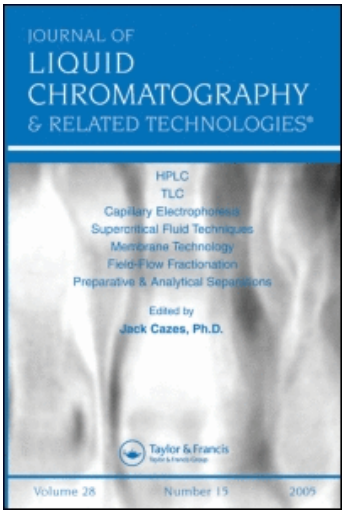

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Preparative Isolation of Catechins from Green Tea Powders Using Intermediate Polar Adsorbent

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Abstract: An adsorption separation method using intermediate polar adsorbent (Amberlite XAD-7HP resin) was applied for isolating a mixture of tea catechins from the green tea powders, where the percentages of content (purities) of the total catechins, comprising the four major catechins: epigallocatechin (EGC), epicatechin (EC), epigallocatechin gallate (EGCG), and epicatechin gallate (ECG), ranged from 13.0 to 16.0 wt%. First, the batch adsorption experiments were performed. The adsorption equilibrium data of the total catechins were fitted closely by a Langmuir isotherm model. Then, the dynamic adsorption and desorption experiments were performed using the column packed with the XAD-7HP resin. In the adsorption step, to avoid the loss of tea catechins, the loading volume was selected according to the elution volume at the break point of the dynamic adsorption curve. In the elution step, under a proper step gradient elution program, the majority of tea catechins were efficiently eluted by the eluent ranged from 20 to 50% ethanol and the fractions were collected as the concentrated product. The purities of the total catechins and EGCG, being the most important catechin, in the concentrated product were ~49 wt% and ~28 wt%, respectively. The contents of the total catechins and EGCG were efficiently concentrated ~3.5 times in high recovery yields (> 75%) using the current column chromatographic method.

Keywords: Catechins, Column chromatography, Green tea, Isolation, Preparative scale

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INTRODUCTION

Green tea is recognized for its high content of tea polyphenols, in particular tea catechins. Recent studies have demonstrated inhibitory effects of green tea against tumor formation and growth.[1,2] This inhibitory activity is believed to be mainly due to the antioxidative and possible antiproliferative effects of polyphenols, mainly catechins, in green tea. The four major catechins of green tea are epigallocatechin (EGC), epicatechin (EC), epigallocatechin gallate (EGCG), and epicatechin gallate (ECG), of which EGCG is the major constituent and possibly the most powerful component.[3]

Current procedures for the isolation of a mixture of tea catechins mainly include liquid-liquid extraction and adsorption separation. In the method of liquid-liquid extraction, the organic solvents used include ethyl acetate, propyl acetate, chloroform, dichloromethane, and ethyl hexanoate.[4–7] However, a few limitations and problems for this method, e.g., large quantities of variable organic solvents (some of them are toxic) are consumed, phase separation is required, and a trace of undesirable solvents, which may be retained in the final products, have been observed. Compared to the above method, the adsorption separation method may be advantageous because of its simple process and low cost. The adsorbents adopted or mentioned in the literature include C-18 bonded silica, acrylic ester such as Amberlite XAD-7HP and polyamide resins, polystyrene-divinylbenzene such as Amberlite XAD-4 and XAD-16HP resins, and polymethacrylate such as TossHaas CG-71 resin.[6–10] In spite of the numerous patents and publications concerning isolation and purification for tea catechins by adsorption separation processes, there still exists a need for processes which are efficient, relatively inexpensive, and easy to scale up into industrialized usage.

Referring to the functional structures as shown in Figure 1, the four major catechins belong to the family of flavan-3-ols.[11] In addition, EGC and EGCG contain an additional phenolic hydroxyl group when compared to EC and ECG, respectively, and EGCG and ECG are gallic acid esters of EGC and EC, respectively. Since the four major catechins possess a wide range of polarity varying from polar to non-polar, intermediate polar adsorbent such as Amberlite XAD-7HP resin is believed to have good adsorption capacities for all of them.

This study is aimed at developing an efficient, economic, and easy to scale up adsorption separation method for the preparative isolation of a mixture of tea catechins from the green tea powders in high purity and high yields. Amberlite XAD-7HP resin was employed to the adsorption separation process with the consideration of their high performance and durability in several similar applications reported by the manufacturer.[12] First, the batch adsorption experiments were performed to find

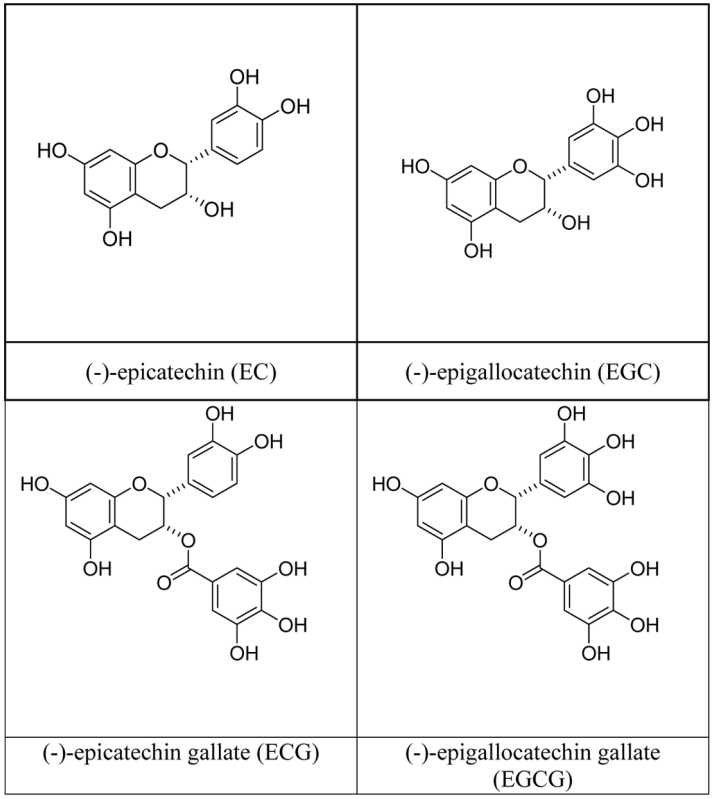

Figure 1. Chemical structures of the four major catechins.

the adsorption equilibrium capacities of the four major catechins. Then, the dynamic adsorption and desorption experiments were performed. In the adsorption step, the dynamic adsorption curve (breakthrough curve) of tea catechins was established to evaluate the suitable loading volume. In the elution step, the dynamic elution curve of tea catechins under a selected step gradient elution program was established and the column performance (in terms of purity, concentration ratio, and recovery yield in the concentrated product) was determined accordingly.

EXPERIMENTAL

Materials

The green tea powders were obtained from Challenge Bioproducts Company (Douliu, Yunlin, Taiwan). They were dry extracts produced

by hot water extraction. Catechins standards including epigallocatechin (EGC, 98% purity), epicatechin (EC, 90% purity), epigallocatechin gallate (EGCG, 95% purity), and epicatechin gallate (ECG, 98% purity) and caffeine (CA, 98.5% purity), were purchased from Sigma (St. Louis, MO, USA). De-ionized water was obtained using a Milli-Q purifier from Millipore (Billerica, MA, USA). Ethanol was bought from J. T. Baker (Phillipsburg, NJ, USA), methanol was from Mallinckrodt (St. Louis, Missouri, USA), and acetic acid was from Merck (St. Frankfurter, Darmstadt, Germany). All the organic solvents used were of HPLC grade and were ultrasonically degassed before use.

Amberlite XAD-7HP polymeric adsorbent with specific surface area of 450 m^2/g and particle diameter of 250–840 μm (20–60 mesh) was from Rohm and Haas (Philadelphia, PA, USA). Based on the "like attracts like" principle,[13] XAD-7HP adsorbent, possessing intermediate polarity due to its both aliphatic and acrylic structure,[12] is effective for adsorbing non-polar compounds from polar solvents and polar compounds from non-polar solvents.

Quantitative Analysis of the Active Ingredients in the Green Tea Powders

The high performance liquid chromatography system included a Jasco Model PU-980 solvent metering pump, a Jasco Model UV-970 UV detector (Tokyo, Japan), a Rheodyne Model 7125 6-way syringe loading valve fitted with a 20 μL sample loop (Cotati, CA, USA), and a Sunway Model 940-CO column oven (Taipei, Taiwan). The HPLC column was Synergi Fusion-RP (250 × 4.6 mm I.D., 4 μm) from Phenomenex (Torrance, CA, USA). All analyses were carried out at 30°C.

In this study, the contents of the four major catechins, i.e., EGC, EC, EGCG, and ECG, were used as the quantitative indices of tea catechins. To analyze the four major catechins and caffeine in the green tea powders, the green tea solution (dissolving tea powders in de-ionized water) was directly analyzed by reversed phase HPLC. Before injection, it was filtrated using a 0.45 μm PVDF disposable syringe filter (Millex-HV, Millipore). Mobile phases consisted of 1% acetic acid in methanol (v/v) (eluent A) and 1% acetic acid in water (eluent B). Elution was carried out at a solvent flow rate of 1.0 mL/min with the following gradient cycle: 20% A for 5 min, 20% A to 15% A over 0.1 min, 15% A for 20 min, and 15% A to 50% A over 35 min. Post run time was 10 min. The peaks were monitored with a UV detector at 280 nm.

Figure 2 shows the corresponding chromatogram of one particular example of the green tea solution where a successful separation of EGC, CA, EC, EGCG, and ECG (listed in the elution order) was obtained. They were then identified by direct comparison with reference standards

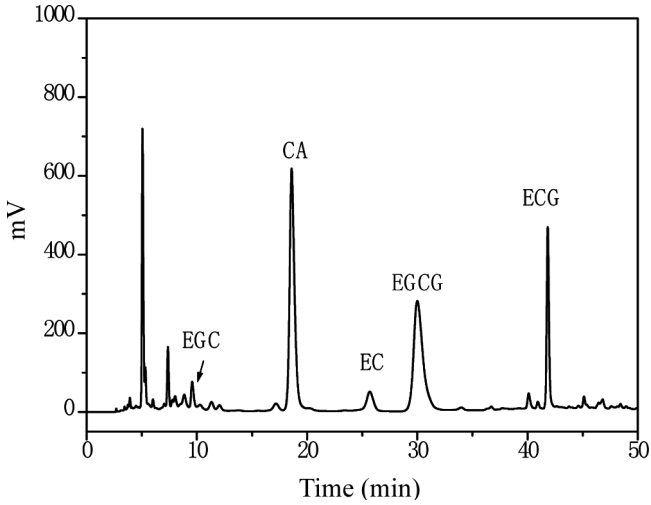

Figure 2. Typical HPLC chromatogram of the green tea solution. Column: Phenomenex Synergi Fusion-RP (250 × 4.6 mm I.D., 4 μm); injection volume: 20 μL; flow rate: 1 mL/min; monitor: UV detector at 280 nm; gradient elution cycle: as described in the text; sample concentration: 8.50 mg/mL; oven temperature: 30°C.

and quantified by using their calibration curves. Multiplying EGC, EC, EGCG, ECG, and CA concentrations by the total solution volume, the contents of EGC, EC, EGCG, ECG, and CA, and the total catechin content (an under-estimated value calculated by only the sum of the contents of EGC, EC, EGCG, and ECG) in the green tea powders were determined.

Adsorption Separation Using Amberlite XAD-7HP Resin

Batch Adsorption Experiments

A volume of 100 mL of green tea solution (dissolved in de-ionized water) with a certain concentration was placed in a 250 mL conical flask. An accurately weighed XAD-7HP resin in a range from 0.20 to 1.80 g was added to the solution. A series of such conical flasks was then shaken at a constant speed of 100 rpm in a 30°C shaking water bath for a sufficient time to reach equilibrium. Then, the XAD-7HP adsorbent was separated by filtering the liquid content through a 0.45 μm PVDF membrane filter (Durapore-HVLP, Millipore). The filtrate was analyzed for the remaining tea catechins concentrations by HPLC. Also, the filtrate was concentrated and the concentrate was weighed to find the mass of concentrate in the filtrate.

The adsorption capacity at equilibrium (mg/g resin), q_e, for each adsorbed species was determined from the difference between the concentration initially added and that which remained after batch adsorption and can be calculated by:

$$q_e = V(C_0 - C_e)/W \quad (1)$$

where C_0 and C_e are the initial and equilibrium concentrations (mg/mL) of each adsorbed species, respectively, and V is the volume (mL) of the solution and W is the weight (g) of the adsorbent.

Dynamic Adsorption and Desorption Experiments

The dynamic adsorption and desorption experiments were performed using a medium pressure liquid chromatograph system, which included a Lab Alliance Model Series II medium pressure pump (Lemont, PA, USA), a Jasco Model UV-970 UV detector (Tokyo, Japan), an ISCO Model 2360 gradient programmer, and a ISCO Model Retriever® 500 fraction collector (Lincoln, NE, USA). A 6.47 g sample of XAD-7HP resin was dry packed in a glass column, sizing 1.5 cm I.D. and 9.7 cm length. The bed volume (BV) of resin was 17.14 mL. The column was located in a water bath, where the temperature was controlled at 30°C.

It is noted that the triangular relationship among the adsorbate, the adsorbent, and the eluent is formed based on the "like attracts like" principle,[13] i.e., whether the adsorbate will stay in the adsorbent or in the eluent depends on the relative strength of attraction between them. Applying the principle to the current system, as the adsorbent possessing intermediate polarity, it can adsorb adsorbates including tea catechins and caffeine from aqueous solutions, and a feed solution at a low ethanol concentration (e.g., $\leq$5%) in the adsorption step is preferred; on the contrary, an eluent at a high ethanol concentration (e.g., $\geq$20%) in the desorption step is favorable.

A whole adsorption separation process, thus, included three steps in sequence: conditioning, adsorption, and elution steps. First, the column was conditioned with de-ionized water. Then, the feed solution was loaded onto the column. Finally, the packed column was eluted with a certain volume of the ethanol aqueous solution whose ethanol percentage varied step gradiently from 0 to 100%. The column was checked to be well regenerated after the step gradient elution step.

The effluent of each step in the adsorption separation process was collected for compositional analysis. Each effluent was concentrated and each concentrate was weighed. Based on the compositions of tea catechins in all effluents, the following column performance was

evaluated. First, the dynamic adsorption curve (breakthrough curve) of tea catechins was established to evaluate the suitable loading volume for the adsorption step. Then, the dynamic elution curve of tea catechins under a selected step gradient elution program was established and the column performance was determined accordingly. The column performance indices, including purity, concentration ratio, and recovery yield of tea catechins, are defined as follows:

$$\text{Purity}(\%) = \text{mass fraction of tea catechins in the concentrated effluent fraction} \tag{2a}$$

$$\text{Concentration ratio} = \text{purity of tea catechins in the concentrated effluent fraction/purity of tea catechins in the green tea solution} \tag{2b}$$

$$\text{Recovery yield} = \text{mass of tea catechins in the concentrated effluent fraction/total mass of tea catechins in the green tea solution} \tag{2c}$$

RESULTS AND DISCUSSION

Quantitative Analysis of Tea Catechins and Caffeine in the Green Tea Powders

The green tea solution was prepared by dissolving 100 mg tea powders in 10 mL de-ionized water and then filtrating it through a 0.45 μm PVDF membrane filter (Durapore-HVLP, Millipore). First, the average concentration of concentrate in five batches of the above green tea solution was calibrated to be 8.50 mg/mL. Then, the contents of the four major catechins and caffeine in each green tea solution were analyzed by HPLC. Averaging the HPLC results of five batches, the percentages of content (purities) of total catechins and caffeine ranged from 13.0 to 16.0 wt% and 4.5 to 5.5 wt%, respectively. The total catechins are composed of the four major catechins with the order of purity being EGCG (ranged from 7.5 to 8.5 wt%) > EGC (ranged from 3.5 to 4.5 wt%), > ECG (ranged from 2.0 to 2.5 wt%), > EC (ranged from 1.5 to 2.0 wt%).

In order to use the green tea solution in the following preparative procedure, it is of interest to know the solubility limit of the green tea powders in water. The solubility limit was characterized by the loss of tea catechins in the concentrated solution as shown in Figure 3, where

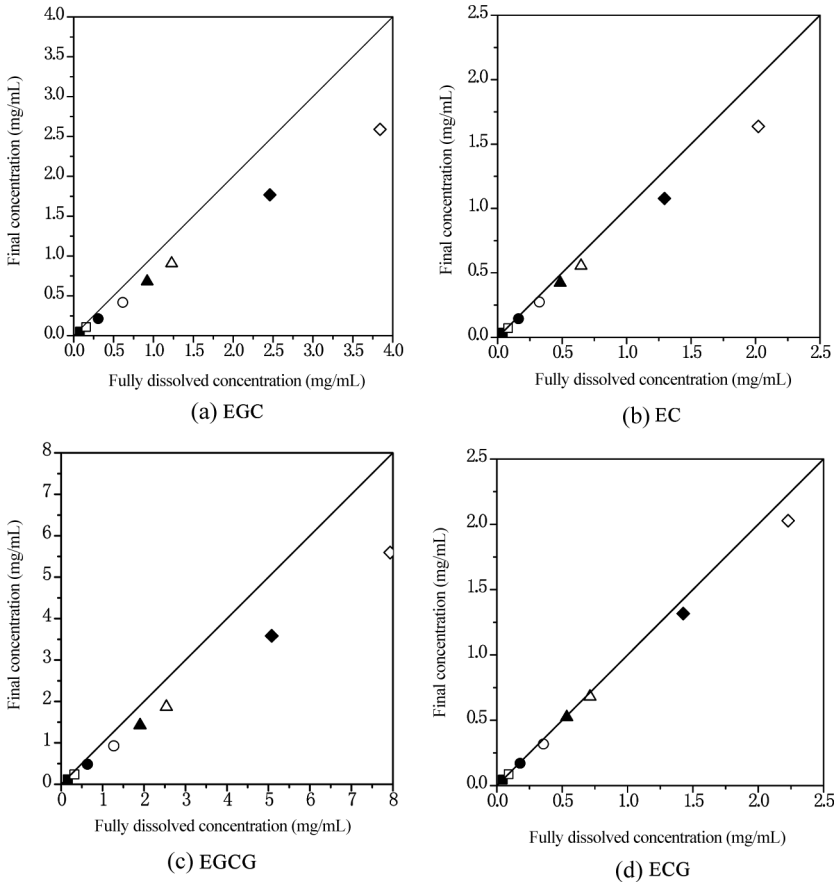

Figure 3. Solubility limit of the green tea powders in water characterized by the loss of tea catechins in the concentrated solution. Type of tea catechins: (a) EGC, (b) EC, (c) EGCG, (d) ECG; prepared concentration of the green tea solution: ■ = 2.02, □ = 4.06, ● = 8.01, ○ = 16.00, ▲ = 24.02, △ = 32.06, ■ = 64.01, □ = 100.02 mg/mL.

the final concentration versus the fully dissolved concentration of each of the four major catechins was plotted. As the prepared concentration of the green tea solution increased, the data deviated more significantly from the diagonal line, which represents the loss of content due to insolubility was increased at higher concentrations. It was then concluded that, in order to reduce the loss of tea catechins, the prepared concentration of the green tea solution should be controlled to be less than 20 mg/mL.

Batch Adsorption Results: Adsorption Equilibrium Isotherms

A series of conical flasks was prepared by adding adsorbent weights of 0.25, 0.56, 0.90, 1.50, and 1.67 g individually to a volume of 100 mL green tea solution with a concentration of 8.50 mg/mL. The flasks were shaken in a 30°C water bath for 4 h. It was proven by the kinetic study that 4 h was a sufficient time for this system to reach equilibrium (data not shown). The equilibrium concentrations, C_e, were analyzed by HPLC and the adsorption capacities at equilibrium, q_e, were calculated by Eq. (1) for the four major and total catechins.

The adsorption isotherms of the four major and total catechins plotted as q_e vs. C_e are presented in Figure 4. It can be seen that the adsorption capacities at equilibrium (q_e) of the four major catechins were in the order: ECG ~EGCG > EC > EGC and, due to the low adsorption capacities of EC and EGC, the isotherms of EC and EGC reached saturation even at low concentrations. It is noted that, based on the "like attracts like" principle,[13] the order of adsorption equilibrium capacity reflects the order of interaction strength and the order of similarity of the polarities between the adsorbent and the adsorbate (tea catechins). Since XAD-7HP adsorbent possesses intermediate polarity and also referring to the elution order of the four major catechins in the reversed phase HPLC column as shown in Figure 2, it is concluded that the polarities of the four major catechins are in the order: EGC (very polar) > EC (polar) > EGCG (intermediate polar) > ECG (non-polar). The polarity order also proved that the gallo catechins (EGC and EGCG) are more polar than the non-gallo catechins (EC and ECG) and the non-gallate

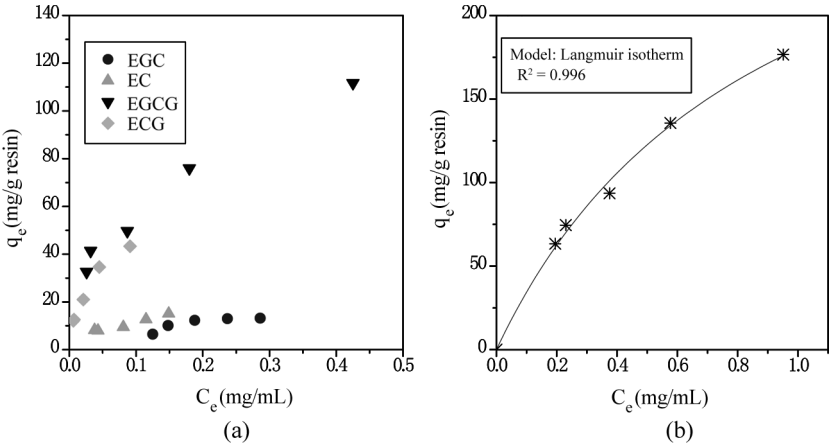

Figure 4. Adsorption equilibrium isotherms of the four major and total catechins on the XAD-7 HP resin: (a) the four major catechins, (b) the total catechins.

catechins (EGC and EC) are more polar than the gallate catechins (EGCG and ECG).

In order to predict the idea loading amount in the following column operation, an isotherm model was used to describe the adsorption equilibrium behavior of the total catechins. The Langmuir isotherm model[14] is shown as follows:

$$q_e = \frac{bq_m C_e}{1 + bC_e} \tag{3}$$

where the two parameters were fitted as $b = 1.127\,\text{mL/mg}$ and $q_m = 340.15\,\text{mg/g}$ resin. The Langmuir isotherm model was found to fit closely with the experimental data of the total catechins, as seen from Figure 4(b), and the regression coefficient (R^2) was calculated to be 0.996.

Dynamic Adsorption Performance

A dynamic adsorption experiment was carried out at a constant flow rate of 2 mL/min using the green tea solution as the feed solution. The concentrations of the concentrate and the total catechins in the green tea solution were 7.50 and 1.021 mg/mL, respectively. No breakthrough for tea catechins at the first 300 mL loading volume was expected (verified later), so the effluent fractions of the adsorption step were collected in the first 300 mL as a whole and then in several intervals after 300 mL, e.g., 100 mL for each interval. The concentrations of the four major catechins in the feed (C_{in}) and all the collected effluents (C_{out}) were analyzed by HPLC. The cumulative loss of each of the four major catechins at a certain cumulative effluent volume $\left(\sum V_{eff}\right)$ was calculated as follows:

$$\text{Cumulative loss}(\%) = \sum V_{eff} C_{out} \Big/ \sum V_{eff} C_{in} \times 100\% \tag{4}$$

The compositional analysis results and the calculated cumulative losses of the four major and total catechins are listed in Table 1 and Table 2, respectively, and their dynamic adsorption curves are plotted in Figure 5. It can be seen that, due to the order of interaction strength of the four major catechins on the XAD-7HP resin being ECG > EGCG > EC > EGC as described in the section above, the cumulative losses of the four major catechins were in the order of EGC > EC > EGCG > ECG.

To avoid the loss of tea catechins from the adsorption step, the breakthrough was set at the point when the cumulative loss of total catechins approached 10%. The loading volume of the adsorption process

Table 1. Compositional analysis results of the four major and total catechins for the dynamic adsorption experiment using the XAD-7HP resin. Column size: 97×15 mm I.D., 250–840 μm; temperature: 30 °C; flow rate: 2 mL/min.

Type of tea catechins	Concentration of the feed, C_{in} (mg/mL)	Concentration of the effluents, C_{out} (mg/mL)					
		0–300 mL	300–400 mL	400–500 mL	500–600 mL	600–700 mL	700–800 mL
EGC	0.218	0.022	0.075	0.107	0.146	0.181	0.219
EC	0.137	0.008	0.027	0.040	0.055	0.069	0.083
EGCG	0.520	0.018	0.057	0.084	0.112	0.134	0.157
ECG	0.146	0.002	0.005	0.009	0.013	0.018	0.023
Total	1.021	0.050	0.164	0.240	0.326	0.402	0.482

was then selected according to the elution volume at the break point. Based on the dynamic adsorption behaviors of Figure 5, the loading volume is estimated to be 450 mL when the column is operated at the feed concentration of total catechins of 1.021 mg/mL and the flow rate of 2 mL/min. However, the loading volume has to be carefully adjusted when the flow rate or the feed concentration changes.

It is worth noting that the mass transfer zones (from the break point to the equilibrium point) of the four major and total catechins were very wide, and the shape of dynamic adsorption curves became an elongated S form. According to the theory of fixed bed adsorption,[15] the length of unused bed (LUB) is defined by:

$$\mathrm{LUB} = \left(\frac{1-\bar{q}'}{q_e}\right) L \tag{5}$$

where L is the column packed length, $\bar{q}'$ is the adsorption capacity at the break point and q_e is the adsorption capacity at equilibrium. For the case of the above column operation, first, substituting the concentration of total catechins in the feed ($C_{in} = 1.021$ mg/mL) into Eq. (3), q_e is estimated to be 182.0 mg/g resin; second, with the break volume of 450 mL and the adsorbent weight of 6.47 g, $\bar{q}'$is estimated to be 71.0 mg/g resin; last, using Eq. (5), LUB is estimated to be 61.0% of L. It is believed that the high percentage (~60%) of unused bed length was formed due to the significant mass transfer resistances from the XAD-7HP resin (particle diameter of 250–840 μm). To achieve a narrower mass transfer zone, a smaller particle size should be used. Therefore, the shape of the dynamic adsorption curves can be steeper, and hence a larger loading amount and a lower LUB can be allowed. However, a higher pressure drop will be needed for a column packed with particles of smaller sizes.

Table 2. Calculated cumulative losses of the four major and total catechins for the dynamic adsorption experiment using the XAD-7HP resin. Column size: 97 × 15 mm I.D., 250–840 μm; temperature: 30 °C; flow rate: 2 mL/min.

	Cumulative mass of the feed, $\sum V_{eff}C_{in}$ (mg)						Cumulative mass of the effluents, $\sum V_{eff}C_{out}$ (mg)						Cumulative loss (%)[a]					
Type of tea catechins	0–300 mL	0–400 mL	0–500 mL	0–600 mL	0–700 mL	0–800 mL	0–300 mL	0–400 mL	0–500 mL	0–600 mL	0–700 mL	0–800 mL	0–300 mL	0–400 mL	0–500 mL	0–600 mL	0–700 mL	0–800 mL
EGC	65.4	87.2	109.0	130.8	152.6	174.4	6.6	14.1	24.8	39.4	57.5	79.4	10.1	16.2	22.8	30.1	37.7	45.5
EC	41.1	54.8	68.5	82.2	95.9	109.6	2.4	5.1	9.1	14.6	21.5	29.8	5.8	9.3	13.3	17.8	22.4	27.2
EGCG	156.0	208.0	260.0	312.0	364.0	416.0	5.4	11.1	19.5	30.7	44.1	59.8	3.5	5.3	7.5	9.8	12.1	14.4
ECG	43.8	58.4	73.0	87.6	102.2	116.8	0.6	1.1	2.0	3.3	5.1	7.4	1.4	1.9	2.7	3.8	5.0	6.3
Total	306.3	408.4	510.5	612.6	714.7	816.8	15.0	31.4	55.4	88.0	128.2	176.4	4.9	7.7	10.9	14.4	17.9	21.6

[a]Cumulative loss (%) $= \sum V_{eff}C_{out} / \sum V_{eff}C_{in} \times 100\%$.

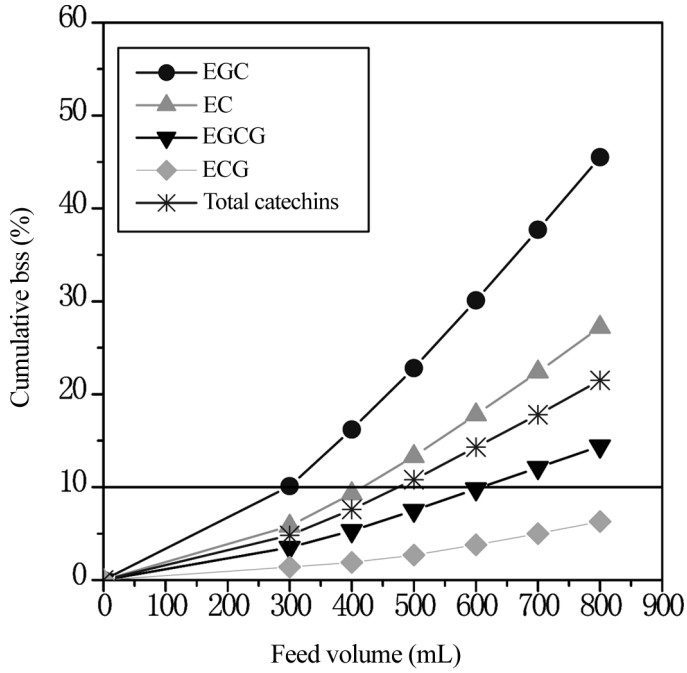

Figure 5. Dynamic adsorption curves of the four major and total catechins using the XAD-7HP column. Column size: 97 × 15 mm I.D., 250–840 μm; temperature: 30°C; flow rate: 2 mL/min; feed concentration of tea catechins, C_{in}: 1.021 mg/mL.

Dynamic Elution Performance

A dynamic elution experiment was then carried out following the adsorption step of 450 mL feed solution with the concentration of total catechins of 1.034 mg/mL. Under a selected step gradient elution program, the column was eluted with a certain volume of the ethanol aqueous solution whose ethanol percentage varied step gradiently from 0 to 100%. The flow rate of 2.0 mL/min was chosen in each of the elution steps. The effluent fractions from the adsorption, and each of the elution steps were collected for compositional analysis.

To ensure a sufficient elution capability, the elution volumes used at various gradient steps were chosen as follows: 100, 120, 120, 140, 140, 100, 80, 70, 70, 60, and 60 mL for the step of 0, 10, 20, 30, 40, 50, 60, 70, 80, 90, and 100% ethanol percentage, respectively. Using the above step gradient elution program, the dynamic elution curves of the four major and total catechins were established and are shown in Figure 6. There are three parts in this figure: the masses of the four major and total

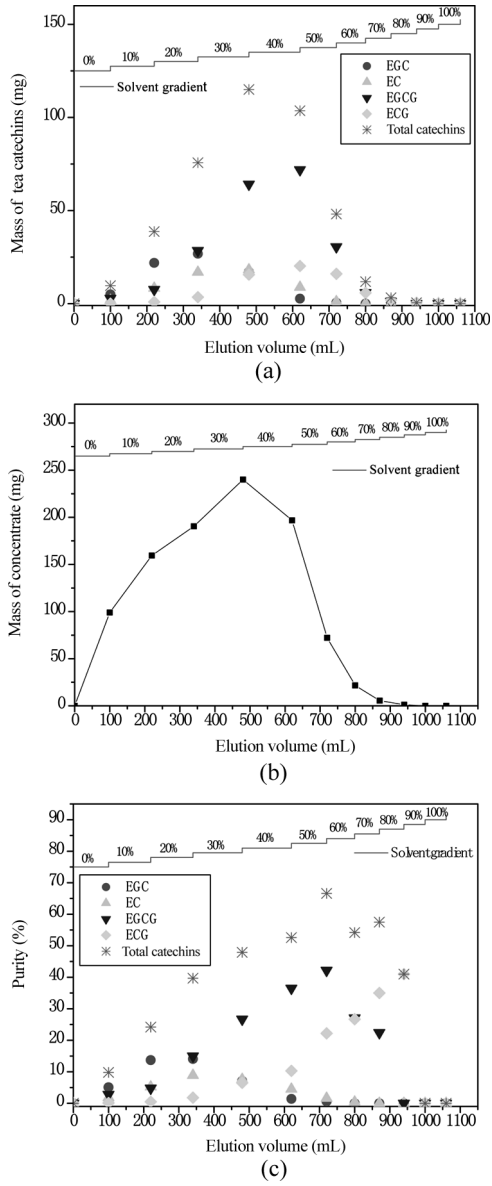

Figure 6. Dynamic elution curves of the four major and total catechins using the XAD-7HP column: (a) masses of the four major and total catechins, (b) mass of concentrate, (c) purities of the four major and total catechins. Column size: same as Figure 5; feed solution: 450 mL; feed concentration: 1.034 mg/mL; feed flow rate: 2 mL/min; elution solution: 0, 10, 20, 30, 40, 50, 60, 70, 80, 90, 100% aqueous ethanol; elution flow rate: 2 mL/min; temperature: 30°C.

catechins, the mass of concentrate, and the purities of the four major and total catechins versus the elution volume, as shown in parts (a)-(c), respectively. The result of part (a) shows that the majority of tea catechins were efficiently eluted by the eluent ranged from 20 to 50% ethanol. The results of parts (b) and (c) show that some more polar impurities were eluted separately by 0 and 10% ethanol, which contributed to an effect for the concentration of tea catechins. However, some impurities with similar polarity as tea catechins were eluted together with tea catechins by 20 to 50% ethanol, which limited the purity of tea catechins collected at this range.

It is worth noting that, as shown in Figure 6(a), there existed only a slight difference in the retentions of the four major catechins. The elution was in the order of first EGC, second EC, third EGCG, and last ECG, which proved again the interaction strengths of the four major catechins on the XAD-7HP adsorbent are in the order of ECG > EGCG > EC > EGC as described in the above section.

Column Performances

The column performances, in terms of the purity, concentration ratio, and recovery yield of tea catechins and the purity and removal ratio of caffeine, are presented in Table 3. It can be seen that, in the adsorption step, the loss of the total catechins was less than 10% when the selected volume (450 mL) of feed solution was loaded. Since nearly 70% of the total mass of concentrate was removed without too much loss of tea catechins (<10%) in the adsorption step, the contents of the total catechins were already isolated nearly 3.0 times (concentration ratio = ~3.0) after the adsorption step and were further isolated by an efficient separation from the other impurities in the elution step. Summing up all the effluent fractions collected from the adsorption and each of the elution steps, the mass of concentrate was around 3272 mg and the mass of the total catechins was around 444 mg. When comparing the collected amounts with their loading amounts, the errors in mass balances of the concentrate and the total catechins were about <5%. The mass conservation was satisfactory and the column was proven to be sufficiently regenerated.

The fractions eluted by 20 to 50% ethanol were collected as the concentrated product. Summing up the four effluent fractions, the mass of concentrate was around 700 mg, the mass of caffeine was around 55 mg, and the masses of the total catechins and EGCG were around 342 mg and 195 mg, respectively. As the unwanted component in the concentrated product, the purity and removal ratio of caffeine were ~8 wt% and ~63%. Nevertheless, as the desired components, the purities of the total catechins and EGCG in the concentrated product were ~49 wt%

Table 3. Column performances of the XAD-7HP column

Step of column operation	Operating volume (mL)	Concentrate		Total catechins			Caffeine	
		Mass (mg)	Mass ratio (%)[b]	Mass (mg)	Recovery yield (%)[c]	Purity (%)[d]	Mass (mg)	Purity (%)[e]
Adsorption[a]	450	2286.0	68.1	38.0	8.2	1.7	31.3	1.4
Step-gradient elution								
0%EtOH	100	99.0	2.9	9.7	2.1	9.8	14.5	14.7
10%EtOH	120	159.5	4.8	38.7	8.3	24.2	41.3	25.9
20%EtOH[f]	120	190.5	5.7	75.7	16.3	39.7	36.9	19.4
30%EtOH[f]	140	240.0	7.1	115.0	24.7	47.9	15.9	6.6
40%EtOH[f]	140	196.8	5.9	103.6	22.3	52.6	1.9	0.9
50%EtOH[f]	100	72.2	2.2	48.1	10.3	66.6	0.3	0.3
		Product: 699.5	Product: 20.9	Product: 342.4	Product: 73.6	Product: 48.9	Product: 55.0	Product: 7.9
60%EtOH	80	21.7	0.6	11.8	2.5	54.2	0.0	0.0
70%EtOH	70	5.5	0.2	3.2	0.7	57.5	0.0	0.0
80%EtOH	70	1.2	0.0	0.5	0.1	41.0	0.0	0.0
90%EtOH	60	0.0	0.0	0.0	0.0	0.0	0.0	0.0
100%EtOH	60	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Total	—	3272.4	97.5	444.3	95.5	—	142.1	—

[a]Feed solution: loading volume = 450 mL; concentration of concentrate = 7.46 mg/mL; concentration of the total catechins = 1.034 mg/mL; purity of the total catechins = 13.86%.

[b]Mass ratio of concentrate (%) = (fractional mass of concentrate/total mass of concentrate) × 100%.

[c]Recovery yield of the total catechins (%) = (fractional mass of the total catechins/total mass of the total catechins) × 100%.

[d]Purity of the total catechins (%) = (fractional mass of the total catechins/fractional mass of concentrate) × 100%.

[e]Purity of caffeine (%) = (fractional mass of caffeine/fractional mass of concentrate) × 100%.

[f]The fraction was collected as a part of the concentrated product.

and ~28 wt%, respectively. The contents of the total catechins and EGCG were efficiently concentrated ~3.5 times in high recovery yields (>75%).

CONCLUSIONS

The adsorption separation method using intermediate polar adsorbent (Amberlite XAD-7HP resin) was verified to be an efficient way for isolating a mixture of tea catechins from the green tea powders. The major findings from this study are as follows:

1. In the green tea powders, produced by hot water extraction, the purities of total catechins and caffeine ranged from 13.0 to 16.0 wt% and 4.5 to 5.5 wt%, respectively. The total catechins are composed of the four major catechins with the order of purity being EGCG (ranged from 7.5 to 8.5 wt%) > EGC (ranged from 3.5 to 4.5 wt%) > ECG (ranged from 2.0 to 2.5 wt%) > EC (ranged from 1.5 to 2.0 wt%).
2. The batch adsorption results show that the adsorption equilibrium capacities of the four major catechins were in the order of ECG ~ EGCG > EC > EGC. It proved that the interaction strengths of the four major catechins on the XAD-7HP adsorbent are in the order of ECG > EGCG > EC > EGC and the polarities of the four major catechins are in the order of EGC (very polar) > EC (polar) > EGCG (intermediate polar) > ECG (polar). The adsorption equilibrium data of the total catechins were fitted closely by a Langmuir isotherm model, which was used to predict the ideal loading amount in the column operation.
3. To avoid the loss of tea catechins, the loading volume of the adsorption process was selected according to the elution volume at the break point of the dynamic adsorption curve. For a column packed with 6.47 g XAD-7HP resins (250–840 μm diameter) and sizing 1.5 cm I.D. and 9.7 cm length, the loading volume is estimated to be 450 mL when the column is operated at the feed concentration of total catechins of 1.021 mg/mL and the flow rate of 2 mL/min. The length of unused bed (LUB) is estimated to be 61.0% of the column packed length. A larger loading amount and a lower LUB are allowed if the particle size of XAD-7HP resins is reduced, but at the expense of a higher pressure drop.
4. Under a proper step gradient elution program for the XAD-7HP column, the eluent composition for an efficient elution of the majority of tea catechins ranged from 20 to 50% ethanol. However, some impurities with similar polarity as tea catechins were eluted together with tea

catechins by 20 to 50% ethanol, which limited the purity of the total catechins collected at this range.

5. The purities of the total catechins and EGCG, in the concentrated product were ~49 wt% and ~28 wt%, respectively. The contents of the total catechins and EGCG were efficiently concentrated ~3.5 times in high recovery yields (> 75%).

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