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PERFORMANCE OF POLYMER-COATED SILICA C_{18} PACKING MATERIALS PREPARED FROM HIGH-PURITY SILICA GEL

THE SUPPRESSION OF UNDESIRABLE SECONDARY RETENTION PROCESSES

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SUMMARY

Coating with silicone polymer of silica surfaces was shown to be very effective in suppressing the undesirable peak tailing in reversed-phase liquid chromatography caused by hydrogen bonding, ion-exchange and chelate formation processes. Silica particles containing various amounts of metal impurities were derivatized to octadecylsilylated (ODS) silica phases with or without subsequent trimethylsilylation, and to a polymer-coated phase with subsequent introduction of octadecyl groups. The performance of endcapped ODS phases prepared from high-purity silica gel was satisfactory for hydrogen-bond acceptors and protonated amines, but not acceptable for chelating compounds. The extent of tailing seen with chelating compounds depends on the solute structure and the metal content of silica particles. Coating with silicone polymer was more effective than trimethylsilylation of ODS phases for the suppression of tailing for chelating compounds, and afforded alkyl-bonded silica packing materials free from undesirable secondary effects by using metal-free silica particles.

INTRODUCTION

Alkylsilylated silica packing materials such as octadecylsilylated silica gels are used for the separation of a wide range of substances in reversed-phase liquid chromatography (RPLC). These stationary phases, however, sometimes give poor results for compounds containing a protonated amino group, a chelating group or a group capable of forming an hydrogen bond. Improvements have been made in the preparation procedure of RPLC packing materials to eliminate the secondary effects and to enhance the performance of alkylsilylated silica packing materials.

The complexities of silica surface composition have been pointed out[1–8]. The undesirable stationary phase effects were attributed to neutral silanols[9–11], metal impurities[3,12,13] and ion-exchange sites existing on the silica surfaces[2]. The ion-exchange sites can be provided by the dissociation of silanols, especially those of higher acidity than ordinary silanols[4–7,14] or they can be related to metal impurities in silica structures[13].

Kohler *et al.*[14] reported the better surface coverage and the longer life of the stationary phase when chemical bonding is carried out after full hydroxylation of silica surfaces. Thus they showed the possibility of achieving an optimized stationary phase by applying a dense surface coverage on a fully hydroxylated silica gel with a minimum amount of metal impurities.

The current status of silica-based packing materials was recently reviewed by Nawrocki and Buszewski[6] who showed examples of so-called silanol effects and the effect of metal impurities. Some commercially available silica C_{18} packing materials exhibit such problems[15–22]. The utilization of silanol effects for achieving separation is sometimes recommended[10,23]. In general, however, the presence of secondary effects is not desirable for both column efficiency and stationary phase stability.

The importance of stationary phase optimization for RPLC is increasing, because of the increasing demand for separations of substances of biological importance. The separations of these substances often require optimized stationary phases without secondary retention as well as severe separation conditions which sometimes decompose silica-based packing materials. Although some of the undesirable effects may be avoided by using so-called silanol blocking reagents such as amines, there still remain problems due to the complicated mobile phase and irreproducible retention.

Here we report a comparison of the methods of preparation of C_{18}-type silica packing materials with respect to their performance for solutes which are known to interact with silanols, ion-exchange sites or metals. A silicone-polymer coating was found to be more effective in shielding the secondary retention processes than conventional ODS phases with endcapping. The use of pure silica gel with a minimum amount of metal impurities along with polymer coating resulted in a stationary phase that enabled facile elution of protonated amines and chelating compounds.

EXPERIMENTAL

The HPLC system consisted of an LC-6A pump, SPD-6A UV detector and CR-3A data processor (all from Shimadzu, Kyoto, Japan). The column temperature was maintained at 30°C with a water-bath.

Two silica gel batches (particle size 5 μm; Shiseido, Tokyo, Japan) were employed; one (S-I) contained a considerable amount of metal impurities, and the other (S-III) was one of the purest silica gels available. S-III was prepared similary to S-I, after the purification of sodium silicate. S-I and S-III silicas were treated with 2 *M* hydrochloric acid at reflux-temperature for 16 h to produce silica gels of much lower metal contents, S-II and S-IV, respectively.

The metal contents of silica particles were examined by inductively coupled plasma-atomic emission spectrometry (Jobin Ivon, Longjumeau, France). The surface areas and pore size distributions of silica particles were measured with a nitrogen sorption system, Autosorb I (Quantachrome, Syosset, NY, U.S.A.). The pH of silica particles was measured as a 1% (w/w) suspension in water, as described by Engelhardt and Muller[24].

Octadecylsilylation and trimethylsilylation (endcapping) were carried out as described previously[25]. Maximum coverages with octadecylsilyl groups were achieved by repeating the octadecylsilylation (the stationary phases are abbreviated as ODS), and the resulting ODS phases were further trimethylsilylated to produce the ODS-TMS phases. Coating with silicone polymer and subsequent introduction of octadecyl groups were carried out, as previously reported for commercially available Capcellpak C_{18}, to produce polymer-coated C_{18} (PC-C_{18}) phases[26]. Each packing material was packed into a stainless-steel column (10 cm × 4.6 mm I.D.).

The solutes employed in this study to illustrate the contribution of secondary effects include caffeine and theophylline for the hydrogen-bonding effect of neutral silanols, amines including aniline, N-methylaniline and N,N-dimethylaniline, procainamide (PA) and N-acetylprocainamide (NAPA) for the ion-exchange effect. Solutes such as acetylacetone and 8-quinolinol which can form chelates with metals were also used. The structures of some of the solutes are shown in Fig. 1.

The mobile phases were methanol–water mixtures, with buffers for the study of ion-exchange effects and chelate formation. The retention time of uracil was used as dead time t_0. Although uracil is slightly retained in a mobile phase of low methanol content, it has essentially no effects on the comparisons among the stationary phases.

RESULTS AND DISCUSSION

Silica particles and chemical bonding

The surface properties of silica gels and their metal contents are listed in Table I. Acid treatment of silica particles S-I and S-III removes a considerable portion of metal impurities, but not completely, with little effects on the surface area and pore size parameters. Repeated acid treatment of S-II did not reduce the metal content significantly. The remaining metals are supposed to be embedded in the silica structure. The pH value of S-II was lower than that of S-I, implying that silanol metal salts were converted into neutral silanols. The pH values of silicas with low metal contents, S-III and S-IV, were similar to each other. Acid treatment of S-III afforded an extremely pure silica gel, S-IV.

These four kinds of silica gels were derivatized to ODS, ODS-TMS and PC-C_{18}, in order to compare how the purities of silica gel and the chemical bonding method affect the appearance of the secondary retention processes in RPLC. Octadecylsilyla-

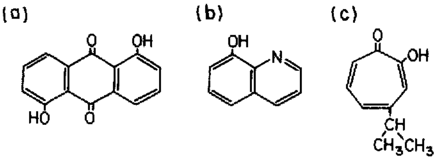

Fig. 1. Structures of solutes capable of chelate formation. (a) 1,5-Dihydroxyanthraquinone, (b) 8-quinolinol, (c) hinokitiol.

TABLE I

PROPERTIES OF SILICA GELS USED

Silica	*Pore diameter (Å)*	*Pore volume (ml/g)*	*Surface area (m^2/g)*	*pH*[a]	*Metal content (ppm)*[b]							
					Na	*Mg*	*Al*	*K*	*Ca*	*Ti*	*Fe*	*Zr*
S-I	134	0.83	273	7.1	308	11	264	42	16	230	154	11
S-II	132	0.86	286	4.9	0	8	188	0	13	162	33	13
S-III	108	0.76	307	5.2	6	0.1	8	0	1	10	15	3
S-IV	113	0.76	281	5.2	0	0	6	0	0	7	1	3

[a] pH of suspension in water.

[b] Other metals were not found in 10 ppm for S-I.

tion was repeated twice under conditions that would achieve maximum surface coverages as reported previously[25]. The surface coverages of the ODS phases, lower than those reported as maximal, may imply the abundance of much smaller pores than average in silica gel.

The first step of the preparation of PC-C_{18} includes the polymerization of 1,3,5,7-tetramethylcyclotetrasiloxane on the silica surface followed by the introduction of octadecyl groups in the presence of a soluble platinum catalyst[26]. Table II lists the carbon contents of the bonded phases. Although the increases in carbon content from silicone polymer-coated silica to PC-C_{18} were similar to those associated with octadecylsilylation, PC-C_{18} phases from pure silicas contained slightly less octadecyl groups than those from silicas with metal impurities prepared under the same reaction conditions.

TABLE II

CARBON CONTENTS OF ALKYLSILYLATED SILICA GEL

Stationary phase	*Silica gel*	*Carbon content (%)*	*Surface coverage ($\mu mol/m^2$)*
ODS	S-I	13.63	2.5
	S-II	13.48	2.4
	S-III	14.58	2.4
	S-IV	14.29	2.6
ODS-TMS	S-I	14.47	–
	S-II	14.67	–
	S-III	14.73	–
	S-IV	14.46	–
PC-C_{18}[a]	S-I	15.74	–
	S-II	13.85	–
	S-III	12.64	–
	S-IV	12.32	–

[a] Increases in carbon content associated with octadecylation are listed, after the polymer coating which resulted in *ca.* 2% carbon contents in each stationary phase.

Hydrogen-bonding effect

The effect of neutral silanols on retention via hydrogen bonding is clearly seen with caffeine as a solute[21]. Caffeine does not have a formal charge in methanol–water mobile phases, but possesses functional groups capable of hydrogen bonding. A correlation between the retention of polar compounds on various C_{18} phases in non-polar eluents in normal-phase mode and the retention of caffeine in reversed-phase mode has been reported[21].

As shown in Table III and in Fig. 2, ODS phases without endcapping resulted in strong retention of caffeine with peak tailing and loss of column efficiency regardless of the starting silica gel. Trimethylsilylation, or so-called endcapping, reduced the retention of caffeine with improvement in the peak shape, as seen with the ODS-TMS phases prepared from any of the silica packings, regardless of the pretreatment or the metal content. Polymer coating was as effective as trimethylsilylation in reducing the silanol effect via hydrogen bonding, although PC-C_{18} from S-IV silica showed a

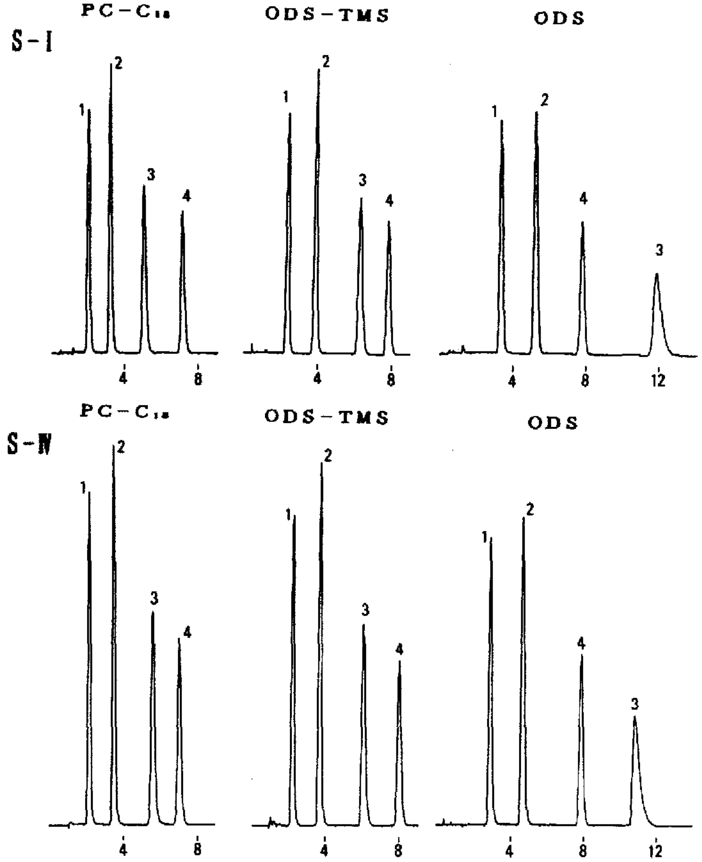

Fig. 2. The effect of the preparation method on the elution of hydrogen-bond acceptors. Mobile phase: methanol–water (20:80). Solutes 1 = theobromine; 2 = theophylline; 3 = caffeine; 4 = phenol. Flow-rate: 1 ml/min. Time scale in min.

TABLE III

RETENTION OF THEOPHYLLINE AND CAFFEINE INDICATING HYDROGEN BONDING

Stationary phase	*Starting silica gel*	$k'(\alpha)^a$	
		Theophylline	*Caffeine*
ODS	S-I	3.78 (0.61)	9.94 (1.61)
	S-II	4.16 (0.60)	10.7 (1.53)
	S-III	3.68 (0.55)	9.86 (1.47)
	S-IV	3.60 (0.53)	9.60 (1.42)
ODS-TMS	S-I	2.54 (0.41)	4.77 (0.77)
	S-II	2.77 (0.40)	5.46 (0.79)
	S-III	2.58 (0.38)	4.93 (0.73)
	S-IV	2.54 (0.38)	4.85 (0.72)
PC-C_{18}	S-I	2.18 (0.36)	4.03 (0.67)
	S-II	2.17 (0.38)	3.98 (0.69)
	S-III	2.31 (0.40)	4.18 (0.72)
	S-IV	2.50 (0.42)	4.60 (0.76)

[a] Mobile phase: methanol–water (20:80). The α values were calculated by dividing the capacity factor (k') of each compound by the k' value of phenol.

slightly larger $\alpha_{\text{caffeine/phenol}}$ value, reflecting the slightly lower surface density of octadecyl groups on this stationary phase.

Cation-exchange effect

As pointed out frequently, a serious secondary effect often termed as the "silanol effect" is observed with protonated amines as the solutes. Since silanols on the silica surface dissociate partially at neutral pH of the mobile phase, the ODS phases containing considerable amounts of residual silanols showed strong retention of protonated amines with severe tailing regardless of the batch of silica gel, as shown in Fig. 3. This is due to the slow ion-exchange process in the hydrophobic stationary phase.

Note that the packing material ODS-TMS from S-I silica showed tailing for PA and NAPA in Fig. 3 in spite of its good performance for caffeine in Fig. 2. The severe tailing with relatively small retention of the amines on ODS-TMS from S-I silica suggests that the slow ion-exchange process is taking place at a small number of ion-exchange sites. The poor accessibility of displacing ions to the ionic sites in hydrophobic stationary phase causes severe tailing[27], unless these ionic sites are completely shielded by the surface alkylsilyl groups. The ODS-TMS phases prepared similarly from S-II, S-III and S-IV silicas showed much better performance for the protonated amines at the neutral pH than the one from S-I silica. This suggests that the cation-exchange effect was not caused only by the participation of silanols.

PC-C_{18} from S-I silica showed much better performance than the corresponding ODS-TMS phase for PA and NAPA, which are alkylamines with pK_a above 9. Polymer coating seems to be more effective than endcapping of the ODS phases in shielding the secondary retention caused by the surface properties of silica gel.

As is the case with the performance of the ODS-TMS phase for PA and NAPA

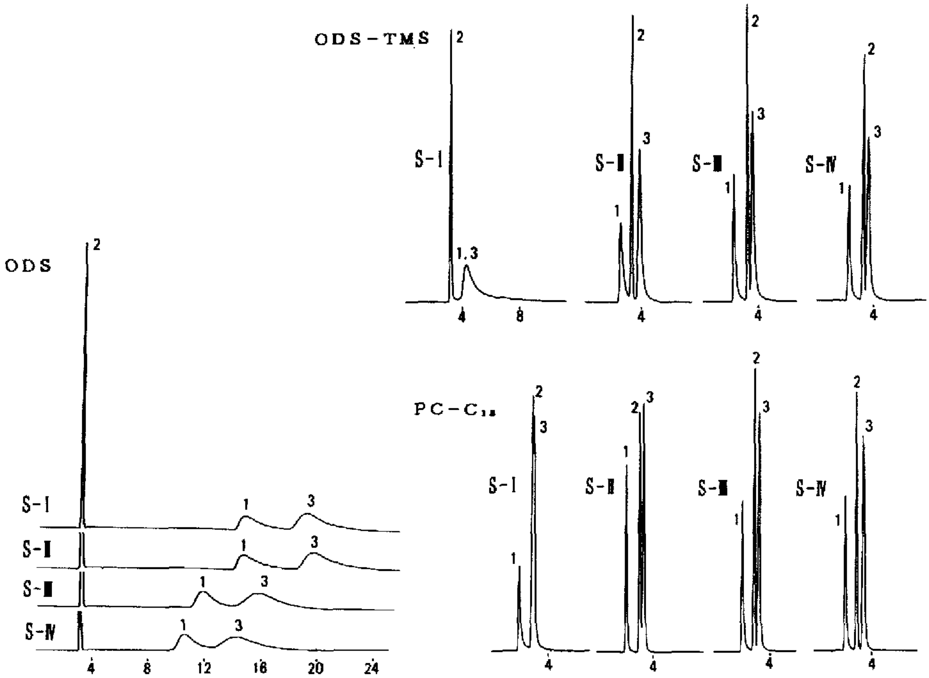

Fig. 3. Chromatograms of procainamide (PA) and N-acetylprocainamide (NAPA). Mobile phase: 0.02 *M* phosphate buffer–methanol (60:40), pH 7.6. Solutes: 1 = PA; 2 = phenol; 3 = NAPA. Flow-rate: 1 ml/min. Time scale in min.

at pH above 7, the results of amine elution at pH below 3 cannot simply be explained by the participation of ordinary silanols. At pH below 3, very few silanols should dissociate, because the pK_a of silica is reported[6] to be about 7.1. At the acidic pH values, the peak shapes of aniline derivatives having pK_a of 4–5 were worst on the ODS phase from S-I silica among the twelve stationary phases. Interestingly, the peak shape was much better on the ODS stationary phases prepared from S-II, S-III and S-IV silicas in this order, as shown in Fig. 4. This was also the case with ODS-TMS phases. It appears that the performance for protonated amines at acidic pH is related more to the amount of metals than to the number of silanols[21]. Note that all the ODS-TMS phases showed good performance for caffeine, implying the presence of few residual silanols, as shown in Fig. 2 and Table III.

As shown in Fig. 4, the ODS phases prepared from S-III and S-IV showed much better performance than those prepared from S-I and S-II in spite of the similar amount of residual silanols on these phases, as indicated by the similar hydrogen-bonding effects in Fig. 2 and the similar ion-exchange effects in Fig. 3 for all the ODS phases without endcapping. The results imply that ordinary silanols are not responsible; rather some other ion-exchange sites are responsible for the peak tailing of protonated amines at pH below 3. The ion-exchange sites can be provided by the presence of the metals[13] or silanols of higher acidity than ordinary silanols[4–7,14].

As simple acid treatment is reported to be inadequate to remove isolated sila-

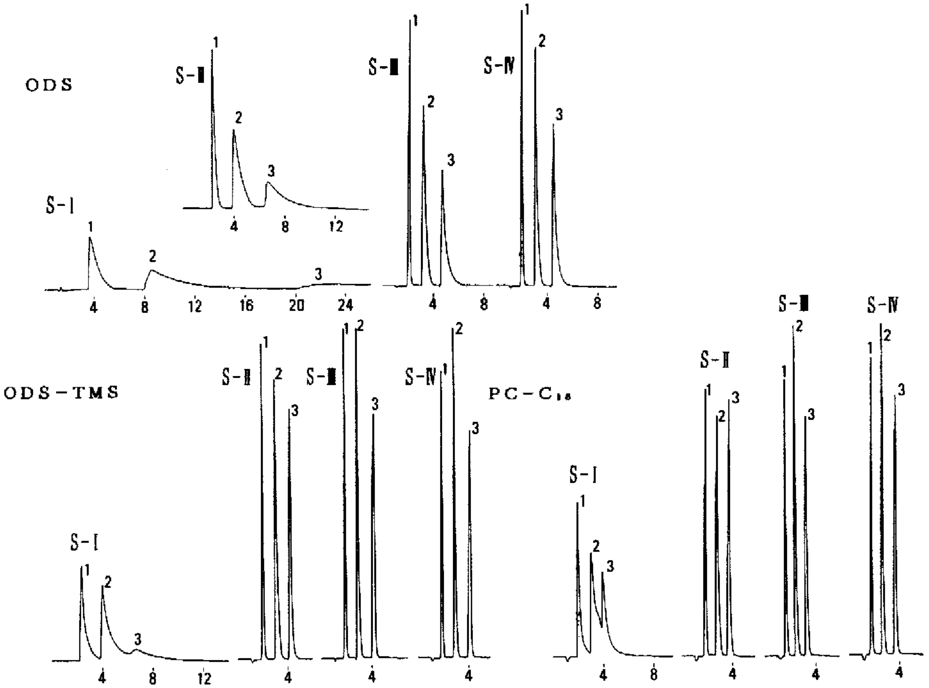

Fig. 4. Chromatograms of aniline derivatives. Mobile phase: 0.02 *M* phosphate buffer–methanol (95:5), pH 2.7. Solutes: 1 = aniline; 2 = N-methylaniline; 3 = N,N-dimethylaniline. Flow-rate: 1 ml/min. Time scale in min.

nols[14], the present results, revealing the better performance with acid-treated silica gels, seem to show that tailing of protonated amines at pH below 3 is related, al least partially, to the metal impurities on the silica surface. This is possible if the presence of metal impurities increases the acidity of silanols[13], or the acidity is provided on the basis of binary metal oxide structures[28,29]. Further studies are required to elucidate the source of the ion-exchange sites at acidic pH, whether they are provided by isolated silanols or by the presence of metal impurities.

The PC-C_{18} phase from any silica gel performed better than the corresponding ODS-TMS phase, indicating that polymer coating can shield the effect of ion-exchange sites on the silica surface more effectively than simple endcapping. Figge *et al.*[30] also reported an excellent performance of polymer-coated silica packing materials for basic substances.

Chelate formation

Verzele and Dewaele[31] reported that metal impurities in silica gel contributed to the peak tailing of carboxylic acids, based on the electrostatic interaction as well as chelate formation. The present results show that metal impurities give rise to the most serious secondary effect by chelate formation.

As shown in Fig. 5, ODS and ODS-TMS phases from S-I and S-II silicas did not give reasonable peak shape for solutes that form chelates, such as 1,5-dihydroxyan-

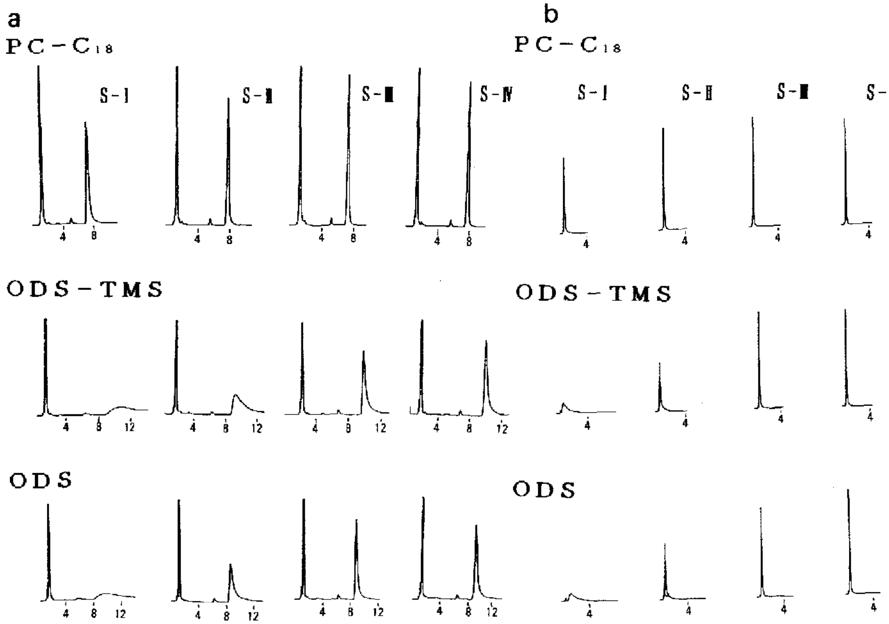

Fig. 5. Chromatograms of chelating compounds. (a) 1,5-Dihydroxyanthraquinone; (b) Acetylacetone. Mobile phase: 0.02 *M* phosphate buffer–methanol (30:70), pH 7.6. Flow-rate: 1 ml/min. Time scale in min.

thraquinone or acetylacetone. The ODS-TMS phases were not better than the ODS phases. This is understandable, because alkylsilylation reactions occur on the silanols and attached alkylsilyl groups cannot directly cover the metals which are supposed to be embedded in the silica structure. Note that undesirable peak tailing was related to the amount of metal impurities in silica gel. ODS and ODS-TMS phases prepared from S-III and S-IV silicas gave acceptable results. Here again the PC-C_{18} phase showed much better results than the corresponding ODS-TMS phase, indicating the better shielding of metal sites by the polymer layer.

The extent of peak distortion by chelate formation depends strongly on the type of solutes. Some of the worst examples can be seen in Fig. 6. Hinokitiol and 8-quinolinol were not eluted from any of the ODS and ODS-TMS phases as acceptable peaks. As hinokitiol does not contain any potentially basic nitrogen atoms, this solute specifically reveals the effect of chelate formation with surface metals, while those containing aromatic nitrogen atoms may interact with highly acidic silanols.

In the case of secondary retention processes in RPLC, very poor results were usually obtained when the solute possesses polar groups at only one part of the molecule able to interact with silica surface sites and the rest of the molecule is hydrophobic. When two pairs of polar groups are located at the opposite ends of the molecule, or when the molecule is not so hydrophobic, the effect of chelate formation is relatively small, as seen in Fig. 5.

Such a case is seen with the ion-exchange process in the chromatography of protonated amines. At pH below 3, PA and NAPA, with polar groups at both ends of the molecule, can be eluted without much peak tailing from ODS phases prepared

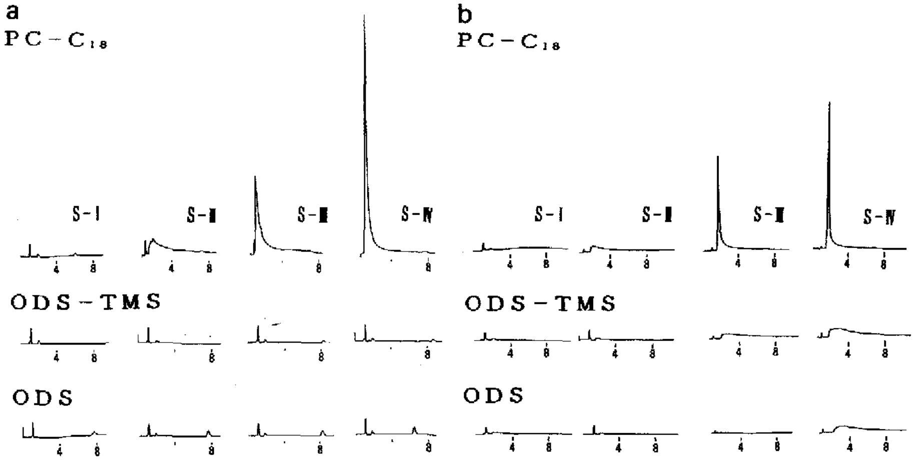

Fig. 6. Chromatograms of chelating compounds. (a) 4-Isopropylcycloheptatrienolone (hinokitiol); (b) 8-quinolinol. Mobile phase: 0.02 *M* phosphate buffer–methanol (30:70), pH 7.6. Flow-rate: 1 ml/min. Time scale in min.

from S-II, S-III and S-IV, whereas the peaks of aniline derivatives with a polar group at one end of the molecule were accompanied by tailing on these stationary phases at the same pH, as shown in Fig. 4. These observations are related to the stability of the ion pair or the chelate in the hydrophobic environment in the stationary phase. Localization of polar groups within a molecule causes an excessive secondary effect, if the interaction between the polar groups and the surface site on the silica is possible. The elution of solutes used in Fig. 6 represents the most sensitive test for stationary phases with respect to the presence and the shielding of metal impurities on alkylsilylated silica packing materials.

The results in Fig. 6 indicate that the polymer coating of silica gel containing a minimum amount of metal impurities and subsequent octadecylation seems to be the way to eliminate the most serious secondary effects of metal impurities in silica gel. Such stationary phases are free from the effects of hydrogen bonding with silanols, electrostatic interactions with ion-exchange sites and chelate formation with metal impurities, and are expected to be suitable for most separations in reversed-phase chromatography.

CONCLUSION

Coating with silicone polymer is more effective than trimethylsilylation of ODS phase, in suppressing the secondary retention processes in RPLC. A polymer-coated C_{18} phase prepared from silica gels with a minimum amount of metal impurities showed excellent performance for compounds known to interact with conventional silica-ODS stationary phases via hydrogen bonding, electrostatic interaction and chelate formation.

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