Journal of Pharmaceutical and Biomedical Analysis 56 (2011) 713–720

Contents lists available at ScienceDirect

Journal of Pharmaceutical and Biomedical Analysis

journal homepage: www.elsevier.com/locate/jpba

Preparation of amino-modified active carbon cartridges and their use in the extraction of quercetin from *Oldenlandia diffusa*

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ARTICLE INFO

Article history:
Received 16 February 2011
Received in revised form 12 July 2011
Accepted 14 July 2011
Available online 22 July 2011

Keywords:
Oldenlandia diffusa
Extraction
Quercetin
Modified active carbon
Adsorption isotherm

ABSTRACT

Polyethyleneimine (PEI) and ethylenediamine (EDA) as modifiers were bonded on active carbon (AC) surface for specific selective extraction of quercetin from *Oldenlandia diffusa*. The characteristics of the modified AC materials that were obtained were investigated by field emission-scanning electron microscopy (FE-SEM) and Fourier transform infrared spectrometer (FT-IR). The interactions between quercetin and the AC materials were investigated by fitting the static adsorption data to four linear and nonlinear adsorption isotherm models. Of these four models, the Langmuir–Freundlich adsorption isotherm was proved the best for investigating quercetin on AC materials. Scatchard analysis was used to evaluate the binding properties of the AC materials for quercetin. Solvent extraction and solid-phase extraction (SPE) were optimized, and the effect of the mobile phase pH was investigated to improve the performance for the separation of quercetin on high performance liquid chromatography (HPLC). The results from the validation of the proposed analytical method demonstrated that the EDA-modified AC was the most suitable SPE cartridge for the purification of quercetin from *O. diffusa*.

1. Introduction

Oldenlandia diffusa is a commonly used herb in traditional Chinese medicine for the treatment of disease [1,2]. It contains many beneficial bioactive components such as phytosterols, ursolic acid, and quercetin [3–5]. These active ingredients are used to treat a variety of diseases such as hepatitis, tonsillitis, appendicitis, urethral infection and cancers [6–8]. Quercetin (Fig. 1) is a polyphenolic flavonoid found in many plants and is used as a nutritional supplement [9]. It is the aglycone form of several other flavonoid glycosides such as rutin and quercitrin and exists as glucosides in fruits and vegetables [10,11]. As well as helping in the prevention of cancers, it has antioxidant, anti-histamine, and anti-inflammatory effects [12–14].

Ultrasonic-assisted extraction (UAE) has been used for the extraction of bioactive compounds from plants, and can effectively expedite extraction and increase its efficiency [15]. However, the methods of purifying the target compounds from the solvent extract need to be improved. Solid-phase extraction (SPE) is the most used target-substrate pretreatment of complicated matrices [16], with research ongoing to find suitable SPE materials for specific applications.

Active carbon (AC) is a commonly used porous adsorbent material. It has a very large surface area and mechanical resistance suitable for adsorption or chemical reactions [17,18]. It is widely used in gas and water purification, sewage treatment, gas mask filters and many other applications [19–22]. Sufficient activation of AC for useful applications may be generated by its high surface area, but further chemical treatment can enhance its adsorbing properties. According to the purpose, the AC surface textures and compositions can be modified to enhance their efficiency. A common modification involves binding functional groups to its surface [23]. Polyethyleneimine (PEI) is a polymer with a large quantity of amino groups on linear macromolecular chains. Ethylenediamine (EDA) is a strongly basic amine and is widely used in chemical syntheses. Both can be used to modify AC surfaces for the selective extraction of plant compounds [21,24].

In this work, PEI- and EDA-modified AC cartridges were fabricated for the SPE of quercetin from *O. diffusa*. The modified AC was characterized by field emission-scanning electron microscopy (FE-SEM) and Fourier transform infrared (FT-IR) spectrometer. The interactions between quercetin and AC were investigated by fitting the static adsorption data to four linear and nonlinear adsorption isotherms. The AC materials were further examined by high performance liquid chromatography (HPLC) with UAE .

2. Experimental

2.1. Reagents and materials

O. diffusa powders were bought from a local market (Incheon, Korea). AC (30–89 μm, average specific surface area, 1150 m^2/g)

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doi:10.1016/j.jpba.2011.07.014

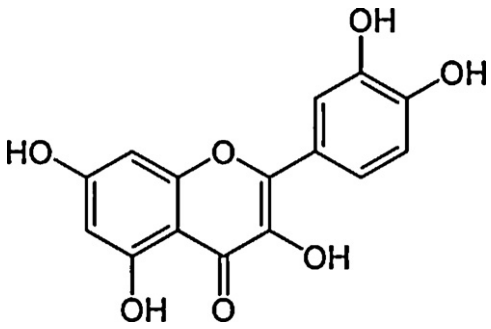

Fig. 1. Chemical structure of quercetin.

and quercetin were purchased from Sigma (St Louis, MO, U.S.A.). EDA was purchased from Fluka (Buchs, Switzerland.). PEI was purchased from Supelco (Bellefonte, PA, USA). Methanol, ethanol, chloroform and acetonitrile (ACN) were obtained from Duksan Pure Chemical Co., LTD (Ansan, Korea). All the other reagents used in the experiment were HPLC or analytical grade. Double distilled water was filtered with a vacuum pump (Division of Millipore, Waters, U.S.A.) and filter (HA-0.45, Division of Millipore, Waters, U.S.A.) before use. All the samples were filtered by using a filter (MFS-25, 0.2 μm TF, WHATMAN, U.S.A.) before injection into the HPLC system.

2.2. Apparatus

The chromatographic system consisted of a Waters 600 s Multi solvent Delivery System, Waters 616 liquid chromatography (Waters Associates, Milford, MA, U.S.A.), a Rheodyne injector (20 μL sample loop) and a variable wavelength 2487 UV dual channel detector. Autochro-2000 software (Younglin Co. Ltd., Korea) was used for data acquisition. The analysis was performed on an OptimaPak C_{18} column (5 (m, 150 × 4.6 mm, i.d., RS tech Corporation, Daejeon, Korea) with a guard column (10 × 4.6 mm, i.d.) packed with C_{18} materials. Deionized water was filtered with a vacuum pump (Division of Millipore, Waters, U.S.A.) and a filter (HA-0.45, Division of Millipore, Waters, U.S.A.) before use. All the samples were filtered by using a filter (MFS-25, 0.2 μm TF, WHATMAN, U.S.A.) before injection into the HPLC system. All of the glassware for preparation of the samples and standard solutions was washed with deionized water and acetone and then dried at room temperature.

2.3. Preparation of the modified AC

The PEI- and EDA-modified AC were both prepared by wet impregnation. In typical reactions, 4.0 g PEI or 5.0 mL EDA were mixed with 40.0 mL methanol in a flask under stirring for 30 min, to which 5.0 g dry blank AC (30–89 μm) was then added. The mixtures were continuously stirred for 1 h, and then placed in a water bath for polymerization at 80 °C under reduced pressure (500 mmHg). Polymerizations were allowed to proceed for 24 h before the treated AC was filtered and washed with water to remove any excess PEI or EDA. The modified AC was dried overnight at 50 °C.

2.4. Characteristics of the modified AC

The blank AC, and PEI- and EDA-modified AC materials were dried at 50 °C overnight. The microstructures of these materials were observed by FE-SEM (S-4200 model, Hitachi, Japan,) operated at 15 kV.

FT-IR spectra were obtained employing a VERTEX 80V FT-IR Vacuum Spectrometer (Bruker, Karlsruhe, Germany) with a deuterated L-alanine doped triglycine sulfate (DLaTGS) detector. Samples (blank AC, and PEI- and EDA-modified AC) were scanned as KBr disks (1%, *w/w*); resolution: 4 cm^{-1}. The data region was 400–4000 cm^{-1}, operation in auto mode and the number of scans per spectrum 16. Spectra were obtained in transmission and absorption mode for the identification and the quantitative determination, respectively. Two milligrams of sample was mixed with 200 mg KBr and ground gently with an agate pestle and mortar under an infrared lamp and afterwards was pressed into a 13-mm diameter disk by applying 10 tons pressure for 2 min.

2.5. Sample preparation and chromatography

Stock standard quercetin solutions were prepared by dissolving the drug in methanol to achieve a final concentration of 1,000 mg/L. A series of standard solutions was then prepared at nine different concentrations from 0.50 to 100.0 mg/L. HPLC separation was conducted using ACN–H_2O–H_3PO_4 = 25:75:0.05 (pH = 5.5, *v/v/v*) as the mobile phase with a flow rate of 0.7 mL/min and detection was carried out at 360 nm.

2.6. Adsorption of quercetin on the modified AC

The static adsorption method was performed on the blank AC, and PEI- and EDA-modified AC materials, respectively, with 0.02 g of modified AC particles being placed in several flasks. Quercetin standard solutions (1.0 mL) with nine different concentrations were added into the flasks, and the suspension was stirred for 48 h at 25 °C to ensure complete adsorption of quercetin on AC particles. The supernatant solution was then collected, filtered and injected into the HPLC system at 25 °C. The adsorption quantity of quercetin on the modified AC was calculated by subtracting the unabsorbed concentrations from the initial concentrations of quercetin.

The adsorption quantity of quercetin on amino-modified AC is determined as follows:

$$Q = \frac{(C_0 - C) \times V}{M} \quad (1)$$

where Q (mg/g) is the adsorption quantity of quercetin on the modified AC at equilibrium, C_0 (mg/L) the initiator concentration, C (mg/L) the unabsorbed concentration, V (L) the volume of the sample solvent, and M (g) the mass of AC particles. Q was obtained by Eq. (1), after which the experimental parameters were estimated and compared with the equilibrium isotherms by linear and nonlinear regression analysis. The experimental adsorption isotherms were fitted to the linear, Langmuir, Freundlich and Langmuir–Freundlich models. This process was accomplished by using the solver function in OriginPro 7.5 software (OriginLab Corporation, MA, U.S.A.) and varying the fitting parameters to reach a value of 1 for the squared correlation coefficient (r^2).

2.7. Extraction of quercetin

In a typical procedure, 2.0 g *O. diffusa* powder samples were dissolved in 0.03 L of each of chloroform, ethanol, methanol and water. Quercetin underwent UAE at 25 °C for 20, 40, 60, 80 and 100 min. After stirring, the extracts were sonicated and filtered. Finally, the extracts were concentrated to a volume of 10.0 mL using a rotary evaporator.

The blank and modified AC (0.2 g) were packed into empty polypropylene cartridges (50 × 8.0 mm, i.d.), and preconditioned with dichloromethane and ACN, respectively. The extract solutions (0.5 mL) were loaded into the SPE cartridges and washed with 5.0 mL water to remove interfering substances. The extracts were then eluted with 2.0 mL ACN. The filtrates were evaporated to dryness and reconstituted in 0.5 mL mobile phase for further HPLC

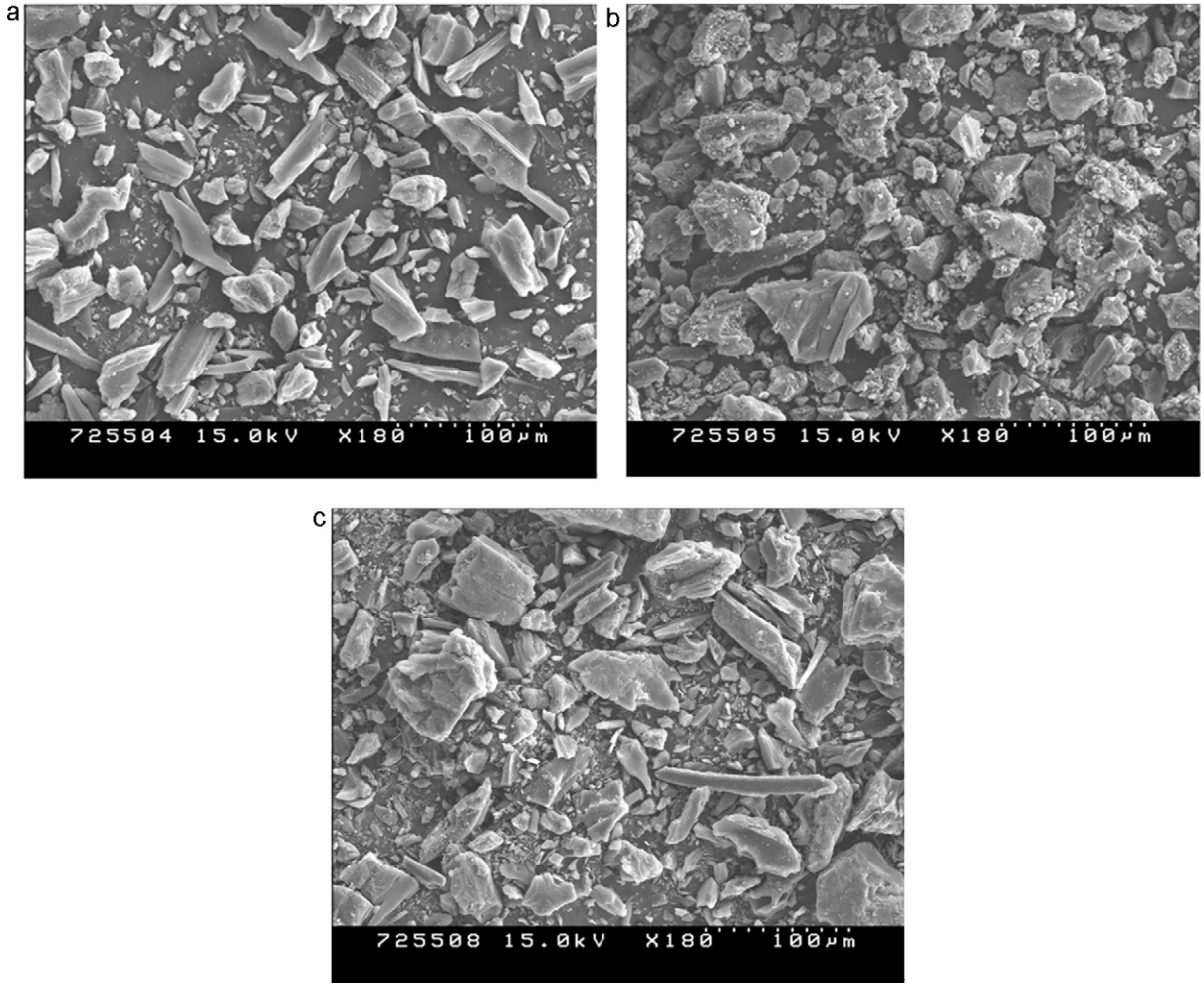

Fig. 2. FE-SEM images of three AC materials (30–89 μm) (a, blank AC; b, PEI-modified AC; c, EDA-modified AC).

analysis. The SPE cartridge was washed with ACN for subsequent use.

3. Results and discussion

3.1. Characteristics of the modified AC

AC is a porous material with high surface area, and further chemical treatment can enhance its selective absorbing properties. PEI and EDA are both commonly used alkaline solvents in chemical reactions. In order to improve the selectivity of AC, PEI and EDA were bonded as modifiers onto the AC surfaces for the selective extraction of quercetin from *O. diffusa*. By comparing the weights of the modified AC with the original blank AC, the contents of the alkaline modifiers were estimated as 65.3 and 38.9 mg/g for PEI and EDA, respectively.

The microstructures of AC materials are evaluated by FE-SEM. FT-IR analysis provided rapid acquisition of a large amount of spectral data in a wide spectral range, and it is used to further verify the exits of bonded functional groups on AC materials. FE-SEM images of the blank (Fig. 2a), and PEI- (Fig. 2b) and EDA-modified AC (Fig. 2c) show the surface morphology changes of AC materials, and Fig. 3 showed the FT-IR absorbance spectrums of three AC materials. 1000–1350 cm^{-1} is the characteristic of C–N stretching vibration region, and 1400 cm^{-1} is the stretching vibration peak of methylene of the alkaline modifiers. 1340–1465 cm^{-1} and 2850–3000 cm^{-1} are the characteristics of C–H flexural and stretching vibration regions, respectively. Moreover, the peak of 3430 cm^{-1} is caused by some adsorbed water molecules on the AC materials. These results indicate that the alkaline modifiers were bonded to the AC surface

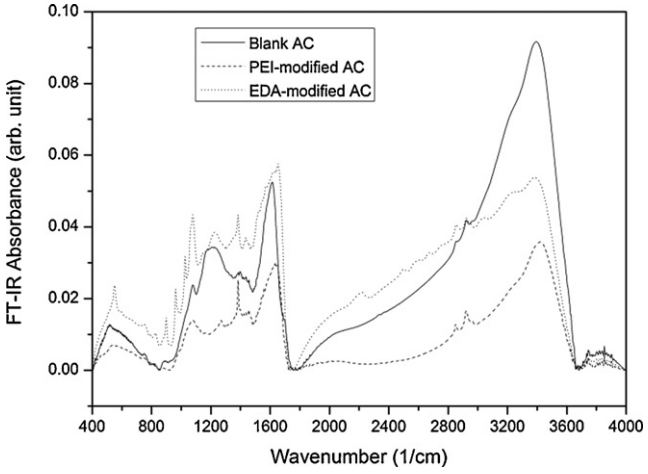

Fig. 3. FT-IR absorbance spectrums of three AC materials.

Table 1
Adsorption quantity (*Q*, mean ± SD, *n* = 3) of quercetin on AC materials at 25 °C.

C (mg/L)	*Q* (mg/g)		
	Blank AC (*M* ± SD)	PEI modified AC (*M* ± SD)	Ethylenediamine modified AC (*M* ± SD)
0.50	0.0147 ± 0.0014	0.0137 ± 0.0012	0.0156 ± 0.0015
0.80	0.0278 ± 0.0029	0.0225 ± 0.0020	0.0267 ± 0.0026
1.00	0.0372 ± 0.0033	0.0368 ± 0.0027	0.0411 ± 0.0051
5.00	0.1883 ± 0.0051	0.1767 ± 0.0063	0.2089 ± 0.0084
10.00	0.4017 ± 0.0178	0.4389 ± 0.0123	0.4412 ± 0.0161
30.00	0.6874 ± 0.0236	0.6553 ± 0.0155	0.7828 ± 0.0223
50.00	0.8567 ± 0.0251	0.8694 ± 0.0242	1.0256 ± 0.0345
80.00	1.1402 ± 0.0336	1.2874 ± 0.0385	1.4582 ± 0.0467
100.00	1.1976 ± 0.0412	1.3452 ± 0.0374	1.6932 ± 0.0579

with irreversibly bound amino groups. To demonstrate the interactions between quercetin and the modified AC, static adsorption was performed as a further test.

3.2. Adsorption isotherm models

Static adsorption was performed on the blank AC, and PEI- and EDA-modified AC materials with 1.0 mL quercetin standard solutions on 0.02 g modified AC particles in a flask. The adsorption quantities, *Q* (mg/g, Mean ± SD, *n* = 3), of quercetin on the AC materials at 25 °C are shown in Table 1.

The adsorption isotherm describes how the adsorbed molecules were distributed between the liquid and solid phases at equilibrium, and can provide qualitative information about the solute-adsorbent interaction at constant temperature [17,25]. In this study, the following four isotherm models were used to describe the results: linear (2), Langmuir (3), Freundlich (4) and Langmuir–Freundlich (5) [26,27]:

$$Q = aC + b \tag{2}$$

$$Q = \frac{aC}{1 + bC} \tag{3}$$

$$Q = aC^{1/c} \tag{4}$$

$$Q = \frac{aC^c}{1 + bC^c} \tag{5}$$

where *Q* (mg/g) is the adsorption quantity of quercetin on the AC materials at equilibrium, *C* (mg/L) the equilibrium concentration of the solute in the liquid phase, *a* the maximum adsorption capacity, *b* the apparent dissociation constant, which represents the affinity between the solute and adsorbent, and *c* a parameter. The linear equation is an algebraic equation in which each term is a single, constant variable. The Langmuir equation quantitatively describes the buildup of a layer of molecules on an adsorbent surface as a function of the concentration of the adsorbed material in the liquid in which it is in contact. The shape of this isotherm is a gradual positive curve that flattens to a constant value. The Freundlich equation is a curve relating the concentration of a solute on the surface of an adsorbent to the concentration of the solute in the liquid with which it is in contact. The Langmuir–Freundlich equation combines the Langmuir and Freundlich equations.

The fitting results, i.e., isotherm parameters and the coefficient of determination, r^2, are shown in Table 2. Fig. 4 shows the experimental points of the quercetin adsorption equilibrium isotherm curves on the blank AC (Fig. 4a), PEI-modified AC (Fig. 4b) and EDA-modified AC (Fig. 4c) at 25 °C. Table 2 shows that the Langmuir–Freundlich Equation (5) gave better correlation results than the other three isotherm equations, and it was therefore considered the most suitable for the adsorption of quercetin on the three AC materials. The

Table 2
Parameters in adsorption isotherm of AC materials.

Material	Adsorption equation no.	Parameters			r^2
		a	*b*	*c*	
Blank AC	(2)	0.0161	0.0158	–	0.9282
	(3)	0.0413	0.0248	–	0.9940
	(4)	0.0946	–	1.7818	0.9886
	(5)	0.0601	0.0285	0.8351	0.9956
PEI modified AC	(2)	0.0176	0.0109	–	0.9405
	(3)	0.0344	0.0156	–	0.9842
	(4)	0.0807	–	1.6168	0.9879
	(5)	0.0672	0.0131	0.7227	0.9889
Ethylenediamine modified AC	(2)	0.0210	0.0106	–	0.9537
	(3)	0.0353	0.0115	–	0.9912
	(4)	0.0799	–	1.5097	0.9961
	(5)	0.0752	0.0040	0.6951	0.9962

r^2 values of the Langmuir–Freundlich equation are 0.9956, 0.9889 and 0.9962 for the three AC materials, respectively. The results showed that the equilibrium data were better fitted by the three-parameter models than by the two-parameter models.

3.3. Binding properties of the modified AC [28,29]

The binding properties of the AC materials were analyzed by finding their maximum binding capacities and dissociation constants. The data of static adsorption experiments were further processed with the Scatchard equation:

$$\frac{Q}{C_{\text{free}}} = \frac{Q\,\text{max} - Q}{K_{\text{D}}} \tag{6}$$

where *Q* (mg/g) is the adsorption quantity of quercetin on the modified AC at equilibrium, Q_{max} the maximum binding capacity, C_{free} the free analyte concentration at equilibrium and K_{D} the dissociation constant.

As shown in Fig. 5, the Scatchard plots of quercetin on the blank (Fig. 5a), PEI-modified (Fig. 5b) and EDA-modified AC (Fig. 5c) were not linear, suggesting that the binding sites in these AC materials were heterogeneous with respect to the affinity for quercetin. The two distinct sections within the plot that can be regarded as linear support the reasonable assumption that the binding sites can be classified into two distinct groups with specific binding properties. The values of K_{D} and Q_{max} are shown in Table 3.

3.4. Optimization of extraction variables

3.4.1. Effect of extraction solvent

The tested extraction solvents were chloroform, ethanol, methanol and water. Two grams *O. diffusa* powder was dissolved in 0.03 L of each solvent for 60 min at 25 °C with an ultrasonic power of 80 W. The results are shown in Fig. 6a. Methanol was the best solvent for extracting quercetin from *O. diffusa.*

3.4.2. Effect of extraction time and ultrasonic power

For further optimization of quercetin extraction, different extraction times (20, 40, 60, 80 and 100 min) and ultrasonic powers (20, 40, 60, 80 and 100 W) of UAE were tested using methanol as the extraction solvent. The extraction times were tested with 80 W ultrasonic power and ultrasonic powers were tested for 60 min each. In Fig. 6b and c, the extracted amounts of quercetin increased as the extraction time was increased from 20 to 60 min and as the ultrasonic power was increased from 20 to 80 W. As no obvious increase of quercetin extraction yield was observed beyond 60 min and 80 W, extraction with methanol under these conditions

Table 3
The results of Scatchard analysis.

Material	Binding site	Linearity	K_D	Q_{max}
Blank AC	1	$Q/C_{free} = 0.1028 + 0.2588Q$	−3.8640	−0.3973
	2	$Q/C_{free} = 0.0708 - 0.0461Q$	21.6826	1.5343
PEI modified AC	1	$Q/C_{free} = 0.0607 + 0.6400Q$	−1.5625	−0.0948
	2	$Q/C_{free} = 0.0518 - 0.0239Q$	41.806	2.164
Ethylenediamine modified AC	1	$Q/C_{free} = 0.1218 + 0.5924Q$	−1.6880	−0.2056
	2	$Q/C_{free} = 0.0715 - 0.0287Q$	34.904	2.4939

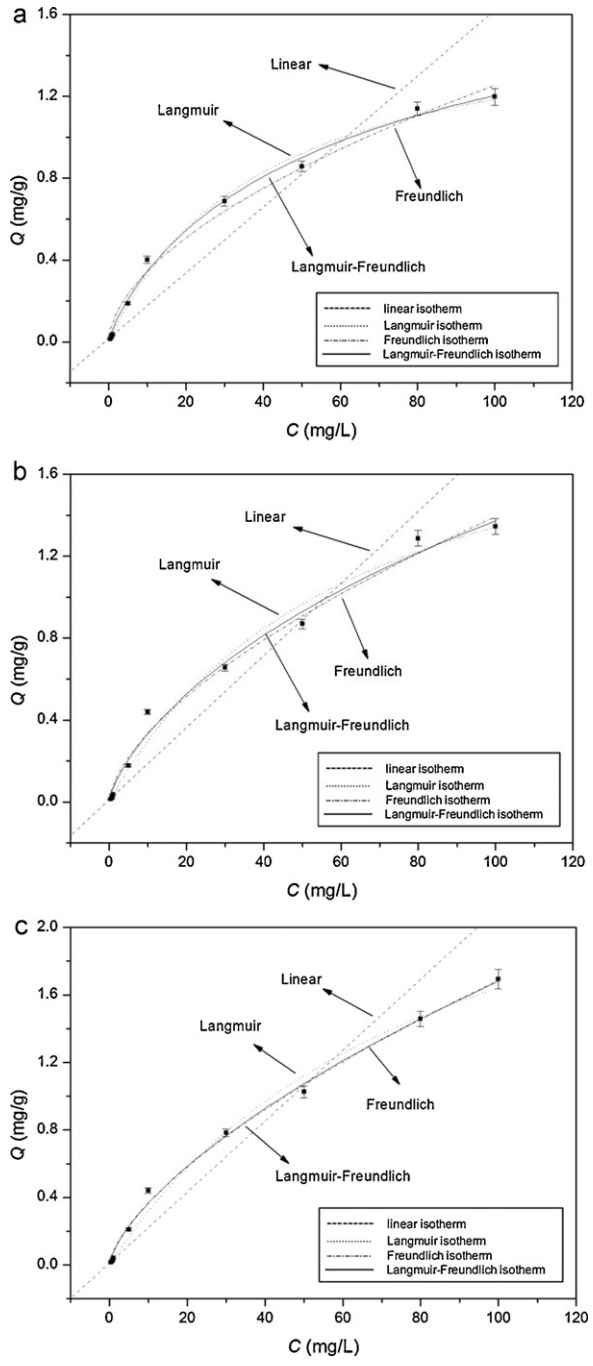

Fig. 4. Comparison of different isotherm models for quercetin adsorption on three AC materials (a, blank AC; b, PEI-modified AC; c, EDA-modified AC).

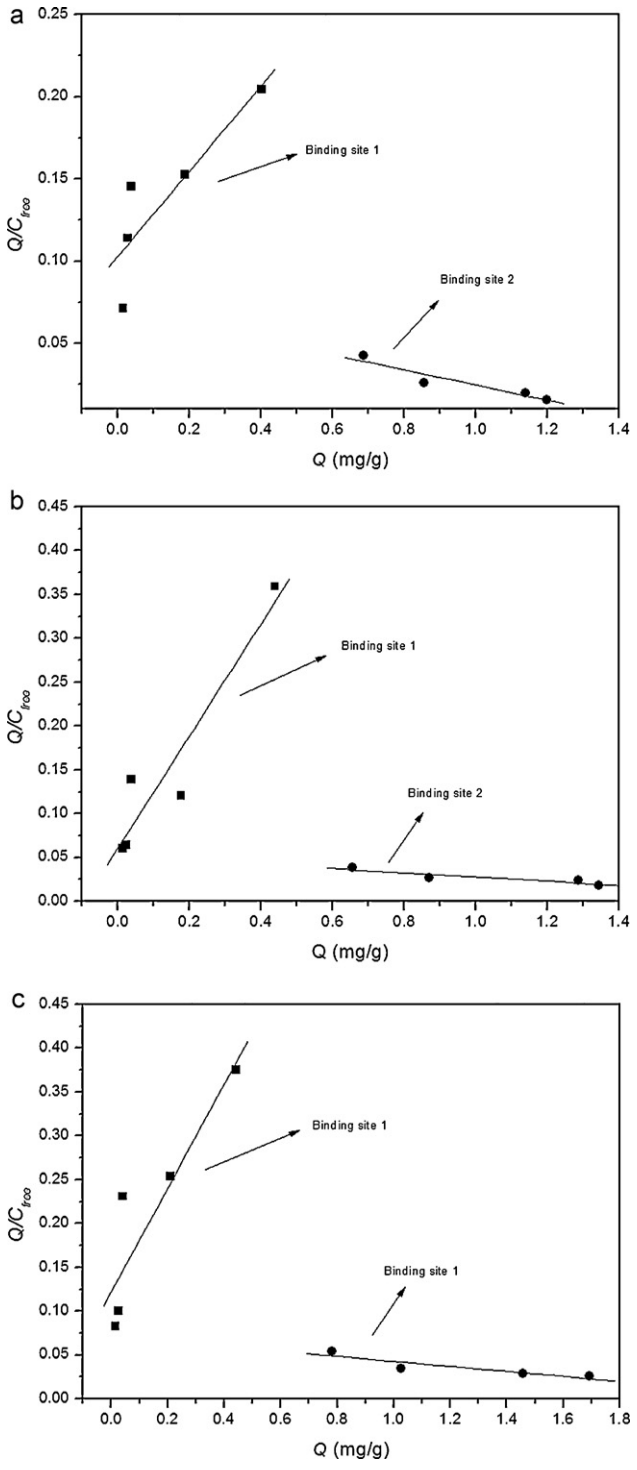

Fig. 5. Scatchard analysis plots of quercetin on three AC materials (a, blank AC; b, PEI-modified AC; c, EDA-modified AC).

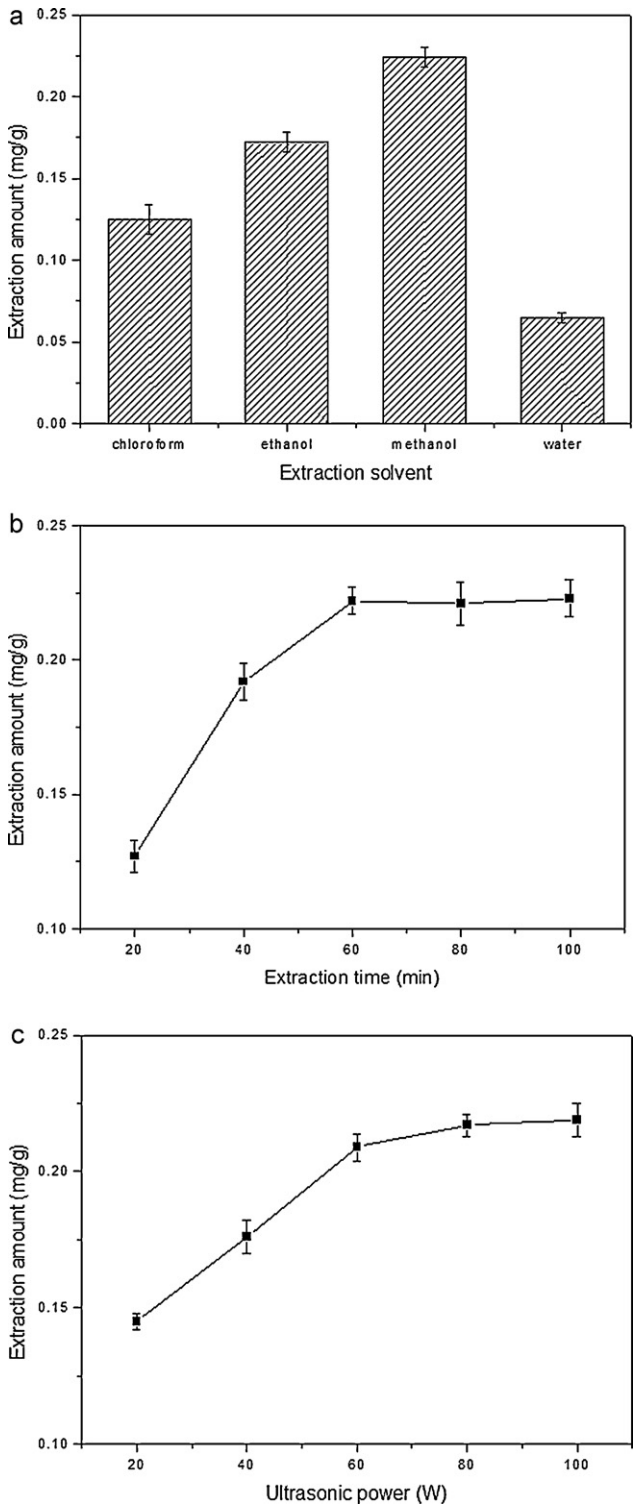

Fig. 6. Effects of extraction solvents (a), extraction time (b) and ultrasonic power (c) on the extracted amounts of quercetin.

was considered the most efficient considering the time and energy costs.

3.5. Separation of quercetin on HPLC

Separation was achieved on a C_{18} column (5 (m, 150 × 4.6 mm, i.d.) and the effects of different mobile phase composition and pH were investigated. First, the proportion of ACN (75, 50, 25 and 10%)

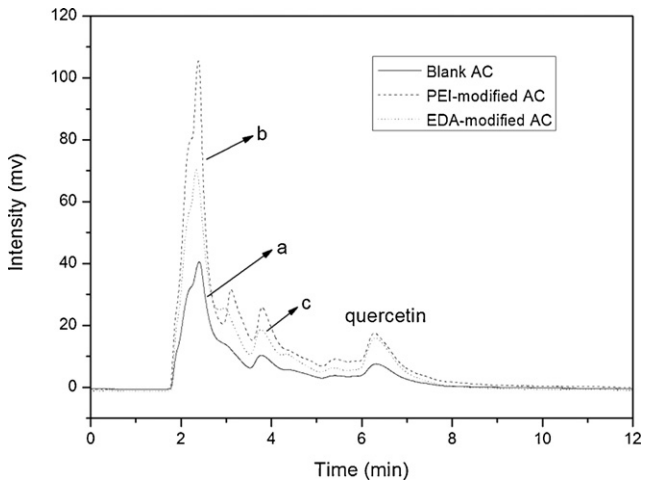

Fig. 7. Chromatogram of methanol microwave-extract from *O. diffusa* (a, blank AC; b, PEI-modified AC; c, EDA-modified AC) (Mobile phase: ACN–H_2O–H_3PO_4 = 25:75:0.05 (pH = 5.5, *v/v/v*), flow rate: 0.7 mL/min, column: C_{18} column (150 × 4.6 mm, i.d.), UV: 360 nm.)

in the mobile phase was tested at the flow rate of 0.7 mL/min. To improve peak shape and separation efficiency further, the pH of the mobile phase (6.5, 6.0, 5.5, 5.0, 4.5, 4.0 and 3.5) was investigated and controlled by adding H_3PO_4. The separation efficiency and resolution results revealed an optimum mobile phase composition of ACN–H_2O–H_3PO_4 = 25:75:0.05 (pH = 5.5, *v/v/v*) with UV detection at 360 nm. The effect of mobile phase flow rate on the HPLC system was also tested at flow rates ranging from 0.4 to 2.0 mL/min. The relationship between flow rate and column backpressure was investigated with the optimum mobile phase. The tradeoff between time and efficiency was considered optimized at the elution flow rate of 0.7 mL/min^{-1}.

3.6. Optimization of SPE conditions

The extract solutions (0.5 mL) were loaded into SPE cartridges packed with 0.2 g of each of the AC materials (Fig. 7a–c). Different solvents (water, ethanol, methanol, ACN, and acetone) were used to wash the loaded SPE cartridges in order to remove interfering substances from the solvent extracts. Water removed the interfering substances efficiently without washing quercetin out of the SPE cartridge. Therefore, water was used as the washing solvent, and different volumes of water (from 1.0 to 8.0 mL) were tested to find the optimum volume. The removal of interfering substances was not improved at volumes of water greater than 5.0 mL. Because ACN has a strong eluting capacity for quercetin, 2.0 mL ACN was used for elution.

As shown in Fig. 7, the blank AC did not have specific selectivity of quercetin, and had a lower recovery rate of quercetin than the PEI- and EDA-modified AC. The chromatographic peaks of quercetin were similar in Fig. 7b and c, indicating that the PEI- and EDA-modified AC were interacting with quercetin. Moreover, the EDA-modified AC removed the interfering substances from extract samples more efficiently than the PEI-modified AC did. Therefore, the EDA-modified AC was considered the more suitable SPE material for the purification of quercetin from *O. diffusa*, with an extraction yield of 0.198 mg/g.

3.7. Validation of the proposed analytical method

Calibration curves were constructed from chromatographic peak areas measured three times at nine concentrations ranging from 0.50 to 100.0 mg/L. A good linear correlation

Table 4
Intra-day and inter-day precisions, accuracies and recoveries of quercetin with three different concentrations.

	C (mg/L)	Intra-day RSD (%)	Inter-day RSD (%)	SPE recovery (%)	Method recovery (%)
Quercetin	5.00	5.51	5.78	76.54	100.36
	30.00	4.98	4.97	81.33	98.75
	80.00	3.88	4.17	86.18	104.69

equation for quercetin was obtained: $Y = 44.42X + 5.61$ ($n = 9$, $r^2 = 0.9996$).

Repeatability assays were calculated as relative standard deviations (RSDs) and were carried out by injecting standard solutions of quercetin five times on the same day and over five consecutive days. The intra- and inter-day RSDs of the proposed method were less than 5.51 and 5.78%, respectively. The sensitivity of a method is expressed by its limits of detection (LOD) and of quantification (LOQ). The standard solutions of quercetin were diluted and injected until the LOD (0.15 mg/L) and the LOQ (0.45 mg/L) were obtained at signal/noise ratios of 3 and 10, respectively. SPE recoveries were calculated by adding three concentrations of quercetin standard solutions to the extracts and comparing the extraction yields before and after SPE, with obtained SPE recoveries ranging from 76.54 to 86.18%. The method's recoveries (98.75–104.69%) were tested to investigate the reliability of the developed method (Table 4).

4. Conclusions

In this study, PEI- and EDA-modified AC cartridges were synthesized for the SPE of quercetin from *O. diffusa*. The highlight of this paper is to improve the selectivity of this cheap porous AC material. FE-SEM and FT-IR revealed that amino modifiers were bonded on the AC surface with an irreversibly bound amino group. The Langmuir–Freundlich adsorption isotherm was shown to be the best model for investigating the interactions between quercetin and the AC materials. Scatchard analysis of binding capacities and dissociation constants was used to evaluate the binding properties of the AC materials. The results showed that the EDA-modified AC was the most suitable SPE cartridge for the purification of quercetin from *O. diffusa*. At the optimum HPLC and SPE conditions, the extraction yield was maximized at 0.198 mg/g. These results support the potential of this type of anion-modified AC sorbent for the separation and purification of bioactive compounds from plant extracts.

In our previous work [30], a SPE method was developed for the extraction and determination of quercetin and myricetin from *C. obtusa* using an ionic liquids-based monolithic cartridge. Comparing with the proposed study, the purposes of these two studies are similar, but the types and structures of SPE materials are different. The amino-modified active carbon materials have the advantages of low cost, easy preparation, high surface areas and high specific selectivity. Ionic liquids-based monolithic cartridge also has many advantages, such as rapid, low pressure and large volume processing. In conclusion, they are both potential selective stationary phase materials, and these two SPE methods were successfully developed for purification of bio-active compounds from natural plants.

Acknowledgment

This research was supported by the Basic Science Research Program through the National Research Foundation (NRF) of Korea funded by the Ministry of Education, Science and Technology (2011-0010673).

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