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Journal of Chromatography B, 858 (2007) 220–226
JOURNAL OF CHROMATOGRAPHY B
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Direct process integration of extraction and expanded bed adsorption in the recovery of crocetin derivatives from *Fructus Gardenia*

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Received 24 May 2007; accepted 30 August 2007
Available online 6 September 2007

Abstract

A process that integrated an extraction tank (EXT) and an expanded bed adsorption (EBA) into a new system EXT-EBA for direct purifying crocetin derivatives from *Fructus Gardenia* was described. Conditions were set to allow the extraction and purification in a single step. A comparison between the integrated process and the conventional process to purify crocetin derivatives was presented. The integrated process resulted in 52.79% recovery of crocin compared to 24.12% in the conventional process. The process time and solvent used were decreased in the integrated process. The result suggests that the EXT-EBA integrates extraction, clarification, and purification in a single step, greatly simplifying the process flow and reducing the cost and time of extraction and purification of crocetin derivatives from *Fructus Gardenia*.

Keywords: Crocetin derivatives; *Fructus Gardenia*; Process integration; Expanded bed adsorption

1. Introduction

Gardenia Jasminoides (Ellis.) is widely cultivated in Asia, especially in the south of China. In traditional Chinese medicine (TCM), *Fructus Gardenia*, the dried ripe fruit of *Gardenia Jasminoides* (Ellis.), is used to cure patients with inflammation, viral encephalitis, hepatitis, tonsillitis, tracheitis and high fever. Crocetin derivatives, a group of water-soluble carotenoid esters, have been considered as being responsible for these beneficial efficacies on human health. Its effective components were considered to be crocin and 13*Z*-crocin (Fig. 1) [1], which have been shown to have antioxidant [2] and antitumor effects [3]. Crocetin derivatives mainly exit in *Crocus sativus* L. (crocin, 1.5%) [4] and *Fructus Gardenia* (crocin, 0.11%) [5]. However, *Crocus sativus* L. is expensive (about $1000/kg) and rare while *Fructus Gardenia* is cheap (about $1/kg) and quite familiar in Asia. Obviously, it is an economical way to extract crocetin derivatives from *Fructus Gardenia*.

Various conventional strategies for the purification of crocetin derivatives from *Fructus Gardenia* have been developed [6,7]. Most of them were as follows: extraction, centrifugation, filtration, concentration, alcohol sedimentation, filtration again, concentration again, then packed bed separation to get purified product. The characters of these processes were multistep, discontinuous, time and labor consuming. Therefore these conventional methods might result in a significant loss of product.

An important aspect of effective components purification from TCM was the development of simplified schemes for extraction and separation. Process integration can improve the recovery of product and lead to less cost and time [8]. Expanded bed adsorption (EBA), a unique integrated technique, combined clearing up, concentration and adsorptive purification in a single

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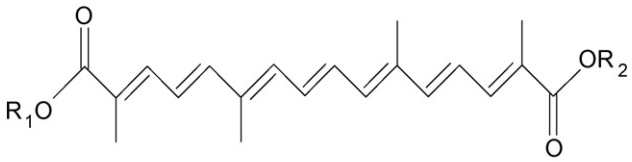
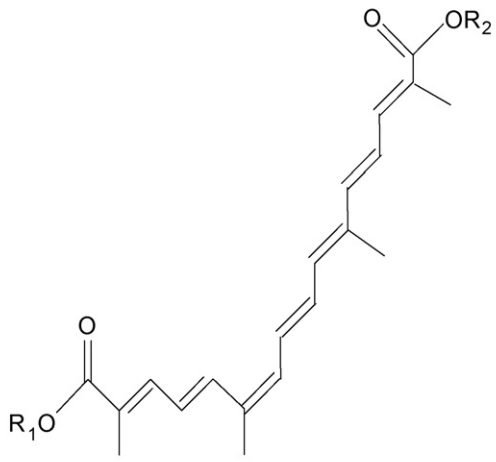
doi:10.1016/j.jchromb.2007.08.041



1 crocin



2 13*Z*-crocin
(R_1=R_2=β-D-gentiobiosyl)

Fig. 1. Structures of crocin and 13*Z*-crocin.

step that permitted crude feed stock to be loaded into the chromatographic column without any pretreatment [9]. The process of EBA possesses the dual advantages of simultaneous solid debris removal and product capture in one step, which consists of five procedures: equilibration, adsorption, wash, elution and regeneration, sequencely [10]. Compared to the conventional methods, it could dramatically increase process efficiency in terms of less process steps [11], higher productivity [12], shorter overall process time [13], decreased work cost and running cost [14]. Direct product sequestration (DPS) had the advantage of minimizing product degradation and improving recovery rate, which was realizable by the operation of product-selective adsorbents in an external loop [15]. Integrated with fluidized bed, DPS showed a promising prospect in products purification. Recently, the development of a simplified process for the simultaneous disruption and direct selective purification of intracellular proteins from unclarified yeast disruptate have been investigated [16].

To our knowledge, although the use of EBA technology in the processing of herbs and botanicals appeared [17], the integrated method of extraction and purification of active components from herbs and botanicals has been little studied. In the present study, EBA was as the capture step directly after the extraction, which was to form an EXT-EBA system. The experiments were presented with a single step process compared with the conventional multi-step process. It was characterized that the EXT-EBA system was simple in equipments and continuous in process, which would lead to a higher recovery of product and be more cost-effective.

2. Materials and methods

2.1. Reagents and materials

Fructus Gardenia (crocin, 0.21%) was purchased from a local drug store. Crocin (>99% purity) was from the National Institute of China for the Control of Pharmaceutical and Biological Products (Beijing, China). Methanol and acetonitrile (Mallinckrodt Baker company, USA) used for HPLC and LC–ESI–MS analysis were of chromatographic grade. Ultrapure water (18.2 MΩ) was prepared with a Milli-Q water purification system (Millipore, USA). Macroporous resin, HPD-100 styrene DVB, was obtained from CangZhou Bon Chemical Company. The adsorbents had a particle diameter of 300–1200 μm and an estimated wet density of 1.01 mg/mL that was measured by a pycnometer.

2.2. Standard and samples preparation

The stock solution of crocin standard was prepared to 1.0 mg/mL in 10% (v/v) aqueous methanol. Then it was diluted with the same solvent to give five different concentrations for the calibration curves. Each concentration was analyzed in triplicate.

The dried fruits of *Fructus Gardenia* were smashed to be about 3 mm in length. In the conventional multi-step process, 5 g of powdered *Fructus Gardenia* was extracted with 50 mL of pure water in a refluxing bath for 60 min, repeated three times. The extracted solutions were combined and centrifugated at 6000 rpm for 10 min using a centrifuge (Mikro 22R, Hettich Zentrifugen, Germany). Then the extract was filtrated with a filtering flask. After that, the supernatant extract was concentrated to one-fifth of its original volume in a rotary evaporator (Rotavapor R-200, BÜCHI, Switzerland) at 55 °C. And then 80 mL of 95% ethanol was added for sedimentation to remove impurities that were insoluble in ethanol. The solution was kept at 4 °C for 24 h. After sedimentation, the solution was filtrated to remove the sediment and was concentrated to 20 mL for packed bed separation. In the integrated process, 5 g of powdered *Fructus Gardenia* was put into an extraction tank, and then the extract was directly loaded into the EBA column.

2.3. Packed bed chromatography

Packed bed chromatography was performed at ambient temperature. HPD-100 adsorbents, equilibrated with water, were packed as 100 mL volumes in a 26 mm × 460 mm column. A volume of 20 mL clarified extract was loaded at a velocity of 0.6 mm/s. Column was washed with 30% ethanol until the A_{254} fell to the base line values. Crocetin derivatives were eluted with 5 BVs (bed volumes) of 95% ethanol. The elution fractions were collected with an auto-collector (Fraction collector B-684, BÜCHI, Switzerland).

2.4. Expanded bed adsorption

A glass column (26 mm × 460 mm) was used for all expanded bed experiments, which had a top adaptor and a bottom flow dis-

tributor. The bottom inlet was packed with glass beads (5 mm, diameter) to distribute the inlet flow. The top adaptor was separated from the adsorbents by a nylon mesh (500 μm). During the experiments, the top adapter in the column was lowered to be just above the surface of the expanded adsorbents to minimize the dead volume of the system. EBA experiment was also conducted at ambient temperature. A volume of 100 mL HPD-100 resin, the same adsorbents with the packed bed experiment, was packed in the expanded bed to give a settled bed height of 20 cm. Following conditioning, the bed was equilibrated with water at a velocity of 0.6 mm/s to give a stable height, where $H/H_0 = 2.1$. Adsorption of crocetin derivatives was performed by recirculating the extract from the extraction tank to the expanded bed. After adsorption, the bed was washed with water upwardly and then eluted with a packed bed mode using 30% ethanol until the A_{254} fell to the base line values.

2.5. Bed expansion

Test of bed expansion was carried out with three different concentrations of ethanol (e.g., 0%, 24% and 44%, v/v) with various viscosities to simulate viscous feed stocks. Different concentrations of ethanol were introduced upwardly into the column, and the flow rate was adjusted from 0 to 22 mL/min. A 30-min interval was set for the stable bed height at different flow rates before recording.

2.6. Integration of expanded bed with the extraction unit

For the continuous process, an expanded bed was integrated with an exaction tank for the recovery of crocetin derivatives reaching the maximum. The laboratory-scale EXT-EBA system of expanded bed and extraction unit is illustrated in Fig. 2, which consists of two three-port valves, a four-port valve, a peristaltic pump (BT00-600M, Longer Pump Company, China), an extraction tank, a cooler (home-made), a heater (home-made), an auto-collector and an EBA column (home-made, 25 mm i.d.). Before extraction, adsorbents were equilibrated with an upward flow of water. Integrated expanded bed adsorption of crocetin derivatives was achieved by recirculating whole extraction from extraction tank through expanded bed column with a flow rate of 0.6 mm/s.

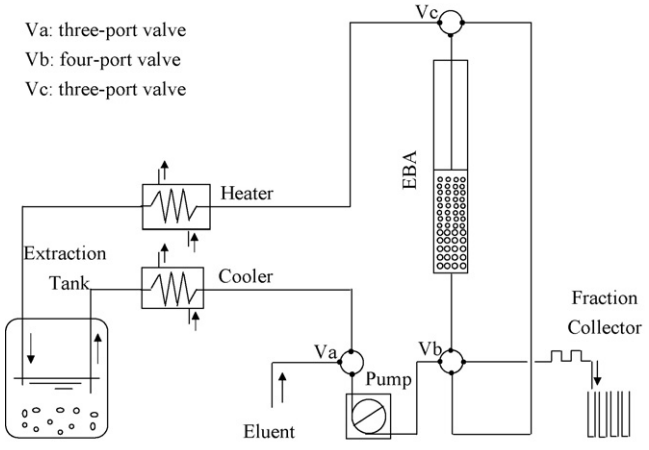


Fig. 2. Schematic diagram of EXT-EBA system.

2.7. HPLC and LC–ESI–MS analysis

The purified crocetin derivatives was analyzed by HPLC on a Waters 2695 high performance liquid chromatography (Waters, USA) equipped with 2996 photodiode array detection set at 440 nm. Separation was carried out using a phenomenex luna C_{18} column (250 mm × 4.6 mm i.d., 5 μm, USA), and the column temperature was kept at 35 °C. Injection volume was 10 μL, and a binary mobile phase consisted of solvent A, 0.3% aqueous formic acid and solvent B, methanol–acetonitrile (9:1, v/v) at a flow rate of 0.8 mL/min. The gradient elution program was as published in the former paper [18]: 0 min, 10% B; 0–15 min, 40% B, hold for 3 min; 18–35 min, 100% B, hold for 5 min; 40–45min, 10% B (v/v). The identification of the purified crocetin derivatives was performed by LC–ESI–MS on an Agilent 1100/MSD (Agilent Corp., Santa Clara, CA, USA).

3. Results and discussion

3.1. Optimization of elution conditions

The sample for chromatography optimization was prepared as in Section 2.2. Prior to the packed bed purification, centrifugation, filtration, and concentration were chosen for extraction clarification. Optimization of the elution conditions of crocetin derivatives was conducted in a packed bed column (26 mm × 460 mm) with 100 mL of HPD-100 resin. A volume of 20 mL *Fructus Gardenia* extraction was loaded into the column. Table 1 shows the effect of varying the ethanol concentration on the percentage of crocin eluted. The percentage of crocin eluted was taken as a relative measure of the total amount of crocin, defined as 100%. During the elution optimization, the concentration of ethanol was varied between 0 and 95%. As a result, an elution ethanol with 95% concentration provided the highest recovery.

3.2. Packed bed purification of crocetin derivatives

Packed bed purification of crocetin derivatives was performed on a column (26 mm × 460 mm) containing 100 mL of HPD-

Table 1
Optimization of elution condition of crocetin derivatives[a]

Concentration of ethanol in elution (%)	Crocin eluted (%)
0	0
15	0
30	4.41
65	93.43
95	100

[a] Total amount of crocin applied to column is defined as 100%.

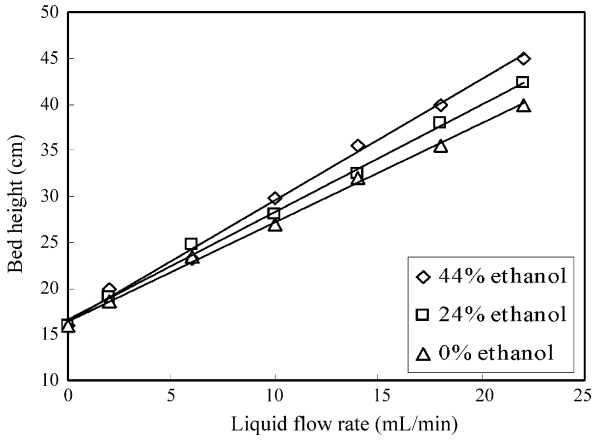


Fig. 3. Bed expansion characteristics for the HPD-100 in 26 mm × 460 mm column at 20 °C. H_0 = 16 cm.

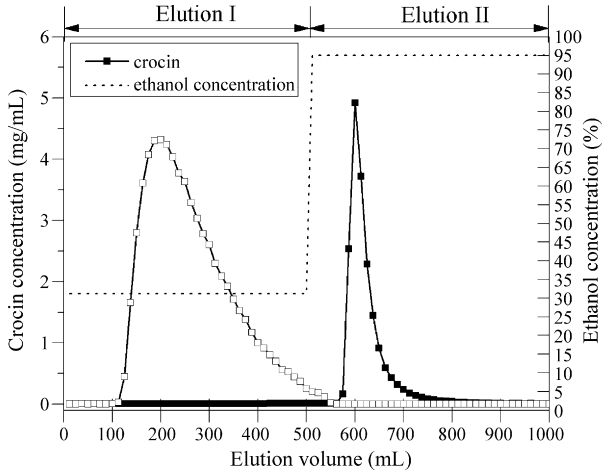


Fig. 4. Elution profile of crocin (solid line) and elution steps (dashed line).

100 adsorbents, which corresponded to a sedimented bed height of 20 cm. Five grams of *Fructus Gardenia* was prepared as in Section 2.2. The chromatographic program used was as follows: 30% ethanol, 5 BVs; and 95% ethanol, 5 BVs. The flow rate was 3 mL/min. These conditions were applied to the elution step in integrated process.

3.3. Bed expansion characteristics

Before processing feed stock with expanded bed, determination of the bed expansion characteristics was necessary, i.e., the influence of liquid physical properties on the expansion. The bed expansion characteristics for the HPD-100 resin are shown in Fig. 3. The ratio of height of the expanded bed to the settled bed height H/H_0 increases linearly at the same flow rate with increasing ethanol viscosity.

It was known that the Richardson–Zaki equation (Eq. (1)) [19] conducted an extensive investigation of the expansion behavior of particles at different velocity.

$$u = u_t \varepsilon^n \tag{1}$$

The average bed voidage can be estimated from the following equation:

$$\varepsilon = 1 - (1 - \varepsilon_0)\frac{H_0}{H} \tag{2}$$

where ε_0 is the packed-bed voidage. Its value was determined to be 0.40 for HPD-100, which was measured in a 25-mL measuring cylinder. Values of the Richarson–Zaki coefficients n, and the apparent terminal velocities of the particles u_t were calculated from linear regression of plots of $\ln(u)$ versus $\ln(\varepsilon)$, and the results are listed in Table 2. For the adsorbents had a mean diameter of 750 μm, the apparent terminal velocities of the particles u_t should be calculated from Allen's equation (Eq. (3)) [20] and values are shown in Table 2.

$$u_t = 0.27\sqrt{\frac{d(\rho_s - \rho)g}{\rho}Ret^{0.4}} \tag{3}$$

It was found that the theoretical terminal velocity u_t was different with the value of that from bed expansion experiment. This is probably because of the theoretical terminal velocity u_t from the Allen's equation with a mean particle diameter, while there was a large size dispersion of the adsorbents in the experiments.

3.4. Direct product sequestration of crocetin derivatives

Based on the optimized conditions above, the purification of crocetin derivatives was carried out by the integrated process. Five grams of powdered *Fructus Gardenia* was decocted in the extraction tank with 50 mL of pure water. Then the unclarified extract was pumped to the expanded bed at a flow rate of 18 mL/min. After loading of the crude extract and washing with pure water, the bed was settled down and eluted with a flow rate of 3 mL/min. Elution (elution I) with 30% of ethanol was used to remove the undesired components. Another elution (elution II) was performed with a step gradient up to 95% ethanol to purify crocetin derivatives. A typical elution profile (Fig. 4) of crocin detected at 440 nm showed a peak during elution II. Most of bound crocin was eluted after the concentration of ethanol reached 95%.

Table 2
Richardson–Zaki equation parameters in different fluidizing solution

Fluidizing solution	H_0 (cm)	u_t (Exp.) (mm/s)	u_t (Cal.) (mm/s)	Richardson–Zaki factor (n)
Water	16.0	2.93	5.19	5.22
24.0% ethanol	16.0	2.83	6.20	5.53
44.0% ethanol	16.0	2.79	10.75	5.77

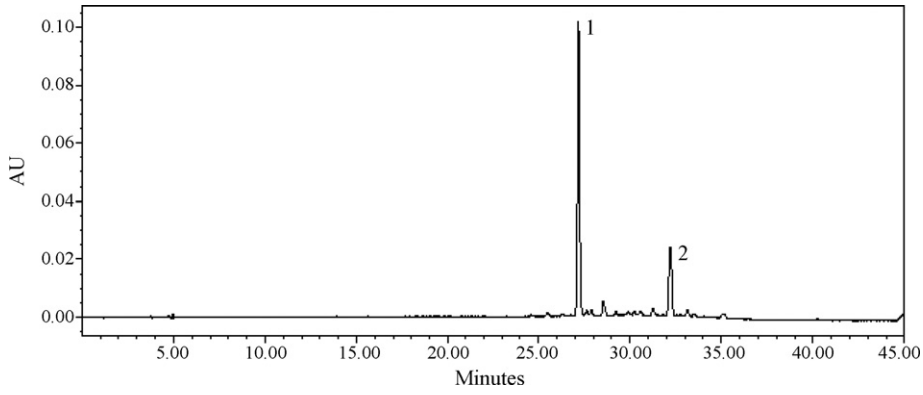


Fig. 5. The chromatogram of crocetin derivatives extract. The peaks in chromatogram was identified by LC–MS–MS as following: (1) crocin; (2) 13*Z*-crocin.

Table 3
Quantitative determination results of crocin in the two different processes

	Content of crocin in *Fructus Gardenia* (%)	Content of crocin in residue (%)	Content of crocin in purified extract (%)
Integrated process	0.21	0.016	2.93
Conventional process	0.21	0.016	1.39

3.5. Quantitative determination and identification of purified crocetin derivatives

A good chromatographic separation of sample (Fig. 5.) from the peak in elution II (Fig. 4.) shows that crocetin derivatives extract from *Fructus Gardenia* was achieved by EXT-EBA system. The chromatogram shows the presence of two peaks. Table 3 shows the quantitative determination results of crocin in the two different separation processes. The contents of crocin in residue in the two processes were at the same level (0.016%), which showed the 50 min continuous extraction had the same extracting effect with the conventional process. And the crocin contend in the extract of the integrated process was over 2-folder higher than the conventional process.

The standard compound crocin was analyzed in loop injection in order to optimize the MS conditions. It was shown that the negative ion mode was more sensitive than the positive ion mode. MS detection was then operated in negative ion mode in the scan range from *m/z* 200 to 1200. The LC–ESI–MS of peak 1 and peak 2 (Fig. 6.) in the negative mode gave the same *m/z* as that for crocin and 13*Z*-crocin [1].

3.6. Comparison with the conventional process

In this study, purification efficiency was compared between a conventional process and an integrated process. Fig. 7 shows the flow sheets of two different processes. In the conventional process, the first seven steps did not contribute any purifica-

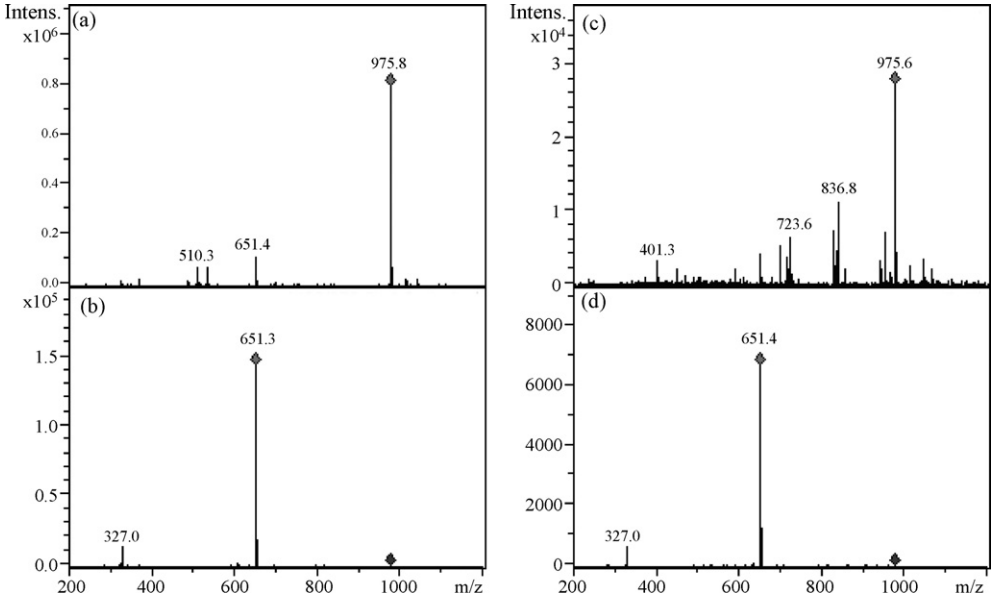


Fig. 6. The MS and MS/MS spectra of crocin and 13*Z*-crocin: (a) MS spectrum of *m/z* 976 of crocin; (b) MS/MS spectrum of *m/z* 976 of crocin; (c) MS spectrum of *m/z* 976 of 13*Z*-crocin; (d) MS/MS spectrum of *m/z* 976 of 13*Z*-crocin.

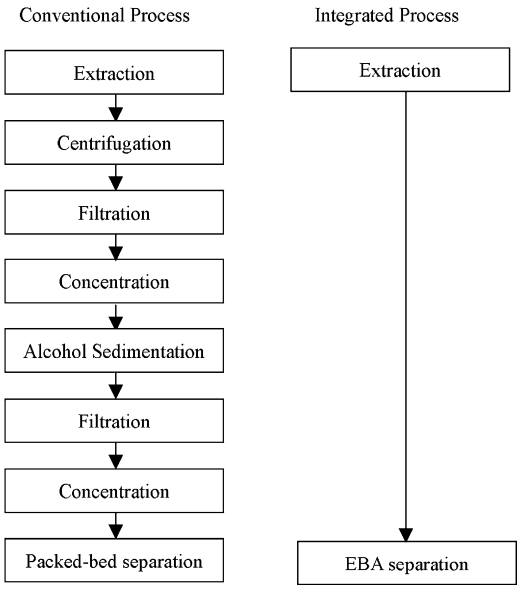


Fig. 7. Comparison of flow sheets in two different processes.

tion of crocetin derivatives but supply the sample for the packed bed separation. However, the integrated process replaced the seven steps, followed by one integrated extraction and chromatographic purification step, which totally simplified the process.

Purification results of crocetin derivatives by integrated process used in this research and the conventional method were listed (Table 4). The same amount of *Fructus Gardenia* (5.0 g) and the same volume of absorbents (100 mL) were used. 5.51 mg crocin was obtained in the integrated process, while 2.52 mg in the multi-step process. According to Table 4, the recovery rate of crocin was 2.2-fold higher in DPS process than in the conventional process (52.79% and 24.12%, respectively); the lower recovery rate obtained in the conventional method seems to be caused by multi-step process.

The purification time of DPS was decreased from 32 to 4 h, which was only 12.5% of that in the conventional one. Moreover, the contents of crocin in gardenia residue in the two processes were at the same level (Table 4), which showed that the 50 min dynamic extraction had the same extracting effect with the conventional extraction.

Furthermore, the volume of water and ethanol used in DPS was less than the conventional process (Table 4), which indicated a great cost-effect in a large-scale production.

Up to now, few studies of the extraction and purification of effective components from herbs and botanicals by using EXT-EBA system have been reported. Although it has to be noticed that the crocetin derivatives purified by EXT-EBA was a mixture of crocin and 13*Z*-crocin, the results in this paper supply a promising way for its extraction and purification.

Table 4
Purification result of crocin by using integrated process and conventional process

Parameters	Integrated process	Conventional process
Numbers of steps	1	8
Recovery of crocin (%)	52.79	24.12
Process time (h)	4.0	32.0
Volume of water used (mL)	500.0	800.0
Volume of ethanol (95%) used (mL)	400.0	600.0

Table 5
Purification result of salvianolic acid B by using integrated process and conventional process

Parameters	Integrated process	Conventional process
Sample	100.0 g *S. miltiorrhiza*	100.0 g *S. miltiorrhiza*
Numbers of steps	1	8
Recovery of salvianolic acid B (%)	81.43	71.25
Process time (h)	6.0	32.0
Volume of water used (mL)	1600.0	3600.0
Volume of ethanol (95%) used (mL)	870.0	270.0

3.7. Adaptation of the EXT-EBA system to the other herbal medicine

When an efficient method for the purification of a herbal medicine was developed, it would be appreciated to be as efficient for other herbs. We have challenged the versatility of EXT-EBA system with the purification of salvianolic acid B from the roots of *S. miltiorrhiza*. Table 5 shows the purification results of salvianolic acid B by using the integrated and conventional methods. According to Table 5, the recovery rate of salvianolic acid B was 10% higher in integrated process than in the conventional process. The purification time of DPS was only 18.75% of that in the conventional one. The integrated process was adapted to allow the swift purification of various herbal medicines.

4. Conclusion

The EXT-EBA system integrated extraction and purification process is successfully used to extract and purify crocetin derivatives from *Fructus Gardenia* in terms of higher recovery, less process time and less solvent used, compared with the conventional process. This system is also adaptable to the purification of salvianolic acid B from the roots of *S. miltiorrhiza*. The results show the EXT-EBA is a better system than others for recovery of effective components from herbs and it is more economically viable than the other purification methods.

Nomenclature

A_{254}	UV absorptance at 254 nm
d	mean adsorbent bead diameter (m)
g	gravitational acceleration (m/s^2)
H	bed height (cm)
H_0	packed-bed height (cm)
n	Richardson–Zaki coefficient, dimensionless
Re_t	Reynolds number, dimensionless

u liquid superficial velocity (m/s)
u_t particle terminal velocity (m/s)

Greek letters
ε bed voidage, dimensionless
ε_0 packed-bed voidage, dimensionless
ρ density of liquid (kg/m^3)
ρ_s density of solid (kg/m^3)

Acknowledgements

This research was financially supported by the 11th Five-Year Key Programs for the Science and Technology Ministry of China (No. 2006BAI08B04-01), the National Natural Science Foundation of China National Science Foundations of China (No. 20475031) and Tsinghua University Yuyuan Foundation (No. 20240000529), National Basic Research Program of China (973 program, No. 2007CB714505) and Key Project of the Department of Science and Technology of Shanghai (No. 05DZ19709).

References

[1] A. Zalacain, S.A. Orrdoudi, E.M. Díaz-Plaza, M. Carmona, I. Blázquez, M.Z. Tsimidou, G.L. Alonso, J. Agric. Food Chem. 53 (2005) 9337.
[2] T. Ochiai, S. Ohno, S. Soeda, H. Tanaka, Y. Shoyama, H. Shimeno, Neurosci. Lett. 362 (2004) 61.
[3] J. Escribano, G.-L. Alonso, M. Coca-Prados, J.-A. Fernández, Cancer Lett. 100 (1996) 23.
[4] Y. Deng, Z.G. Guo, Z.L. Zeng, Z. Wang, China J. Chin. Mater. Med. 27 (2002) 565.
[5] C.F. Wang, J. Lu, Chin. J. Pharm. Anal. 17 (1997) 321.
[6] S. Pfister, P. Meyer, A. Steck, H. Pfander, J. Agric. Food Chem. 44 (1996) 2612.
[7] T.Q. Pham, F. Cormier, E. Farnworth, V.H. Tong, M.R. Van Calsteren, J. Agric. Food Chem. 48 (2000) 1455.
[8] K. Schgerl, Biotechnol. Adv. 18 (2000) 581.
[9] H.A. Chase, Trends Biotechnol. 12 (1994) 296.
[10] G.E. McCreath, H.A. Chase, C. Lowe, J. Chromatogr. 659 (1994) 275.
[11] Y. González, N. Ibarra, H. Gómez, M. González, L. Dorta, S. Padilla, R. Valdés, J. Chromatogr. 784 (2003) 183.
[12] C. Cabanne, A.M. Noubhani, A. Hocquellet, F. Dole, W. Dieryck, X. Santarelli, J. Chromatogr. 818 (2005) 23.
[13] F. de Lamotte, J. Chromatogr. 818 (2005) 29.
[14] E. Bodo, A. Durieux, C. Saint-Hubert, R. Lavallée, J.-M. Boufflette, J.-P. Simon, Biotechnol. Lett. 28 (2006) 1033.
[15] G.E. Hamilton, F. Luechau, S.C. Burton, A. Lyddiatt, J. Biotechnol. 79 (2000) 103.
[16] T.C. Liang, A. Lyddiatt, J. Biotechnol. 119 (2005) 436.
[17] W.D. Chen, Y. Wang, L. Zha, G. Ma, Z. Su, Biotechnol. Lett. 26 (2004) 1233.
[18] Y. Xu, J. Cao, Y.M. Wang, G.A. Luo, Acta Pharmacol. Sin. 38 (2003) 543.
[19] J.F. Richardson, W.N. Zaki, Trans. Inst. Chem. Eng. 32 (1954) 35.
[20] C. Chai, G. Zhang, Fluidization of Chemical Fluent and Heat Transfer, Chemical Industry Press, Beijing, 2000, p. 212.