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Retention and enantioselectivity of 2-arylpropionic acid derivatives on an avidin-bonded silica column Influence of base materials, spacer type and protein modification

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Abstract

The influences of pore sizes of base materials, spacer type and protein modification on the chiral resolution of 2-arylpropionic acid derivatives on avidin (AVD)-bonded silica materials were investigated. With regard to the pore sizes of the base silica materials, 120-Å materials gave higher enantioselectivity and resolution than 300-Å materials. With regard to spacer type, aminopropyl (AP)-silica gels activated by N,N′-disuccinimidyl carbonate (DSC) and N,N′-disuccinimidyl suberate (DSS) and glycerylpropyl (GP)-silica gels activated by 1,1′-carbonyldiimidazole (CDI) were compared. The AP-silica gels activated by DSC gave the highest protein coverage and resolution. Modified AVD-bonded materials were prepared by reaction with glutaraldehyde, glyceraldehyde and benzaldehyde. The retentive and enantioselective properties of 2-arylpropionic acid derivatives on these modified AVD columns were compared with those on an unmodified AVD column. The retentions of 2-arylpropionic acid derivatives on the modified AVD columns were shorter than those on the unmodified AVD column, whereas the modified AVD columns gave lower or approximately equal enantioselectivity compared with the unmodified AVD column. These results may be attributed to a decrease in the number of ion-exchange sites for retention and chiral recognition, and/or changes in protein conformation as a result of modification.

1. Introduction

Glycoprotein-bonded stationary phases including α_1-acid glycoprotein [1], ovomucoid (OVM) [2], avidin (AVD) [3] and cellobiohydrolase [4] have been developed for the separation of enantiomers. AVD, of molecular mass 68 300 and isoelectric point (pI) 10.0, is well known to biochemists because of its strong binding with biotin [5]. AVD-bonded silica materials were developed by Miwa et al. [3] and showed excellent chiral recognition for 2-arylpropionic acid derivatives. It is well known that many factors such as physical properties of base silica materials, spacer type and bonding method affect the resolution of enantiomeric pairs on protein-bonded stationary phases [6–9]. In this study, we investigated the influences of these factors on the retention, enantioselectivity and resolution of 2-

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arylpropionic acid derivatives on unmodified and modified AVD-bonded silica columns.

2. Experimental

2.1. Reagents and materials

1-Butanol, 2-butanol, *tert.*-butanol, 1-propanol, 2-propanol, ethanol, methanol and acetonitrile of HPLC grade were obtained from Wako (Osaka, Japan). Ibuprofen, ketoprofen, flurbiprofen, fenoprofen calcium and pranoprofen were kindly donated by Kaken Pharmaceutical (Tokyo, Japan), Chugai Pharmaceutical (Tokyo, Japan), Yamanouchi Pharmaceutical (Tokyo, Japan) and Yoshitomi Pharmaceutical (Osaka, Japan). The structures of these compounds are shown in Fig. 1. AVD proteins from egg white were kindly donated by Eisai (Tokyo, Japan). N,N′-Disuccinimidyl carbonate (DSC) and 1,1′-carbonyldiimidazole (CDI) were purchased from Sigma Chemical (St. Louis, MO, USA) and N,N′-disuccinimidyl suberate (DSS) from Pierce (Rockford, IL, USA). Silica gels (Ultron-120, particle diameter 5 μm, pore size 120 Å, specific surface area 300 m^2/g, and Ultron-300 particle diameter 5 μm, pore size 300 Å, specific surface area 100 m^2/g) were obtained from Shinwa Chemical Industries (Kyoto, Japan). Other solvents and reagents were used as received.

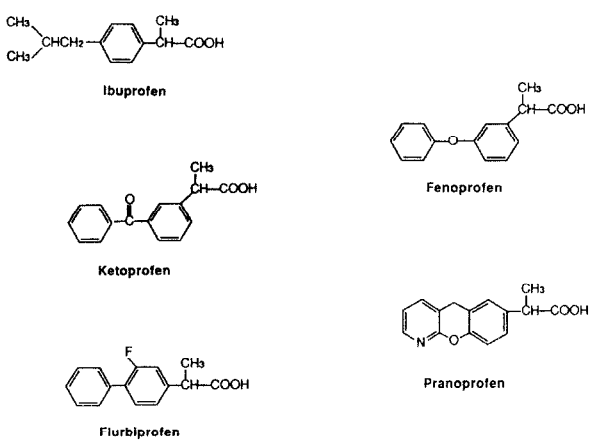

Fig. 1. Structures of the 2-arylpropionic acid derivatives.

Water purified with a Nanopure II unit (Barnstead, Boston, MA, USA) was used for the preparation of the eluent and sample solutions.

Preparation of silica gels having different spacer types

Silica gels (5 g) were dried in vacuo over P_2O_5 at 150°C for 6 h and the dry silica gels were added to 120 ml of dry toluene. The mixture was heated to reflux until all the water had been removed as an azeotrope into a Dean–Stark-type trap. Next, 3-aminopropyltrimethoxysilane and 3-glycidoxypropyltrimethoxysilane, corresponding to 10 μmol/m^2 of the specific surface area, were added and reacted for 8 and 48 h, respectively. The reaction mixture was cooled to room temperature, filtered and washed with toluene and methanol. The isolated silica gels were dried in vacuo over P_2O_5 at 60°C for 2 h. 3-Aminopropyl (AP)-silica gel was used for the activation reaction described below. To the silica gels reacted with 3-glycidoxypropyltrimethoxysilane, 80 ml of perchloric acid (pH 3.0) were added and the mixture was refluxed for 4 h. The reaction mixture was cooled to room temperature, filtered and washed with water and methanol. The isolated 3-glycerylpropyl (GP)-silica gel was dried in vacuo over P_2O_5 at 60°C for 2 h and used for the activation reaction.

Activation of silica gels having amino or hydroxyl groups

The AP-silica gels were activated with DSC or DSS. Five grams of the gels were slurried in 70 ml of acetonitrile and reacted with 5 g of DSC or 7.2 g of DSS for 24 h at 30°C. The reaction mixture was filtered and washed with acetonitrile and methanol. The GP-silica gels were activated by CDI. Five grams of the gels were slurried in 30 ml of dioxane. To the mixture, 4 g of CDI dissolved in 20 ml of dioxane were added and reacted for 4 h at 30°C. The reaction mixture was filtered and washed with dioxane and methanol. Both activated silica gels were dried in vacuo over P_2O_5 at 60°C for 2 h.

Preparation of AVD-bonded materials

AVD-proteins were bound to the DSC or DSS-activated AP-silica gels as follows: 2 g of the DSC or DSS-activated silica gels were slurried in 20 m*M* phosphate buffer (pH 6.8). To the mixture, 1 g of AVD-proteins dissolved in 20 ml of the same buffer was added slowly and stirred for 15 h at 30°C. Similarly, AVD was bound to CDI-activated silica gels as follows: 2 g of the CDI-activated GP-silica gels were slurried in 20 m*M* phosphate buffer (pH 7.8). To the mixture, 1 g of AVD-proteins dissolved in 20 ml of the same buffer was added slowly and stirred for 15 h at 30°C. Then both the reaction mixtures were filtered, washed with water and methanol and dried in vacuo over P_2O_5 at 40°C for 6 h.

Preparation of AVD-BENZ, AVD-DIOL and AVD-GA materials

The AVD-proteins were bound to aminopropylsilica gels (120-Å base silica) activated by DSC. The materials were then further modified with benzaldehyde, glyceraldehyde and glutaraldehyde.

Three grams of the AVD materials were added to 50 ml of 20 m*M* phosphate buffer (pH 7.5), then 200 mg of sodium cyanoborohydride, 200 mg of zinc sulfate and 50 mg of benzaldehyde dissolved in 1 ml of dioxane were added. After adjusting the pH to 7.5, the mixture was slowly rotated at 30°C for 15 h. Then the mixture was filtered and washed with water and methanol. The isolated materials (AVD-BENZ) were dried in vacuo over P_2O_5 at 40°C for 6 h.

Three grams of the AVD materials were added to 50 ml of 20 m*M* phosphate buffer (pH 7.5), then 200 mg of sodium cyanoborohydride, 200 mg of zinc sulfate and 250 mg of D-glyceraldehyde were added. After adjusting the pH to 7.5, the mixture was slowly rotated at 30°C for 15 h. Then the mixture was filtered and washed with water and methanol. The isolated materials (AVD-DIOL) were dried in vacuo over P_2O_5 at 40°C for 6 h.

Three grams of the AVD materials were added to 50 ml of 100 m*M* ammonium dihydrogenphosphate buffer solution (pH 4.4) and sonicated for 5 min, then 300 μl of 5% glutaraldehyde solution were slowly added. The mixture was slowly rotated at 30°C for 15 h, filtered and washed with 100 m*M* ammonium dihydrogenphosphate buffer solution (pH 4.4), 50 m*M* sodium phosphate buffer (pH 7.0), water and methanol. The isolated materials (AVD-GA) were dried in vacuo over P_2O_5 at 40°C for 6 h.

The unmodified and modified AVD-bonded materials were packed into a 100 × 4.6 mm I.D. stainless-steel column by the slurry packing method.

2.2. Instrumentation

Chromatography

The HPLC system used was composed of an LC-9A pump, an SPD-6A spectrophotometer, an SIL-6B autoinjector, a C-R4A integrator and an SCL-6B system controller (all from Shimadzu, Kyoto, Japan). The flow-rate was maintained at 0.8 ml/min. Detection was performed at 220 or 254 nm.

Capacity factors (k'), enantioseparation factors (α), resolutions (R_s) of racemates were calculated. All separations were carried out at 25°C using a CO-1093C column oven (Uniflows, Tokyo, Japan).

The eluents are prepared by using phosphoric acid–sodium dihydrogenphosphate or sodium dihydrogenphosphate–disodium hydrogenphosphate and organic modifier, whose content was calculated to give all of the eluents equal eluotropic strength, as reported by Iredale et al. [10]. The eluents used are specified in the figures and tables.

Elemental analysis

The elemental analysis of the AVD-bonded silica materials was performed using a Model NCH-12 analyser (Sumika Chemical Analysis Service, Osaka, Japan) for nitrogen or ion chromatography combined with the oxygen-flask method for sulfur.

Sample preparation

A known amount of a racemic solute was dissolved in methanol or water and the solution was diluted with the eluent to desired concen-

tration. A 20-μl aliquot of the sample solution was loaded on to a column. The amount loaded was 0.2–0.5 μg.

3. Results and discussion

3.1. Surface coverages of AVD-proteins

Table 1 shows the surface coverages of AVD-proteins on the AVD-bonded materials with different pore sizes of base silica materials and spacer type. With regard to comparison of the base silica gels having 120- and 300-Å pore sizes, the latter materials showed about 2.5-times higher surface coverages of the AVD-proteins. Only the outer surfaces of the former materials could be utilized for the binding of AVD, the molecular mass of which is 68 000. However, the amounts of AVD-proteins bound on the former materials were higher than those on the latter materials. With regard to comparison of spacer type, the AP materials activated by DSC had higher surface coverages than those activated by DSS, where C_6 carbon chains remain after cross-linking of the AP-silica and protein. The GP materials activated by CDI showed about one quarter of the surface coverages of the proteins compared with the AP materials activated by DSC. Even if one takes into account that the surface coverages of AP phases are double those of GP phases, DSC or DSS is more suitable than CDI as a cross-linking agent for binding of AVD. These results reveal that the largest amounts of AVD are bound to the 120-Å silica gels activated by DSC.

3.2. Effect of base silica materials and spacer for chiral recognition

Table 2 shows the influence of the pore sizes of base silica materials and spacer type on the retention, enantioselectivity and resolution of 2-arylpropionic acid derivatives on the AVD-bonded materials. With regard to comparison of base silica gels having 120- and 300-Å pore sizes, the former material showed slightly better enantioselectivity and resolution than the latter. This could be due to the binding of slightly larger amounts of the proteins for the former materials. The AP materials activated by DSC and DSS showed good enantioselectivity for all the 2-arylpropionic acid derivatives tested, whereas ibuprofen, ketoprofen and fenoprofen were not resolved on the GP materials activated by CDI. The AP materials activated by DSC showed better enantioselectivity and resolution than those activated by DSS. Previously, we reported [9] that ovomucoid-bonded materials using DSC and CDI as the cross-linker showed excellent enantioselectivity for a wide range of solutes, whereas the materials using DSS showed slight

Table 1
Comparison of surface coverages of AVD- and modified AVD-bonded materials

Spacer[a]	Cross-linker[b]	Pore size of base silica (Å)	AP or GP phase		Avidin phase	
			Carbon content (%)	Surface coverage[c] (μmol/m²)	Sulfur content (%)	Surface coverage[d] (μmol/g)
AP	DSC	120	3.23	3.3	0.16	3.1
AP	DSC	300	1.25	3.6	0.13	2.5
AP	DSS	120	3.23	3.3	0.13	2.5
GP	CDI	120	3.18	1.6	0.04	0.8

[a] AP and GP are aminopropyl and glycerylpropyl phases, respectively.
[b] DSC, DSS and CDI are N,N'-disuccinimidyl carbonate, N,N'-disuccinimidyl suberate and 1,1'-carbonyldiimidazole, respectively.
[c] Estimated from the nitrogen elemental analysis data.
[d] Estimated from the sulfur elemental analysis data.

Table 2
Comparison of base silica materials and spacer type with respect to retention, enantioselectivity and resolution of various solutes on an unmodified AVD-bonded material

Column	Ibuprofen			Ketoprofen			Flurbiprofen			Fenoprofen			Pranoprofen		
	k'_1	α	R_s	k'_1	α	R_s	k'_1	α	R_s	k'_1	α	R_s	k'_1	α	R_s
DSC-120	9.43	1.76	5.45	16.6	2.51	7.51	16.1	2.09	7.99	16.2	1.85	6.69	53.6	1.33	3.54
DSC-300	5.11	1.58	2.79	8.42	2.01	4.76	10.7	1.97	3.89	8.96	1.72	3.40	26.7	1.27	2.08
DSS-120	7.11	1.71	3.42	11.5	2.06	5.61	12.5	1.94	4.73	11.9	1.81	4.77	33.4	1.12	1.15
CDI-120	2.10	1.00		3.28	1.00		10.3	1.12	1.20	4.58	1.00		5.74	1.22	1.62

HPLC conditions: column, an unmodified AVD-bonded material packed into a 100 × 4.6 mm I.D. stainless-steel column; eluent, 20 m*M* phosphate buffer (pH 7.0) containing 2.14% ethanol; flow-rate, 0.8 ml/min.

enantioselectivity only for oxprenolol. It was thought that superfluous achiral interactions of a solute with the hydrophobic spacer might diminish the chiral interactions of a solute with the OVM-protein. It is interesting that the AVD-bonded materials activated by DSS gave excellent enantioselectivity for 2-arylpropionic acid derivatives. These results reveal that a suitable

Table 3
Effect of eluent pH on retention and enantioselectivity of 2-arylpropionic acid derivatives on unmodified and modified AVD-bonded materials

Column	Compound	pH 6.0[a]		pH 6.5[a]		pH 7.0[a]		pH 7.5[a]	
		k'_1	α	k'_1	α	k'_1	α	k'_1	α
AVD	Ibuprofen	9.95	1.82	6.47	1.89	4.78	1.94	3.48	2.01
	Ketoprofen	20.5	1.98	13.4	2.03	9.98	2.08	7.44	2.15
	Flurbiprofen	19.6	2.36	12.9	2.45	9.76	2.56	7.12	2.55
	Fenoprofen	18.9	1.70	12.6	1.72	9.58	1.75	7.06	1.80
	Pranoprofen	73.9	1.24	49.5	1.24	35.8	1.27	26.8	1.26
AVD-BENZ	Ibuprofen	9.68	1.68	6.25	1.73	4.54	1.79	3.32	1.86
	Ketoprofen	17.3	1.87	11.5	1.93	8.46	1.99	6.30	2.06
	Flurbiprofen	20.0	1.98	12.7	2.05	9.29	2.13	6.58	2.18
	Fenoprofen	17.0	1.61	11.1	1.65	8.29	1.69	6.15	1.75
	Pranoprofen	66.3	1.15	42.2	1.18	30.4	1.19	22.5	1.20
AVD-DIOL	Ibuprofen	9.98	1.66	6.04	1.71	3.97	1.75	2.76	1.80
	Ketoprofen	18.1	1.85	10.8	1.90	7.32	1.98	5.24	2.11
	Flurbiprofen	20.3	2.08	12.1	2.13	7.90	2.18	5.43	2.21
	Fenoprofen	17.2	1.58	10.6	1.62	7.20	1.66	5.03	1.73
	Pranoprofen	64.8	1.16	37.4	1.19	25.3	1.20	18.3	1.19
AVD-GA	Ibuprofen	8.78	1.79	5.26	1.79	3.89	1.90	2.89	1.94
	Ketoprofen	15.3	1.90	10.8	1.93	7.59	2.01	5.78	2.04
	Flurbiprofen	16.9	2.25	11.3	2.40	8.46	2.41	6.20	2.47
	Fenoprofen	14.7	1.60	9.93	1.61	7.52	1.64	5.59	1.71
	Pranoprofen	58.5	1.23	38.1	1.26	26.3	1.32	20.6	1.28

All materials were prepared by using aminopropylsilica gels (120-Å base silica) activated by DSC. HPLC conditions: column, unmodified and modified AVD-bonded materials packed into a 100 × 4.6 mm I.D. stainless-steel column; eluent, 20 m*M* phosphate buffer containing 2.07% *tert.*-butanol; flow-rate, 0.8 ml/min.
[a] Buffer pH.

spacer should be selected for binding of a protein.

On the other hand, Oda et al. [11] reported that DSS is a better spacer than DSC for direct sum injection assays for drug enantiomers on an AVD-bonded material. However, our results reveal that DSC is a better spacer than DSS for the chiral resolution of 2-arylpropionic acid derivatives on the AVD-bonded materials.

3.3. Effect of eluent pH on retention and enantioselectivity of 2-arylpropionic acid derivatives on unmodified and modified AVD-bonded materials

The AVD proteins were bound to aminopropylsilica gels (120-Å base silica) activated by DSC and used in the following experiments. The amino groups of the AVD materials were partially reacted with benzaldehyde, D-glyceraldehyde and glutaraldehyde. However, it was difficult to detect any differences before and after modification. Table 3 shows the effect of eluent pH on the retention and enantioselectivity of 2-arylpropionic acid derivatives on AVD, AVD-BENZ, AVD-DIOL and AVD-GA columns, with 20 m*M* phosphate buffer containing 2.07% *tert.*-butanol as the eluent. The capacity factors (k'_1) of the first-eluted enantiomers of 2-arylpropionic acid derivatives on the AVD-bonded columns decreased with an increase in eluent pH. Taking into account the p*I* of AVD of 10.0 and the pK_a of 2-arylpropionic acid derivatives of 4.0–4.5, the bound AVD-protein and solutes are positively and negatively charged, respectively, over the eluent pH range tested. These results reveal that hydrophobic and electrostatic interactions should play an important role in the retention properties of solutes on the AVD-bonded columns. The highest enantioselectivity (α) of these compounds was attained at an eluent pH of 7.0 or 7.5.

On the modified AVD-bonded columns, the k'_1 of 2-arylpropionic acid derivatives was slightly decreased except for flurbiprofen on the AVD-BENZ column and ibuprofen and flurbiprofen on the AVD-DIOL column at an eluent pH of 6.0 (Table 3). The α values of these compounds were almost the same or decreased except for separation of pranoprofen on the AVD-GA column. The retentive and enantioselective tendencies obtained with the modified AVD columns were similar to those with the unmodified column. Fig. 2 shows the chiral resolution of pranoprofen on the unomodified and modified AVD columns. The k'_1 value of pranoprofen decreased on the AVD-BENZ, AVD-DIOL and AVD-GA columns compared with that on the unmodified AVD column. The AVD-GA column gave a higher enantioselectivity than the unmodified AVD column under the conditions employed. These results reveal that a decrease in the number of ion-exchange sites for retention and chiral recognition, and/or changes in protein

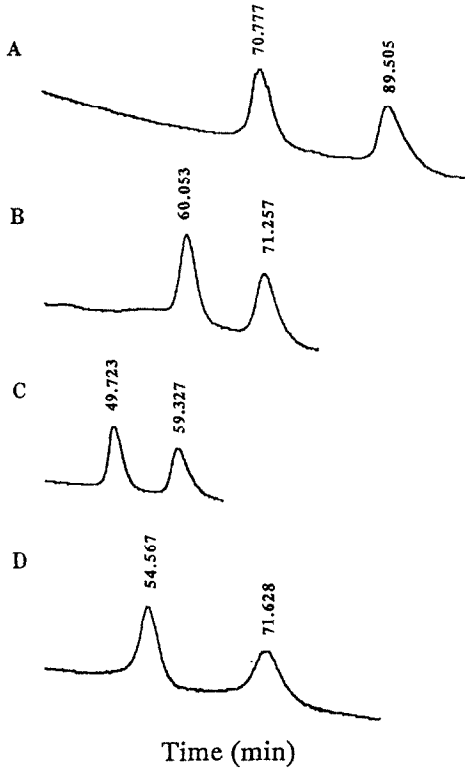

Fig. 2. Chiral resolution of pranoprofen on (A) AVD, (B), AVD-BENZ, (C) AVD-DIOL and (D) AVD-GA columns. HPLC conditions: column, unmodified and modified AVD-bonded materials packed into a 100 × 4.6 mm I.D. stainless-steel column; eluent, 20 m*M* phosphate buffer containing 2.07% *tert.*-butanol; flow-rate, 0.8 ml/min; injection volume, 20 μl (20 μg/ml).

conformation could occur as a result of modification.

3.4. Effect of organic modifier on enatioselectivity of 2-arylpropionic acid derivatives on unmodified and modified AVD-bonded materials

Table 4 shows the effects of *tert.*-butanol content on the retention and enantioselectivity of 2-arylpropionic acid derivatives on the AVD, AVD-BENZ, AVD-DIOL and AVD-GA columns, with 20 m*M* phosphate buffer (pH 7.0) containing *tert.*-butanol as the eluent. With an increase in *tert.*-butanol content, the α values of ibuprofen, ketoprofen and fenoprofen decreased on the all columns, whereas with an increase in *tert.*-butanol content, those of flurbiprofen and pranoprofen increased. It is interesting that the retentions of both flurbiprofen and pranoprofen enantiomers decreased with an increase in *tert.*-butanol content, but the enantioselectivities of flurbiprofen and pranoprofen increased. Similar results were obtained with the use of 2-propanol as an organic modifier.

Table 5 shows the effects of type of organic modifier on the retention and enantioselectivity of 2-arylpropionic acid derivatives on the AVD, AVD-BENZ, AVD-DIOL and AVD-GA columns, with 20 m*M* phosphate buffer (pH 7.0) containing various organic modifiers as the eluent. The use of *tert.*-butanol as the organic

Table 4
Effect of organic modifier content on retention and enantioselectivity of 2-arylpropionic acid derivatives on unmodified and modified AVD-bonded materials

Column	Compound	*tert.*-Butanol content (%)					
		1.04		2.07		4.14	
		k'_1	α	k'_1	α	k'_1	α
AVD	Ibuprofen	7.23	1.92	4.78	1.94	2.44	1.87
	Ketoprofen	15.6	2.26	9.98	2.08	4.68	1.82
	Flurbiprofen	13.6	2.35	9.76	2.56	5.40	2.68
	Fenoprofen	14.3	1.97	9.58	1.75	4.77	1.51
	Pranoprofen	57.5	1.16	35.8	1.27	16.0	1.44
AVD-BENZ	Ibuprofen	6.85	1.87	4.54	1.79	2.54	1.66
	Ketoprofen	13.0	2.20	8.46	1.99	4.26	1.72
	Flurbiprofen	12.8	2.09	9.29	2.13	5.72	2.13
	Fenoprofen	12.3	1.90	8.29	1.69	4.58	1.45
	Pranoprofen	48.8	1.12	30.4	1.19	13.6	1.33
AVD-DIOL	Ibuprofen	6.33	1.82	3.97	1.75	2.19	1.64
	Ketoprofen	12.1	2.23	7.32	1.98	3.78	1.17
	Flurbiprofen	11.5	2.11	7.90	2.18	4.96	2.20
	Fenoprofen	11.2	1.90	7.20	1.66	3.97	1.42
	Pranoprofen	44.0	1.10	25.3	1.20	12.1	1.33
AVD-GA	Ibuprofen	6.01	1.95	3.89	1.90	2.13	1.77
	Ketoprofen	11.9	2.17	7.59	2.01	3.81	1.73
	Flurbiprofen	12.2	2.25	8.46	2.41	5.02	2.47
	Fenoprofen	11.4	1.86	7.52	1.64	4.04	1.42
	Pranoprofen	42.0	1.21	26.3	1.32	12.6	1.44

All materials were prepared by using aminopropylsilica gels (120-Å base silica) activated by DSC. HPLC conditions: column, unmodified and modified AVD-bonded materials packed into a 100 × 4.6 mm I.D. stainless-steel column; eluent, 20 m*M* phosphate buffer (pH 7.0) containing *tert.*-butanol; flow-rate 0.8 ml/min.

Table 5
Effect of type of organic modifier on retention and enantioselectivity of 2-arylpropionic acid derivatives on unmodified and modified AVD-bonded materials

Column	Organic modifier	Ibuprofen		Ketoprofen		Flurbiprofen		Fenoprofen		Pranoprofen	
		k'_1	α	k'_1	α	k'_1	α	k'_1	α	k'_1	α
AVD	Methanol	17.5	1.65	24.6	2.25	20.1	2.26	22.3	2.03	78.9	1.14
	Ethanol	9.43	1.76	16.6	2.51	16.1	2.09	16.2	1.85	53.6	1.33
	1-Propanol	3.09	1.69	5.36	1.56	7.64	2.54	5.42	1.39	17.2	1.54
	2-Propanol	5.60	1.90	10.9	1.98	11.5	2.55	10.6	1.72	36.0	1.33
	1-Butanol	2.40	1.70	4.24	1.57	5.96	2.64	4.43	1.35	13.1	1.62
	2-Butanol	2.77	1.75	5.11	1.65	6.67	2.63	5.17	1.42	16.5	1.52
	tert.-Butanol	4.78	1.94	9.98	2.08	9.76	2.56	9.58	1.75	35.8	1.27
	Acetonitrile	10.1	1.09	9.06	1.74	14.2	1.82	9.17	1.56	30.5	1.33
AVD-BENZ	Methanol	14.3	1.59	18.6	2.22	16.5	2.05	17.7	2.05	65.8	1.08
	Ethanol	8.25	1.65	13.0	2.10	13.2	2.03	12.4	1.88	45.8	1.13
	1-Propanol	2.97	1.44	4.62	1.53	7.11	1.95	4.85	1.37	14.5	1.38
	2-Propanol	4.94	1.76	8.58	1.95	9.77	2.08	8.55	1.70	30.3	1.19
	1-Butanol	2.35	1.49	3.73	1.51	5.90	2.07	4.09	1.32	10.9	1.46
	2-Butanol	2.77	1.54	4.58	1.60	6.70	2.06	4.89	1.39	14.4	1.37
	tert.-Butanol	4.54	1.79	8.46	1.99	9.29	2.13	8.29	1.69	30.4	1.19
	Acetonitrile	8.38	1.00	7.01	1.72	11.5	1.54	7.11	1.53	24.3	1.21
AVD-DIOL	Methanol	14.9	1.54	18.5	2.26	16.7	2.07	17.2	1.99	60.2	1.08
	Ethanol	8.41	1.61	12.7	2.14	13.3	2.06	12.5	1.87	42.1	1.13
	1-Propanol	2.90	1.45	4.69	1.53	6.86	2.02	4.84	1.35	14.5	1.37
	2-Propanol	4.81	1.69	8.25	1.96	9.36	2.12	8.11	1.69	28.5	1.19
	1-Butanol	2.17	1.54	3.59	1.51	5.50	2.13	3.87	1.31	10.4	1.46
	2-Butanol	2.62	1.53	4.40	1.59	6.18	2.13	4.61	1.37	13.6	1.37
	tert.-Butanol	3.97	1.75	7.32	1.98	7.90	2.18	7.20	1.66	25.3	1.20
	Acetonitrile	8.61	1.00	6.99	1.73	11.7	1.53	7.07	1.52	22.7	1.21
AVD-GA	Methanol	13.8	1.56	18.5	2.10	16.0	2.22	16.9	1.97	61.2	1.13
	Ethanol	7.55	1.71	12.3	2.11	12.6	2.25	11.9	1.85	43.3	1.19
	1-Propanol	2.55	1.64	4.27	1.52	6.60	2.39	4.51	1.34	13.9	1.51
	2-Propanol	4.49	1.84	8.10	1.93	9.24	2.38	8.09	1.66	28.2	1.27
	1-Butanol	1.99	1.69	3.33	1.52	5.40	2.50	3.70	1.30	9.92	1.70
	2-Butanol	2.31	1.24	4.08	1.60	6.02	2.54	4.36	1.37	13.1	1.47
	tert.-Butanol	3.89	1.90	7.59	2.01	8.46	2.41	7.52	1.64	26.3	1.32
	Acetonitrile	7.66	1.07	6.77	1.73	11.1	1.71	6.86	1.52	23.2	1.27

All materials were prepared by using aminopropylsilica gels (120-Å base silica) activated by DSC. HPLC conditions: column, unmodified and modified AVD-bonded materials packed into a 100 × 4.6 mm I.D. stainless-steel column; eluent, 20 m*M* phosphate buffer (pH 7.0) containing an organic modifier, with concentrations of 2.47% for methanol, 2.14% for ethanol, 2.03% for 1-propanol and 2-butanol, 2.00% for 2-propanol and 1-butanol, 2.07% for *tert.*-butanol and 2.86% for acetonitrile; flow-rate, 0.8 ml/min.

modifier gave the highest enantioselectivity for the chiral resolution of ibuprofen on the unmodified and modified AVD-bonded columns. However, using acetonitrile the ibuprofen enantiomers were slightly resolved on the AVD-bonded columns but were not resolved on the modified AVD columns. For flurbiprofen, the enantioselectivities obtained with alkanol modifiers were better than those with acetonitrile on the unmodified and modified AVD columns. For ketoprofen and fenoprofen, the use of methanol, ethanol, 2-propanol and *tert.*-butanol gave better enantioselectivities with the unmodified and modified AVD columns. However, alkanols such as 1-propanol, 1-butanol and 2-butanol, having long alkyl chains, were not good organic modifiers for ketoprofen and fenoprofen. For pranoprofen, 1-propanol, 1-butanol and 2-butanol were better organic modifiers on the unmodified and modified AVD columns. Selection of a suitable organic modifier is an important factor in achieving good resolution.

Throughout the study, we used two columns packed with unmodified AVD materials, whereas only one column packed with modified materials was used. The results suggest that the modified AVD column is more stable than the unmodified column, as reported previously for a modified OVM column [12].

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