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Retention behaviour of products extracted from oriental medicine formulations in high-performance liquid chromatography on octadecyl acrylate-modified silicas

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Abstract

In this work, octadecyl acrylate (ODA) having a reactive group at one side of the terminal groups were synthesized and evaluated using the extract products in oriental medicine formulations by high-performance liquid chromatography (HPLC). By elemental analysis as well as IR and NMR spectroscopy, the average degree of polymerization of ODA-modified silicas were determined to be 19. Using acetonitrile–water mixtures as the eluent, *ginsenosides Rb1* and *Rg1* in commercial *Panax Ginseng* or *Red Ginseng* were separated on ODA-modified silicas, but with different degrees of resolution comparing with ODS-modified silicas.

Keywords: Pharmaceutical analysis; Silica, bonded; Octadecyl acrylate-modified silica; Ginsenosides

1. Introduction

Chemically modified phases are used mostly in HPLC. Thus, it is very important to investigate the characteristic retention mechanism, preferentially as supports for chemically bonded phases. These materials consist of organic functional groups, such as octadecyl, octyl, ethyl and phenyl groups, bonded to silicas. In previous papers [1–8], we have suggested that the important parameters of silica with respect to the number of accessible alkylamino or phenyl groups per 1 nm^2 are the pore diameter and the specific surface area. Then, we have assumed that the hydrogen-bonded silanol groups on the silica surface have an inhibitory effect on the retention of the solute and that the retention effect was due mainly to free silanol groups on the silica surface [9]. Moreover, we have also studied the preparation of ODS-modified silicas or glasses, and evaluated their performance with endcapping in the HPLC [7]. On the other hand, the identification of drugs and determination of their concentration, especially in oriental medicine formulations and for forensic science purposes, require several types of column gels for HPLC as well as several types of column gels for gas chromatography [10–18]. And now, the octadecyl acrylate (ODA)-modified silica has been previously evaluated in our laboratory to determine its characteristic structure [19,20]. And then, if we get logical characteristic information of new ODA-modified silicas, it will be easy to choose preparative

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conditions for better columns. However, there have been few reports of the chromatographic behaviour on ODA-modified silicas in HPLC columns. Therefore, in the continuing evaluation of these ODA-modified silicas, the analysis of extract products in oriental medicine formulations using HPLC were compared with ODS-modified HPLC column gels, using *ginsenosides Rb1* and *Rg1* in commercial *Panax Ginseng* or *Red Ginseng* and some components of extract products in oriental medicine formulations.

2. Experimental

2.1. Reagent and materials

Octadecylacrylate, 3-mercaptopropyltrimethoxysilane, azobisisobutyronitrile, octadecyldimethylchlorosilane, *p*-hydroxyacetophenone, *sennoside A, hesperidin, naringin, paeoniflorin, glycyrrhizin, ginsenosides Rb1* and *Rg1* were obtained from Wako (Osaka, Japan). The other reagents and organic solvents were of analytical-reagent grade.

2.2. Porous silicas

Porous silicas were prepared in our laboratories, and were determined to be; mean pore diameter 12.1 nm, mean particle size distribution 5.5 μm, specific surface area 378 ml/g, and pore volume 1.14 ml/g, using an MOD-220 porosimeter (Carlo Erba, Milan, Italy) and SA-1000 surface-area, pore-volume analyser (Shibata, Tokyo, Japan).

2.3. ODA-modified silicas

As described previously [19,20], octadecylacrylate and 3-mercaptopropyltrimethoxysilane (20:1 molar ratio) were dissolved in ethanol. Azobisisobutyronitrile (0.1%, w/w, for monomers) was added to the solution at 80°C. The mixture was stirred for 6 h at 80°C under an N_2 gas atmosphere. The white precipitates obtained were gathered by filtration, washed successively with methanol and acetone and dried in vacuo. The product was immobilized on porous silicas by mixing in tetrachloromethane at 80°C. After stirring for 24 h, the silica suspension was filtered with a glass filter (1 μm), washed several times with chloroform, carbon tetrachloride and dried in vacuo, finally producing ODA-modified silicas for HPLC. The average degree of polymerization of ODA-modified silicas was determined to be 19 by elemental analysis as well as IR and NMR spectroscopies.

2.4. ODS-modified silicas

As described previously [9,21–22], 7 g of dried silica was added to 70 ml of a 3.4% solution of octadecyldimethylchlorosilane in dry toluene containing 3 ml of triethylamine. The silica suspension was refluxed for 5 h, filtered through a glass filter (1 μm), washed several times with toluene, chloroform, methanol and acetone and then dried in vacuo at 70°C for 2 days. The carbon contents of the treated silicas were determined to be 16.4% by elemental analysis using an elemental analyzer as ODA-modified silicas.

2.5. Apparatus

The HPLC measurements were carried out on a Twincle instrument (Jasco, Tokyo, Japan), equipped with a Uvidec-100 IV variable-wavelength detector (Jasco, Tokyo, Japan) and a column of 150×4.6 mm I.D., packed with ODA- or ODS-modified silicas.

2.6. Sample preparation

As previously described [18], 1.5 g of *Panax Ginseng* or *Red Ginseng* were extracted with 100 ml of acetonitrile–distilled water (7:3). The mixture was refluxed in a hot water bath for 30 min, and was mixed ultrasonically (45 W, 38 kHz, 10 min). After centrifugation (1000 *g*, 10 min), the upper phase was filtered through a membrane filter (0.22 μm), and was rewashed with 10 ml of acetonitrile–distilled water (7:3). After the solvent was evaporated off, the residue was dissolved in 200 ml of distilled water. And the aqueous layer was washed with 100 ml of diethyl ether to produce the fatless solvent. And then, the solvent was extracted with *n*-butanol (30 ml×3). The total butanol layer was washed with 1% aqueous potassium hydroxide (30 ml×2), and evaporated off under low temperature, carefully. The remaining

residue was made up to volume with acetonitrile in a 20 ml volumetric flask, then an aliquot of the upper phase was injected onto the HPLC system. Results were reported as averages of triplicate determinations.

Results and discussion

The distribution of the pore diameter and the mean particle size were shaped sharp and narrow in the chart of original porous silicas.

The chemical structure of ODA-modified silicas were determined by ^{1}H-NMR spectroscopy. As described previously [19,20], the average degree of polymerization was also estimated to be 19 by ^{1}H-NMR spectroscopy ($SiOCH_3$: δ=3.58 ppm, $C(=O)OCH_2$: δ=4.15 ppm in C^2HCl_3). The resolution value (R_s) of *paeoniflorin* in *Paeoniae Radix* versus *p*-hydroxyacetophenone on ODA- or ODS-modified silicas were determined to be 4.10 and 9.65 under the HPLC conditions [mobile phase: acetonitrile–distilled water (1:4), flow-rate: 0.3 ml/min, detection: 230 nm UV, column: 150 mm×4.6 mm I.D.], respectively. And then, the (R_s) value of *naringin* versus *hesperidin* in *Citrus Unshiu Peel* were determined to be 3.57 and 3.85 under the HPLC conditions [mobile phase: acetonitrile–distilled water–acetic acid (20:80:2), flow-rate: 1 ml/min, detection: 285 nm UV, column: 150 mm×4.6 mm I.D.].

Fig. 1 shows the correlations between the retention factors of some components of ethical extract products in oriental medicine formulations on ODA-modified silica versus ODS-modified silica.

The retention behaviour of *naringin*, *hesperidin* in *Citrus Unshiu Peel* (as the models of oxygen–heterocyclic compounds), *glycyrrhizin* in *Glycyr-*

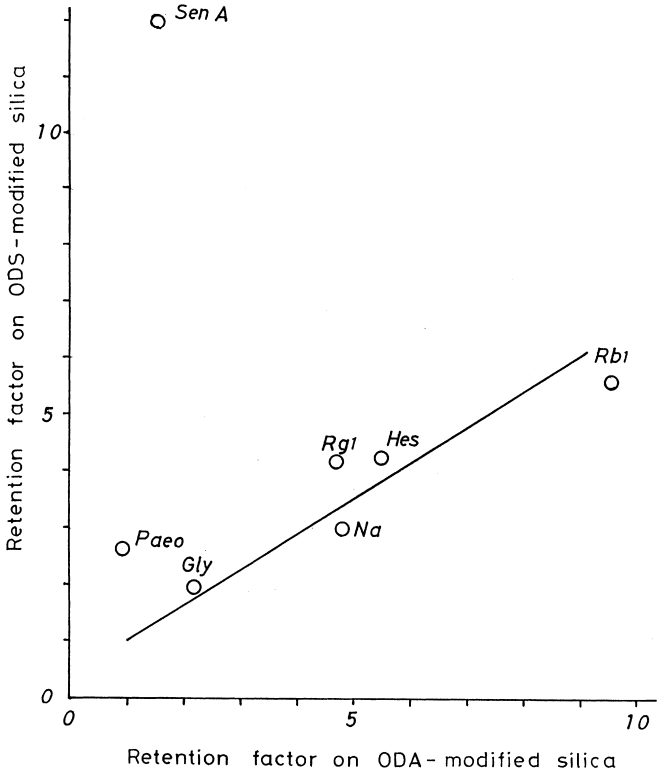

Fig. 1. The correlations between the retention factors of some components of ethical extract products in oriental medicine formulations on ODA-modified silica versus ODS-modified silica. Rb1: *ginsenoside Rb1*, Rg1: *ginsenoside Rg1*, Sen A: *sennoside A*, Na: *naringin*, Hes: *hesperidin*, Gly: *glycyrrhizin*, Paeo: *paeoniflorin*.

rhiza glabra (as a model of pentacyclic triterpenoids), and *ginsenosides* Rg_1 and Rb_1 in *Panax Ginseng* (as the models of saponins) were a slightly linear relationship for most of the sample compounds, on ODA-modified silica versus ODS-modified silica. However, on ODA-modified silica versus ODS-modified silica, the retention behaviour was not linear for *paeoniflorin* in *Paeonia albiflora* (as a model of modified monoterpens) and *sennoside A* in *Cassia angustifolia* (as a model of bimolecular anthraquinoids). It was apparent that the retention was not primarily dependent on the hydrophobic effect but seemed to be greatly dependent on the presence of highly-oriented comb-like structures and their effect on selectivity of ODA-modified silica [19,20], while comparing with the ODS-modified silica.

Figs. 2–4 show typical liquid chromatograms obtained with *ginsenosides Rb1* and *Rg1* in commercial *Panax Ginseng* on ODA-, ODS-modified silica, and commercial Nucleosil $5C_{18}$ column. As can be seen in Figs. 2–4, *ginsenosides Rb1* and *Rg1* in commercial *Panax Ginseng* were separated on ODA-modified silicas as well as ODS-modified silicas (of course, including commercial Nucleosil $5C_{18}$ column). And then, *ginsenosides Rb1* and *Rg1* in commercial *Red Ginseng* were separated on ODA-modified silicas.

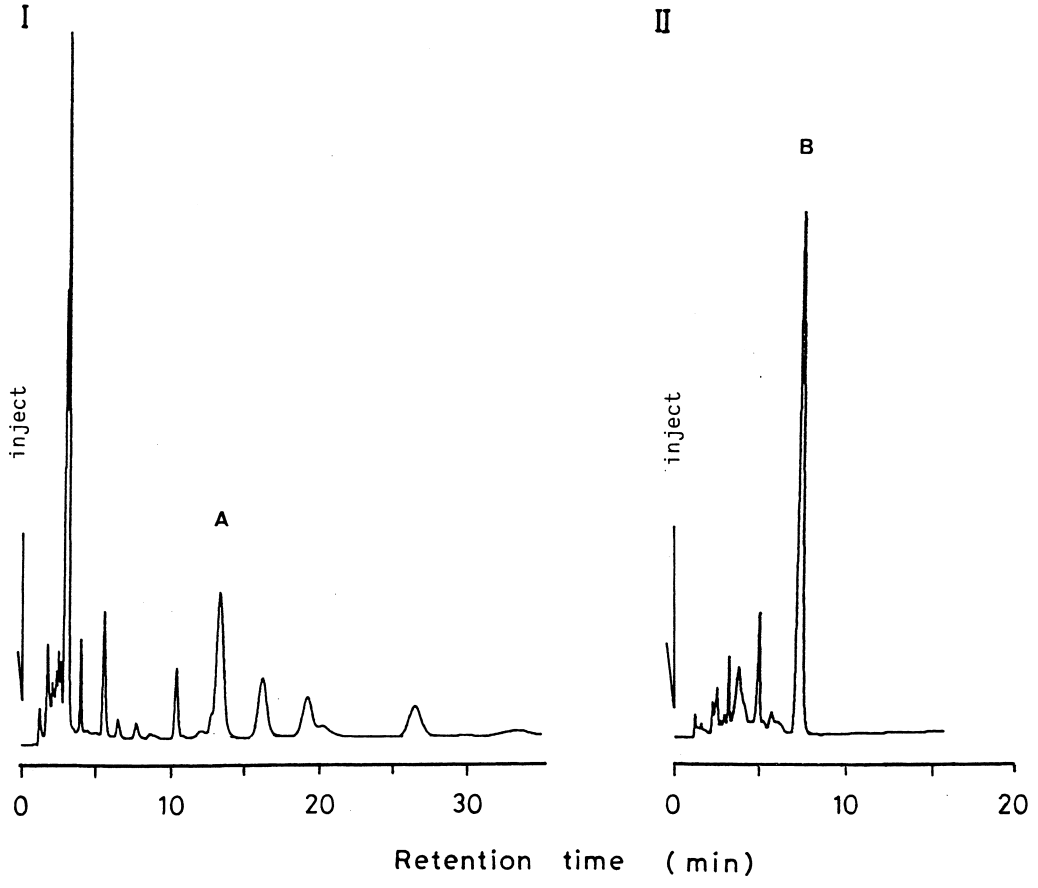

Fig. 2. The chromatographic behaviour of *ginsenoside Rb1* and *Rg1* in commercial *Panax Ginseng* on ODA-modified silica. (A) *Ginsenoside Rb1.* (B) *Ginsenoside Rg1.* HPLC conditions: (I) Acetonitrile–distilled water (33:67). (II) Acetonitrile–distilled water (25:75). Column: 150 mm×4.6 mm ID. Flow-rate: 1.0 ml/min. Detection: 203 nm UV.

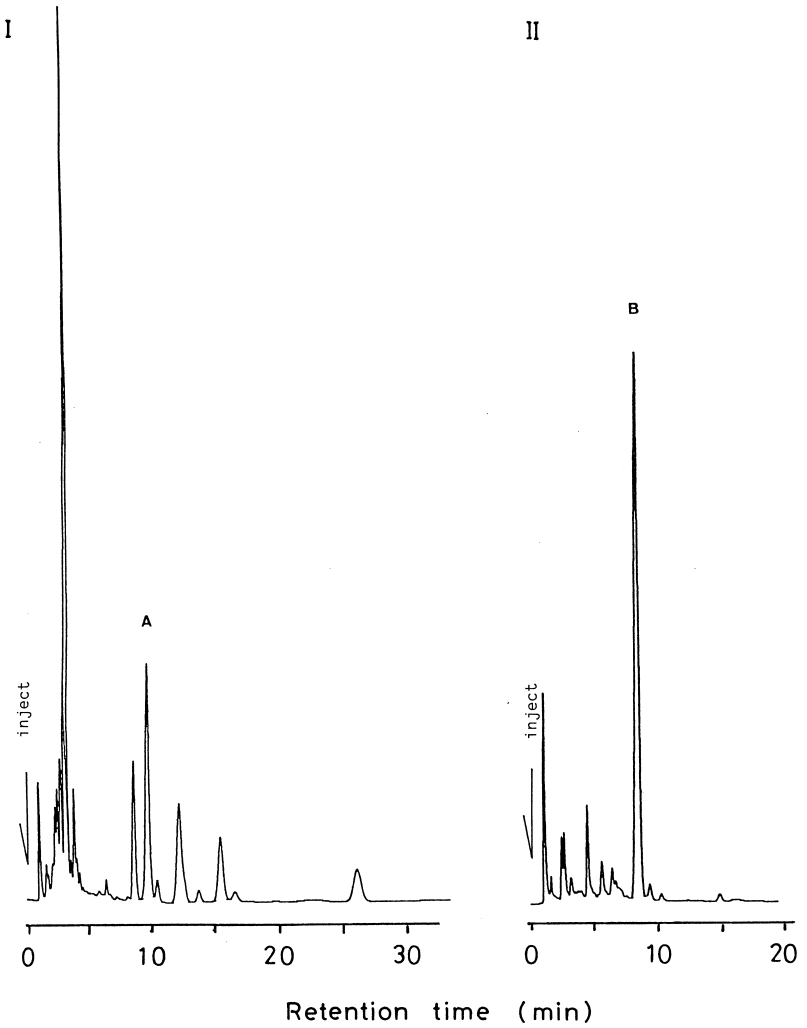

Fig. 3. The chromatographic behaviour of *ginsenosides Rb1* and *Rg1* in commercial *Panax Ginseng* on ODS-modified silica. (A) *Ginsenoside Rb1.* (B) *Ginsenoside Rg1.* HPLC conditions: flow-rate: 0.7 ml/min. Other conditions as in Fig. 2.

Table 1 shows the *ginsenosides* Rg_1 and Rb_1 contents in commercial *Panax Ginseng* and *Red Ginseng*.

These results also show that some components of extract products in oriental medicine formulations can be separated on ODA-modified silica as well as ODS-modified silica.

It is concluded from the present investigation that it is not sufficient to evaluate column gels solely on the basis of the carbon content of chemically bonded reversed-phase materials, because it is possible for polyfunctional silanes such as the trimethoxysilyl-terminated poly ODA to attach at the reactive end of a previously anchored group. The pore size distribution of the silica supports, the amount of residual silanol groups [23], the metal impurities in silica supports [24–28], the secondary or tertiary interactions [29] with endcapping, the bulkiness of the ligands bonded to the silica and the molecular size of the solute must also be considered. And then,

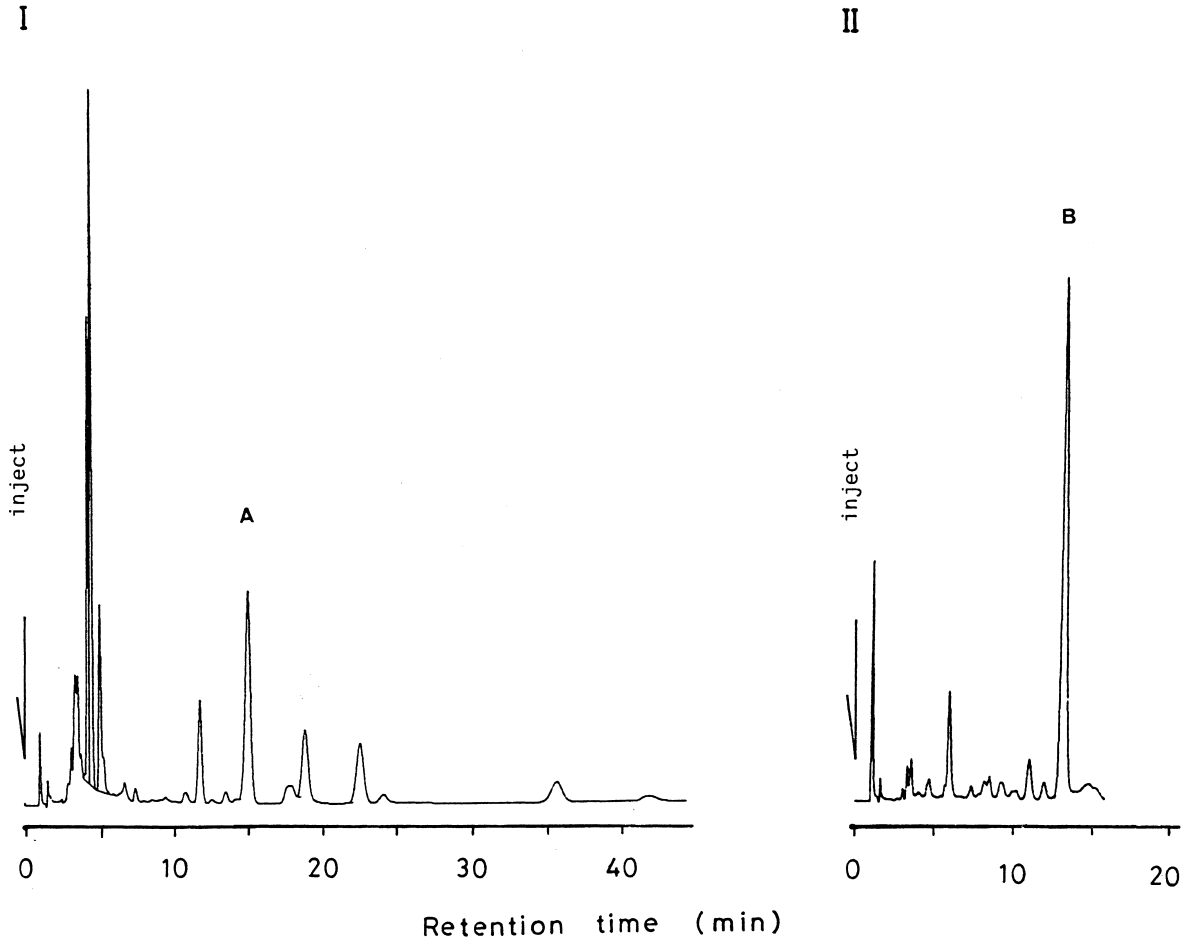

Fig. 4. The chromatographic behaviour of *ginsenosides Rb1* and *Rg1* in commercial *Panax Ginseng* on commercial Nucleosil $5C_{18}$ column. (A) *Ginsenoside Rb1.* (B) *Ginsenoside Rg1.* HPLC conditions: column: 250 mm×4.6 mm I.D. Other conditions as in Fig. 2.

Table 1
Ginsenosides Rg_1 and Rb_1 contents in commercial *Panax Ginseng* and *Red Ginseng* (n=12)

Sample		*Ginsenoside* Rg_1 (%)	*Ginsenoside* Rb_1 (%)	Recovery (%)	RSD (%)
Panax Ginseng	Sample 1	0.401	0.320	96.1–98.4	2.08
	Sample 2	0.292	0.334	96.5–98.8	2.34
	Sample 3	0.282	0.290	95.2–98.6	2.33
Red Ginseng	Sample 4	0.400	0.454	94.4–97.3	2.16
	Sample 5	0.363	0.453	94.6–98.6	2.20
	Sample 6	0.361	0.375	93.5–96.5	2.25

this paper is one of a part of a summary for new ODA-modified silicas and a hint of column preparation, also an idea to view in the future on HPLC columns.

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