



## Review

# Silica gel-based monoliths prepared by the sol–gel method: facts and figures

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There is a great deal of interest in continuous beds as stationary phases for both HPLC and CEC. There are various ways to prepare monoliths, by polymerization of organic species or by polymerization of silicon alkoxides. The former method has recently been reviewed, while silica based monoliths are now commercially available. The purpose of this paper is to deal with the problems associated with silica based monoliths. The most important problem is obviously the cracking and the shrinkage of the bed during drying. The second problem is monolith cladding. Much literature has been published but no definitive solution is available and thus a wide research area remains open. Monoliths are a compromise between loadability, permeability and mass transfer kinetics. Due to the better mass transfer properties of a monolithic skeleton over distinct particles, high flow rates and high speed separations are possible.

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**Keywords:** Reviews; Monolithic columns; Sol–gel; Columns; Stationary phases, LC; Stationary phases, CEC; Continuous beds

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## 1. Introduction

Column technology in HPLC has now reached a high standard and high reproducibility. Column performance is expressed as the number of theoretical plates per unit time,  $N/t$ , and the number of theoretical plates per unit pressure drop, i.e. the separation impedance,  $\Delta P = (\Delta P/N)(t_0/N)(1/\eta) = h^2\phi$ , where  $\Delta P$  is the pressure drop,  $N$  the plate count,  $t_0$  the retention time of the inert solute,  $\eta$  the viscosity of the mobile phase,  $h$  the reduced plate height and  $\phi$  the column resistance factor.  $E$  allows for comparison of different chromatographic supports of different geometry. For conventional HPLC columns with reduced plate height,  $h=2$ , the smallest value for  $E$  is 3000–4000. According to Darcy's law, the pressure drop is inversely proportional to the square of the particle diameter. Consequently, small particles have been used in a short column for fast separations and better column efficiency, based on smaller eddy diffusion and shorter diffusion path length. Increasing the flow rate to speed up the separation process is limited by the resulting increasing back pressure which might be associated with a loss in efficiency. To reach higher and higher efficiencies one may use ultra high pressures as did Jorgenson [1] or turn to electrochromatography (CEC) [2]. An increase in column performance can also be achieved by increasing the column permeability. Knox and Bristow [3] recognized the potential advantages of monolithic columns more than 30 years ago. Porous solid columns can exhibit high performance if they have small size skeletons and relatively large through-pores.

Most columns utilized in CEC or in micro HPLC are prepared by packing fused-silica capillaries of 20–100  $\mu\text{m}$  I.D. with porous or non-porous functionalized particles of 2–10- $\mu\text{m}$  diameter. The performance and stability of such particulate packings greatly depend on the retaining frits at column end keeping the packing in place. These frits must retain the packing particles and possess a porous structure so as to allow uniform mobile phase flow through the whole cross-section. It is difficult to reproducibly prepare a highly permeable and mechanically strong end frit by sintering. Heat generated in the sintering process partially destroys the stationary phase. End frits reduce the column separation efficiency and are

often responsible for bubble formation during analysis. The presence of bubbles causes a drop in current and the run must be interrupted. Problems are similar in planar chromatography which may turn into planar electrochromatography [4]. Preparation of a continuous bed is obviously a convenient way to solve the problem. The use of monolithic columns as an alternative to packed capillaries has already been reported in GC [5]. In the late 1990s there was great interest in monoliths devoted to either LC or CEC and several extensive reviews appeared on monolithic columns [6–10].

Four approaches have been utilized to prepare continuous beds:

- (i) polymerization of an organic monomer with additives,
- (ii) formation of a silica based network using a sol–gel process,
- (iii) fusing the porous particulate packing material in a capillary by a sintering process, and
- (iv) organic hybrid materials.

At present, the first two quoted procedures are the most popular. Organic monoliths have been successfully produced on a laboratory scale but are not yet widely commercially available. Silica based monoliths seem to give more practical results and the number of applications is continuously increasing. The fourth approach is being thoroughly studied by organic chemists and looks promising. Since the above quoted reviews [6–10] provide a lot of information, the purpose of this paper is mainly to focus on some aspects of silica based monoliths from standpoint of sol–gel chemistry and give a short overview of the variety of procedures. It is mainly devoted to those who want to be involved in a topic which seems as rich as the bonding of alkyl moieties on silica particles.

## 2. Organic polymers

Hjerten and Liao [11] were the first to report a monolith from a compressed polyacrylamide gel with the purpose of separating proteins. The idea was not totally new since Hansen and Sievers [12] had published a preliminary paper in 1974.

In their well documented review, Zou et al. [9] distinguished: rods based on polystyrenes; rods based

on polymethacrylates; rods based on polyacrylamides; and monolithic molecularly imprinted polymers.

In a recent review [13], Oberacher and Huber examined the use of monolithic capillary columns prepared by copolymerization of styrene and divinylbenzene inside a 200- $\mu\text{m}$  I.D. fused-silica capillary to analyse nucleic acids by HPLC–MS.

The polymer based monoliths are almost exclusively prepared by radical polymerization (thermal or UV). For example, in a single one step procedure, a solution of acrylic monomers including piperazine as cross linking agent was polymerized in a fused-silica capillary pretreated with 3-(trimethoxysilyl)propyl methacrylate [14]. Preparation of a compressed continuous bed for conventional and micro HPLC in normal-phase mode has been extensively studied by Frechet and colleagues [15–25]. They devised a rigid polyacrylamide-based monolithic column containing butyl methacrylate for separation of proteins by hydrophobic interaction chromatography [18]. They also used a monolithic poly(styrene-co-divinyl benzene) [20]. Molded rigid monolithic columns confined in untreated silica capillaries were prepared by copolymerisation of mixtures of butyl methacrylate, ethylene dimethacrylate, and 2-acrylamido-2-methyl-1 propanesulfonic acid in the presence of a porogenic solvent [19]. The same group attached a chiral moiety to the surface [22,23]. The goal is to prepare a stationary phase for electrochromatography with further application to separations on a chip [25]. The reader is referred to the review published by Svec et al. [22]. Recent papers on the preparation of methacrylate monolithic columns for capillary liquid chromatography [26] or electrochromatography [27] have appeared. Hoegger and Freitag [28] treated the capillary walls with 3-(trimethoxysilyl)propyl methacrylate before performing in situ the methacrylamide polymerization. Zhang and El Rassi [29] devised a polyacrylamide monolithic phase with bonded dodecyl ligands and sulfonic acid groups to separate moderately polar solutes by CEC; peptide analysis in capillary LC–MS was performed on surface alkylated polystyrene monolithic columns by Huang et al. [30]. An alternative approach is the use of ring opening metathesis polymerization (ROMP) as pioneered by Novak and Grubbs [31] and extensively used by Buchmeiser and colleagues [7,32].

This method of monolith preparation produces phases with excellent pH stability but they may shrink or swell when exposed to different mobile phases; another shortcoming is the mechanical stability of the packing with pressure. Due to convective flow these phases are well suited for protein and peptide analysis and a review on the topic has appeared [33] where 200 000 plates per meter were claimed.

Commercially available monolithic columns of polymer based supports can be obtained from BIA (Slovenia). They are highly cross-linked porous rigid monolithic polyglycidylmethacrylate styrene–divinylbenzene polymers [34–38] and applications have been published especially as regards preparative purposes. Bio-Rad Laboratories and Sartorius produce acrylamido based polymeric columns [39,40].

### 3. Silica based monoliths

Sol–gel technology has been utilized to create surface bonded coatings. Engelhardt and Cunat-Walter [41] used sol–gel chemistry to create an olefinic sublayer for subsequent polymerization with acrylamide to prepare polyamide coated open tubular columns for capillary electrophoresis. Guo and Colon [42] bound retentive alkyl ligands in open tubular columns for LC and CEC through this technique. Hayes and Malik [43] filled a capillary with an alkoxide solution and performed hydrolysis. Condensation of tetrahydroxysilane with surface silanols of the inner walls and derivatized fused-silica surface with Ucon (a polyethylene oxide) yields a claimed uniform coating. Historically, papers on polymer based monoliths appeared before those on silica based monoliths, but more research has been carried out on inorganic matrix. The successful preparation of silica-based rod columns was reported by Nakanishi and Soga in 1991 [44,45]. By combining the sol–gel reaction with phase separation and a subsequent solvent exchange treatment, double-pore silica gel (macropores and mesopores) monoliths were prepared. The real breakthrough was the mastering of both the macropores and the mesopores. Silica rods thus prepared were released on the market by Merck. In situ derivatization was

performed with octadecyldimethyl-(*N,N*-diethyl aminosilane) followed by end capping with hexamethyl disilazane. The problem of contact with the wall had to be overcome and Merck produced a PEEK column. The invention of monolithic silica based columns can be regarded as a major technological change in column technology.

### 3.1. Sol–gel based monoliths

The basic sol–gel process involves the sequential hydrolysis and polycondensation of alkoxy silicon derivatives (e.g. tetraethyl orthosilicate (TEOS), or tetramethyl orthosilicate (TMOS)), in aqueous acid or base with a mutual cosolvent. Non-silica aerogels are notably weak and fragile in monolithic form, however the synthesis of high porosity monolithic alumina aerogels has been recently described [46] and hydrolysis of aluminium tri-*sec*-butoxide in acetone was studied by NMR [47]. The first step is the formation of a sol. A sol is a colloidal suspension of solid species in a liquid. The sol is converted into a gel through polycondensation of the sol leading to a wet structure. A gel is a biphasic medium from the isotropic and progressive densification. It is a porous network in a liquid. Hydrogels are formed from aqueous solutions whereas alcogels are formed from alcoholic solutions. The process of gelation starts with aggregation of particles or polymers into fractal clusters, then the clusters interpenetrate to some extent and finally link together to form an infinite network [48]. Xerogels are dried by evaporation of the liquid, and aerogels are usually obtained by removal of solvents in supercritical conditions. In silica aerogels there is less than 2% silicon dioxide and 98% air.

#### 3.1.1. Starting material

Sol–gel precursors are mainly silicon alkoxides which can be obtained in a high degree of purity whereas potassium silicate is very difficult to purify. Tetramethoxysilane (TMOS) undergoes a more rapid hydrolysis than tetraethoxysilane (TEOS) and was extensively used by a Japanese team to produce monoliths [49–53]. Wagh et al. [54] compared the aerogels obtained from three different precursors: TEOS, TMOS and PEDS (polyethoxydisiloxane) and claimed that TMOS yields narrow and uniform pores

and higher surface area than TEOS. Methyltriethoxy silane (MTES) was advocated by Colon et al. [55] for the more flexible network produced from its gelling. A mixture of TMOS and MTES was also promoted by Harreld et al. [56] and Ishizuka et al. [57]. Adding methyltrimethoxysilane to TMOS or dimethyldiethoxysilane to TEOS [58] increases the hydrophobicity of the aerogel and shifts the pore size distribution towards larger pore radii [59]. Functional groups on silicon atoms which impart the hydrophobic properties to the solids are atoms showing high electronegativity such as F (Fig. 1). One of the sol–gel precursors may be a mixture of alkoxy-silanes, one of them being a functionalized moiety such as aminopropyltriethoxysilane [60], (3-(2-aminoethylamino)propyltrimethoxysilane (EDAS) [61] or *N*-octyltriethoxysilane as proposed by Rodriguez and Colon [62] or Constantin and Freitag [63] who performed a copolymerization process. Guo et al. used a mixture of TEOS and polydimethylsiloxane ( $M_w=4200$ ) [64]. According to Mansur et al. [60], a combination of different silane agents during synthesis of the gels can satisfactorily be used to produce materials containing specifically designed chemical functionalities. Einarsrud et al. studied the hydrolysis of polyethoxydisiloxane [65]. To prepare monolithic sol–gel columns with surface bonded ligands, Malik and Hayes [66] utilized two sol–gel precursors and a deactivation reagent (phenyldimethylsilane) to produce a monolith.

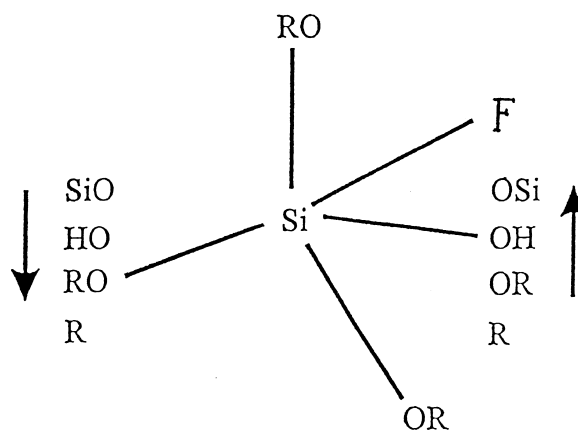


Fig. 1. Donor acceptor characteristics of alkoxy-silanes. Arrow on left: increase of electron donor (acid) properties; arrow on right: increase of electron acceptor (basic) properties.

### 3.1.2. Sol preparation and hydrolysis

Numerous procedures are described in the literature and there are many different methods (Table 1). It must be pointed out that most of these procedures are for other purposes than preparing monoliths for chromatography. In any case the starting silicon derivative is dissolved in a solvent, an aqueous solution (acid or base) reacts and gelation is produced within a certain period of time. TEOS, TMOS or other silicon alkoxides are not soluble in water. They are dissolved in alcohol to produce an homogeneous solution and water is then added. In their preparation of column frits, Zhuang and Huang [67] used methylene chloride to dissolve MTES. Dai et al. [68] promoted ionic liquids. Einarsrud et al. [65] dissolved the acid catalyst in ethyl acetoacetate and added this mixture to polyethoxydisiloxane. Hydrolysis of silicon alkoxides is a versatile technique which can produce different materials according to the different parameters and to the acid catalysis or base catalysis reaction. It may produce silica spheres or silica gels depending upon the experimental conditions.

The critical point is the ratio Si:H<sub>2</sub>O, i.e. the proportions of TMOS (or TEOS) and water which yields different products. With concentrated ammonia, silica colloids (Stoeber colloids) [69] can easily be prepared by the procedure described by Nakanishi and Takammiya [70] and Osseo-Asare and Arriagada [71]. Unger et al. [72] produced monodispersed silica microspheres by hydrolysis in an alkaline solution. Karmakar et al. [73] observed that any acid–water mixture irrespective of the type of acid (formic acid, acetic acid, propanoic acid, pentanoic acid, hydrochloric acid, nitric acid, sulphuric acid, orthophosphoric acid) can produce silica microspheres from TEOS with a water:TEOS ratio in the range 1.0–1.5 for strong acids and 1.5–4.0 for weak acids. They argued from FTIR spectra that four membered siloxane rings (Si<sub>4</sub>O<sub>12</sub>) are formed in the heavier liquid. Nicolaon and Teichner [74] carried out a study of the influences of water and TMOS concentration when producing aerogels exhibiting high porosity and concluded that the water quantity should be 2–5-fold the stoichiometric proportion with a TMOS concentration in the alcohol of ~5–10%. The molar ratio of H<sub>2</sub>O:Si(OR)<sub>4</sub> in the sol should be at least 2:1 to approach complete hy-

drolysis of the alkoxide. By increasing water concentration, chemical reactions are accelerated and gelation times decrease.

Hydrolysis is performed with a catalyst. Three procedures are proposed: acid catalysis, base catalysis and two-step catalysis. It is generally agreed that under acid catalysis, entangled linear or randomly branched chains are formed in silica sols whereas under base catalysis, it is easy to form a network of uniform particles in the sol. Acid catalysis is performed with HCl, H<sub>2</sub>SO<sub>4</sub>, HNO<sub>3</sub>, HF, oxalic acid, formic acid, and acetic acid. A typical volume ratio is TEOS:C<sub>2</sub>H<sub>5</sub>OH:H<sub>2</sub>O:acid 1:3:4:0.002. Gelation times are generally longer when the pH of the sol is low. Kirkbir et al. [75] observed that HF catalysis yields the highest pore volume and pore diameter but the gel is weak. They also promoted double acid catalysis [76] with HF and either HCl, HNO<sub>3</sub> or H<sub>2</sub>SO<sub>4</sub>. Hydrolysis of TMOS at 70–80 °C in an open vessel accelerates condensation and reduces the amount of liquid by expelling excess methanol through distillation [77]. Acid-catalyzed hydrolysis can be stimulated by sonication [78]. Klemperer et al. [79] studied the products of the hydrolysis of Si(OMe)<sub>4</sub> (3 mol) in methanol (14 mol) by water (1.8 mol) containing hydrochloric acid (0.05 mol) after a period of 5 h. The mixture of polysilicate species was then esterified with diazomethane to convert hydroxyl groups. Analysis with capillary GC and <sup>29</sup>Si NMR evidenced a great number of species: (Si<sub>2</sub>O(OMe)<sub>6</sub>), (Si<sub>3</sub>O<sub>2</sub>(OMe)<sub>8</sub>), (Si<sub>4</sub>O<sub>4</sub>(OMe)<sub>8</sub>), (Si<sub>4</sub>O<sub>3</sub>(OMe)<sub>10</sub>), (Si<sub>5</sub>O<sub>5</sub>(OMe)<sub>10</sub>), (Si<sub>5</sub>O<sub>5</sub>(OMe)<sub>10</sub>), (Si<sub>5</sub>O<sub>4</sub>(OMe)<sub>12</sub>), (Si<sub>6</sub>O<sub>6</sub>(OMe)<sub>12</sub>), (Si<sub>6</sub>O<sub>5</sub>(OMe)<sub>14</sub>), (Si<sub>7</sub>O<sub>7</sub>(OMe)<sub>14</sub>), (Si<sub>7</sub>O<sub>6</sub>(OMe)<sub>16</sub>), (Si<sub>8</sub>O<sub>8</sub>(OMe)<sub>16</sub>), and (Si<sub>8</sub>O<sub>7</sub>(OMe)<sub>18</sub>).

Base catalysis usually involves dilute ammonia 10<sup>-2</sup> M [80]. Under base catalysis it is easy to form a network of uniform particles in the sol, and the resulting pore volume is quite large. Under base catalysis condensation kinetics are faster than hydrolysis kinetics (Fig. 2). The two-step procedure was proposed by Brinker et al. [81] and used by others [82–84]. In a typical procedure [80] TMOS, ethanol, H<sub>2</sub>O and HCl are mixed in the volume ratio 4:4:1:1 × 10<sup>-2</sup> and this first solution is then mixed with H<sub>2</sub>O and ammonia in the volume ratio 9:1:4 × 10<sup>-2</sup>. The addition of NH<sub>4</sub>OH as a second catalyst to

Table 1  
Sol-gel preparation

Silicon derivative	Solvent	Water	Catalyst	Porogen	Reference	Remark
TEOS	EtOH					
1 <sup>a</sup>	3	4	HCl 0.02			
1	3	4	HNO <sub>3</sub> 0.02			
1	3	4	H <sub>2</sub> SO <sub>4</sub> 0.02		[75]	
1	3	4	Oxalic acid 0.02			
1	3	4	HF 0.02			
TMOS	MeOH					
1 <sup>a</sup>	0.5–3	2–20	CH <sub>3</sub> CO <sub>2</sub> H 0.06–1 <i>M</i> NH <sub>4</sub> OH 0.02–1 <i>M</i>		[74]	
TMOS	Ionic liquid		HCO <sub>2</sub> H		[68]	
1 <sup>a</sup>	1		2			
MTES	CH <sub>2</sub> Cl <sub>2</sub>		TFA		[67]	Addition of silica gel
0.75 <sup>a</sup>	2	0.1				
TEOS	EtOH		HCl 12 × 10 <sup>-3</sup> <i>M</i>			
C <sub>8</sub> TEOS						
107 μl <sup>b</sup>	168 μl	60 μl			[55]	
TEOS/C <sub>8</sub> TEOS			HCl 0.11 <i>M</i>		[63]	
500 μl <sup>b</sup> /282 μl	200 μl	93 μl	11 μl			
TEOS	EtOH		HCl 4.8 × 10 <sup>-6</sup> <i>M</i>	PEG	[88]	
1 g <sup>b</sup>	1 ml	0.86 μl				
TMOS	EtOH		HCl 10 <sup>-2</sup> <i>M</i>		[59]	
1 <sup>a</sup>	4	1				
TMOS	MeOH		NH <sub>4</sub> OH 10 <sup>-2</sup> <i>M</i>		[80]	
1 <sup>a</sup>	4	4				
TMOS	MeOH		NH <sub>4</sub> OH 3.7 × 10 <sup>-3</sup> <i>M</i>		[92]	Addition of TMOS
1 <sup>a</sup>	14	4				
PEDS	Ethyl acetate		HF 21 N		[65]	
1 <sup>b</sup>	1		0.02%			
TMOS			CH <sub>3</sub> CO <sub>2</sub> H 0.01 <i>M</i> then washing with NH <sub>4</sub> OH	PEO	[87]	TMOS addition in CH <sub>3</sub> CO <sub>2</sub> /PEO
45 ml						
TEOS	EtOH		NH <sub>4</sub> OH 0.18 <i>M</i>		[93]	Other silicon derivatives
a						
TEOS	EtOH		NH <sub>4</sub> OH (27%), HCl 1 <i>M</i>		[82]	Double catalysis
1 <sup>b</sup>	20		2.8/3.2			
TMOS	EtOH		HCl 10 <sup>-2</sup> <i>M</i> /NH <sub>4</sub> OH 10 <sup>-1</sup> <i>M</i>		[83]	Double catalysis
4 <sup>b</sup>	4	1				
TMOS			CH <sub>3</sub> CO <sub>2</sub> H 10 <sup>-2</sup> <i>M</i>	PEO	[57]	9 g Urea
0.45 <sup>b</sup>						

<sup>a</sup> Molar ratio.<sup>b</sup> Volume ratio.

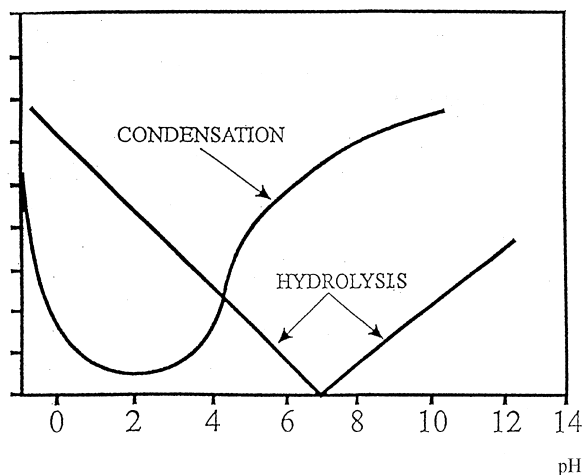


Fig. 2. Scheme of relative reaction kinetics of alkoxy silanes versus pH.

a sol initially catalyzed by HCl can increase the rate of condensation reactions and reduce the gelation time. A two-step procedure involving urea was recently proposed by Ishizuka et al. [85]. Gelation can take place on wide time scale: seconds, minutes, hours, days, or months. Gelation times can be followed by measurement of viscosity (Fig. 3); the gel exhibits a Newtonian viscosity in the initial state

and then transforms into a viscoelastic gel. An alcoholic solution of triethoxysilane  $\text{H}(\text{Si}(\text{OEt})_3)$  yields gelation without any catalyst reagent. The hydrolysis does not induce the cleavage of Si–H bonds [86].

The aggregation process yields mass fractal structures. The fractal dimension is of paramount importance. A small fractal dimension indicates a highly porous sol–gel network; high values are indicative of micropores.

### 3.1.3. Additives

The pore size and the mechanical properties of gels can be varied with the addition of polyethylene glycol (PEG) to the sol. PEG is a porogen which acts as a through-pore template and solubilizer of the silane reagent. This has been done by Nakanishi et al. [87], Judenstein et al. [88] and by Martin et al. [89] who claimed that high concentrations of PEG weaken the solid matrix whereas a small concentration of PEG strengthens the matrix. The pore size of macroporous silica aerogel can be controlled by varying the concentration of water soluble polymer. Narrow and more uniform pore size distribution was observed with the addition of glycerol which acts as a drying additive since it prevents further reaction of

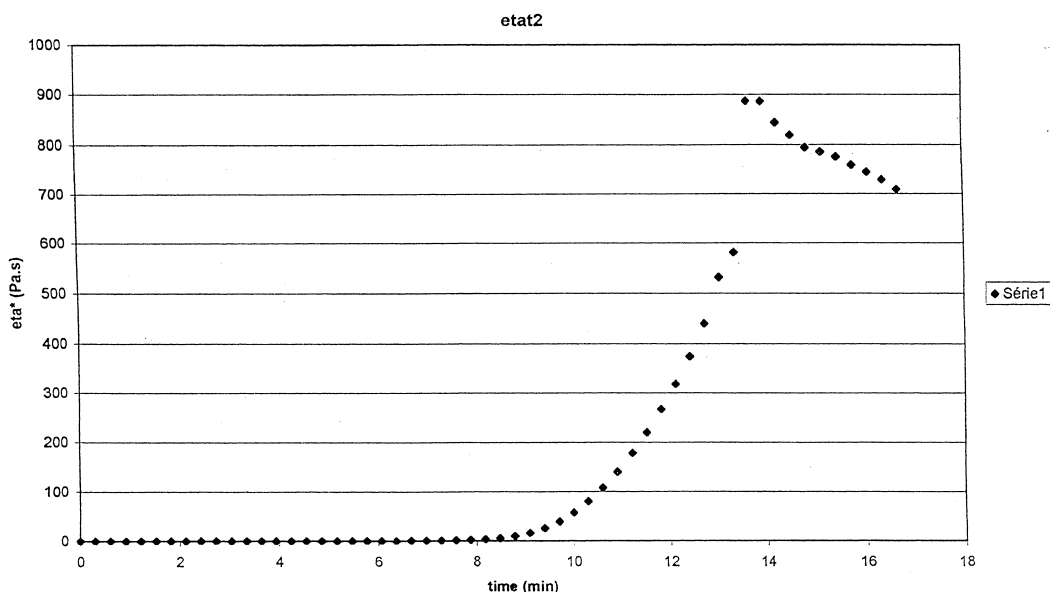


Fig. 3. TEOS gelation (double catalysis) followed by viscoelastic measurement (Contraves rheometer).

water [90]. Use of a surfactant in water–ethanol solution produces surfactant templated aerogels [91]. Since the hydrophobic properties of TMOS based monoliths are increased by incorporating MTES as a synthesis component [92], Alié et al. [93–95] considered the incorporation of other silanes to be equivalent to the incorporation of additives. Differences in reactivity are obvious (Fig. 1). Consequently, the nucleation mechanism is related to the difference in reactivity between the main reagent (TMOS, TEOS, or TPOS) and the additive. They examined the behavior of 3-(2-aminoethylamino)propyltrimethoxysilane (EDAS), 3-aminopropyltriethoxysilane (AES), 3-aminopropyltrimethoxysilane (AMS), propyltrimethoxysilane (PMS) and 3-(2-aminoethylamino)propyltriethoxysilane (EDAES). The main reagent is either TMOS, TEOS, or TPOS. A nucleation mechanism by the additive takes place. When the additive contains methoxy groups, it reacts first to form particles to which the main reagent condenses in a later stage. When both the additive and the main reagent contain an ethoxy group, there is no nucleation mechanism by the additive. Amine or alkyl groups only influence gelation time. Addition of silica spheres (aerosil) in the solution before gelation strongly affects the aggregation mechanism; two fractal structures coexist. Table 1 lists some typical procedures. The main feature is the use of very similar catalysts, e.g. HCl in acid catalysis and ammonia in base catalysis.

### 3.1.4. Aging

Washing in H<sub>2</sub>O/EtOH increases the liquid permeability of the solid part of the gel by a dissolution–reprecipitation process for silica. Aging in a siloxane solution increases the stiffness and strength of the alcogel by adding new monomers to the silica network and by improving the degree of siloxane cross linking; conversely this step will reduce the permeability [96]. Einarsrud et al. [97,98] have reported strengthening of the silica gels aged in TEOS, water, and ethanol solutions. Gels are washed with a 20% water–ethanol solution for 24 h at 60 °C, then an aging solution (70%TEOS:ethanol, v/v) is used for 6–72 h at 70 °C followed by washing with ethanol and heptane. Data from small angle neutron scattering show only a slight increase in the volume fractal dimension of the porous gel network. The

same group demonstrated that washing in a water solution increases the permeability of the gels by dissolution–reprecipitation [65] (Ostwald ripening). Silylation removes Si–OH surface groups by promoting silica polycondensation resulting in a decrease of pore size.

We can notice that in many papers the gel is aged without indication of the procedure. Subsequent drying is performed carefully to prevent cracking.

### 3.1.5. Drying

Drying of the gel is the critical step. Drying is governed by capillary pressure. During drying, shrinkage of the gel occurs due to capillary pressure. It is the gradient in capillary pressure within the pores that leads to mechanical damage; the capillary tension developed during the drying may reach 100–200 MPa [99] with consequent shrinkage and cracking. Silica gel may decrease in volume by as much as a factor 10 as it dries. The extent of shrinkage is governed by the balance between capillary pressure  $P_c$ , and modulus of the solid matrix:

$$P_c = -2\gamma_{(LV)} \cos \theta / r_h$$

where  $\gamma_{(LV)}$  is the surface tension of the pore liquid at the liquid vapor interface,  $\theta$  is the contact angle of the liquid, and  $r_h$  is the hydraulic pore radius:

$$r_h = 2V_p / S_p$$

where  $V_p$  and  $S_p$  are pore volume and surface area, respectively. They are critical parameters.

Three phases are present: liquid, solid and gas. When liquid evaporates from the pores of a gel and when the contact angle,  $\theta$ , between the liquid and the network is  $<90^\circ$ , concave liquid–vapor menisci form at the exterior surface of the body so that the liquid goes into tension and the solid network of the gel is compressed. When  $\gamma_{(SG)} > \gamma_{(SL)}$ , the liquid ascends the pore by capillary rise, the solid/gas interface is replaced by a solid/liquid interface, and a concave meniscus is observed. If  $\theta = 0$ , or  $\rightarrow 0$ , the capillary liquid is stressed, and the solid network is compressed. When  $\gamma_{(SL)} > \gamma_{(SG)}$ , a convex meniscus is observed, if  $\theta = \pi$  or  $\rightarrow \pi$ , there is no compression of the gel (Fig. 4). The small pore size can induce fracture during drying due to enormous capillary forces.



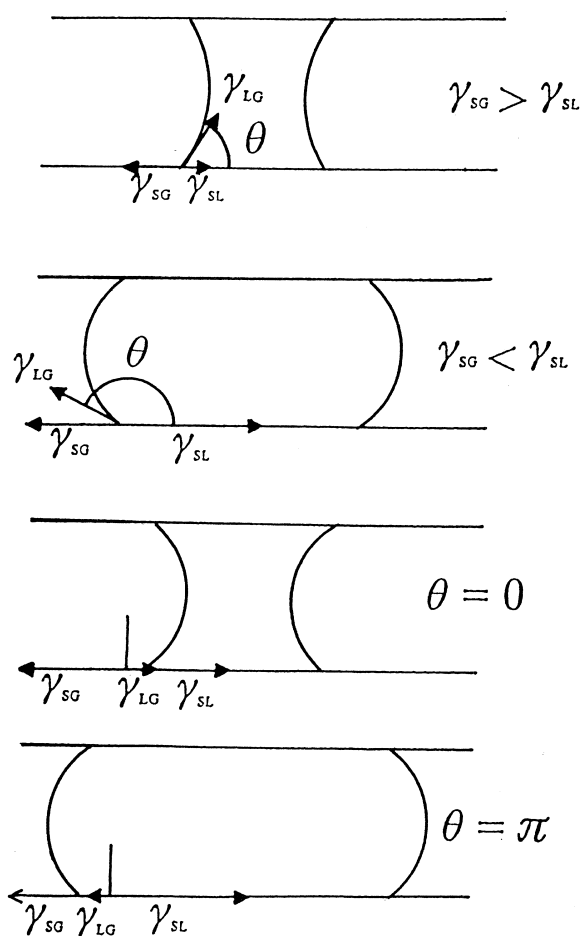


Fig. 4. Formation of menisci during drying: concave or convex meniscus on wetting angle.  $\gamma_{(L,V)}$ , surface tension (liquid/vapor);  $\gamma_{(S,G)}$ , surface tension (solid/gas);  $\gamma_{(S,L)}$ , surface tension (solid/liquid);  $\theta$ , wetting angle.

There are many drying methods: replacing the alcohol by washing with liquid  $\text{CO}_2$  and drying above the critical point; replacing the alcohol with water followed by washing with, for example, acetone and liquid  $\text{CO}_2$  and supercritical drying at  $35^\circ\text{C}$  and 8.5 MPa in an autoclave [100]; changing the pore fluid (solvent exchange) and drying; drying at ambient pressure [97].

The surface of the solid network of silica gels is not modified by supercritical drying in  $\text{CO}_2$  since no surface tension exists and the extent of dissolution redeposition is not significant. Ionic liquids exhibit

negligible vapor pressure and can stabilize the network. They are further removed using a solvent.

Efforts have been directed towards understanding drying phenomena above the critical temperatures and pressures but much less towards understand the behavior of gels dried below the critical conditions. By drying at ambient pressure, the surface tension between liquid and vapor cannot be avoided. Stress within the gel is proportional to the viscosity of the pore liquid and the drying rate and inversely proportional to the permeability of the wet gel. The important parameters are: the pristine gel strength, the pore size of the wet gel, and the solvent used in drying. The small pore size can induce fracture during drying due to enormous capillary forces. The pore liquid is under enormous tension when the pore size is smaller than  $200 \text{ \AA}$ . On the other hand, when the pore size is larger than  $200 \text{ \AA}$ , the shrinkage will be less and cracking will be less likely to occur [59]. Conversely in some cases small pore size gels ( $40 \text{ \AA}$ ) are easier to dry than larger pore size gels, which is explained by a theory of cavitation [99]. Manipulation of pore size distribution can be done through the drying solvent.

Kirkbir et al. [96] observed that a threshold pressure exists above which the shrinkage is negligible. Below the threshold point, the capillary pressure overcomes the strength and the structure collapses. Above the threshold point, the strength is always higher and the shrinkage becomes negligible. The threshold point depends on sol composition. Shrinkage is negligible for wet gels dried in isobutanol and 2-pentanol at chamber pressures as low as 1.8 MPa. The surface tension of isobutanol is two times higher than that of ethanol. As a result the gels will experience twice the capillary pressure in isobutanol. However shrinkage is negligible. We found that shrinkage is negligible for wet gels dried in 2-pentanol at 1.8 MPa and  $300^\circ\text{C}$ . This can be explained by silica solubility (Table 2), and structural modification since the process may induce an increase in the network connectivity by formation of new siloxane bonds. The variation in silica solubility may not be sufficient to explain the variation in shrinkage with type of alcohols. Moreover, *n*-alcohols promote higher  $S_p$ , higher porosity, and smaller pore diameter. Dieudonne et al. [80] claimed that thermal treatment of silica gels under alcohol in

Table 2  
Silica solubility in *n*-alkanols

Methanol (mg/l)	1890	
Ethanol (mg/l)	164	
Propanol (mg/l)	8	
	20°	205°
Pore liquid	$\gamma_{(LV)}$ dyne/cm	$P_v$ $\gamma_{(LV)}$ $P_c$
Ethanol	22.4	3.2    2.5    0.7
Isobutanol	22.6	1.8    7.2    1.9
2-Pentanol	24.0	1.0    6.1    1.6
Isooctane	18.8	1.0    3.8    1.0

$\gamma_{(LV)}$ , surface tension at different temperatures;  $P_c$ , estimated capillary pressure;  $P_v$ , vapor pressure.

an autoclave induces textural transformations of the solid network on a nanoscopic scale.

Smith et al. [101] developed a model to predict gel shrinkage from which it turns out that the number of process variables which can be utilized to control shrinkage is rather limited (pore fluid, drying rate, and initial density), which modifies the gel structure. Alaoui et al. [102] densified aerogels by isostatic compression but making chromatographic columns with this technique looks difficult. Sglavo et al. [103] dried a gel under relative humidity for 150 days!

### 3.2. Monolith information

The bulk density is measured from the weight to volume ratio. Main parameters to control are: porosity, pore radius and specific surface.

The surface area is measured by  $N_2$  adsorption. The Kelvin equation for cylindrical pores establishes a relationship between pore radius and relative pressure ( $P/P_0$ ) utilized in the  $N_2$  adsorption–desorption technique. Estimates of specific pore volume are obtained from the amount of nitrogen adsorbed by the sample in the range  $0.994 < P/P_0 < 0.999$ . The total run time needed to measure a well equilibrated isotherm increases with mesoporosity.

The mean pore radius,  $r$ , is calculated through  $r = 2V_p/S_p$  (BET).

Mercury intrusion analyses can be performed, but porosimetry data are affected by isostatic compression of the monolith at pressures up to  $\sim 80$  MPa, a problem discussed by Alié et al. [104]. Compression of the monolith causes constriction of the pores until

the silica structure stiffens to form a rigid structure to a point at which the mercury can intrude into the shrunken pores. Inverse size exclusion chromatography (ISEC) is more informative since pore size distribution is estimated from a calibration curve drawn with molecular probes; the theory is described in Ref. [105]. ISEC allows the pore size distribution to be examined. Al-Bokari et al. [106] determined the porosities of monolithic columns (from Merck); the porosity of a conventional column is  $\sim 0.62$  whereas the observed porosity on the monolithic column is  $\sim 0.85$ . The internal porosity of a monolithic column appears smaller than that of a conventional column but the authors mentioned that it may depend on the definition.

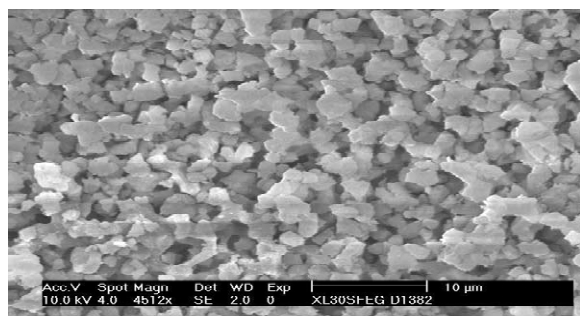
To obtain good chromatographic performances the ratio (through-pore/skeleton size) is of primary importance.

The bulk structure is studied mainly by scattering techniques.

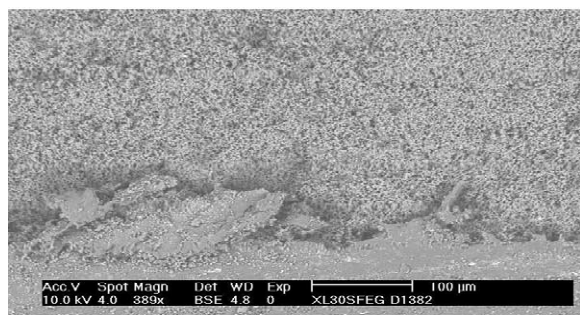
Fractal dimension is determined through the analysis of SAXS data. The fractal dimension (usually found around 2) is the signature of the linkage between the polymeric clusters. The evolution with time of the SAXS intensity provides information on the kinetics of aggregation of TEOS derived silica sols; it gives the scattering exponent and the average radius of giration of the clusters. Ultra small angle X-ray scattering and atomic force microscopy can be used to check densification.  $^{29}\text{Si}$  MAS NMR can be used to identify and characterize the early stages of condensation reactions. These methods (SAX, NMR) of investigation are difficult to apply to the study of large monoliths and to the study of chromatographic columns.

Scanning Electron Microscopy (SEM) or field emission gun scanning electron microscopy are used to study the morphology of monoliths. The available figures in the literature usually display a cross section of the monolith which does not provide information on the homogeneity of the whole skeleton. Tanaka et al. [10] recently displayed both cross section and longitudinal SEM pictures of monoliths. We display a SEM picture (Fig. 5) of a longitudinal section of a Chromolith™ exhibiting the homogeneity of the skeleton size.

The gelation process can be studied by following the time evolution of the viscoelastic properties



(a)



(b)

Fig. 5. Two SEM pictures of Chromolith (longitudinal section), performed in the Centre Pluridisciplinaire de Microscopie électronique de Saint Jérôme, Marseille. (a) Core of the monolith (20- $\mu\text{m}$  scale); (b) PEEK cladding (bottom of the picture).

through dynamic oscillatory measurement with a controlled stress rheometer (Fig. 3).

Aerogels obtained by alcohol supercritical drying are hydrophobic as are those from TMOS–MTES; hydrophobic properties are determined from contact angle measurements.

The three-dimensional structure is studied by laser scanning confocal microscopy.

### 3.3. Cladding

It is necessary to encase the rod. Possible loss of contact with the wall and the subsequent by-pass of eluent are problems which have to be overcome. Malik and Hayes [66] patented a method for the fabrication of monolithic beds embedded in a fused-silica capillary. The reagent system involves two sol–gel precursors, a deactivation reagent, one or more solvents and a catalyst. The first sol–gel precursor is a polymethoxysilane, the second is *N*-

octadecyldimethyl(3-(trimethoxysilyl)propyl) ammonium chloride, phenyldimethylsilane is the deactivation reagent and trifluoroacetic acid is the selected catalyst. The fused-silica capillary is hydrothermally treated prior to filling with the sol solution. According to the method, the products of hydrolysis can undergo polycondensation: (i) between hydrolyzed products of the same original precursors; (ii) between hydrolyzed products of two different original precursors; (iii) between the hydrolyzed products of either precursor with the silanol groups of the silica surface.

Some authors display cross-sectional views of monoliths. In their frit making procedure, Zhang and Huang [67] showed that packing particles are tightly fixed onto the capillary wall to form a mechanically stable layer.

The commercial breakthrough came with silica rods from Merck [107–112] that turned to Chromolith™. The monolith is prepared according to the Nakanishi and Soga procedure [44,45] which enables control of the bimodal pore structure (2- $\mu\text{m}$  through-pores and 13-nm mesopores). The silica sorbent is then encased in a PEEK plastic cover and the silica sorbent is derivatized in situ. The PEEK plastic cover is shrink wrapped onto the silica rods to ensure that there is no void space between the silica and the PEEK material.

## 4. Particulate-alkoxide gels

Horvath and colleagues [113] packed fused-silica capillaries with porous  $\text{C}_{18}$  silica microspheres which they subjected to thermal treatment at 360 °C to produce a column with porous silica based monolithic packing. After sintering, the monolithic packing was reoctadecylated in situ. It is noteworthy that Horvath and colleagues also produced a monolith prepared from silanized fused-silica capillaries of 75- $\mu\text{m}$  I.D. by in situ copolymerization of divinylbenzene either with styrene or vinylbenzyl chloride in the presence of a suitable porogen [114].

Some investigators have synthesized gels by dispersing  $\text{SiO}_2$  particles in alkoxide solutions. Toki et al. [115,116] prepared 15 cm  $\times$  15 cm  $\times$  0.5 cm glass plates by sintering particulate-alkoxide gels dried at 60 °C for 10 days and found that porosity increased

with increasing SiO<sub>2</sub> particle content. Dulay et al. [117] prepared a monolith by immobilization of C<sub>18</sub> particles in a sol–gel matrix prepared in situ in a capillary tube with subsequent drying at high temperature.

This procedure is very difficult to reproduce. Tang et al. [118,119] filled a capillary tube with a CO<sub>2</sub> slurry of C<sub>18</sub> silica particles, treated the material with propylsulfonic acid to modify the surface and bound the particles together using a sol–gel formation process (the alkoxide was in fact a mixture of TMOS and ethyltrimethoxysilane). Zhang and Huang [67] prepared an on column frit from a sol made with methyltriethoxysilane, water, methylene chloride and trifluoroacetic acid in which they suspended silica particles.

To control the porosity of the organic based monolithic polymers, solvents are used as porogenic agents. Use of silica beads as sacrificial material for the templating of porosity eliminates the need for porogenic solvent. Chirica and Remcho [120] prepared such monolithic columns with 3-(trimethoxysilyl)propyl methacrylate which reacts on the silanol groups of the wall while the methacrylate group is the anchor for the butyl methacrylate (or another monomer) to be synthesized in a polymerization reaction.

A hybrid procedure was devised by the group of Zare [121–123]. Methacryloxypropyltrimethoxysilane (MPTMS) contains both methacrylate and alkoxysilane groups, and is used to prepare a photopolymerized sol–gel in a single step reaction. A mixture of MPTMS, hydrochloric acid and water is stirred in the dark, toluene, which will act as a porogen, is then added, and finally the photoinitiator. The solution is filled into a capillary and irradiated. It has been shown that the hydrochloric acid concentration is critical for the rigidity of the columns and the ratio of monomer solution to porogen controls the through-pore size and the surface area and thus the separation capabilities.

## 5. Bonding

During the sol–gel polymerization process, Davis and Katz [124] incorporated imprint molecules consisting of aromatic rings carrying one, two or three

3-aminopropyltriethoxysilane side groups. Removal of the aromatic cores created framework cavities in which the aminopropyl groups were anchored. Chen et al. [125] grafted L-phenylalaninamide and other chiral selectors on the surface of the monolith through available silanols. Kang et al. [126] used a coating procedure to prepare a Chirasil beta DEX.

The bonding of alkyl ligands must be performed after monolith preparation.

## 6. Performances

Monoliths can be used in HPLC or CEC. We can distinguish between commercially available columns and those only described in papers. In Chromoliths, there are large through-pores which are comparable to the interstitial voids of a particle packed column and mesopores of the same size and volume as in porous particles. Models of liquid flow through monolith have been devised by Liapis and colleagues [127–129].

As far as silica based monoliths are concerned, preliminary chromatographic data on silica rods were given by Minakuchi et al. [52], Ishizuka et al. [130], Cabrera and Lubda [131] who emphasized the separation of test solutes at very high flow rates. They insisted upon separation impedance and low pressure drop, allowing fast separations.

For conventional HPLC column with reduced plate height  $h=2.0$  at a minimum and  $\phi=700$ –1000, the smallest value for separation impedance  $E$  is 3000–4000. Great differences with monolithic rods were demonstrated by Minakuchi et al. [52] since a 7-fold decrease in  $E$  was observed. Impedances as low as 300–700 were observed. Fig. 6 compares the separation impedance of conventional columns versus Chromolith. Recent results with fused-silica capillaries [136] are impressive since a value of 100 was obtained. The coupling of many columns in series is possible.

Bidlingmaier et al. [132] displayed the Van Deemter curve of a Chromolith prototype column; they obtained a 13- $\mu\text{m}$   $H_{\text{min}}$  at a linear velocity of 2.8 mm s<sup>-1</sup> corresponding to a flow rate of 3 ml min<sup>-1</sup>. Permeability is  $1.0 \times 10^{-10}$  cm<sup>2</sup> which is six times lower than that observed with a conventional column.

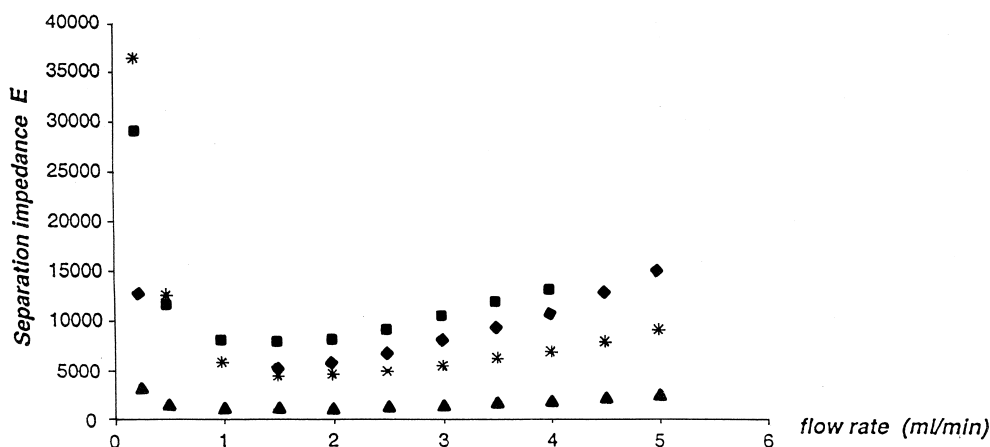


Fig. 6. Comparison of the separation impedance of conventional columns versus Chromolith. ▲, Chromolith; ◆, 35×4.6 mm column, 3  $\mu\text{m}$ ; ∗, 50×4.6 mm column, 3  $\mu\text{m}$ ; ■, 50×4.0 mm column, 3  $\mu\text{m}$  (courtesy of Merck KgA).

Mc Calley [133] compared conventional microparticulate packed columns and a monolith from Merck (Chromolith). He used the Dorsey Foley equation to determine column efficiency. He found that the

monolith gave similar values of  $H_{\text{min}}$  for benzene to the 5- $\mu\text{m}$  phase.  $H_{\text{min}}$  remains practically constant over the range 2–5  $\text{ml min}^{-1}$  due to the favorable diffusion characteristics of the monolith; as a conse-

Table 3  
HPLC performances of  $\text{C}_{18}$  monolithic silica columns

$H_{\text{min}}$ ( $\mu\text{m}$ )	$U_{\text{opt}}$ ( $\text{mm s}^{-1}$ )	Column diameter (mm)	Solute	Ref.
13	2.8	4.6	Alkylbenzenes	[132]
10	2.6	4.6	Benzene	[133]
9	1.5	4.6	Benzene	[134]
7.5–13	0.8–0.5	4.6	Amylbenzene	[135]
8–10	1–2	4.6–7.0	Hexylbenzene	[10]
12	0	0.05	Alkylbenzenes	[136]

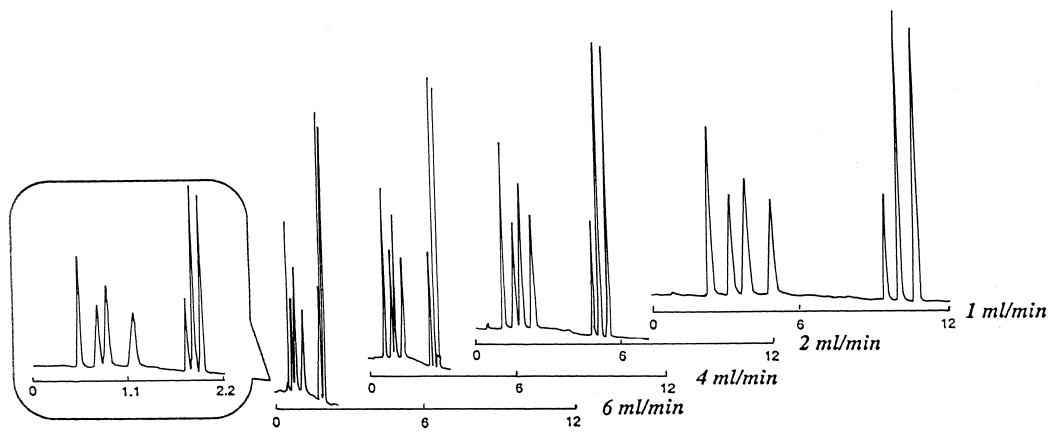


Fig. 7. Separation of alkyl benzenes at different flow rates, Chromolith  $\text{C}_{18}$  column (courtesy of Merck KgA).

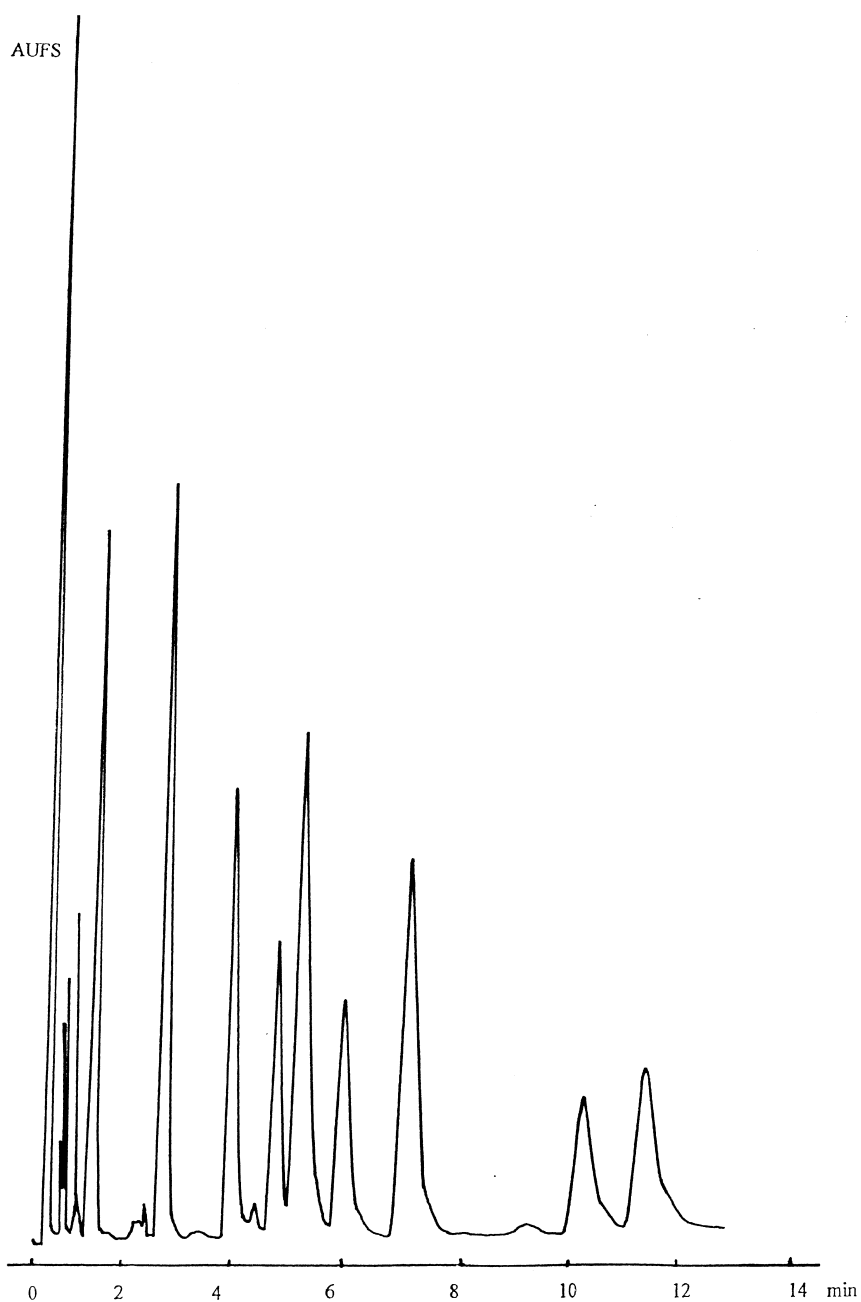


Fig. 8. Separation of some psychotropic drugs on Chromolith  $C_{18}$ . Chromolith Performance column,  $100 \times 4.6$  mm, flow rate  $3.2 \text{ ml min}^{-1}$ . Mobile phase: phosphate buffer  $10^{-4} \text{ M}$ , pH 3.2:acetonitrile (65:35) v/v. Elution order: 9-OH risperidone, risperidone, reduced haloperidol, haloperidol, desmethyl cyamemazine, cyamemazine, desmethyllevomepromazine, levomepromazine, desmethyl chlorpromazine, chlorpromazine.

quence at high flow rate ( $5 \text{ ml min}^{-1}$ ),  $H$  is almost 3.5 times lower than on the  $5\text{-}\mu\text{m}$  phase. These data are in accordance with data from Bidlingmaier but are different from those published by Sinz and Cabrera [134] and those published by Kele and Guiochon [135]. With two columns of different diameter (4.6–7.0 mm), Tanaka et al. [10] observed similar plate heights (Table 3). In capillary HPLC, Ishizuka et al. [136] reported 100 000 plates on a 130 cm long column,  $H_{\text{min}}$  is  $12 \mu\text{m}$  and the separation impedance is as low as 100–200.

Band spreading does not increase with velocity when high interparticle Peclet number is observed. Kele and Guiochon extensively studied the performances of six Chromoliths which they found very similar, thus emphasizing the reproducibility of the manufacturing process. They observed a very low  $C$  term when the solute is unretained and a higher value ( $C=2$  to 3) for retained solutes. Ishizuka et al. [136] also observed the dependence of plate height on retention factor. Values of  $A$  and  $C$  terms increase as the solute is more retained. They performed measurements of the peak efficiencies from the peak width at half height. Measured permeabilities lie in the range  $7.1\text{--}9.2 \times 10^{-10} \text{ cm}^2$  which is more than twice as low as for conventional columns. In this mode, a tremendous increase in flow rate is possible, as demonstrated by Fig. 7 which shows the separation of alkyl benzenes at  $6 \text{ ml min}^{-1}$ . Flow rates up to  $9 \text{ ml min}^{-1}$  are possible but careful attention must be paid to the instrument and especially the detector. Due to the reduction of back pressure it is possible to use high percentages of buffer in the binary mobile phase to perform fast analysis of solutes exhibiting a wide range of retention without performing a gradient. Fig. 8 shows the separation of some psychotropic drugs together with the main metabolites. New possibilities are offered in preparative liquid chromatography: a separation of diastereomers has been published [137].

The  $A$  term is quite large compared to conventional columns. Leinweber et al. [138] assumed an independent contribution from mechanical and non-mechanical dispersion mechanisms. They evidenced diffusion in mesopores and calculated an equivalent dispersion particle diameter  $d_{\text{disp}}$ .  $d_{\text{disp}}$  can be regarded as an equivalent length scale which solute molecules travel in stagnant zones of the porous

medium.  $d_{\text{disp}}$  can be approximated by regression of the  $C$  term of the Van Deemter equation with a set of solutes (a homologous series is well suited). The hydraulic permeability can be characterized by an equivalent particle diameter  $d_{\text{perm}}$  which has been found to be  $\sim 15 \mu\text{m}$ .

## 7. Conclusion

Since the pioneering work of Tswett, liquid chromatography columns are filled with particles the diameter of which has decreased with the years to withstand the high pressures required to operate the columns at optimum velocities.

The advent of continuous beds represents a new generation and will be the major topic in future. Considering silica based monoliths it will lead to continuous improvements. Much information can be obtained from papers dealing with the fabrication of silica aerogels; it is interesting to watch papers still appearing on  $\text{C}_{18}$  bonded silica particles!

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