (Table 1). This order is in accordance with the order of ILs' hydrogen-bonding basicity strength.[40] No obvious regulation was found for the influence of variation in the alkyl chain length of imidazolium-based ILs with $[PF_6]^-$ anion (Table 1), which may due to the fact that variation in the alkyl chain length of the IL had a contrary effect on its hydrogen-bonding basicity and hydrophobicity.[41]

Effect of pH Value of the Aqueous Phase. In Figure 3, it is seen that the distribution coefficients of all the SIAs in [BMIm][PF_6]–water biphasic system increased with the decrease of aqueous pH value, similar to the results reported for the partitioning of picric acid between [BMIm][PF_6] and water.[19] These results are related to the charged characteristics of SIAs at different pH conditions: SIAs exist as neutral molecules at acidic conditions and will partially transform into anionic form at basic conditions, leading to higher solubility of SIAs in more basic aqueous solutions,[42] consequently weakening

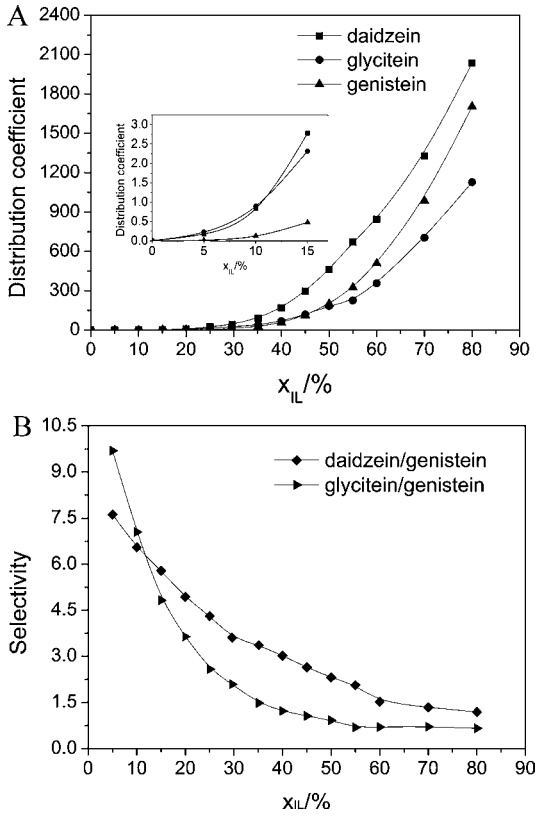

Figure 4. Effect of the initial mole fraction of [EMIm]Br in the extraction solvent on the separation of the SIAs: (A) distribution coefficient; (B) selectivity. The initial concentrations of daidzein, glycitein, and genistein in ethyl acetate were 0.14, 0.06, and 0.08 mg mL^{-1}, respectively. The volume ratio was 1:1. The temperature was 303 K.

Table 2. Distribution Coefficients and Selectivities of the SIAs in Hydrophilic IL/Water–Ethyl Acetate Biphasic Systems at 303 and 313 K[a]

	distribution coefficient			selectivity	
extractant	Dai	Gly	Gen	Dai/Gen	Gly/Gen
		303 K			
[EMIm][BF_4]	1.02 ± 0.04	1.22 ± 0.03	0.27 ± 0.01	3.8	4.5
[EPy][BF_4]	0.98 ± 0.02	1.20 ± 0.03	0.27 ± 0.01	3.6	4.4
[EMIm]Br	0.86 ± 0.02	0.92 ± 0.02	0.13 ± 0.02	6.6	7.1
[EPy]Br	0.75 ± 0.03	0.77 ± 0.02	0.11 ± 0.00	7.1	7.4
[EMIm]Cl	0.71 ± 0.02	0.62 ± 0.03	0.10 ± 0.03	7.4	6.4
[HOEtMIm]Cl	0.22 ± 0.00	0.20 ± 0.01	0.03 ± 0.00	7.7	7.1
water	0.0060 ± 0.0001	0.0076 ± 0.0004	0.0008 ± 0.0001		
		313 K			
[EMIm][BF_4]	1.10 ± 0.03	1.25 ± 0.04	0.30 ± 0.02	3.7	4.2
[EPy][BF_4]	1.09 ± 0.02	1.28 ± 0.03	0.30 ± 0.01	3.6	4.3
[EMIm]Br	1.09 ± 0.03	1.08 ± 0.03	0.18 ± 0.01	6.0	6.0
[EPy]Br	0.92 ± 0.03	0.89 ± 0.03	0.15 ± 0.01	6.1	5.9
[EMIm]Cl	1.34 ± 0.02	1.01 ± 0.01	0.22 ± 0.01	6.1	4.6
[HOEtMIm]Cl	0.28 ± 0.00	0.22 ± 0.01	0.04 ± 0.00	7.6	5.9
water	0.0073 ± 0.0005	0.0089 ± 0.0003	0.0011 ± 0.0000		

[a]The initial mole fraction of IL in the extraction solvent was 10%. The initial concentrations of daidzein (Dai), glycitein (Gly), and genistein (Gen) in ethyl acetate were 0.14, 0.06, and 0.08 mg mL^{-1}, respectively. The volume ratio was 1:1.

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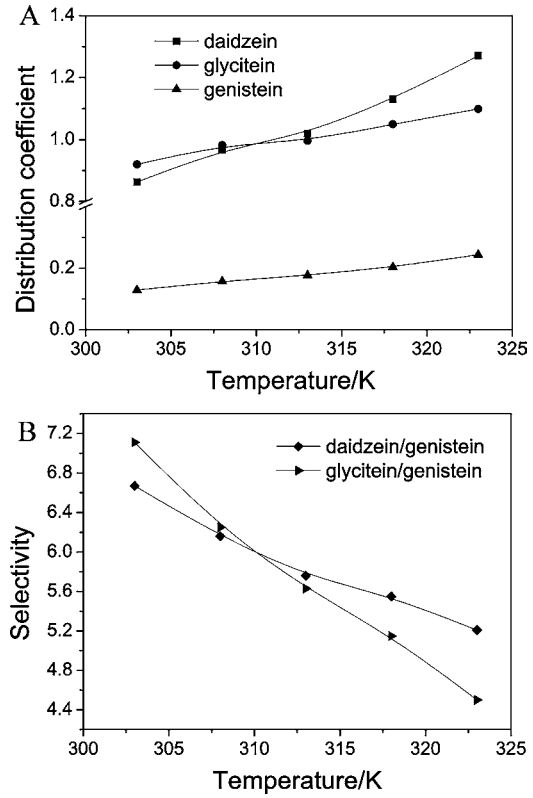

Figure 5. Effect of temperature on the separation of SIAs: (A) distribution coefficient; (B) selectivity. The initial concentrations of daidzein, glycitein, and genistein in ethyl acetate were 0.14, 0.06, and 0.08 mg mL^{-1}, respectively. The initial mole fraction of [EMIm]Br in the extraction solvent was 10%. The volume ratio was 1:1.

the interaction between IL and SIAs. As a result, both the distribution coefficients and the selectivity data decreased with increasing pH value of the aqueous phase.

Extractive Separation of SIA Homologues with Hydrophilic IL/Water−Ethyl Acetate Biphasic Systems. As the solubility of SIA in water is rather low, solvents that dissolve more SIAs, such as methanol, ethanol, and ethyl acetate,[43] should be more appropriate for large-scale production, because the low solubility in water could cause low extraction capacity as well as too large distribution coefficients of SIAs in the hydrophobic IL−water biphasic system, which are not beneficial for the separation of the homologues. Finally, ethyl acetate was chosen as solvent, not only for the high solubility of SIA in it but also for its relatively low cost and better ability to form biphasic systems than other solvents. ILs with Cl^- and Br^- as anions were selected to form biphasic systems with ethyl acetate because they are immiscible with ethyl acetate, and the relatively strong hydrogen-bonding basicity of Cl^- and Br^- may improve their affinity to SIAs.[17] As ILs with halide anions are solid at room temperature, water was used as diluent in association with ILs to form biphasic system with ethyl acetate.

Effect of the Chemical Structure of IL. The distribution coefficients of SIAs in hydrophilic IL/water−ethyl acetate biphasic systems are listed in Table 2. Extraction solvent without IL was also studied for comparison. The distribution coefficients of all the SIAs in the IL-containing biphasic systems were found to be almost 2 orders of magnitude higher than those in water−ethyl acetate biphasic systems, confirming the notable interactions between the SIAs and IL. Comparing the selectivities in Tables 1 and 2 shows that better separation efficiency, as a combination of more appropriate distribution coefficients (not too high) and higher selectivities, was achieved with hydrophilic IL/water−ethyl acetate biphasic systems than with the formerly studied hydrophobic IL−water biphasic systems.

The kind of IL's anion has a significant effect on the extractive separation. The data revealed in Table 2 show that the biphasic systems containing ILs with $[BF_4]^-$ anion exhibited higher distribution coefficients and lower selectivities than other systems under the same experimental conditions, and the highest selectivities for the SIA homologues were obtained in the biphasic systems containing ILs with halide anions. The research on the phase equilibrium manifests that the [EMIm][BF_4]/water−ethyl acetate system has relatively higher mutual solubilities than the biphasic systems containing IL with halide anion, which causes a low selectivity for the SIA homologues.

The structure of IL's cation also affects the extractive separation of the SIAs. When the anion is the same (Br^-), the

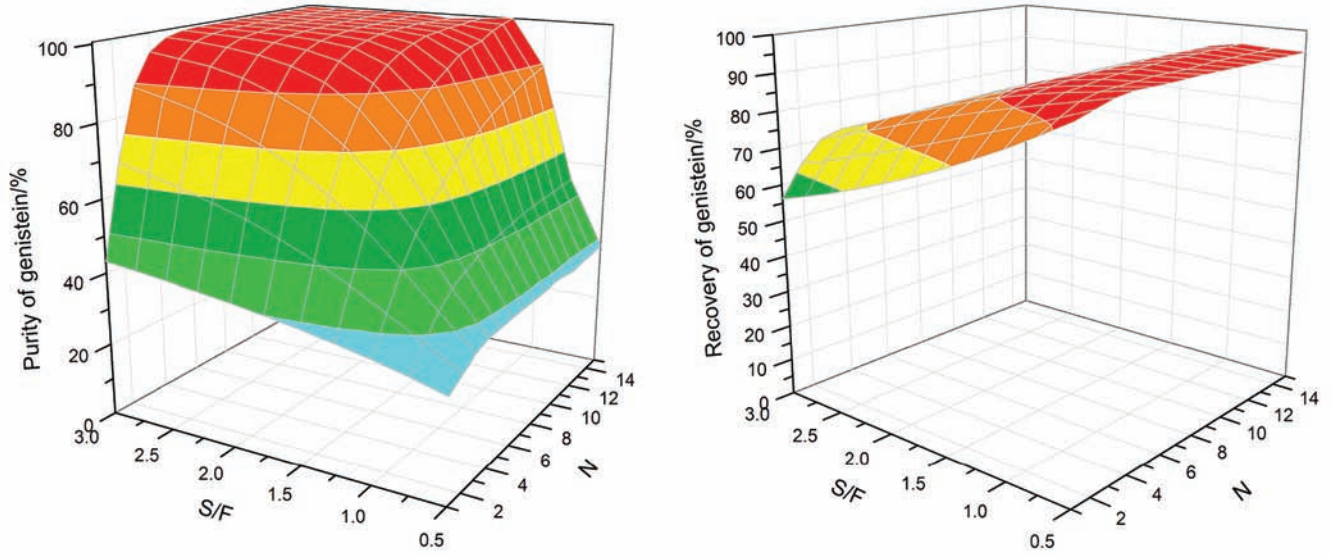

Figure 6. Calculated purity and recovery of genistein versus the solvent-to-feed ratio (S/F) and the number of extraction stages (N) with countercurrent extraction.

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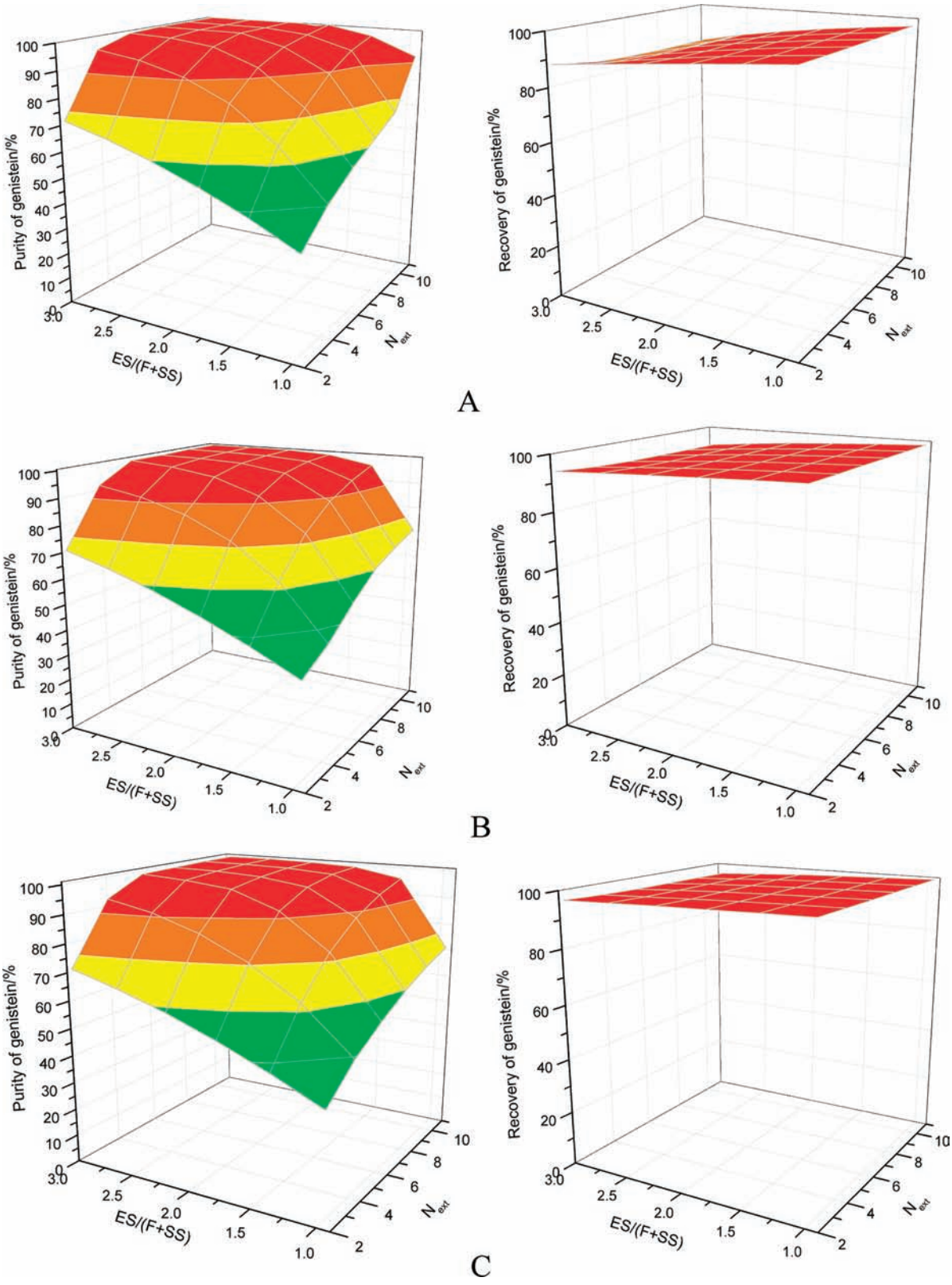

Figure 7. Calculated purity and recovery of genistein versus the flow ratio (ES/(F + SS); ES, F, and SS represent the flow rates of extraction solvent, feed, and scrubbing solvent, respectively) and the number of stages of the extraction section (N_{ext}) with fractional extraction. The flow ratio of the scrubbing section (scrubbing solvent-to-extraction solvent ratio) was set at 0.33. The numbers of stages of the scrubbing section were 2, 3, and 4 for panels A, B, and C, respectively.

distribution coefficient of SIAs obtained with [EPy]Br are slightly smaller than the values from [EMIm]Br, and the selectivities are similar. However, when an additional polar hydroxyl group was introduced into the 1-ethyl-3-methylimidazolium cation, the distribution coefficient of SIAs has a drastic decrease and the selectivity slightly increases.

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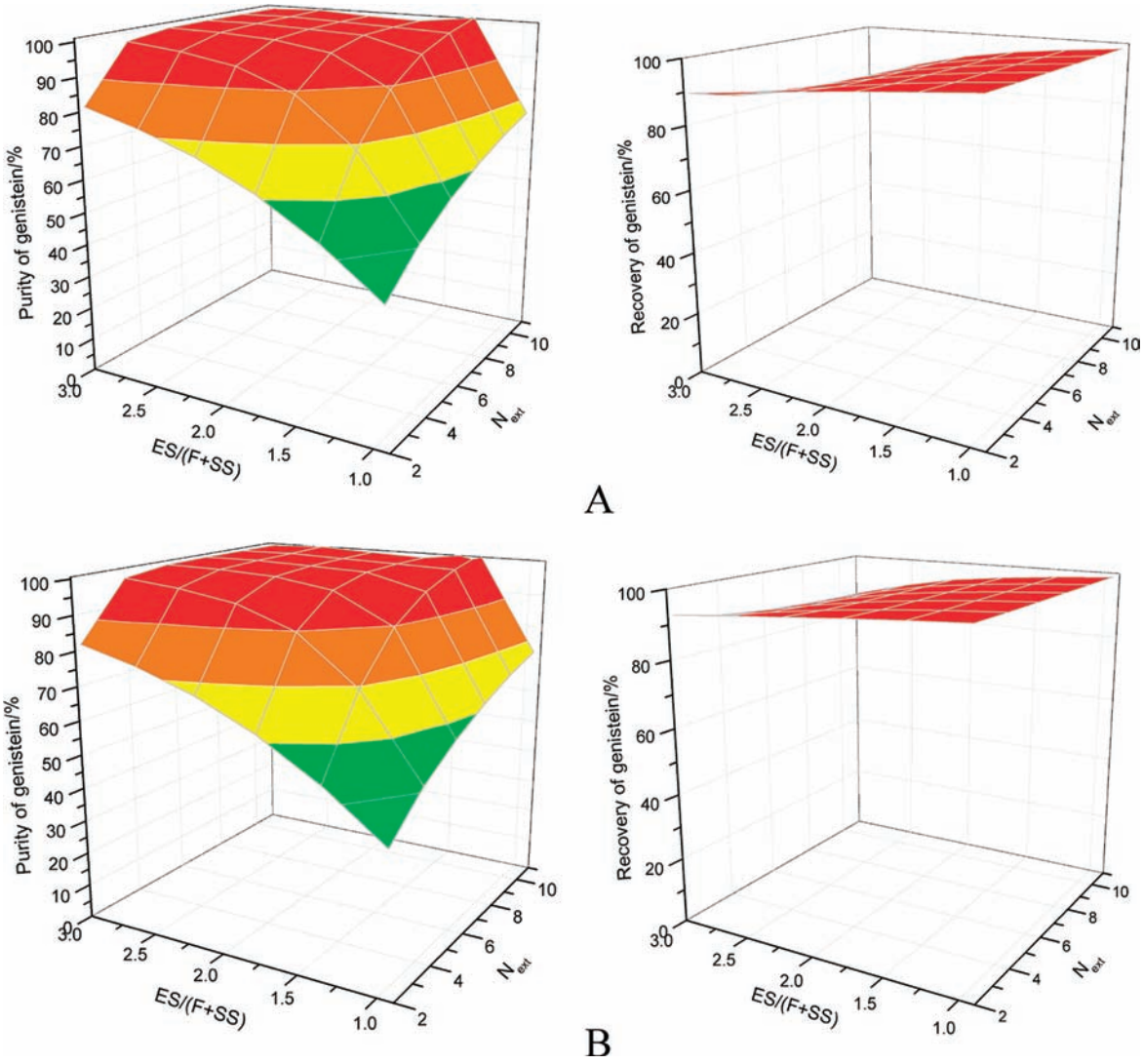

Figure 8. Calculated purity and recovery of genistein versus the flow ratio (ES/(F + SS); ES, F, and SS represent the flow rate of extraction solvent, feed, and scrubbing solvent, respectively) and the number of stages of the extraction section (N_{ext}) with fractional extraction. The flow ratio of the scrubbing section (scrubbing solvent-to-extraction solvent ratio) was set at 0.25. The numbers of stages of the scrubbing section were 4 and 5 for panels A and B, respectively.

Effect of the Initial Concentration of IL in the Extraction Solvent. Figure 4A shows plots of the distribution coefficients of SIAs against the initial mole fraction of [EMIm]Br (x_{IL}) in the extraction solvent. It is clear that for dilute aqueous IL solutions (x_{IL} < 11%), the order of distribution coefficients of SIAs was genistein < daidzein < glycitein. As IL concentration increased, the distribution coefficients of daidzein and genistein increased more sharply than that of glycitein, and the distribution coefficient changed to daidzein < glycitein < genistein in decreasing order when the IL concentration was >50%. The phenomenon is caused by the following reasons: at the dilute IL concentration range, the hydrophobicity of SIAs dominated their distribution behavior, and thus similar distribution behavior of SIAs to that in hydrophobic IL−water and octanol−water biphasic systems was found; as the IL concentration increased, the hydrogen-bonding basicity of the extraction solvent was enhanced and the hydrogen-bonding interaction gradually dominated the distribution behavior of the SIAs, so the order of distribution coefficients of different homologues changed to be in accordance with the order of their hydrogen-bonding acidity.

As seen from Figure 4B, there were dramatic decreases for the selectivity of daidzein and glycitein to genistein during increasing x_{IL}. As the dipolarity/polarizability of the aqueous IL solution has been reported to decrease with the addition of IL into its aqueous solution,[44] the selectivity of the SIAs in the [EMIm]Br/water−ethyl acetate biphasic system is probably determined by the dipolarity/polarizability of the extraction solvent.

Effect of Temperature. The influence of temperature on the extractive separation of SIAs was investigated. The distribution coefficients presented in Figure 5A reveal that the distribution values of the SIAs increased slightly as the temperature increased. Figure 5B reveals that the selectivities of daidzein and glycitein to genistein decreased with increasing temperature. Temperature influences the distribution through various aspects, such as the solubility of SIAs in two phases, the physical and chemical properties of each phase, and the mutual solubilities of the biphasic system.

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Continuous Multistage Extraction. With the aim to analyze the suitability of the process for a practical production, continuous multistage extractions were performed by both calculations and experiments.

The calculation results presented in Figures 6 and 7 show the purity and recovery of genistein as a function of the solvent-to-feed ratio and the number of extraction stages for both countercurrent extraction and the extraction section of fractional extraction. It is seen that the recoveries of genistein with fractional extraction were overall higher than with countercurrent extraction. The calculation results (Figure 7) obtained under conditions with the same scrubbing solvent-to-feed ratio of the scrubbing section (namely, the scrubbing solvent-to-the extraction solvent ratio) indicate that the purity varied a little and the recovery of genistein increased as the number of scrubbing stages increased. In cases when the scrubbing stages were 4 (Figures 7B and 8A), while the solvent-to-feed ratio of the scrubbing section decreased from 0.33 to 0.25, relatively high purity and low recovery of genistein were observed. It is obvious that both high purity and high recovery of genistein could be achieved under optimized conditions.

Then, countercurrent and fractional extractions were performed experimentally. The countercurrent extraction with five stages was conducted with the feed to solvent ratio of 1:1. The relative purity of genistein obtained was 54.1% and the calculated purity was 62.4% under the same conditions. The calculated and experimentally obtained relative mass fraction of genistein in the SIAs versus the stage number by using fractional extraction is plotted in Figure 9. It is seen that the

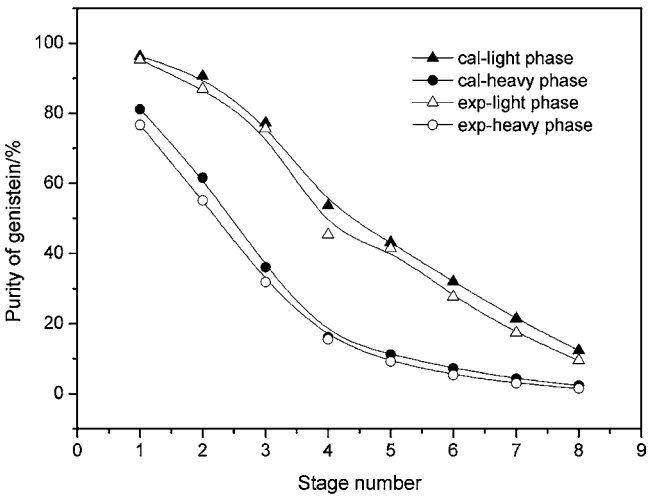

Figure 9. Calculated (cal) and experimental (exp) purity of genistein in the light and heavy phases at different stages. The stages were numbered from the extraction section to the scrubbing section.

experimental data were close to the calculated ones, indicating that the simulation method was reliable. The results show that the relative purity of genistein was 95.3% and the recovery of genistein was >90%. The deviations between the calculated and experimental values were mainly caused by the factual nonconstant distribution coefficients of SIAs and the mutual solubilities of the biphasic system during the experiments.

In conclusion, an IL-based liquid–liquid extraction method was developed for the separation of genistein from its homologues. Selectivities higher than 7.0 together with appropriate distribution coefficients have been obtained in the hydrophilic IL/water–ethyl acetate biphasic system, and the feasibility of this method for a practical process has been demonstrated by continuous multistage extraction studies. Hydrophobic and hydrogen-bonding interactions are supposed to be essential factors affecting the separation efficiency. The regeneration of ILs might be achieved by adjusting the pH values of the aqueous phase in hydrophobic IL–water biphasic system or by changing the IL concentration of the extraction solvent in the hydrophilic IL/water–ethyl acetate biphasic system.

■ ASSOCIATED CONTENT

Supporting Information

Simulation of multistage extraction by "funnel method" and the operating patterns; Figure S1. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

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