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Characteristics of C_4- and C_8-bonded vinyl alcohol copolymer gels for reversed-phase high-performance liquid chromatography

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ABSTRACT

The fundamental characteristics of C_4- and C_8-bonded polymer (C4P and C8P) gels developed for reversed-phase high-performance liquid chromatography and obtained by introduction of butyryl and octanoyl groups, respectively, at the hydroxyl groups of vinyl alcohol copolymers were investigated and compared with the characteristics of a previously developed C_{18}-bonded polymer (ODP) gel obtained in a similar manner and with those of commercial C_4-, C_8- and C_{18}-bonded silica gels.

For both alkyl alcohols and standard proteins, the retention strength on the polymer gels clearly increased with increasing number of carbons in the bonded alkyl group and thus in the order C4P, C8P, ODP. The C4P and C8P gels, like the ODP gel and in clear contrast to the silica gels, displayed excellent tolerance towards acidic, alkaline and buffered eluents. They also exhibited minimal shrinking and swelling effects with variations in eluent polarity, as measured by their solvent regain.

INTRODUCTION

In reversed-phase liquid chromatography (RP-LC), which is now predominant in high-performance liquid chromatographic (HPLC) applications, columns have generally been packed with C_{18}-bonded polymer or silica gels.

Asahipak ODP-50, a commercially available column packed with a hard gel composed of rigid polymers with octadecyl (C_{18}) groups, is known to display little shrinkage and swelling with varying eluent polarity, unlike conventional polymer-based gel columns, and to equal or exceed conventional octadecylsilica (ODS) columns in separation effficiency. It is also known to display excellent tolerance to both acidic and alkaline elution, in contrast to the generally poor chemical stability and short service life of ODS columns[1,2].

In recent years, the need has grown for weakly hydrophobic gels which would allow more efficient analysis of peptides and proteins. C_1-, C_4- and C_8-bonded silica

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gels, with relatively short alkyl groups, have partially met this need, but have also displayed a tendency for even lower chemical stability than the ODS gels and thus are restricted to a narrow range of chromatographic conditions[3,4].

The C_4- and C_8-bonded polymer (C4P and C8P) gels investigated in this study were therefore developed to meet the requirement for weak hydrophobicity while displaying a broader tolerance to eluent pH and composition.

EXPERIMENTAL

C4P and C8P gels were obtained by reaction of *n*-butyryl and *n*-octanoyl chloride, respectively, with the hydroxyl group of a vinyl alcohol copolymer gel having an average particle size of 5 μm. The resulting increases in carbon content were 6% and 10% of the final gel weight, respectively, as determined from the gel weight before and after the reaction. The C4P and C8P gels were packed in stainless-steel columns (150 mm × 4.6 mm I.D.).

Commercially available columns used for comparisons (all 150 mm × 4.6 mm I.D.), were the C_{18}-bonded polymer-based ODP-50 (ODP) and the silica-based Vydac 214TP (C_4) and 218TP (C_8), YMC-AP802 (C_4), AP202 (C_8) and AP302 (C_{18}).

Solvent regain was calculated as $SR = [(W_1 - W_2)/d]/W_2$, where d is the solvent density, W_1 is the measured weight of the gel following immersion in the solvent and centrifugion for removal of excess solvent and W_2 is the measured weight of the gel following its subsequent drying.

The chromatographic equipment consisted of Model 543 degassers (Showa Denko, Tokyo, Japan), 880-PU pump (Japan Spectroscopic, Tokyo, Japan), a Reodyne Model 7125 injector, a Model 875 UV detector (Japan Spectroscopic) and a Model SE-61 refractive index detector (Showa Denko).

Chemicals were obtained from Wako (Osaka, Japan). Organic solvents were of HPLC grade. Alkyl alcohols from Wako, peptides from Peptide Kenkyujo (Osaka, Japan) and proteins from Sigma (St. Louis, MO, U.S.A.) were used without further purification as samples.

RESULTS

Gel properties

The particle size, carbon content attributable to alkyl groups and solvent regain of the C4P, C8P and ODP gels are given in Table I.

The *SR* for a given solvent is a measure of the amount of solvent retained in the gel and is thus an indirect representation of the gel's pore volume when immersed in that solvent. In most gels, solvents of different polarities tend to result in marked differences in SR and thus in pore volume, with a corresponding swelling or shrinking of the gel.

For both the C4P and the C8P gel, the difference between *SR* values in distilled water and acetonitrile was even smaller than that observed for the ODP gel. In view of the known tolerance of the ODP gel to varying solvent polarities, the C4P and C8P gels may also be expected to be amenable to a broad range of eluents.

TABLE I

CHARACTERISTICS OF C4P, C8P AND ODP GELS

Gel	*Particle size (μm)*	*Carbon content (%)*	*Solvent regain (SR) (ml/g)*	
			Distilled water	*Acetonitrile*
C4P	5	6	1.01	1.06
C8P	5	10	0.86	0.92
ODP	5	17	0.68	0.75

TABLE II

k' AND *N* VALUES OF THE C4P, C8P AND ODP COLUMNS

k' and *N* were determined with 10 μl of 1% analyte solution using methanol–water (80:20) as eluent; flow-rate, 0.6 ml/min; detector, refractive index; temperature, 30°C.

Compound	*C4P*		*C8P*		*ODP*	
	k'	*N*	*k'*	*N*	*k'*	*N*
Ethylene glycol		3800		3500		3500
C_4H_9OH	0.12	4000	0.16	4300	0.19	4200
$C_6H_{13}OH$	0.21	4300	0.31	4100	0.40	4700
$C_8H_{17}OH$	0.34	4200	0.57	4500	0.82	5900
$C_{10}H_{21}OH$	0.51	4200	0.98	5200	1.65	6900
$C_{12}H_{25}OH$	0.75	4500	1.69	6100	3.43	7700
$C_{14}H_{29}OH$	1.08	4400	2.90	7700	7.34	8000

TABLE III

k' AND *N* VALUES OF SILICA-BASED COLUMNS

Conditions as in Table II.

Compound	*Vydac 214TP (300 Å, C_4)*		*YMC-AP202 (300 Å, C_8)*		*Vydac 218TP (300 Å, C_{18})*	
	k'	*N*	*k'*	*N*	*k'*	*N*
Ethylene glycol				3900		3500
C_4H_9OH	0.04		0.08	4200	0.08	3400
$C_6H_{13}OH$	0.08		0.18	5000	0.17	3200
$C_8H_{17}OH$	0.14	8500	0.37	5100	0.38	3600
$C_{10}H_{21}OH$	0.23	8100	0.72	5900	0.89	3600
$C_{12}H_{25}OH$	0.39	8500	1.38	7200	2.31	2700
$C_{14}H_{29}OH$	0.65	6100	2.60	9700	7.09	1200

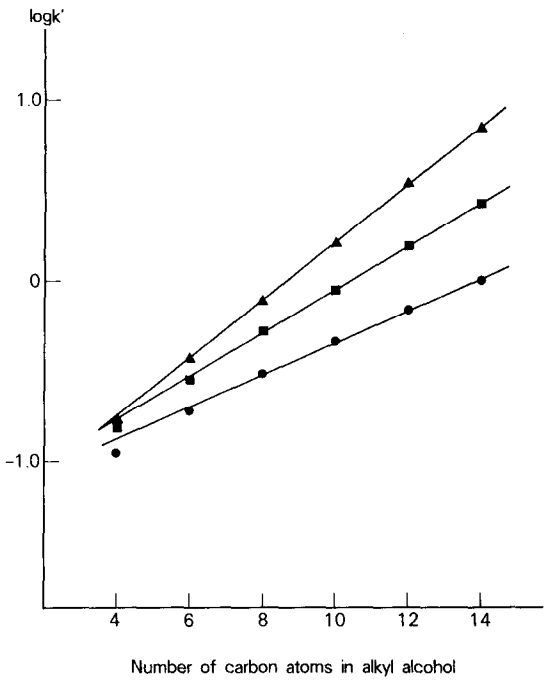

Fig. 1. Relationship between logarithm of capacity factor and carbon number in alkyl alcohol. Columns: (●) C4P; (■) C8P; (▲) ODP. Sample: *n*-alkyl alcohol (n = 4, 6, 8, 10, 12, 14). Eluent: methanol–distilled water (80:20). Flow-rate: 0.6 ml/min.

Retention strength

The capacity factors (k') and numbers of theoretical plates (N) measured for a series of alkyl alcohols in methanol–water (80:20) are given in Table II for the C4P, C8P and ODP columns and in Table III for the silica-based columns. The k' values were calculated as $k' = (V_r - V_o)/V_o$, where V_r is the retention volume of the analyte and V_o is the retention volume of ethylene glycol. As shown in Fig. 1, a close correlation between the k' value and the number of carbon atoms in the alkyl alcohol was observed for all three of the polymer-based gels. Among these gels, the k' value was also observed to rise with increasing number of carbons in the bonded alkyl group, in accordance with Martin's rule; the retention strength was clearly lowest on the C_4-, intermediate on the C_8- and highest on the C_{18}-bonded polymer gel. Similar tendencies were observed for the silica-based gel columns.

As indicated by the chromatograms in Fig. 2, a similar tendency for retention strength to increase with increasing number of carbons in the bonded alkyl group was observed for the standard proteins on the polymer-based gels and on the two silica-based gels which were tested. The differences in retention strength between the C4P, C8P and ODP gels, however, were substantially larger and far more consistent than those between the two silica-based gels.

Stability in acidic and alkaline eluents

Fig. 3 shows chromatograms obtained for a mixture of uracil, methyl benzoate,

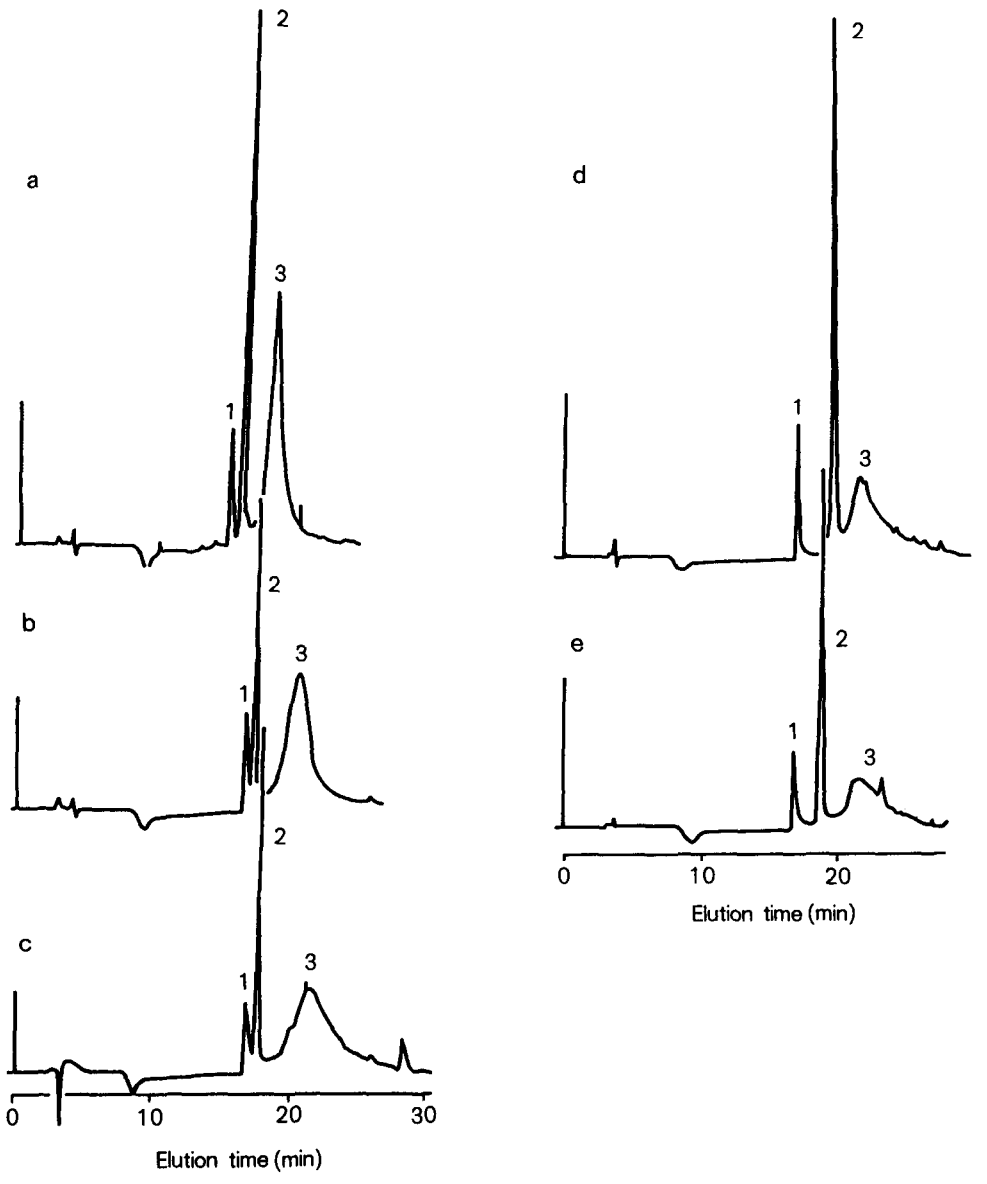

Fig. 2. Chromatograms of standard proteins on columns (a) C4P, (b) C8P, (c) ODP, (d) YMC-AP802 and (e) YMC-AP202. Sample: 1, BSA; 2, chymotrypsinogen A; 3, ferritin. Eluent: A, 0.1% TFA–acetonitrile (90:10); B, 0.1% TFA–acetonitrile (5:95); linear gradient from A to B in 30 min. Flow-rate: 0.6 ml/min. Detector: UV at 280 nm. Temperature: 30°C.

butyl benzoate and hexyl benzoate on the C4P column before and after its exposure to passage of an acidic solution (0.1% trifluoroacetic acid, pH 2) for 140 h. Fig. 4 shows those obtained before and after passage of an alkaline solution (100 m*M* sodium phosphate buffer, pH 9) for 140 h. No decrease in column efficiency occurred

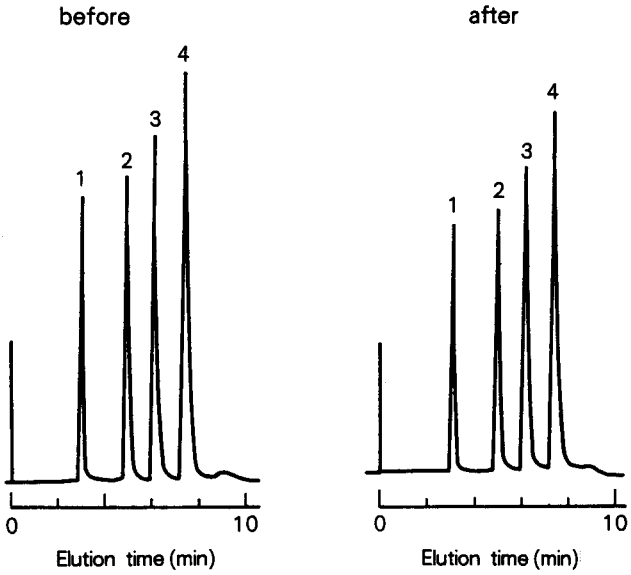

Fig. 3. Chromatograms obtained with C4P before and after passage of acidic solution [0.1% TFA (pH 2.0); flow-rate, 0.6 ml/min, 140 h]. Eluent: acetonitrile–distilled water (65:35). Flow-rate: 0.6 ml/min. Detector: UV at 254 nm. Temperature: 30°C. Peaks: 1 = uracil; 2 = methyl benzoate; 3 = butyl benzoate; 4 = hexyl benzoate.

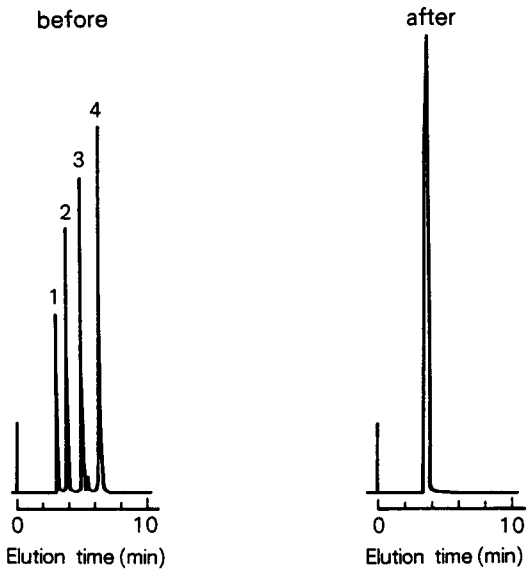

Fig. 4. Chromatograms obtained with C4P before and after passage of alkaline solution [100 m*M* sodium phosphate buffer (pH 9.0); flow-rate, 0.6 ml/min, 140 h]. Conditions as in Fig. 3.

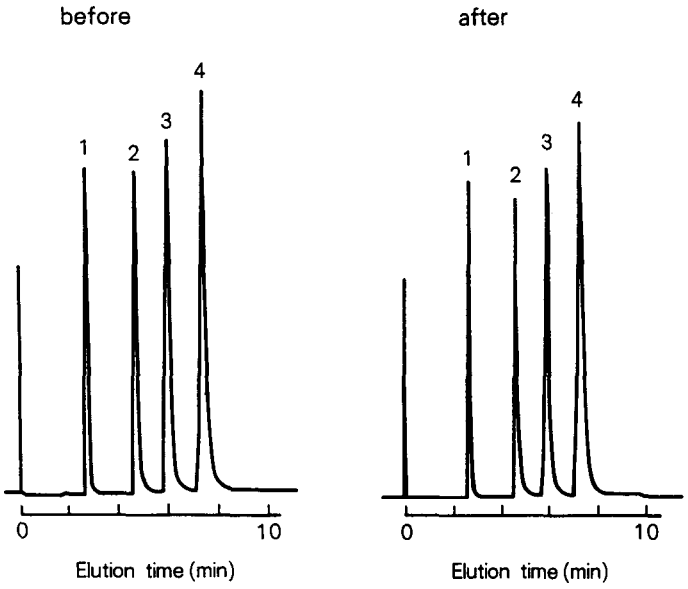

Fig. 5. Chromatograms obtained with YMC-AP802 before and after passage of acidic solution. Conditions as in Fig. 3.

in either instance. These results indicate that the C4P column and presumably the C8P column, like the ODP column, are highly stable in eluents throughout the pH range 2–9.

Silica-based columns are generally known to decrease rapidly in efficiency under acidic conditions and therefore were not tested with solutions of high pH in this study. One C4-bonded silica gel column (YMC-AP802) was tested (Fig. 5), by exposure to a solution of pH 2 in the same manner as described above. The results indicate that this gel is unstable in strongly acidic eluents and also in alkaline eluents, presumably because of separation of its alkyl groups from the base silica.

CONCLUSION

The C4P and C8P gels investigated were developed in order to eliminate the disadvantages of conventional C_1-, C_4- and C_8-bonded silica gels utilized in RP-LC. C_8- and particularly C_4-bonded silica gels are generally known to exhibit short service life under alkaline conditions, because of their tendency for solubility at pH 8 or higher[4]. This study has shown that C_4-bonded silica gel may also exhibit a serious loss in retention strength on exposure to highly acidic solutions, apparently because of separation between the bonded alkyl groups and the silica.

The results indicate that the problem of gel stability has been eliminated with the C4P and C8P gels, owing to the utilization of a vinyl alcohol copolymer rather than silica as the gel base, and that both acidic and alkaline eluents, in a far wider pH range than was previously possible, can be readily used with columns containing these gels.

Among the polymer-based gels, the retention strength for both alkyl alcohols and standard proteins was clearly lowest on the C4P, intermediate on the C8P and highest on the ODP gel, in close correspondence with the number of carbons in their bonded alkyl groups. The C_4-, C_8- and C_{18}-bonded silica gels exhibited a similar tendency for low-molecular-weight samples, but for some of the high-molecular-weight samples showed little or no such tendency.

The C4P and C8P gels may be expected to lead to a substantially expanded range of protein and peptide analyses by RP-LC.

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