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INTERNAL STRUCTURES OF WIDE-PORE PACKING MATERIALS FOR HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY STUDIED BY TRANSMISSION ELECTRON MICROSCOPY*

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SUMMARY

Internal structures of high-performance liquid chromatographic column packing materials, including porous glass particles, silica gels and polymer gels, were studied by transmission electron microscopy. Clear micrographs of the internal structures were obtained for macroporous particles by using ultrathin sections, with staining in the case of polymer gels. Corpuscular structures were seen with silica gels with relatively small pores, while porous glass particles showed a typical spongy structure. Spherical silica particles with large pores in the range 50–500 nm from different sources possess common spongy structures with a difference in the presence of a shell at the outer surface. Several silica packing materials of 30–400 nm nominal pore size were found to be mixtures of two or more kinds of silica particles of different pore sizes, which accounted for the broad, in some instances bimodal, pore size distribution found in nitrogen adsorption measurements and in inverse size-exclusion chromatography. A bimodal pore size distribution was also seen for unmixed macroporous polymer gel particles, with one of the maxima in a small pore size range, presumably due to the presence of micropores formed by the network structures in microgels.

INTRODUCTION

The characterization of porous particles for high-performance liquid chromatography (HPLC) is important in understanding the chromatographic properties of

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different column packing materials and in the development of new ones. Porous packing materials have customarily been subjected to characterization procedures such as measurement of surface area and pore size by nitrogen adsorption, mercury intrusion and size-exclusion chromatography (SEC). These methods provide data for the overall porosity and pore size distribution of porous materials, but do not give information on the shape of the pores, or how the pores penetrate the bulk particle structure.

A variety of structures have been described as the internal structures of porous particles[1,2]. The determination of physical parameters such as pore size and pore size distribution depends on the shape of the pores[2,3]. In studies of the preparation and characterization of reversed-phase materials, geometric factors of silica pores are related to the chromatographic properties of the resulting stationary phase[4,5]. Therefore, it is desirable to include a pore structure study in the characterization of porous packing materials.

With respect to the size and shape of the pores in HPLC column packings, transmission electron microscopy (TEM) seems to give the most straightforward information and to be best suited for such a pore structure study. There have been a few reports on electron microscopic studies of column packing materials[6–15]. A difficulty has been encountered with the thickness of the sample, which hinders the close observation of the internal structures of silica particles with nominal 6–10 nm pores most commonly used in HPLC. Scanning electron microscopy (SEM) may not afford information on internal structures, because some silica particles are covered with a surface layer, or a shell at the outer surface. Tracz and Barna[14] recently reported the preparation of ultrathin samples of porous materials for TEM by ion milling.

It is of great interest to examine directly the actual internal structures of silica and polymer particles from various manufacturers to see the shape of pores, and any possible differences in internal structures produced by the difference in the method of preparation. We report here the TEM observation of ultrathin sections of various packing materials, including silica gel of 30–400 nm nominal pore sizes, porous glass particles and macroporous polymer gels. Clear internal structures obtained by both TEM and SEM will show whether the packing materials actually possess the stated pore size and uniformity. This will help researchers attempting studies on the effect of pore geometry on the physical and chromatographic properties of packing materials.

EXPERIMENTAL

Materials

The following silica particles were purchased: LiChrospher Si 500, Si 1000 and Si 4000 (particle size 10 μm) (Merck, Darmstadt, F.R.G.), Nucleosil 100, 300, 1000 and 4000 (5 μm) (Machery, Nagel & Co., Düren, F.R.G.), Spherisorb 300 (5 μm) (Phase Separations, Norwalk, CT, U.S.A.), Hypersil 300 (5 μm) (Shandon, Runcorn, Cheshire, U.K.) and Vydac TP (10 μm) (Separations Group, Hesperia, CA, U.S.A.). Porous glass particles were prepared at the Institute of Industrial Science of the University of Tokyo. TSK G4000PW and G4000H polymer gels were obtained from Tosoh (Tokyo, Japan). The particles are listed in Table 1.

Samples for TEM were prepared as follows: porous particles were embedded in Spurr epoxy resin (Polysciences, Warrington, PA, U.S.A.), then hardened at 70°C for 8

TABLE I

MACROPOROUS COLUMN PACKING MATERIALS EMPLOYED FOR TEM STUDY

Packing material	*Surface area** (m^2/g)	*Pore size** (*nm*)	*Pore volume** (*ml/g*)	*Particle size** (*μm*)
LiChrospher 500	50 (69**)	50	0.8 (0.90**)	10
1000	20	100	0.8	10
4000	6	400	0.8	10
Nucleosil 300	100 (109**)	30	0.8 (0.81**)	5
1000	25	100	0.8	5
4000	10	400	0.7	5
Hypersil 300	60	30	0.6	5
Spherisorb 300	190	30	1.5	5
Vydac TP	80	30	0.6	10
Porous glass	(386**)	(55***)	(0.72**, 0.49***)	10
TSK G4000H[§]	(333**)	–	1.29 (1.19**)	12
TSK G4000PW[§§]	(64**)	–	(0.64**)	10

* Manufacturer's specification.
** Determined by nitrogen adsorption.
*** Determined by mercury intrusion.
§ Polystyrene–divinylbenzene gel.
§§ Acrylate or methacrylate gel.

h. G4000PW gel was stained with ruthenium tetraoxide vapour[16] prior to epoxy resin impregnation. Samples for SEM were coated with platinum–palladium by ion sputtering. Polystyrene standards were obtained from Pressure Chemicals (Pittsburg, PA, U.S.A.).

Instruments

An H 800 electron microscope (Hitachi, Tokyo, Japan) was operated at 200 kV. A Sorvall MT-6000 ultramicrotome (DuPont, Wilmington, DE, U.S.A.) was used to prepare ultrathin sections of about 80 nm thickness from the embedded porous particles. Electron microscopy was carried out at the Nitto Technical Information Centre. The HPLC system consisted of an 800 PU pump and 875 UV detector (Jasco, Tokyo, Japan), a 98.00 refractive index detector (Knauer, Berlin, F.R.G.) and a 7000A data processer (System Instruments, Tokyo, Japan).

Measurements

HPLC measurements were carried out at 30°C. The inverse SEC method of Knox and co-workers[17,18] was employed to calculate pore size distributions. Nitrogen adsorption measurements were carried out at the Shiseido Toxicological and Analytical Research Centre (Yokohama, Japan).

RESULTS AND DISCUSSION

Clear electron micrographs of pore structures were obtained by employing ultrathin sections of about 80 nm thickness for silica gels, porous glass and polymer gels. As shown below, most of the pores were filled with the epoxy resin. In the

transmission electron micrographs, the light area represents the epoxy resin in the pores and in the interparticle space, and the dark area the skeleton, or ruthenium in the case of G4000PW gel. Channels in particles not perpendicular to the cross-section are shadowed owing to the partial electron adsorption by the skeleton.

Fig.1 shows a comparison of internal structures between porous glass particles and LiChrospher Si 500 silica particles. The pores in the glass particles were round and relatively uniform in size, whereas those in LiChrospher Si 500 were irregularly shaped, and possessed a broad size distribution. In other words, the glass particles possess the features of a spongy system, consisting of channels in solids, whereas the silica particles possess those of a corpuscular system, consisting of aggregates of microstructures[1,2]. In agreement with the microscopic observations, a relatively

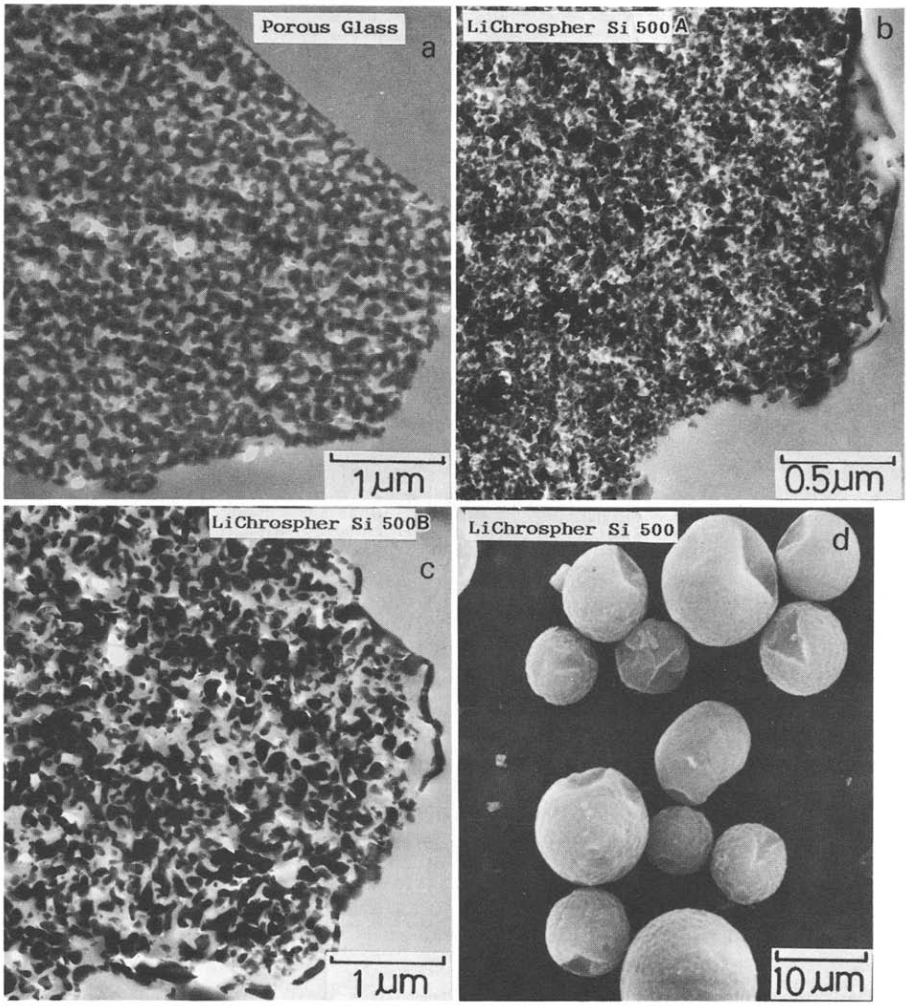

Fig.1. TEM photographs of (a) porous glass particles and (b and c) LiChrospher Si 500 and (d) an SEM photograph of LiChrospher Si 500.

narrow pore size distribution was found with the porous glass particles compared with the silicas except in the micropore region, in nitrogen adsorption measurements and in mercury intrusion, as shown in Fig. 2. Differences in pore sizes determined by different methods can be seen in Fig. 2, where the nitrogen adsorption method gave smaller pores sizes for both silica and glass particles.

Fig. 1b and c were obtained from a single batch of LiChrospher Si 500. The internal structures of the silica particles are evidently different, indicating that the packing material is actually a mixture of two types silica particles, (the two types of particles cannot be made in the same preparation, because no particles in between were found in the batch; the same is true for the mixed particles shown below). One type of particle possesses a pore diameter of less than 30 nm and the other up to 300 nm. The difference cannot be observed by SEM, however, owing to the existence of a shell at the outer surface of this silica (Fig. 1d). Fig. 1b and c show a dark area at the periphery of the spheres in spite of the highly porous structure of the particles. The thickness of the shell is slightly less than 100 nm.

Fig. 3 shows the micrographs of LiChrospher Si 1000. This silica was also found to be a mixture of two types of particles, one with 50–100-nm pores and the other with pores up to 200 nm. The surface structures of LiChrospher Si 1000 and Si 4000 are similar to those of Si 500. The surface shell seems to have been made by heat treatment after the formation of spheres, as shown by the appearance of the sintered structure in Fig. 4c. The particles with higher porosity possess the thicker surface shell. The surface shell developed on highly porous LiChrospher Si 4000 seems to be relatively fragile, because a considerable portion was broken by ultrasonication in about 10 min (Fig. 4b and e), as pointed out by Unger and Gimpel[19]. Therefore, prolonged ultrasonication prior to column packing should be avoided.

Fig. 4d and e shows that LiChrospher Si 4000 consists of two types of silicas, one of which is similar to that in LiChrospher Si 1000. The pore structures in Figs. 3 and 4 have some similarities to those of a spongy system. As mentioned by Unger[2], silica particles can have a spongy structure depending on the method of preparation. Another feature of LiChrospher Si 4000 is the development of some crystallinity of the silica skeleton of particle B, as shown by the moire pattern in Fig. 4e. The crystallinity was lost during the TEM observation. Whether the crystallinity has any effect on chromatographic properties is yet to be studied.

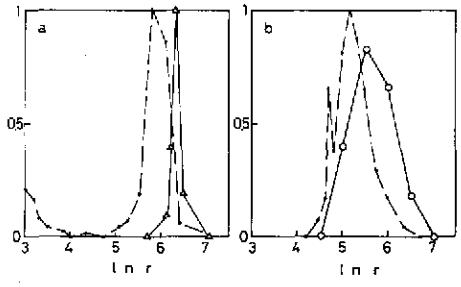

Fig. 2.(a) Pore size distribution of porous glass: solid line, mercury intrusion method; dashed line, nitrogen adsorption method. (b) Pore size distribution of LiChrospher Si 500: dashed line, nitrogen adsorption; solid line, inverse SEC. The vertical axis corresponds to fraction of pore volume, normalized in the case of inverse SEC.

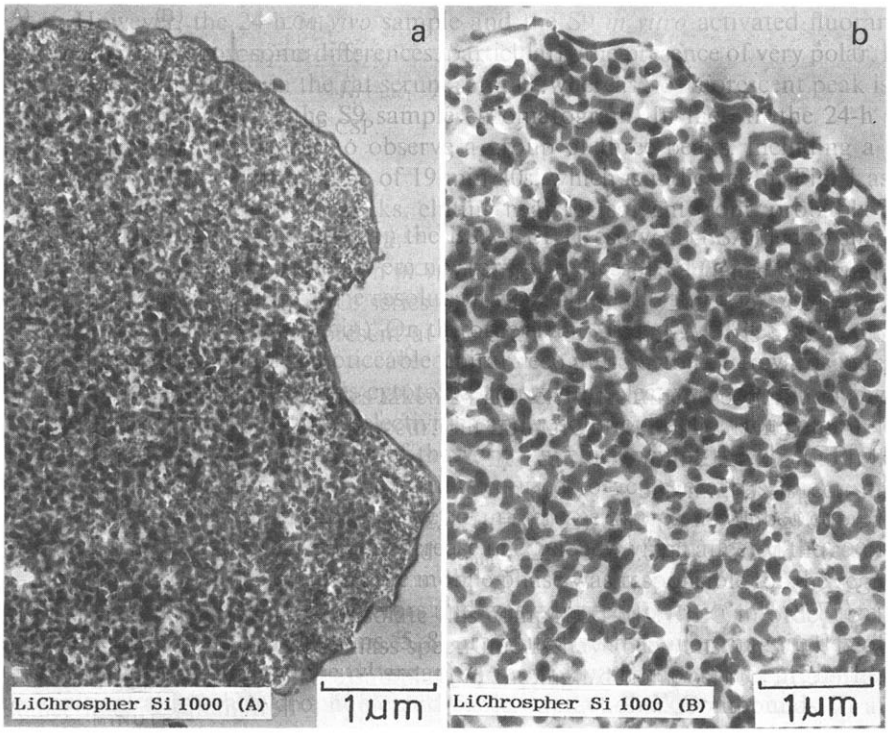

Fig. 3. TEM photographs of LiChrospher Si 1000.

With LiChrospher particles, the presence of the surface shell made it difficult to determine the particle composition, although more than one type of internal structure can be observed with many ultrathin sections prepared from this silica. On the other hand, the internal structures of Nucleosil particles can be examined from the appearance. Fig. 5 shows micrographs of Nucleosil 300. The micrographs showed that at least three kinds of particles having different surface roughness constitute the packing material. About 10% of the particles (C in Fig. 5a) possess a rough surface with a pore size of 50–100 nm, as shown in the insets in Fig. 5b. The other two types of particles in Fig. 5b possess much more compact structures, their pore sizes being smaller than 30 nm. Whereas a corpuscular structure was seen with particles A and B, a spongy structure was seen with particle C, as with the macroporous LiChrospher.

Nitrogen adsorption measurements on Nucleosil 300 showed a bimodal distribution of pore size, one maximum agreeing with that of Nucleosil 100 and the other greater than 50 nm, as shown in Fig. 6a. The SEC calibration graph obtained with standard polystyrenes in tetrahydrofuran for this silica indicates an extremely broad range for permeation of the standards with molecular weight from 500 to 10^6 or above, as shown in Fig. 6c. The pore size distribution curves in Fig. 6b were calculated by the method of Knox and co-workers[17,18]. The calculated pore size distribution of Nucleosil 300 is considerably broader than the others. In Fig. 6b, part of the pore size range of Nucleosil 300 overlaps with those of Nucleosil 100, 1000 and 4000. Smaller

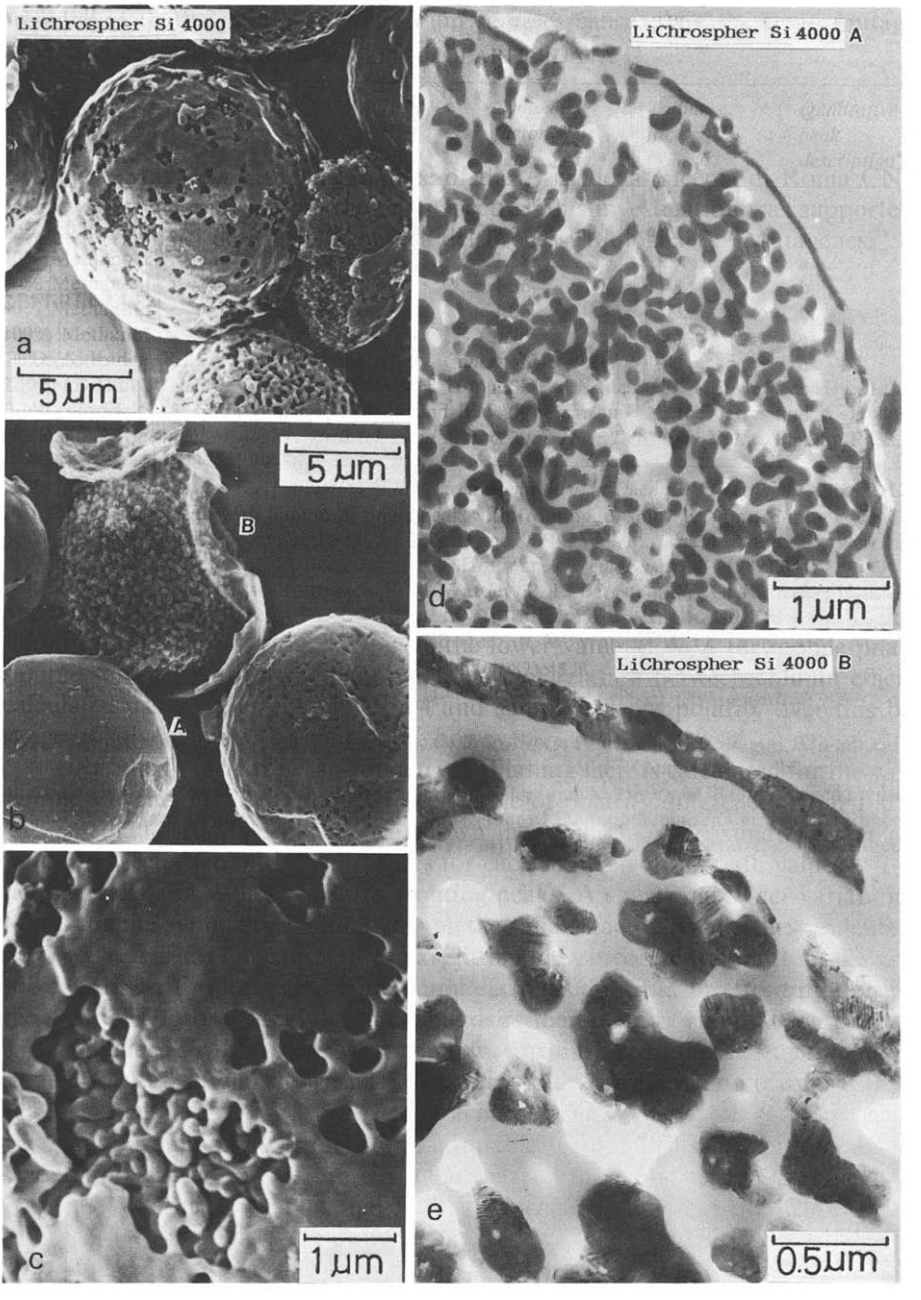

Fig. 4. (a–c) SEM photographs and (d and e) TEM photographs of LiChrospher Si 4000.

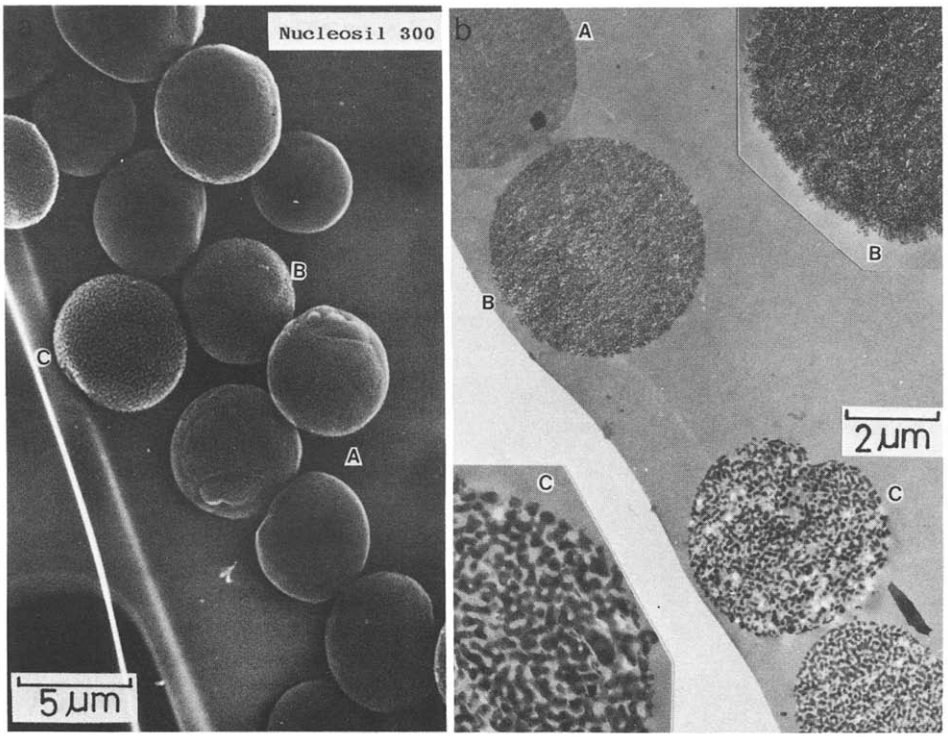

Fig. 5. (a) SEM photograph and (b) TEM photograph of Nucleosil 300. Particles B and C are enlarged 2.5 times in the insets.

pore sizes were obtained from the SEC data by using the method by Halasz and Martin[20,21] but with a similarly broad range of pore size distribution for Nucleosil 300. This silica thus provides a wide linear portion in the molecular weight–elution volume curve. This is a desirable feature in a packing material for SEC, and suitable for the size separation of solutes with a wide molecular weight range. The intention of the manufacturers who offer mixed particles is supposed to be a linear molecular weight–elution volume curve over a wide range for SEC, and the adjustment of the surface area and the pore size distribution within a specification for each batch of commercial products.

Nucleosil 1000 and 4000 were also found to be mixtures of two or three types of silicas, as shown in Figs. 7 and 8. Nucleosil 1000 contained one type of particles (A) that were much more compact than the other (B), which possess a pore size of 200–300 nm. Nucleosil 4000 contained at least three major constituents in comparable amounts, as seen in Fig. 8a. Two (A and B) are similar to those in Nucleosil 300 and 1000. Particle C, with pores up to 500 nm, shows the development of a surface shell to some extent, and some crystallinity of the silica skeleton (Fig. 8c), similarly to LiChrospher Si 4000 in Fig. 4c. Very small amounts of smooth particles (D) are contained in Nucleosil 4000. This type of particle, however, may not be mixed intentionally, because only a few of them were observed.

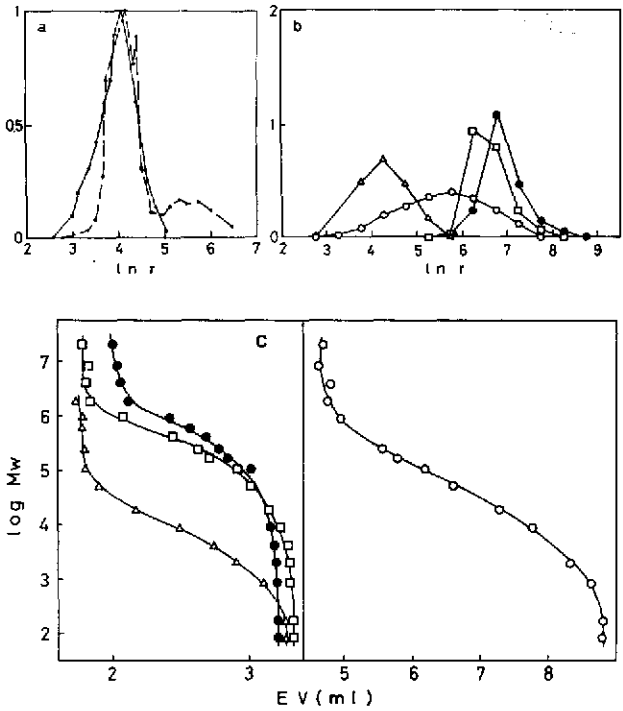

Fig. 6. (a) Pore size distribution of Nucleosil 100 (solid line) and 300 (dashed line) measured by nitrogen adsorption; (b) pore size distribution of Nucleosil 100 (△), 300 (○), 1000(□) and 4000 (●) measured by inverse SEC; (c) molecular weight–elution volume curves obtained on Nucleosil 100, 300, 1000 and 4000. Columns of 25 cm × 4.6 mm I.D. were used for Nucleosil 100, 1000 and 4000, and 25 cm × 8 mm I.D. for Nucleosil 300. The vertical axis in (a) and (b) is as in Fig. 2.

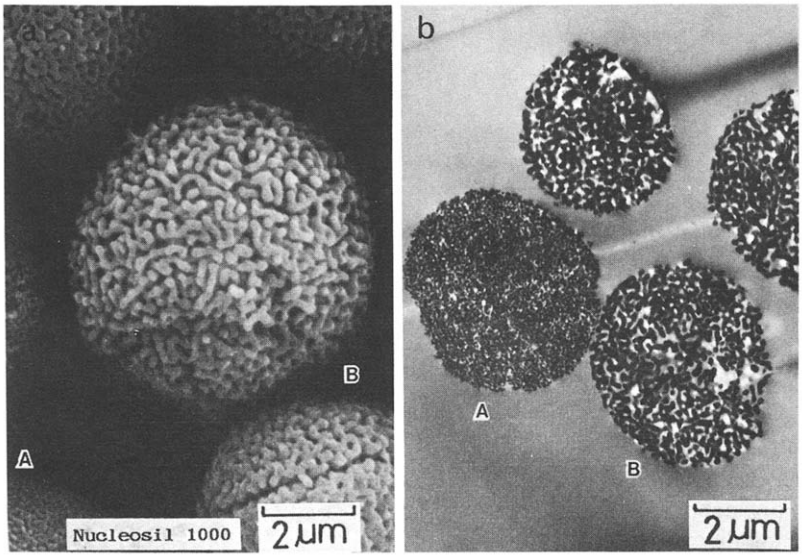

Fig. 7. (a) SEM and (b) TEM photographs of Nucleosil 1000.

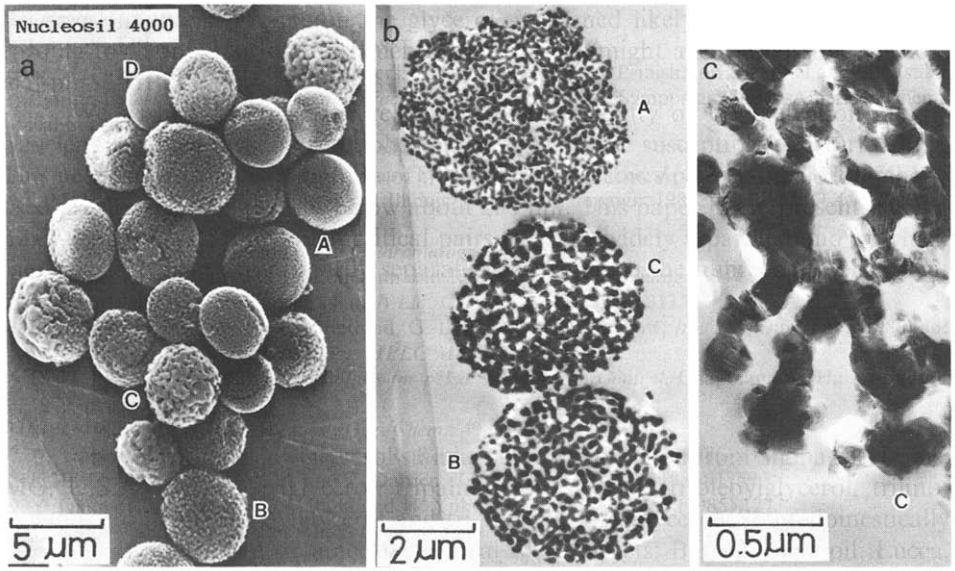

Fig. 8. (a) SEM and (b and c) TEM photographs of Nucleosil 4000.

Not all the commercially available silica particles with a pore size of about 30 nm are mixtures. Hypersil 300, Spherisorb 300 and Vydac TP seemed to consist of one type of silica particles. Among these, Hypersil and Spherisorb showed a typical corpuscular structure, whereas Vydac showed an irregular internal structure, or the presence of a large void, as might be expected from the rough surface structure shown by SEM[22]. The porosity of Spherisorb 300 particles is much higher than those of the other materials, as shown in Fig. 9b and indicated in the pore volume (1.5 ml/g), which may account for the extremely large surface area per unit mass of this packing material.

The transmission electron micrograph of TSK G4000H, a polystyrene–divinylbenzene gel, can be obtained directly without appreciable structural changes of the particle, whereas ruthenium tetraoxide staining was needed for TSK G4000PW, an acrylate or methacrylate gel. Without staining, the TSK G4000PW gel rapidly softened under the electron beam, and disintegration of the particle structure resulted. TSK G4000H particles are aggregates of microspheres, a typical corpuscular system, whereas TSK G4000PW particles possess the features of a spongy system.

The pore structures shown in the micrographs in Figs. 10 and 11 cannot account for the bimodal distribution of pore size seen in the inverse SEC and nitrogen adsorption measurements for these particles (Fig. 12). The pore sizes determined by nitrogen adsorption were smaller than those obtained by inverse SEC, as with silica particles. The large pores found in SEC, nitrogen adsorption and TEM correspond to the nominal pore sizes of these particles. The smaller pores seen only in SEC and nitrogen adsorption measurements are common to most polymer gels[23], and have been used in gel chromatography in the separation of small molecules[24,25]. Such micropores have not been seen with ordinary silica particles for HPLC. The size of these micropores, about 1 nm radius or smaller, suggests that they are formed by the network of cross-linked polymer chains. The results of SEC and nitrogen adsorption imply a biporous

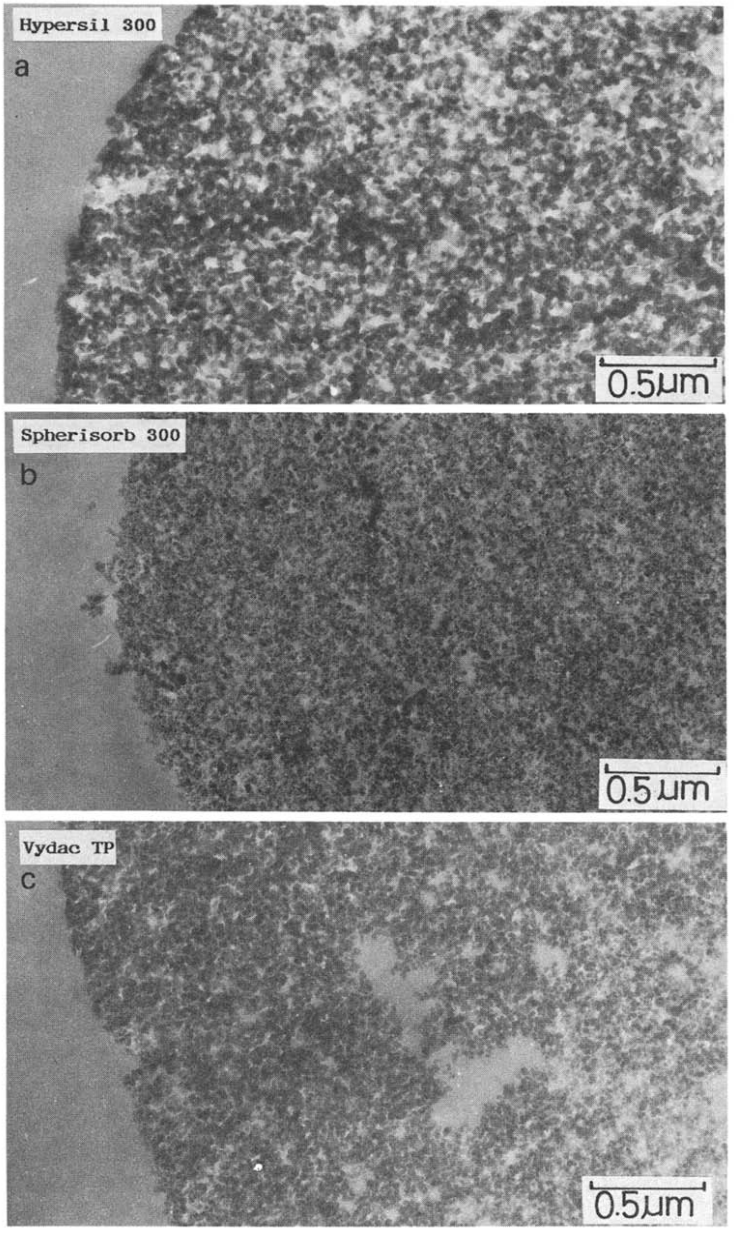

Fig. 9. TEM photographs of (a) Hypersil 300, (b) Spherisorb 300 and (c) Vydac TP.

structure of polymer gels[23], beads composed of aggregates of microporous microgranules. The micropores seem to play an important role in determining both the performance and the shape selectivity, namely selective binding of rigid molecules of a certain size, found in the reversed-phase (RP) HPLC of various hydrocarbons[26,27]. Polymer-based stationary phases showed large differences in performance and in

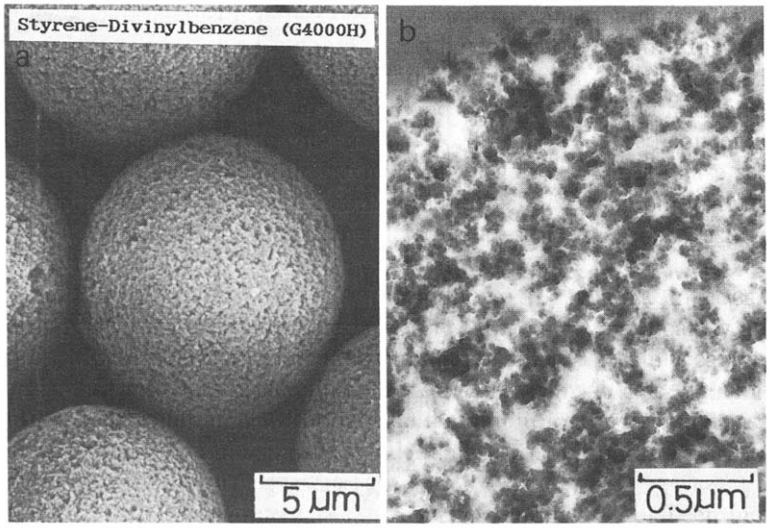

Fig. 10. (a) SEM and (b) TEM photographs of TSK G4000H.

selectivity compared to silica-based phases in RP-HPLC based on solute structures. The relationship between these properties and the pore structures is currently under investigation. These macroporous polymer-based phases are often employed in the separation of large molecules, although they sometimes show poor performance for small molecules depending on the elution conditions[28].

As some packing materials were found to be mixtures, one should be aware whether the packing material in use is a mixture or not when examining the effect of pore size on chromatographic and other properties[29] and when studying the agreement of experimental with theoretical results based on pore models.

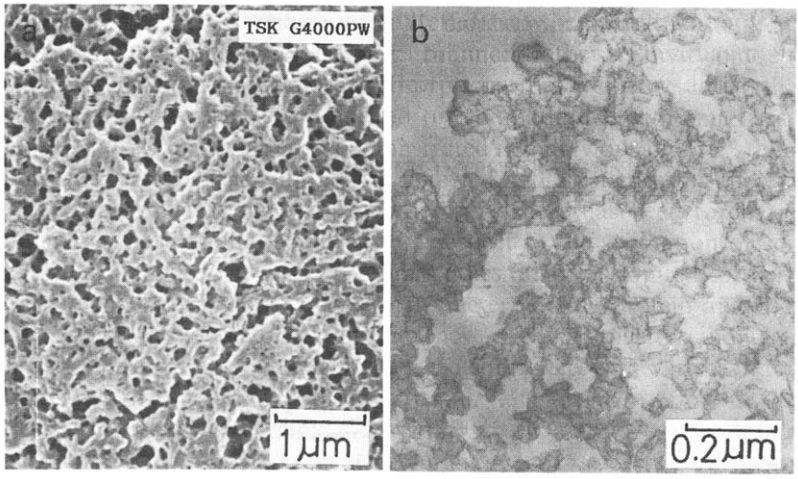

Fig. 11. (a) SEM and (b) TEM photographs of TSK G4000PW.

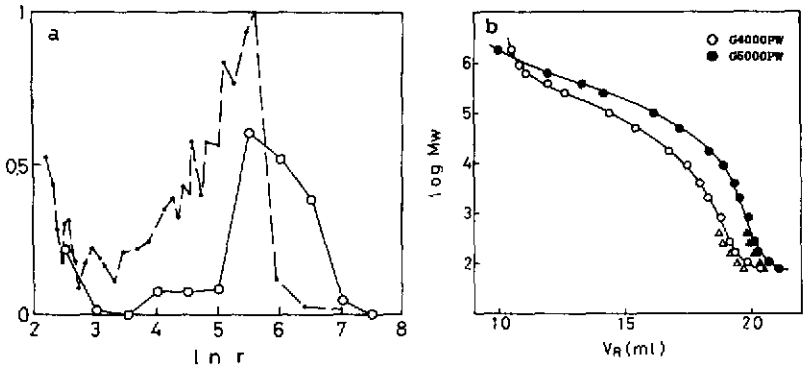

Fig. 12. (a) Pore size distribution of TSK G4000PW measured by nitrogen adsorption (dashed line) and by inverse SEC (solid line), the latter based on the molecular weight–elution volume curve shown in (b). Column, 60 cm × 7.5 mm I.D. Vertical axis as in Fig. 2.

It would be of great interest to examine the effect of the surface shell found with some silica particles on the chromatographic properties and on the determination of pore size and pore size distribution by nitrogen adsorption or mercury intrusion. Direct characterization of porous materials might be possible if the pores are sufficiently large and clear micrographs are obtainable.

CONCLUSION

TEM of ultrathin sections was shown to be a reliable and straightforward method for examining the internal structure of macroporous particles, including silica, glass and polymer gel particles. Some silica particles were covered with a shell due to sintering, which can be broken by excessive ultrasonication in the case of particles with high porosity. Several silica packing materials of 30–400 nm nominal pore size were found to be mixtures of several different types of particles. These packing materials usually give a broad, sometimes bimodal, pore size distribution on measurement by nitrogen adsorption and inverse SEC.

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