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Note

Polyphenol bonded to silica gel, a new alternative for normal-phase high-performance liquid chromatography

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Several papers have been published on the synthesis and properties of filtration media and column packing materials for liquid chromatography to which polyphenols have been bonded or adsorbed. Cellulose powder has been used as a substrate for this purpose[1] and the adsorption capacities for proteins[2–4] and metals on this insoluble material have been reported. Applications include the fining of saké[5], the immobilization of enzymes[6,7] and the separation of proteins[8]. Cellulose, however, cannot withstand the high pressures used in modern high-performance liquid chromatography (HPLC) and bacterial degradation is also a problem. Our purpose was to synthesize similar column packing materials that are resistant to high pressures. The obvious choice is to use silica gel as a solid substrate. Examples of the same approach include acetylamide stationary phase[9,10] for the aqueous size exclusion chromatography of proteins and polypeptides and the Pirkle stationary phases[11] for separations of enantiomers. In this work a new polyphenol-bonded silica gel was developed and its chromatographic characteristics were investigated.

EXPERIMENTAL

A Varian 5020 liquid chromatograph, a Varian LC-50 UV detector, a Varian 9176 recorder and a Valco 7000 p.s.i., 10-μl sample loop injector were used. The column dimensions were 15 and 25 cm × 0.46 cm I.D. The 5 and 10 μm Polyphenol-RSiL and other stationary phases were all irregularly shaped particle materials. Polyphenol-RSiL was developed in collaboration with Alltech-RSL and is available from the latter firm. The amount of organic material bonded to the silica gel in Polyphenol-RSiL, measured by thermogravimetric analysis, was 15%. That the polyphenol is chemically bonded and not physically adsorbed is shown by the absence of polyphenolic material in the washings when Polyphenol-RSiL is rinsed with water.

The mobile phase was hexane–methanol–tetrahydrofuran, all of UV grade. Further details are given in the legends of the figures. The columns were filled by a slurry packing method. The Polyphenol-RSiL was suspended in methanol–water (9:1) and packed into the columns at 400 bar.

RESULTS AND DISCUSSION

The new polar polyphenol-bonded silica gel was compared with silica gel and cyanopropylsilica gel, two common stationary phases for normal-phase HPLC. One of the test mixtures contained chalkone, benzoic acid, aspirin, theophylline, caffeine and catechin, which represent different classes of compounds. No acid or base was added to the mobile phase. Fig. 1a, b and c show the chromatograms of the above mixture obtained on the polyphenol (Polyphenol-RSiL), silica gel (RSiL) and cyanopropylsilica gel (RSiL-CN) stationary phases, respectively. Retention times, capacity factors, efficiencies and asymmetry factors are given in Table I.

The resolution of the first three peaks was best on Polyphenol-RSiL. The polar compounds were eluted with longer retention times on this more polar stationary phase, which possesses silanol in addition to phenolic functions, both interacting with polarized bonds and capable of forming hydrogen bonds with the sample molecules.

The carbonyl oxygens of aspirin are, for example, strongly attracted to the polyphenol phase, as reflected in the greater selectivity factor, α_1 (see Table I). The efficiency of the column was lower than those of the other two stationary phases. The decrease in efficiency is compensated for by the higher selectivity for polar compounds (see also Fig. 2). Peak symmetry was almost perfect on the new column. The

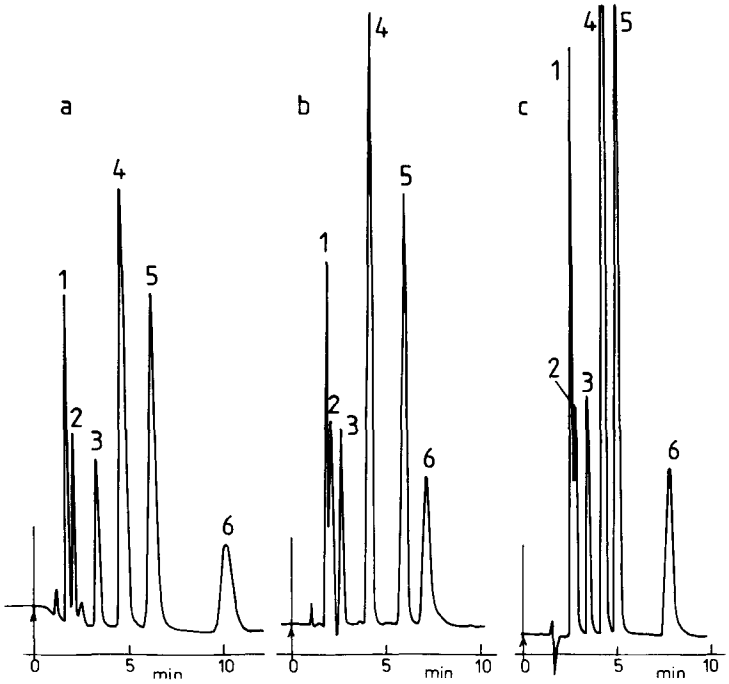

Fig. 1. Separation of a mixture of compounds on three different columns. (a) Column: 10 μm Polyphenol-RSiL (15 × 0.46 cm I.D.). Mobile phase: hexane–methanol–tetrahydrofuran (70:22.5:7.5). Flow-rate: 1 ml/min. Recorder paper speed: 0.5 cm/min. Detector: UV (280 nm). ΔP = 25 atm. Peaks: 1, chalkone; 2, benzoic acid; 3, aspirin; 4, theophylline; 5, caffeine; 6, catechin. (b) Column: 10 μm silica gel (RSiL). Conditions as in (a). (c) Column: 10 μm cyanopropylsilica gel (RSiL-CN). Conditions as in (a).

TABLE I

RETENTION TIMES, CAPACITY FACTORS, EFFICIENCES AND ASYMMETRY FACTORS OF FIG. 1

Peak	*Polyphenol-RSiL*				*RSiL*				*RSiL-CN*			
	t_R *(mm)*	k'	N^*	A^{**}	t_R	k'	N^*	A^{**}	t_R	k'	N^*	A^{**}
1	9.5	0.36	1020	–	9.6	0.60	1413	–	13.0	0.67	1463	–
2	11.2	0.72	1085	–	10.2	0.70	–	–	14.0	0.29	–	–
3	17.0	1.62	1323	–	13.8	1.30	2113	–	12.0	1.18	2502	–
4	24.5	2.77	1299	106	21.0	2.55	2513	100	20.9	1.68	2420	80
5	32.3	3.92	1601	100	29.6	3.93	2950	117	24.7	2.17	2793	100
6	51.1	6.86	1499	120	36.1	5.02	1805	119	38.7	3.92	2053	167
$\alpha_1 = (t'_{R_3}/t'_{R_2})$	2.23				1.86				1.48			

* N = plate number calculated from the peak width at half-height.
** A = asymmetry factor.

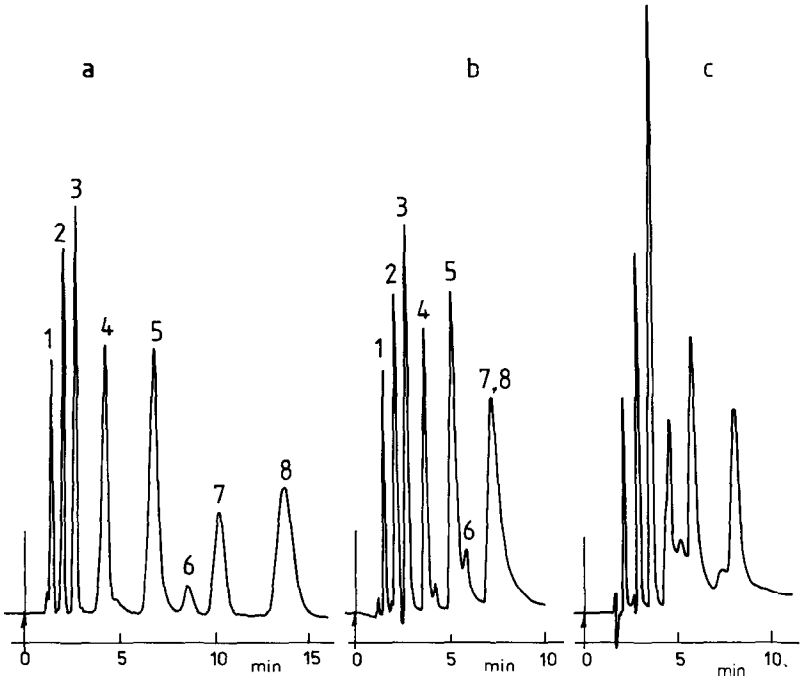

Fig. 2. Separation of phenols. (a) Conditions as in Fig. 1a. Peaks: 1, di-*tert.*-butylphenol; 2, phenol; 3, catechol; 4, pyrogallol; 5, gallic acid; 6, impurity; 7, catechin; 8, quercitrin. (b) Conditions as in Fig. 1b. (c) Conditions as in Fig. 1c.

silica gel also produced good peak shapes, but the cyanopropylsilica gel column gave some tailing of the catechin peak. This tailing could not be reduced by adding citric acid (0.25%) to the mobile phase.

A sample of eight phenolic compounds was analysed on the same columns (Fig. 2). Complete separation was only achieved on Polyphenol-RSiL; quercitrin and catechin were not separated on the other two stationary phases. The impurity of quercitrin appeared as a shoulder in Fig. 2b and c.

To confirm the possibilities of the new stationary phase we analysed some more phenolic compounds. Fig. 3a and b show the analysis of Chinese tannic acid (a polygalloylglucose mixture from *Rhus semialata* plant) on polyphenol-bonded silica gel and on silica gel. The results are very similar. The polyphenol-bonded silica gel stationary phase reveals its higher polarity by the higher eluting strength needed in the gradient to produce the compound peaks in about the same time. The addition of citric acid to the eluents increased the reproducibility of the retention times. When amines were present in the eluent, gradual changes could be introduced, leading eventually to very large increases in retention time. After a polyphenol-bonded silica gel column had been rinsed overnight with 1% triethylamine in tetrahydrofuran, phenolic compounds were no longer eluted. The original column behaviour was restored by washing through a 1% citric acid solution in tetrahydrofuran for the same time. Such complexation of phenolic groups with amines is well known[12].

Tricyclic benzodiazepine antidepressants were studied, and in the normal-

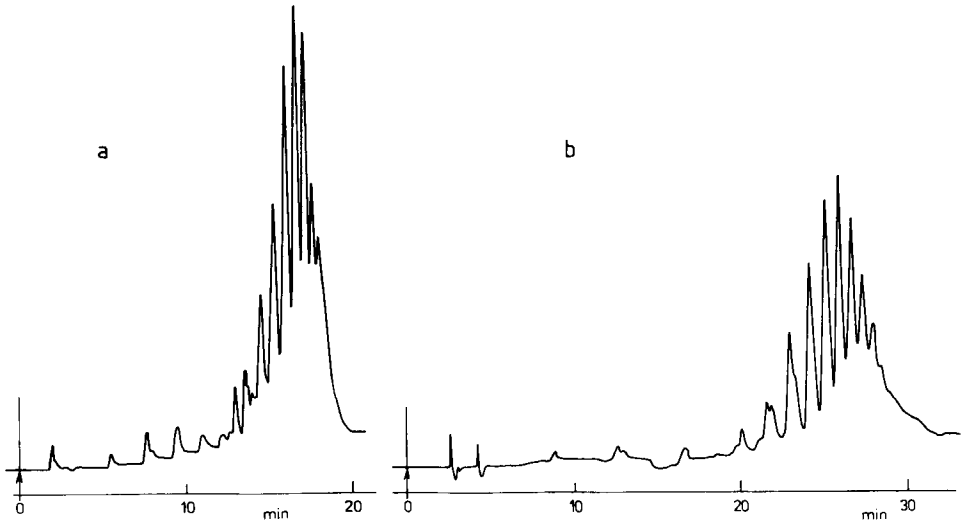

Fig. 3. Separation of components of Chinese tannic acid. (a) Column: 5 μm Polyphenol-RSiL (25 × 0.46 cm I.D.). Mobile phase: start, hexane–methanol–tetrahydrofuran (60:30:10); 20 min, A–B (10:90), where A is hexane and B is methanol–tetrahydrofuran (3:1) containing 0.25% citric acid. Flow-rate: 1 ml/min. Recorder paper speed: 0.5 cm/min. Detection: UV (280 nm). (b) Column: 5 μm silica gel (RSiL) (25 × 0.46 cm I.D.). Mobile phase: start, A–B (35:65); 15 min, A–B (50:50); 30 min, A–B (65:35), where A is hexane and B is methanol–tetrahydrofuran (3:1) containing 0.25% citric acid. Flow-rate: 1 ml/min. Recorder paper speed: 0.5 cm/min. Detection: UV (280 nm).

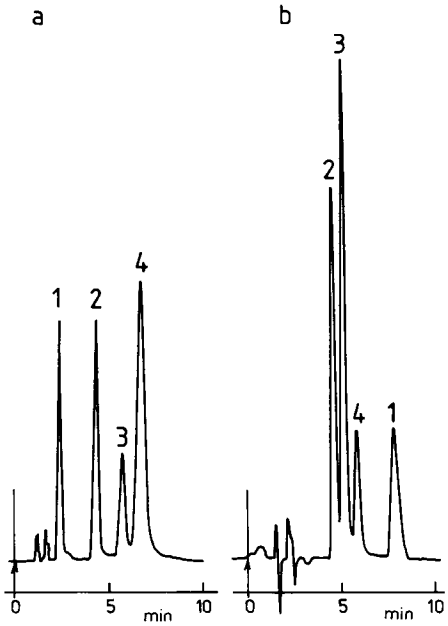

Fig. 4. Separation of benzodiazepine antidepressants. (a) Column: 10 μm Polyphenol-RSiL (15 × 0.46 cm I.D.). Mobile phase: hexane–methanol–tetrahydrofuran (70:22.5:7.5). Flow-rate: 1 ml/min. Recorder paper speed: 0.5 cm/min. Detection: UV (254 nm). Peaks: 1, midazepam; 2, nitrazepam; 3, oxazepam; 4, lorazepam. (b) Column and conditions as in (a) except that 0.25% citric acid was added to the eluent.

phase mode the polyphenol-bonded column gave better separations than the other two columns, on which only two peaks appeared. The selectivity was completely different when an acid is added to the mobile phase (Fig. 4). This large change in selectivity may be useful for the analysis of this kind of mixture.

For all separations mentioned above a high percentage of methanol–tetrahydrofuran in the eluent solvent is worth noting. This is a recent trend in normal-phase HPLC, replacing more usual solvents such as diethyl ether and chloroform with a much smaller amount of modifier. The solvent mixtures as used here give better retention time stability than traditional normal-phase solvent systems.

In conclusion, we have shown that Polyphenol-RSiL is a potentially useful polar stationary phase for HPLC, having greater polarity than silica gel itself in the sense that it has a stronger retention power for polar compounds.

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