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Characterization of an internal-surface reversed-phase silica support for liquid chromatography and its application to assays of drugs in serum

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ABSTRACT

Internal-surface reversed-phase (ISRP) silica supports having N-octanoylaminopropyl phases bound to the internal surfaces of the porous silica and N-(2,3-dihydroxypropyl)aminopropyl phases bound to the external surfaces were synthesized from silica particles differing in nominal pore diameters and specific surface areas. These ISRP supports were characterized with regard to physical and chromatographic properties. The support with an N-octanoylaminopropyl phase coverage of 485 μmol/g and an average pore diameter of 65 Å was the most suitable for the direct-injection determination of hydrophilic or hydrophobic drugs in serum or plasma. Non-steroidal anti-inflammatory (acetylsalicylic acid and salicylic acid) and tricyclic antidepressant drugs (desipramine and nortriptyline) in serum were successfully determined with this support and an acidic eluent.

INTRODUCTION

Recently, special silica supports that exclude macromolecules such as serum proteins without destructive accumulation but retain small molecules such as drugs have been designed for the direct-injection determination of drugs in biological fluids by high-performance liquid chromatography (HPLC)[1–4]. Hagestam and Pinkerton[2,5] designed a so-called internal-surface reversed-phase (ISRP) silica support having a hydrophobic oligopeptide phase and a hydrophilic diol phase, bonded to particles with a pore size of less than 80 Å, as internal and external surfaces, respectively. The widely used ISRP support designed by Hagestam and Pinkerton[2] has glycyl-L-phenylalanyl-L-phenylalanine (GFF) and diol phases as internal and external surfaces, respectively. Although direct serum injection assays have been developed for some hydrophilic substances with the GFF ISRP columns[6–8], the GFF bonded phase cannot retain certain classes of hydrophilic drugs, such as cephalosporins and penicillins, in the recommended eluent pH range of 6.0–7.5 (ref. 4).

Previously, we reported[4] the preparation of a new ISRP silica support having

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N-octanoylaminopropyl and N-(2,3-dihydroxypropyl)aminopropyl phases as internal and external surfaces, respectively, by using a novel enzyme, polymyxin acylase[9]. The aim of this work was to characterize the physical and chromatographic properties of ISRP supports made from porous silicas of various pore sizes and surface areas and to apply them to the direct-injection determination of hydrophilic or hydrophobic drugs in serum.

EXPERIMENTAL

Reagents and materials

(3-Aminopropyl)trimethoxysilane was obtained from Shin-etsu Silicon Chemicals (Tokyo, Japan). Octanoyl chloride, triethylamine, glycidol (2,3-epoxy-1-propanol), acetylsalicylic acid and salicylic acid were purchased from Nacalai Tesque (Kyoto, Japan). Other reagents and organic solvents of analytical-reagent grade and control human serum (Control Serum I) were obtained from Wako (Osaka, Japan). Desipramine and nortriptyline hydrochlorides were kindly donated by Nippon Ciba-Geigy (Takarazuka, Japan) and Dainippon Pharmaceutical (Osaka, Japan),

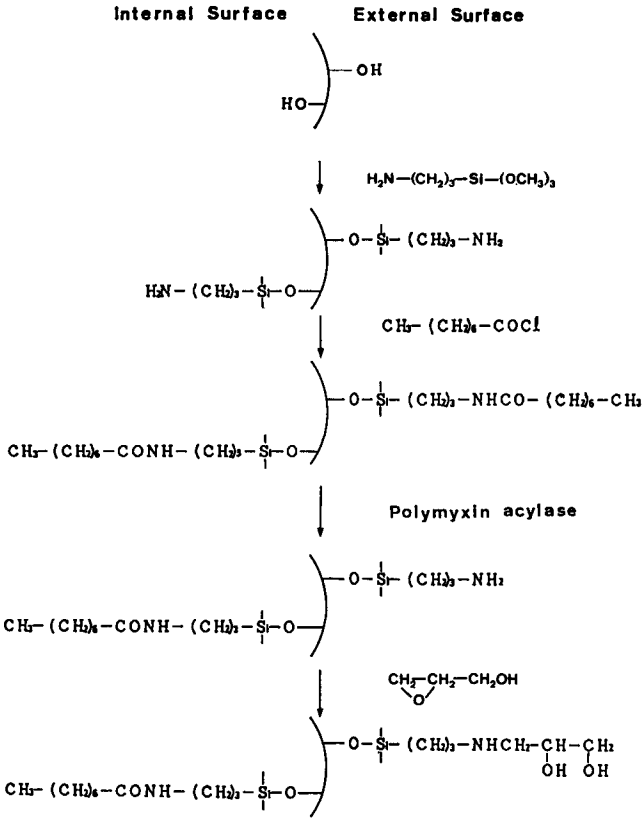

Fig. 1. Synthetic scheme for the preparation of the new ISRP silica support.

respectively. Commercially available porous silicas with particle diameters of *ca.* 5 μm were used: Develosil-60, Develosil-90 (Nomura Chemicals, Seto, Japan) and Spherisorb (Phase Separations, Hauppage, NY, U.S.A.) with nominal pore diameters of 60, 90 and 80 Å and specific surface areas of 500, 400 and 220 m^2/g, respectively.

Water was purified with a Nanopure II unit (Barnstead, Boston, MA, U.S.A.) and used to prepare the eluent and the sample solutions.

Preparation of the ISRP silica support

The supports were synthesized as described previously[4]. Fig. 1 illustrates the synthetic scheme for the preparation of the new ISRP silica support. 3-Aminopropyl groups were attached to the base silica materials by silanization with (3-aminopropyl)trimethoxysilane. The amino groups of both the internal and external surfaces were converted to N-octanoylaminopropyl phases by reaction with octanoyl chloride in the presence of triethylamine. The octanoyl groups on the external surfaces were cleaved with a polymyxin acylase. The deacylated amino groups were converted to a diol phase by reaction with glycidol, as reported previously[4,5]. Thus ISRP silica supports having a hydrophobic N-octanoylaminopropyl phase on the internal surfaces and an N-(2,3-dihydroxypropyl)aminopropyl phase on the external surfaces were obtained.

Instrumentation

The amounts of (3-aminopropyl)trimethoxysilane and octanoyl chloride reacted were determined by elemental analysis of the prepared ISRP silicas using a CHN Corder Type MT-3 (Yanagimoto, Kyoto, Japan). The amount of octanoic acid liberated by enzyme treatment was measured by a gas chromatographic method as reported previously[4].

The prepared ISRP support was packed into 100 × 4.0 mm I.D. and 10 × 4.0 mm I.D. stainless-steel tubes for the analytical and guard columns, respectively, by conventional high-pressure slurry-packing procedures[10].

The chromatographic system consisted of a Model 880-PU pump (Japan Spectroscopic, Tokyo, Japan) equipped with a variable-wavelength detector (875-UV, Japan Spectroscopic). The precolumn (50 × 4.6 mm I.D.) packed with LC-sorb Sp-A-ODS (particle size 25–40 μm) (Chemco Scientific, Osaka, Japan) was inserted between the pump and injector to protect the analytical column from microparticles in the eluent and to saturate the eluent with silica. Samples were injected with a Sil-9A autoinjector (Shimadzu, Kyoto, Japan). The chromatograms were recorded and integrated using a Chromatopac CR-6A (Shimadzu). All separations were carried out at room temperature.

Preparation of human serum sample

Drugs were dissolved in human serum at a known concentration, and an appropriate volume of serum sample was applied to the ISRP support after filtration through a 0.22-μm membrane filter.

TABLE I

CHARACTERISTICS OF COMMERCIAL SILICA PARTICLES USED

All data are those specified by the manufacturers.

Property	*Develosil-60*	*Develosil-90*	*Spherisorb*
Particle diameter, d_p (μm)	5	5	5
Nominal pore diameter, d (Å)	60	90	80
Specific surface area, S (m^2/g)	500	400	220
Specific pore volume, V (cm^3/g)	0.75	0.90	–

RESULTS AND DISCUSSION

Characteristics of the prepared ISRP supports

In a previous study[4], we prepared an ISRP support having N-octanoylaminopropyl and N-(2,3-dihydroxypropyl)aminopropyl phases on the internal and external surfaces by using Devolosil-90 silica as the base silica material. The support had an N-octanoylaminopropyl phase coverage of 1000 μmol/g. We tried to design an ISRP support having a low coverage to permit the eluation of hydrophobic drugs with an eluent including a small percentage of organic modifier. When the surface coverage of N-octanoylaminopropyl phases was decreased using Develosil-90 silica, the ISRP support obtained showed a lower column efficiency. Hence we prepared the ISRP support using Spherisorb silica which has a specific surface area of 220 m^2/g (about half that of Develosil-90 silica). The characteristics of the base silica materials and the prepared ISRP supports are shown in Tables I and II, respectively. The ISRP supports made from Develosil-60, Develosil-90 and Spherisorb silicas had specific N-octanoylaminopropyl phase coverages of 950, 1000 and 485 μmol/g, respectively, after enzymatic cleavage, which correspond to 1.9, 2.5 and 2.2 $\mu mol/m^2$, respectively. Note

TABLE II

CHARACTERISTICS OF THE ISRP SUPPORTS PREPARED

Property	*Develosil-60 ISRP*	*Develosil-90 ISRP*[a]	*Spherisorb ISRP*
Aminopropyl phase coverage (μmol/g)	1810	1840	514
N-Octanoylaminopropyl phase coverage (μmol/g):			
Before cleavage	1050	1110	512
After cleavage	950	1000	485
Cleavage (%)	9.5	9.0	5.3
Pore diameter (Å)	31	50	65
Number of theoretical plates[b] (plates per 10 cm)	2200	4400	3800
Capacity factor of barbital[c]	2.79	2.81	1.83

[a] The same support as in ref. 4.

[b] Number of theoretical plates for barbital in 100 m*M* phosphate buffer–acetonitrile (12:1, v/v) at 0.6 ml/min.

[c] Capacity factor of barbital under the same conditions as in footnote *b*.

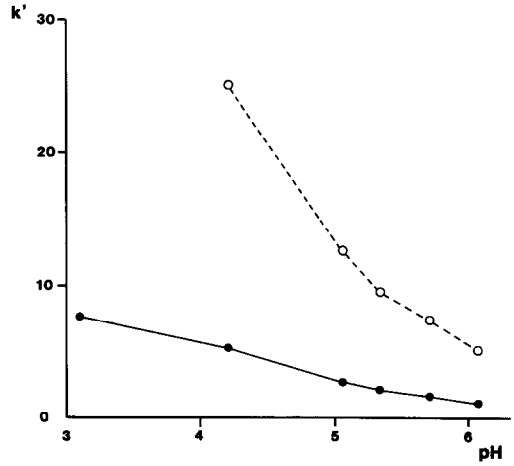

Fig. 2. Dependence of capacity factors of (●) acetylsalicylic acid and (○) salicylic acid on the eluent pH. Column, Spherisorb ISRP (100 × 4.6 mm I.D.); eluent, 100 m*M* phosphate buffer–acetonitrile (10:1, v/v); detection, 254 nm.

that the ISRP supports made from Develosil-90 and Spherisorb silicas have almost the same coverage per square metre of surface area. The low N-octanoylaminopropyl phase coverage of the support made from Develosil-60 could have been due to steric hindrance impeding diffusion of the silanizing and acylating reagents into some pores of the small-pore silica. The ISRP supports made from Develosil-60, Develosil-90 and

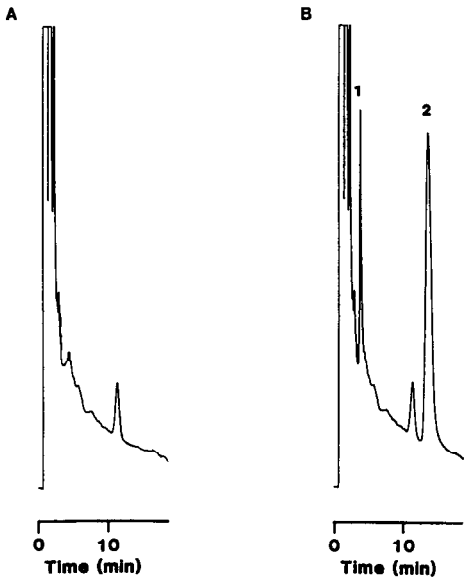

Fig. 3. Chromatograms of (A) control serum and (B) control serum spiked with (1) acetylsalicylic acid (10 μg/ml) and (2) salicylic acid (40 μg/ml). HPLC conditions: column, Spherisorb ISRP (100 × 4.6 mm I.D.); eluent, 100 m*M* phosphate buffer–acetonitrile (10:1, v/v) (final pH 5.1); flow-rate, 0.8 ml/min; detection, 254 nm; injection volume, 10 μl.

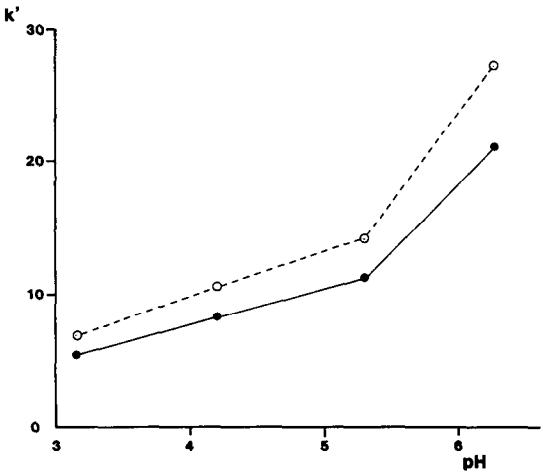

Fig. 4. Dependence of capacity factors of (●) desipramine and (○) nortriptyline on the eluent pH. Column, Spherisorb ISRP (100 × 4.6 mm I.D.); eluent, 100 m*M* phosphate buffer–acetonitrile (4:1, v/v); detection, 254 nm.

Spherisorb silicas gave capacity factors of barbital of 2.79, 2.81 and 1.83, respectively. These results indicate that the capacity of the supports is dependent on the specific N-octanoylaminopropyl phase coverage. The average pore diameters of the ISRP supports prepared from the selected silicas were less than 65 Å when measured by the

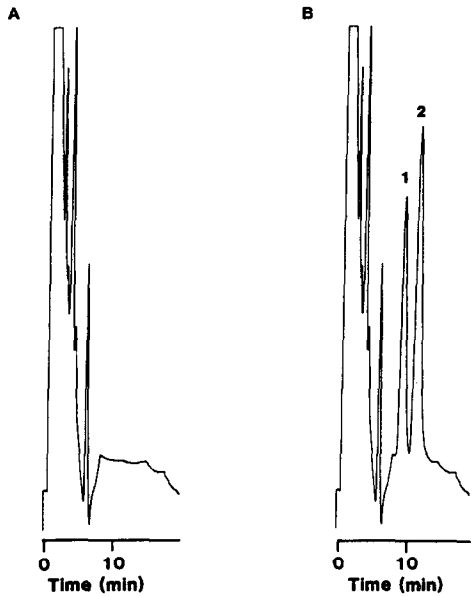

Fig. 5. Chromatograms of (A) control serum and (B) control serum spiked with (1) desipramine (5 μg/ml) and (2) nortriptyline (5 μg/ml). HPLC conditions: column, Spherisorb ISRP (100 × 4.6 mm I.D.); eluent, 100 m*M* phosphate buffer–acetonitrile (4:1, v/v) (final pH 4.2); flow-rate, 0.8 ml/min; detection, 254 nm; injection volume, 20 μl.

TABLE III

REPRODUCIBILITY AND RECOVERY OF DRUGS FROM HUMAN SERUM

The concentrations were 25 μg/ml for salicylic acid and 5 μg/ml for desipramine and nortriptyline. Relative standard deviations (R.S.D.) of five analyses.

Drug	*R.S.D. (%)*	*Recovery (%)*
Salicylic acid	1.23	101
Desipramine	4.25	99.4
Nortriptyline	2.16	101

inverse size-exclusion chromatographic method reported by Cook and Pinkerton[11]; these are small enough to exclude human serum albumin without penetrating into the pores. Although the ISRP support made from Develosil-90 silica (Table II) gave the highest column efficiency, the support made from Spherisorb silica was used for the direct-injection determination of various drugs in serum after taking into account the separation of hydrophobic drugs.

Direct-injection determination of drugs in serum

In a previous paper[4], we reported that serum proteins could be almost completely recovered from our ISRP support, while the recovery from that prepared by Pinkerton and Hagestam was low with an acidic eluent. Also, our support could be used for the direct-injection determination of drugs with an acidic eluent, whereas the latter was limited to the eluent pH range 6.0–7.5. Another aim of this study was to apply the prepared ISRP support to the direct-injection determination of hydrophilic and hydrophobic drugs with an acidic eluent.

Fig. 2 shows the pH dependence of the capacity factors of acetylsalicylic acid and salicylic acid. It can be seen that these capacity factors result from ion exclusion as the pH of the eluent increases. Hence an eluent pH of 5.1 was selected for the direct-injection determination of acetylsalicylic acid and salicylic acid in serum (Fig. 3). Fig. 4 shows the plots of the capacity factors of desipramine and nortriptyline against the pH of the eluent. As these drugs have a secondary amino group in the molecule, the capacity factors decreased with decrease in pH. As shown in Fig. 5, these drugs were separated from serum components using an eluent pH of 4.2 with a short run time. Table III gives the relative standard deviations for the assays of salicylic acid, desipramine and nortriptyline in serum and their recoveries from the serum samples. The drugs were almost completely recovered (99.4–101%) from serum with good reproducibility.

We conclude that the prepared ISRP support can also be used for direct serum injection analysis by separating hydrophilic or hydrophobic drugs with an acidic eluent.

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