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Preparation and characterization of mixed functional phase silica materials using phenyl-, butyl- or octylchlorosilane as a silylating agent

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ABSTRACT

Mixed functional phase (MFP) silica materials having phenyl–diol, butyl–diol and octyl–diol phases were prepared for direct injection analysis of drugs in serum. The MFP materials were synthesized from a porous silica gel of 9 nm pore size in two steps; introduction of a hydrophobic (phenyl, butyl or octyl) phase using the corresponding trichlorosilane as a silylating agent and introduction of a glycerylpropyl (*i.e.*, diol) phase using 3-glycidoxypropyltrimethoxysilane in an aqueous medium (pH 3.5). By changing the density of the hydrophobic and hydrophilic ligands, proteins were completely recovered from the prepared MFP materials in the first injection. The MFP materials can be used for direct injection analysis of hydrophobic and hydrophilic drugs in serum.

INTRODUCTION

Recently, various types of restricted access packings or packings with surface barriers have been developed for sample clean-up or assays of drugs in biological fluids [1–3]. In previous papers [4.5], we reported a synthetic method for mixed functional phase (MFP) silica materials having phenyl, butyl or octyl groups as a hydrophobic phase and diol groups as a hydrophilic phase for direct serum injection assays of drugs. The MFP materials were prepared in three or four steps: introduction of a 3-glycidoxypropyl phase, introduction of a phenyl phase and hydrolysis of the oxirane ring to a diol phase, and three steps plus further introduction of diol phases. In the above method, phenyl-, butyl- or octylalkoxysilane was used as a silylating agent. It is difficult to introduce an octyl phase owing to the low reactivity of the corresponding alkoxysilane. Also, the packing materials obtained had the disadvantages of low column efficiency and bad batch-to-batch reproducibility. Recently, we reported an improved preparation method for an MFP material in two steps; introduction of a phenyl phase and introduction of a glycerylpropyl (*i.e.*, diol) phase [6]. The MFP materials also showed high column efficiency and good batch-to-batch reproducibility.

This paper deals with the preparation of MFP materials having phenyl, butyl or octyl groups as a hydrophobic ligand by using the corresponding trichlorosilane as a silylating agent. They were characterized by physical and retention properties and applied to direct serum injection assays of hydrophobic and hydrophilic drugs.

EXPERIMENTAL

Reagents and materials

Theobromine and bovine serum albumin (BSA) were purchased from Nacalai Tesque (Kyoto, Japan). HPLC-grade acetonitrile was purchased from Kanto Chemical (Tokyo, Japan) and 3-glycidoxypropyltrimethoxysilane, phenyltrichlorosilane, butyltrichlorosilane and octyltrichlorosilane were from Petrtarch Systems (Bristol, PA, USA). Other reagents of analytical-reagent grade and control human serum (Control Serum I) were purchased from Wako (Osaka, Japan). Phenobarbital, phenytoin,

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carbamazepine, theophylline and caffeine were kindly donated by Sankyo (Tokyo, Japan), Nippon Ciba-Geigy (Takarazuka, Japan) and Eisai (Tokyo, Japan). Develosil 90-5 silica gels (particle size 5 μm; pore size 9 nm; specific surface area 400 m^2/g) were obtained from Nomura Chemicals (Seto, Aichi, Japan).

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

Preparation of the MFP silica materials

The MFP packing materials were prepared in two steps as shown in Fig. 1.

Introduction of a phenyl, butyl or octyl phase

Amounts of 4 g of Develosil silica gels were dried *in vacuo* over P_2O_5 at 150°C for 6 h and the dry silica gel was added to 120 ml of dry toluene. The mixture was heated at reflux until all the water had been removed as an azeotrope into a Dean–Stark-type trap. Next, 1.26, 0.40 or 0.17 ml of phenyl-, butyl- or octyltrichlorosilane (equivalent to 2.5, 1.5 or 0.45 μmol, respectively, per square metre of surface area) and 0.77, 0.47 or 0.14 ml of pyridine were added to the mixture. After refluxing for 7 h, the reaction mixture was cooled to room temperature, filtered and washed with toluene and methanol. The isolated silica gel was then dried *in vacuo* over P_2O_5 at 60°C for 2 h. The silica materials thus obtained are termed 2.5Ph, 1.5Bu or 0.45Oc silica, taking into account the number of micromoles of phenyl-, butyl- or octyltrichlorosilane per square metre of surface area used for the reaction.

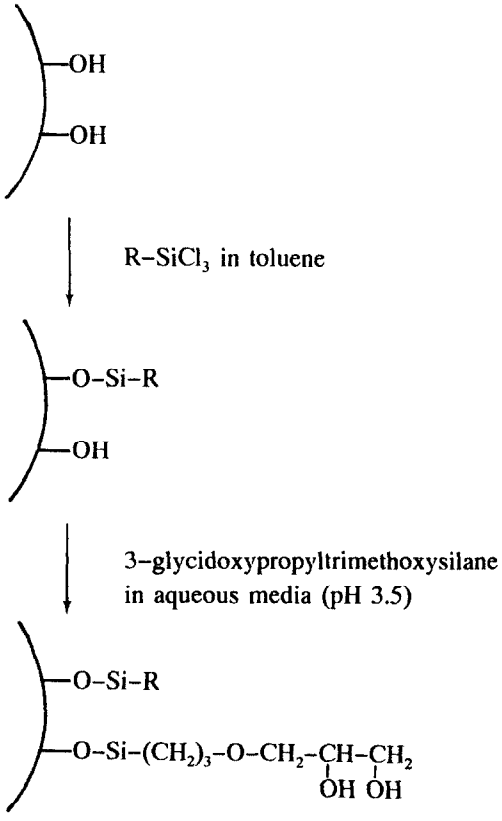

Fig. 1. Synthetic route for the MFP packing materials. R = phenyl, butyl or octyl.

Introduction of a diol phase

To 4 g of the 2.5Ph, 1.5Bu or 0.45Oc silica, 90 ml of queous solutions containing 12.0 ml of 3-glycidoxypropyltrimethoxysilane (34 μmol per square metre of surface area), the pH of which was adjusted to 3.5 by addition of perchloric acid, were added and the mixture was refluxed for 5 h. The mixture was filtered and washed with water and methanol. The isolated silica gel was dried *in vacuo* over P_2O_5 at 60°C for 2 h. The MFP silica materials thus obtained are termed 2.5Ph–34G, 1.5Bu–34G and 0.45Oc–34G silica.

Instrumentation

The reacted amounts of phenyl-, butyl- or octyltrichlorosilane and 3-glycidoxypropyltrimethoxysilane were determined by elemental analysis using an NC-80AUTO analyser (Sumika Chemical Analysis Service, Osaka, Japan).

The prepared MFP materials were packed into 100 × 4.0 mm I.D. stainless-steel tubes by conventional high-pressure slurry-packing procedures.

The chromatographic system was composed of an LC-9A pump, an SPD-6A spectrophotometer, a SIL-6B autoinjector, a C-R4A integrator and an SCL-6B system controller (all from Shimadzu, Kyoto, Japan). The eluents used are specified in the captions of the tables and figures. Detection was performed at 254 or 275 nm. All separations were carried out at ambient temperature.

Preparation of human serum samples

Drugs were dissolved in human serum at a

TABLE I

CARBON CONTENTS AND SURFACE COVERAGES OF THE MFP SILICA PACKING MATERIALS HAVING PHENYL–DIOL PHASES

Silica	Carbon content (%)	Surface coverage (μmol/m^2)	
		Hydrophobic phase	Diol phase
2.5Ph	5.33	2.12	–
2.5Ph–17G	8.94	2.12	1.49
2.5Ph–34G	9.79	2.12	2.30
2.5Ph–68G	11.63	2.12	2.68

known concentration and an appropriate volume of the serum sample was applied to the MFP material after filtration through a 0.22-μm membrane filter (Nippon Millipore, Tokyo, Japan).

Recovery of BSA from the MFP silica material

The recovery of BSA in the first injection from the MFP silica material was determined by measuring the UV absorbance at 280 nm after injection of 100 μl of BSA (10 mg/ml) sample as reported previously [6].

RESULTS AND DISCUSSION

Preparation of the MFP materials having phenyl, butyl or octyl phases as a hydrophobic ligand

Previously, we reported [6] that by using silica of 9-nm pore size as a starting material and preparation in two steps (introduction of a phenyl phase and introduction of a diol phase), the MFP packing materials obtained showed high column efficiency and good batch-to-batch reproducibility. In this study, we prepared the MFP materials having phenyl, butyl or octyl phases as a hydrophobic ligand by the same method except that phenyl-, butyl- or octyltrichlorosilane was used as a silylating agent.

Table I shows the carbon contents and surface coverages of MFP materials having phenyl–diol phases. The introduction of phenyl phases was kept constant by reaction with 2.5 μmol/m^2 of phenyltrichlorosilane with addition of pyridine as a basic catalyst. The amounts of diol phases introduced were varied by changing the amount of 3-glycidoxypropyltrimethoxysilane used for the reaction from 17 to 68 μmol/m^2.

Table II shows the average pore diameters and column efficiencies of the MFP packing materials having phenyl–diol phases and the recovery of BSA in the first injection. It was assumed that the hydrophobic phases introduced were not hydrolysed in

TABLE II

AVERAGE PORE DIAMETERS AND COLUMN EFFICIENCIES OF THE MFP SILICA PACKING MATERIALS HAVING PHENYL–DIOL PHASES AND RECOVERY OF PROTEINS

Silica	Average pore diameter[a] (nm)	Column efficiency[b] (plates per 10 cm)	Recovery of proteins in first injection[c] (%)
2.5Ph–17G	5.0	3700	90
2.5Ph–34G	5.7	4600	100
2.5Ph–68G	5.2	2600	100

[a] Measured by the inverse size-exclusion chromatographic method.

[b] Number of theoretical plates for carbamazepine under the following high-performance liquid chromatography (HPLC) conditions: column, 100 mm × 4.0 mm I.D. packed with MFP silica; eluent, 100 m*M* phosphate buffer (pH 6.9)–acetonitrile (85:15, v/v).

[c] A 100-μl portion of bovine serum albumin (10 mg/ml) was injected on to the column under the HPLC conditions as in footnote *b*.

TABLE III

CARBON CONTENTS AND SURFACE COVERAGES OF THE MFP SILICA PACKING MATERIALS HAVING BUTYL–DIOL AND OCTYL–DIOL PHASES

Silica	Carbon content (%)	Surface coverage (μmol/m^2)	
		Hydrophobic phase	Diol phase
1.0Bu–34G	7.42	0.95	2.58
1.5Bu–34G	7.42	1.76	2.03
2.5Bu–34G	7.71	2.89	1.41
0.3Oc–34G	7.16	0.35	2.58
0.45Oc–34G	7.79	0.51	2.69
0.6Oc–34G	8.61	0.79	2.74

the process of introduction of diol phases, and that diol phases were introduced as a monomeric layer. By introduction of hydrophobic and hydrophilic ligands, the average pore diameter, measured by the inverse size-exclusion chromatographic method reported by Cook and Pinkerton [7], was decreased from 9 to 5–6 nm. The recovery of proteins from the MFP materials in the first injection was 100% except for the 2.5Ph–17G silica, from which proteins were completely recovered in the second injection. The number of theoretical plates (N) was 3700, 4600 and 2600 for carbamazepine for the 2.5Ph 17G, 2.5Ph–34G and 2.5Ph–68G silicas, respectively, packed into a 100 × 4.6 mm I.D. column. Hence the amount of 3-glycidoxypropyltrimethoxysilane used for the reaction was determined to be 34 μmol/m^2.

Table III shows the carbon contents and surface coverages of MFP materials having butyl–diol or octyl–diol phases, where the amount of 3-glycidoxypropyltrimethoxysilane used for the reaction was kept constant at 34 μmol/m^2. Table IV illustrates the same data as in Table II for butyl–diol and octyl–diol phase materials.

These results reveal that the butyl- and octyltrichlorosilane used for the reaction are completely introduced into the silica surface. To recover BSA completely from the MFP materials in the first injection, the amounts of butyl-, and octyltrichlorosilane used for the reaction were 1.5 and 0.45 μmol/

TABLE IV

AVERAGE PORE DIAMETERS AND COLUMN EFFICIENCIES OF THE MFP PACKING MATERIALS HAVING BUTYL–DIOL AND OCTYL–DIOL PHASES AND RECOVERY OF PROTEINS

Silica	Average pore diameter[a] (nm)	Column efficiency[b] (plates per 10 cm)	Recovery of proteins in first injection[c] (%)
1.0Bu–34G	3.8	1400	100
1.5Bu–34G	4.6	2000	100
2.5Bu–34G	4.5	4500	30
0.3Oc–34G	5.7	2300	100
0.45Oc–34G	5.2	2800	100
0.5Oc–34G	5.1	4800	60

[a–c] See footnotes to Table II.

TABLE V

RETENTION PROPERTIES OF ANTICONVULSANT DRUGS AND METHYLXANTHINE DERIVATIVES ON THE MFP SILICA PACKING MATERIALS

Type	Compound	Capacity factor (k')		
		2.5Ph–34G	1.5Bu–34G	0.45Oc–34G
Anticonvulsant drugs[a]	Phenobarbital	1.26	0.95	0.76
	Phenytoin	4.87	3.45	2.59
	Carbamazepine	6.61	4.51	3.92
Methylxanthine derivatives[b]	Theophylline	2.57	0.86	0.75
	Theobromine	3.70	0.93	0.79
	Caffeine	6.82	1.38	1.17

[a] Capacity factors were measured under the following chromatographic conditions: eluent, 100 m*M* phosphate buffer (pH 6.9)–actonitrile (85:15, v/v); flow-rate, 0.6 ml/min.

[b] Capacity factors were measured under the following chromatographic conditions: eluent, 100 m*M* phosphate buffer (pH 6.9)–acetonitrile (23:1, v/v); flow-rate, 0.6 ml/min.

m^2, respectively. Assuming that hydrophilic phases were introduced into phenyl–diol, butyl–diol and octyl–diol materials with almost the same ligand density, the highest ligand density of phenyl phases could be obtained, resulting in complete recovery of proteins in the first injection. This is due to the length and width (*i.e.*, shape) of the substituted hydrophobic ligand on a silanol group: phenyl and butyl phases have maximum lengths of 6.28 and 6.17 Å, respectively, and maximum widths of 4.81 and 5.94 Å, respectively, according to the Sterimol parameter reported by Verloop *et al.* [8]. This indicates that to avoid interaction of hydrophobic ligands with proteins, the butyl–diol materials need a lower hydrophobic ligand density than the phenyl–diol materials when almost the same hydrophilic ligands are introduced. As octyl groups have a maximum length and width of 10.27 and 8.85 Å, respectively, the ligand density should be much smaller than with phenyl and butyl groups. If hydrophilic ligands such as polyoxyethylene groups were used instead of 3-glycerylpropyl (*i.e.*, diol) phases, a higher density of hydrophobic ligands and/or longer ligands such as octadecyl groups could be introduced, as reported by Perry *et al.* [9] and Desilets *et al.* [10] for semi-permeable surface (SPS) materials.

Retention properties of MFP materials having phenyl, butyl or octyl phases as a hydrophobic ligand

Table V shows the retention properties of anticonvulsant drugs and methylxanthine derivatives on the 2.5Ph–34G, 1.5Bu–34G and 0.45Oc–34G silicas. The anticonvulsant drugs were well retained on the all MFP packing materials, whereas 2.5Ph–34G silica was the most suitable for the separation of methylxanthine derivatives, which should be retained by hydrophobic and π-electron interactions. These retention properties were comparable to those on the MFP materials prepared in three or four steps. These results suggest that MFP materials having phenyl–diol phases could be good candidates for direct serum injection assays of hydrophobic and hydrophilic drugs.

Direct injection analysis of drugs in serum

Fig. 2A, B and C show chromatograms from the direct injection analysis of the anticonvulsant drugs phenobarbital, phenytoin and carbamazepine in human serum on the 2.5Ph–34G, 1.5Bu–34G and 0.45Oc–34G silicas, respectively. These drugs were eluted following the elution of serum proteins in the void volume, and were well separated from the background components of serum. The MFP materials prepared in two steps could be used for about 500 repetitive injections of 20-μl serum samples (total 10 ml of serum sample) without a decrease in column efficiency or increase in back-pressure.

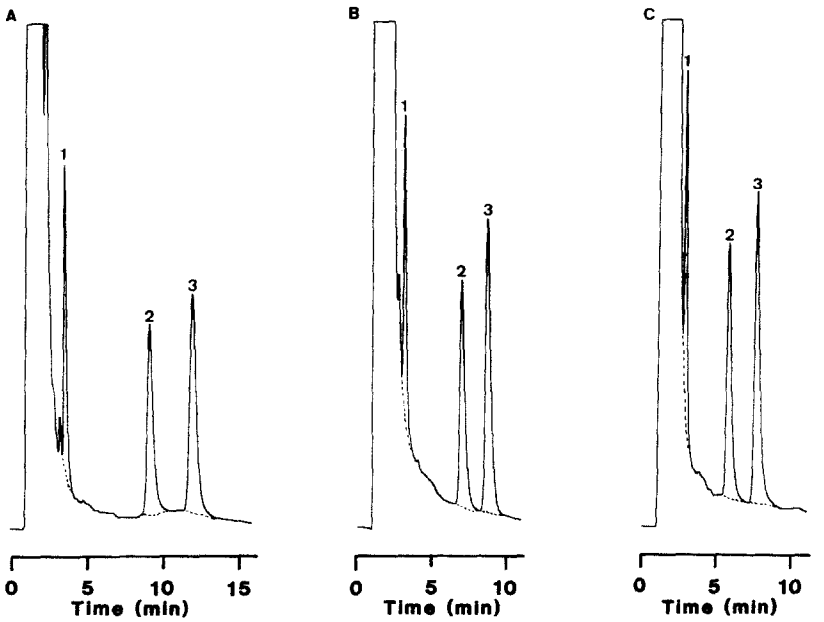

Fig. 2. Chromatograms of control serum spiked with (1) phenobarbital (20 μg/ml), (2) phenytoin (50 μg/ml) and (3) carbamazepine (10 μg/ml) on (A) 2.5Ph–34G, (B) 1.5Bu–34G and (C) 0.45Oc–34G silicas. Chromatographic conditions: column, 100 × 4.0 mm I.D.; eluent, 100 m*M* phosphate buffer (pH 6.9)–CH_3CN (10:1, v/v); flow-rate, 0.6 ml/min; detection, 254 nm; injection volume, 20 μl. Dotted lines indicate serum blank.

CONCLUSION

MFP silica materials having hydrophobic and diol phases were prepared in two steps; introduction of a hydrophobic (phenyl, butyl or octyl) phase using the corresponding trichlorosilane as a silylating agent and introduction of a diol phase using 3-glycidoxypropyltrimethoxysilane in an aqueous medium. The method can be applied to the preparation of MFP packing materials having various functionalities, and the MFP materials could be utilized for direct injection assays of drugs in serum for the purposes of therapeutic drug monitoring and biopharmaceutical studies.

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REFERENCES

1 J. Haginaka, *Trends Anal. Chem.*, 10 (1991) 17.
2 T. C. Pinkerton, *J. Chromatogr.*, 544 (1991) 13.
3 K. K. Unger, *Chromatographia*, 31 (1991) 507.
4 J. Haginaka and J. Wakai, *Chromatographia*, 29 (1990) 23.
5 J. Haginaka, J. Wakai and H. Yasuda, *J. Chromatogr.*, 535 (1990) 163.
6 J. Haginaka and J. Wakai, *Anal. Sci.*, submitted for publication.
7 S. E. Cook and T. C. Pinkerton, *J. Chromatogr.*, 368 (1986) 233.
8 A. Verloop, W. Hoogstraaten and J. Tipker, in E. J. Ariens (Editor), *Drug Design*, Vol. 8, Academic Press, New York, 1976, Ch. 4.
9 J. A. Perry, L. J. Glunz, T. J. Szczerba and J. D. Rateike, *LC · GC*, 8 (1990) 832.
10 C. P. Desilets, M. A. Rounds and F. E. Regnier, *J. Chromatogr.*, 544 (1991) 25.