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Preparation and evaluation of octadecyl-treated porous glasses

Application to the determination of methotrexate in serum

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ABSTRACT

The retention and selectivity of methotrexate (MTX) in serum were studied by high-performance liquid chromatography on octadecyl-treated porous glasses and silicas. From elemental analysis data for carbon, the maximum number of bonded octadecyl surface groups per gram (mean pore diameter 153 Å, specific surface area 193 m^2/g, pore volume 0.83 ml/g) in octadecyl-treated glass was calculated to be 0.131×10^{21}. MTX in human serum could be separated on both glasses and silicas with methanol–acetate buffer mixtures as eluents.

INTRODUCTION

Chemically bonded stationary phases are the most widely used column packing materials for reversed-phase high-performance liquid chromatography (HPLC). These materials consist of organic functional groups, such as octadecyl, octyl, ethyl and phenyl groups, bonded to silicas. In previous papers[1–4], we suggested that the important parameters of silica with respect to the number of accessible alkylamino or phenyl groups per 100 $Å^2$ are the pore diameter and the specific surface area. In investigations of bioavailability, and also in pharmacokinetic and forensic science studies, several types of gel are used. Therefore, we have now considered how the chromatographic properties of octadecyl- and phenyl-modified gels are important in HPLC or micro-HPLC columns; a few reports of HPLC analyses on octadecyl-treated glass columns in physical and chemical research have been reported[5–9]. Furthermore, we studied the HPLC determination of methotrexate (MTX) in human serum.

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EXPERIMENTAL

Reagents

Octadecyldimethylchlorosilane (ODS) was obtained from Petrach Systems (Bristol, PA, U.S.A.) and MTX from Sigma (Poole, U.K.). Four types of porous glasses (1G, 2G, 3G and 4G) and four types of porous silicas (1S, 2S, 3S and 4S) differing in their mean particle size, mean pore diameter, specific surface area and pore volume (Table I), were prepared in our laboratories. The other reagents and organic solvents were of analytical-reagent grade.

TABLE I

CHARACTERISTICS OF ORIGINAL GLASSES AND SILICAS

Sample[a]	*Mean particle size (μm)*	*Mean pore diameter (Å)*	*Specific surface area (m^2/g)*	*Pore volume (ml/g)*
Glass 1G	5.8	153	193	0.83
Glass 2G	8.2	335	69	0.57
Glass 3G	8.5	577	57	0.84
Glass 4G	8.7	731	47	0.81
Silica 1S	8.0	164	197	1.20
Silica 2S	8.8	507	78	1.05
Silica 3S	9.7	728	52	1.11
Silica 4S	9.6	787	50	1.10

[a] The designations are for convenience and have no commercial significance.

Apparatus

HPLC measurements were carried out on a Twincle instrument (Jasco, Tokyo, Japan), equipped with a Uvidec-100 IV variable-wavelength detector (Jasco, Tokyo, Japan) and a column of 150 × 4.6 mm I.D., packed with ODS-treated glass or silica.

Stationary phase and elemental analysis

As described previously[1–4], 7 g of dried glass 1G, 2G, 3G or 4G or silica 1S, 2S, 3S or 4S were added to 70 ml of a 3.4% solution of ODS in dry toluene containing 3 ml of triethylamine. The glass or the silica suspension was refluxed for 5 h, filtered through a glass filter (1 μm), washed several times with toluene, chloroform, methanol and acetone and then dried *in vacuo* at 70°C for 2 days. The final products are listed in Table II as 1G-ODS, 2G-ODS, 3G-ODS, 4G-ODS, 1S-ODS, 2S-ODS, 3S-ODS and 4S-ODS, respectively. The characteristics of these materials are also given in Table II. The carbon contents of the treated glasses or silicas were determined by elemental analysis using an MT-3 CHN elemental analyzer (Yanagimoto, Kyoto, Japan). The specific surface areas, mean pore diameters and pore volumes of the column glasses and silicas were determined with an MOD-220 porosimeter (Carlo Erba, Milan, Italy) and an SA-1000 surface area/pore volume analyser (Shibata, Tokyo, Japan), and the data are given in Table II.

Column preparation

The column glasses or silicas were packed into stainless-steel columns (150 × 4.6 mm I.D.) by the slurry technique.

Procedure

According to the Brimmell and Sams method[10], a 25-μl volume of internal standard (aminopterin, 40 mg/l in water, with 200 μl of 1 *M* sodium hydroxide added to aid solution) was added to 225 μl of human serum in a microtube and centrifuged in a microcentrifuge at 10 000 *g* for 10 min. Acetone (250 μl) was added as protein precipitant and mixed, using a vortex mixer. The mixture was again centrifuged for 60 s. A volume of 300 μl of the supernatant was transferred to a second microtube containing 300 μl of butan-1-ol and 400 μl of diethyl ether and the procedure of mixing and centrifugation for 60 s was repeated. The supernatant was discarded, then 10–30 μl of the remaining solution were injected onto the column.

RESULTS AND DISCUSSION

Fig. 1 shows the pore diameter *vs.* pore volume plots obtained for the original glasses (glass-1G); the average pore diameter and pore volume were determined as 153 Å and 0.83 ml/g, respectively.

From the elemental analyses of glasses and silicas treated with ODS, the number of bonded octadecyl surface groups per gram or per 100 Å^2 were calculated by the previously described procedure. The results are given in Table II. As can be seen, an

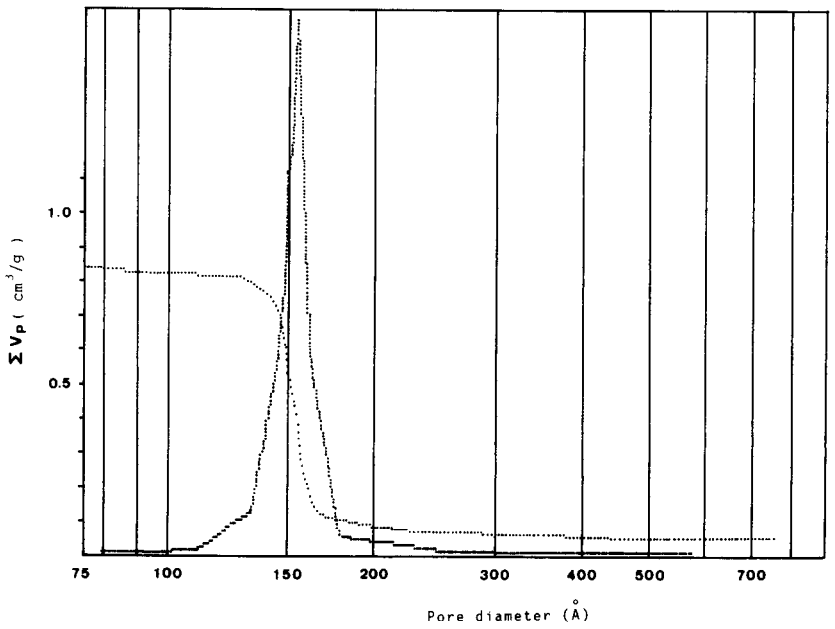

Fig. 1. Pore diameter (Å) *versus* pore volume (cm^3/g) of the original glass (glass 1G).

TABLE II

CHARACTERISTICS OF TREATED GLASSES AND SILICAS

Treated gel	*Specific surface area (m^2/g)*	*Carbon content (%)*	*Average pore diameter (Å)*	*Pore volume (ml/g)*	*No. of surface groups per gram ($\times 10^{21}$)*	*No. of surface groups per 100 Å^2*
1G-ODS	107	5.73	131	0.80	0.131	1.44
2G-ODS	46.4	2.92	328	0.57	0.073	1.58
3G-ODS	43.9	2.71	530	0.70	0.068	1.55
4G-ODS	33.7	2.20	699	0.62	0.055	1.63
1S-ODS	146	11.0	130	0.81	0.276	1.89
2S-ODS	59.7	1.71	485	0.97	0.043	0.72
3S-ODS	42.7	1.44	688	0.96	0.036	0.85
4S-ODS	39.8	1.27	733	1.00	0.032	0.80

increase in the specific surface area of glass or silica increases the number of bonded surface groups per gram, but does not change the number of bonded surface groups per 100 Å^2.

The retention time of MTX was 13.2 min on 1G-ODS. The limit of detection for MTX was $8.0 \cdot 10^{-8}$ *M*, at which concentration the peak area was three times the noise level of the system. However, real minimum detectable concentration of samples, prepared as described under Experimental, was $1.5 \cdot 10^{-7}$ *M*.

A chromatogram for a human serum sample taken 1 h after intraveneous infusion of MTX is shown in Fig. 2, which corresponds to the experiments with 600 mg of MTX in Fig. 3. Blanks were prepared from human serum from MTX-free subjects, and no interfering peaks were observed at the retention times of the compounds of

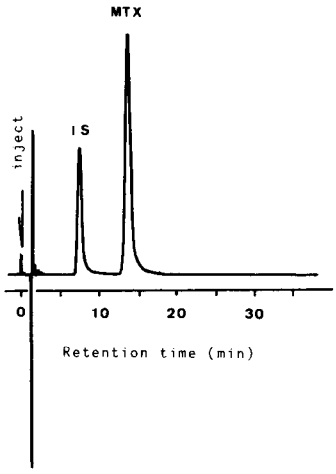

Fig. 2. Liquid chromatogram of human serum 1 h after intraveneous infusion of methotrexate. Peaks: MTX = methotrexate; IS = internal standard (aminopterin). Sample, human serum sample 1 in Fig. 3; column, 1G-ODS, 150 × 4.6 mm I.D.; mobile phase, methanol–0.01 *M* acetate buffer (11:39) (pH 3.8); flow-rate, 1.5 ml/min; detection, UV (305 nm).

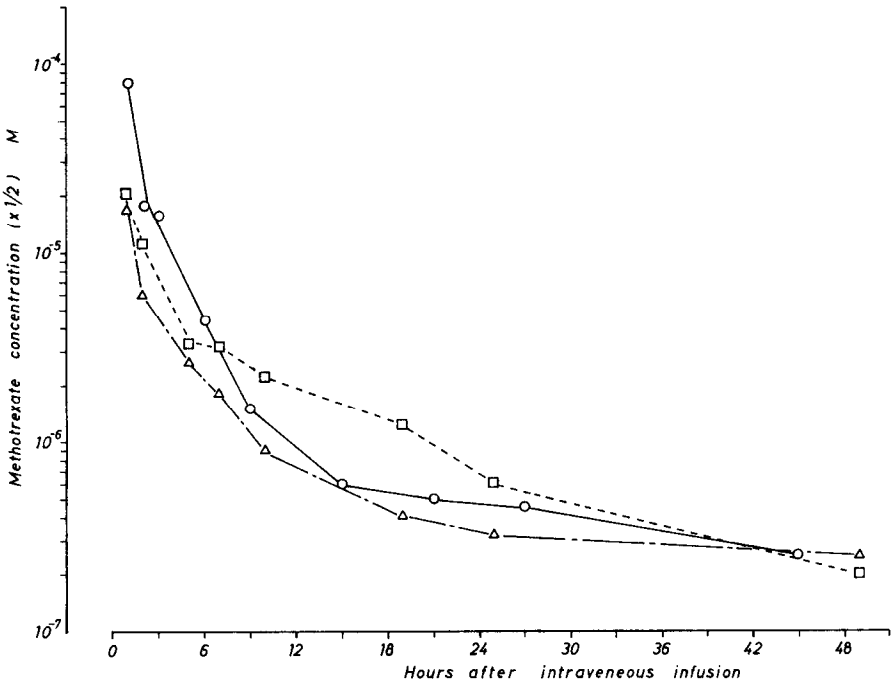

Fig. 3. Changes in methotrexate concentration in human serum with time after intraveneous infusion of 600 mg of methotrexate. Conditions and solutes as in Fig. 2. ○ = Sample 1; △ = sample 2; □ = sample 3.

interest. The MTX was separated on both the glasses and silicas studied, but with different degrees of resolution and elution orders. These results show that the ODS-modified glasses could be useful column materials for HPLC.

It can be concluded that it is not sufficient to evaluate column gels only from the point of view of the carbon content of the chemically treated reversed-phase materials; one should also consider the pore-size distribution of the support glass, the bulkiness of the ligand bonded to the glass and the molecular size of the solute before any precise statement is made.

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