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# Designing monolithic double-pore silica for high-speed liquid chromatography

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**Abstract**

A novel type of silica-based chromatographic column without particle-packed structure has been recently developed. A monolithic silica gel having continuous gel skeletons and through-pores together with open mesopores is the key material of this 'rod column'. The principle and procedure of designing the built-in pore structure is explained. © 1998 Elsevier Science B.V.

*Keywords:* Silica rods; Pore structure; Stationary phases, LC

## 1. Introduction

For nearly two decades, columns packed with spherical particles of 5 μm have been most widely used for practical HPLC [1]. Since the reduction of particle size leads to an increase in the column efficiency in exchange for an increase in the pressure drop, the 5-μm particle has been a compromise between these incompatible factors. One of the ways of circumventing this limitation is to fabricate a column made of a single piece of a porous solid with a built-in structure consisting of thin skeletons of continuous solid-phase and a relatively high fraction of through pores. Several examples of such monolithic columns made of organic polymer (polymer rods) have been reported and proven to be effective in the high-speed separation of relatively large molecular mass species such as proteins [2,3]. Recently, Minakuchi et al. reported the fabrication and evaluation of monolithic silica columns (silica rods) prepared via a so-called sol–gel process based on the hydrolytic polycondensation of alkoxysilanes [4–6]. The analytical performance so far evaluated on the silica rods is far better than those of conventional particle-packed columns even for small molecules. The present paper aims to briefly introduce the principle of controlling the porous structure of the silica rods, focusing particularly on the formation mechanism of micrometer-range continuous silica skeletons and through-pores.

## 2. Experimental

The monolithic silica gels for rod columns were prepared as follows: Tetramethoxysilane (TMOS) was added to a solution of poly(ethylene oxide) (PEO: molecular mass=10 000; Aldrich, Milwaukee,

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Table 1
Compositions of the preparation mixtures, the concentration of ammonium hydroxide and the micrometer-range pore characteristics of the heat-treated silica gels

| Sample code size | Composition | | Skeleton size (μm) | Through-pore size (μm) |
|---|---|---|---|---|
| | PEO (g) | TMOS (ml) | | |
| a | 9.4 | 45 | 2.31 | 3.39 |
| b | 9.8 | 45 | 1.58 | 2.23 |
| c[a] | 10.2 | 45 | 1.16 | 1.73 |
| d | 10.4 | 45 | 1.06 | 1.26 |
| e | 11.6 | 40 | 0.97 | 1.66 |
| f[a] | 10.2 | 45 | 1.12 | 1.68 |
| g | 8.8 | 50 | 1.41 | 1.64 |
| h | 7.0 | 55 | 1.59 | 1.53 |

[a] Samples a–d and e–h were prepared in different batches so that the resultant structure is slightly different for the samples c and f prepared from an identical starting composition.

WI, USA) in 0.01 *M* acetic acid (100 ml) and stirred at 0°C for 30 min. The composition of the reaction mixture was varied as shown in Table 1 so that silica rods of various skeleton sizes or skeleton/through-pore size ratios were produced. The resultant homogeneous solution was poured into a cylindrical polycarbonate mould and allowed to react at 40°C. The gelation occurred within 2 h and the gelled sample was subsequently aged at the same temperature for 1 day. Wet silica rods thus formed, were washed with distilled water, then immersed in an aqueous ammonium hydroxide solution in order to tailor the mesopore structure. Evaporation drying and heat-treatment were successively performed accompanying no serious deformation or fracture of gel specimens, which lead to the decomposition of organic constituents and stabilization of the surface of hydrophilic silica gel.

The micrometer-range gel skeletons and through-pores were examined by scanning electron microscopy (SEM, S-510 Hitachi, Japan) using the flat fractured surface of the sample specimens. The pore-size distribution was determined by the mercury intrusion (Poresizer 9320, Micromeritics, USA) and nitrogen adsorption (ASAP 2000, Micromeritics) methods, using heat-treated gel samples. The details of surface modification by octadecylsilyl and methylsilyl groups and those of evaluation of chromatographic performance are described in the preceding papers [4,7].

## 3. Results and discussion

### *3.1. Formation of micrometre-range continuous gel skeletons and through pores*

In the preceding studies on the polymerization-induced phase separation of inorganic sol–gel systems [8–11], it has been revealed that: (1) Inorganically polymerizing systems indeed undergo a phase separation, specifically a spinodal decomposition, driven by the increased free energy of mixing due either to enthalpic or entropic contribution in the Flory–Huggins' formulation. (2) The spinodal decomposition leads to the formation of transient co-continuous phase domains which coarsen with an elapse of time. (3) The inorganic sol–gel transition to form an oxide network can freeze the transient co-continuous domains as a permanent gel morphology (Fig. 1).

The domain size frozen in the gel depends on how far the coarsening of the domains can proceed until they are frozen-in by the sol–gel transition. In the present case, the increase in the concentration of PEO in the starting composition leads to retarded onset of phase separation relative to the sol–gel transition. As a result, the phase domains are frozen in the earlier stage of the coarsening, and finer domains are observed in gelled samples.

The effect of varying the PEO concentration is illustrated by SEM photographs (Fig. 2) and pore-

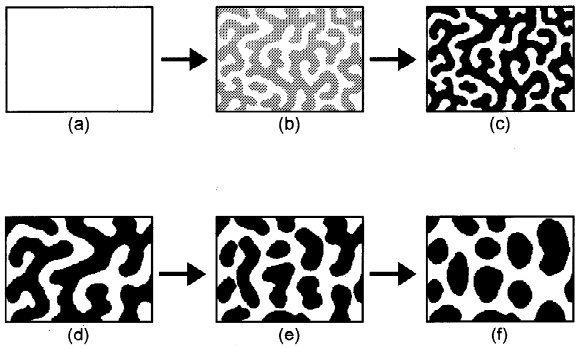


Fig. 1. Early stages (a–c) and coarsening stages (d–f) of the structure development process by the spinodal decomposition.

size distribution determined by the mercury intrusion measurements (Fig. 3).

The volume fraction of through-pores in the dried gels directly corresponds to the volume fraction of the fluid phase in the phase separated wet gels. In the present PEO–silica-solvent quasi ternary system, the gel phase is mainly composed of polymerized silica and PEO while the solvent mixture comprises almost all of the fluid phase. As a result, the solvent fraction plays a dominant role in determining the through-pore volume of the gel samples, similarly to the case of fabricating styrene–divinylbenzene porous polymer beads by adjusting the additive solvent fraction. Experimental results show that the volume fraction

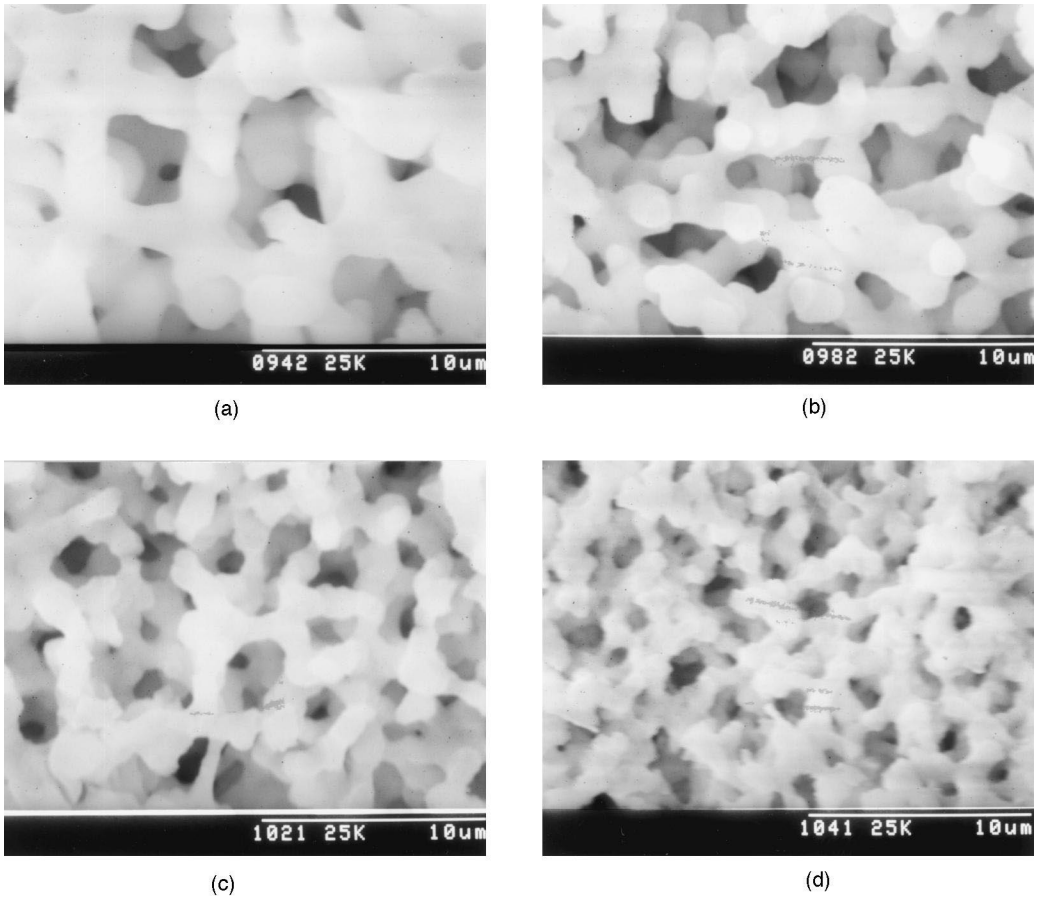


Fig. 2. SEM photographs of heat-treated gel samples prepared from the compositions a–d in Table 1. The bars denote 10 μm in all photographs.

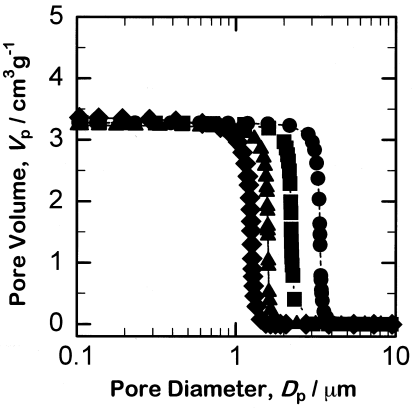


Fig. 3. Pore-size distribution of gel samples identical to those shown in Fig. 2. (a) ●, (b) ■, (c) ▲, (d) ♦.

of through-pores can be as high as 80% depending on the coarsening behaviour of the viscoelastic gel-phase domains. If the co-continuous domains retain appreciable dynamic elasticity until the sol–gel transition, a web-like domain with thin and elongated branches and nodes connecting three or more branches can be obtained [13].

The effect of varying the solvent fraction is shown as the pore size distribution determined by the mercury intrusion measurements (Fig. 4). Pores with almost identical median size and varied volume fraction can be designed simply adjusting the solvent fraction. The maximum volume fraction of through-pores can be as high as 80%. However, in the extreme cases of very high and low solvent fractions,

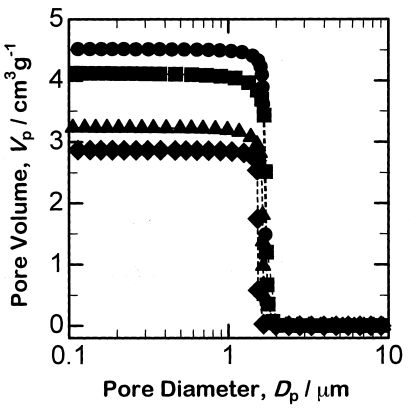


Fig. 4. Pore-size distribution of gel samples prepared from the compositions e–h in Table 1. (e) ●, (f) ■, (g) ▲, (h) ♦.

aggregates of gel particles with very low connectivity and a monolithic gel matrix without detectable micrometer-range continuous pores are formed, respectively, which are no more suitable for rod columns.

Thus, the two compositional parameters, the concentration of PEO and the fraction of solvent, are conveniently used to control the size and volume fraction of through-pores almost independently to each other.

### *3.2. Tailoring nanometer-range pores in the continuous gel skeleton*

The mesopore structure of the wet gel is tailored by exchanging the fluid phase with an external solution. The median size of the mesopores mainly depends on the temperature and pH of the exchanged solution. The reorganization behaviour of nanometer-range silica network is well explained by the Ostwald ripening mechanism, so that the controlling principle of mesopores is common with that for colloidally generated oxide particles [12]. The substantially faster kinetics of the reorganization than that in colloidal particles might be due to the fact that the as-prepared wet silica gel has a much finer and weaker siloxane network structure. The chromatographically important size range of mesopores, between 5 and 25 nm median diameter, can be covered by adopting 0.001 to 1 *M* ammonium hydroxide solution with the temperature up to 120°C. It should be noted that micrometer-range structures of gel skeletons and through-pores are essentially unaffected by the solvent exchange treatments. Although the structural reorganization takes place almost in the wet stage [14], subsequent drying and heat-treatment give chemically and mechanically stabilized double-pore structure to the monolithic gel. Here the term double-pore denotes that continuous open pores are prepared in the discrete size ranges of micrometres and nanometres.

### *3.3. Pressure drop and column efficiency*

The typical silica rods thus prepared exhibit through-pore size: 1.6 μm and volume >60% and mesopore size: 14 nm and volume ca. >15% with a corresponding surface area of about 400 m$^2$/g. The

mesopore volume is slightly reduced after octadecylsilylation. It has been confirmed that the silanol groups in the normal-phase evaluation and octadecylsilyl modified surface in the reversed-phase evaluation are chromatographically equivalent to the conventional high purity silica particles.

The HETP (height equivalent to a theoretical plate) vs. mobile phase velocity relationships examined for alkylbenzenes and insulin revealed that HETP only weakly depended on the mobile phase velocity compared with conventional particle-packed columns having comparable interstitial void size [4]. The small size of silica skeleton relative to the particle diameter is a reasonable explanation for the weakened mobile phase velocity dependence of HETP, which suggests that the advantage of the silica rods becomes increasingly obvious in the high-speed analyses.

The pressure drop can be drastically reduced for silica rods which exhibit much higher through-pore volume than that of particle packed columns. For example, silica rods with through-pores of 1.5–1.8 μm exhibited less than half the pressure drop of that of columns packed with 5-μm particles. When the total column efficiency is evaluated by the separation impedance which is defined by the product of the reciprocal number of theoretical plates per unit pressure drop, the reciprocal number of theoretical plates per unit time and the reciprocal mobile phase viscosity, the impedances for silica rods are at most half that of particle-packed columns and increases only slightly with the mobile phase velocity [6]. As the velocity increases, the impedance for particle-packed columns increases steeply to result in nearly an order of difference from that for silica rods.

## 4. Conclusion

Silica gels with well-defined double-pore structure can be synthesized utilizing the phase separation in the polymerizing sol–gel system and the reorganization of nanometer-range structures by the Ostwald ripening. The silica gel monolith thus obtained can suitably be applied as monolithic columns (silica rods) for HPLC. The silica rods exhibit the weak dependence of HETP on mobile phase velocity as well as the very low pressure drop, which enables one to perform a high-speed analysis with the conventional HPLC apparatus.

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