

CHROM. 23 261

Review

Silica: backbone material of liquid chromatographic column packings

ALAIN BERTHOD

Laboratoire des Sciences Analytiques, Université de Lyon 1, UA CNRS 0435 (J. M. Mermet), 69622 Villeurbanne (France)

(First received April 4th, 1990; revised manuscript received February 21st, 1991)

ABSTRACT

More than 90% of column packings used in normal- and reversed-phase liquid chromatography (LC) are based on silica. This paper reviews the general properties of silica (SiO_2), the backbone material for LC column packings. First, the properties of the chemical SiO_2 are reported. A description of the crystallography and surface state of silica and the natural and artificial origin of silica is given. Next, the chemical properties of silica are surveyed. Acid–base properties, surface hydroxyl or silanol properties, silica derivatization reactions and the effect of hydrofluoric acid are detailed. The physico-chemical properties, particle shape and size, specific surface area and porosity with pore size and pore volume are given. The chromatographic requirements are outlined: pH and mechanical resistance, surface area, pore size and pore volume related to solute retention and selectivity, particle size related to efficiency and column permeability and carbon content and bonding density for reversed-phase LC packings. The properties of silica are tabulated listing the characteristics of several commercially available packings used for analytical normal- and reversed-phase LC.

CONTENTS

1. Introduction	2
2. The chemical SiO_2	3
2.1. Crystallography and surface state	3
2.1.1. Crystalline phases	3
2.1.2. Surface silanols	3
2.1.3. Silanol assessment	4
2.2. Origin of amorphous silica	6
2.2.1. Natural silica: diatomites	7
2.2.2. Liquid-phase synthesis of silica: silicate hydrolysis	7
2.2.3. The gas-phase route for synthesis of amorphous silica: pyrogenic silica	9
2.3. Chemical properties of synthetic silica	10
2.3.1. Water adsorption and desorption	10
2.3.2. Water solubility	11
2.3.3. Acid–base properties	11
2.3.4. Chemical reactions involving surface silanols	12

3. Physico-chemical properties of silica	14
3.1. Particle size	14
3.1.1. Definition	14
3.1.2. Particle size determination	15
3.2. Surface area and porosity	15
3.2.1. Surface area	15
3.2.2. Porosity	16
3.2.3. Relationship between surface area and porosity	17
4. Applications to LC column packings	18
4.1. Particle size, pressure and efficiency	18
4.2. Polarity, surface, porosity and surface modification	19
4.2.1. Surface silanols and polarity	19
4.2.2. Silica surface and porosity	23
4.2.3. Carbon content and bonding density	24
4.3. Chemical and physical chromatographic requirements	24
4.4. Examples	26
5. Conclusions	26
6. Symbols	26
References	27

1. INTRODUCTION

Today, more than 90% of column packings used in normal- and reversed-phase liquid chromatography (LC) are based on silica. Silica exists in various forms with the stoichiometric composition SiO_2 . Silicon, atomic number 14, atomic weight 28.08, makes up 25.7% (w/w) of the Earth's crust, and it is the second most abundant element, being exceeded only by oxygen, which constitutes 49.2%. Silicon is not found free in nature, but occurs chiefly as the oxide and as silicates. Interestingly, carbon, the first element of Group IVA of the Periodic Table, is the most important element in the living world. Silicon, the second element of Group IVA, with the same external electron layers (s^2p^2 with sp^3 hybridization) is the most important element in the lithosphere. More than 55% (w/w) of the Earth's crust is made up of silicon oxide and silicates. Granite, sand, asbestos, feldspar, clay, mica and flint are a few of the common silicate minerals. Quartz, rock crystal, amethyst, agate, jasper and opal are less common or rare silicon-containing stones used in jewelry [1].

In contrast of the widespread use of silica as LC column packings, its physico-chemical structure and chemical properties are often neglected by chromatographers. The books by Unger [2] and Snyder [3] can still be regarded as basic references in this field. The chemistry of silica is extensively described in the book by Iler [4].

The silica used for LC column packings is essentially porous and non-crystalline with the general formula $\text{SiO}_2 \cdot x\text{H}_2\text{O}$. Water is chemically bound in a non-stoichiometric amount, forming the Si-OH silanol groups most important for LC packing use [2,3]. Silanols are responsible for the polar character of silica packings used in normal-phase LC. They are used to graft organic moieties. This treatment changes the silica surface and allows to obtain the bonded phases used in reversed-phase and other modes of LC.

This paper reviews the physico-chemical attributes of silica, focusing on its use as an LC column packing. The chemical SiO_2 is the topic of Section 2. The crystallography and surface state of silica are briefly described; the origin of amorphous silica is

given and the chemical properties of SiO_2 are surveyed. Section 3 deals with the physico-chemical properties and structure of silica: particle size, surface area, and porosity. Part 4 considers the relevance of such properties in LC applications.

2. THE CHEMICAL SiO_2

2.1. *Crystallography and surface state*

As already stated, the silica used for LC column packings is mostly non-crystalline. Crystalline silica has a well defined network of Si and O atoms. The surface of the crystal is partially hydrated and bears some silanol groups.

2.1.1. *Crystalline phase*

Temperature changes induce crystallographic changes of silica. This can be simplified as follows: the crystalline quartz form is stable from room temperature to 870°C , the quartz form evolves into tridymite between 870 and 1470°C and from 1470°C up to the melting point (*ca.* 1700°C) the crystalline form is cristobalite. Fused silica can be moulded and cooled into silica glass, an amorphous form. Silica glass has good mechanical and optical properties, with a low coefficient of thermal expansion and high transparency in the ultraviolet, visible and near-infrared regions of the electromagnetic spectrum. Quartz is the most common crystalline form. There are more than 35 crystalline silica polymorphs known, with nine different crystalline forms of tridymite and at least three forms of cristobalite [5,6].

Such a variety of crystalline forms of silica exists because silicon and oxygen atoms can be arranged in octahedra (Fig. 1A) or in tetrahedra (Fig. 1B). The most stable forms, quartz and cristobalite, contain the tetrahedral structure with an Si–O bond length of 0.162 nm and an O–O distance of 0.264 nm.

It is possible to obtain hydrated pseudo-crystalline forms of silica by acid hydrolysis of crystalline silicates. The acid makes soluble the metallic ions of the silicate which are washed out. After rinsing with water and drying with acetone, a hydrated silica retaining the original silicate structure is obtained. For example, asbestos or chrysotile is a fibrous crystalline magnesium silicate. A fibrous "crystalline" hydrated silica is obtained by acid hydrolysis of asbestos [7].

Silicas used in chromatography do not produce any X-ray spectrum as they are amorphous.

2.1.2. *Surface silanols*

The surface of most silica forms is covered by hydroxylated silanol (Si–OH) groups. Fig. 2 shows the possible types of surface silanols. Two hydroxyl groups on two vicinal silicon atoms are vicinal silanols (Fig. 2, region 1). When they are borne by the same silicon atom, the two groups are termed geminal silanols (Fig. 2, region 2). Fig. 2, region 3, shows an isolated or free silanol group [2].

The surface of a crystalline form of silica is covered mainly by isolated silanol groups. Amorphous silica, with a porous structure and highly disordered surface, bears the three kinds of silanol arrangements as shown in Fig. 2.

Silanol groups can form hydrogen bonding with water molecules. The more elevated the surface silanol concentration, the greater is the hydrophilic character of

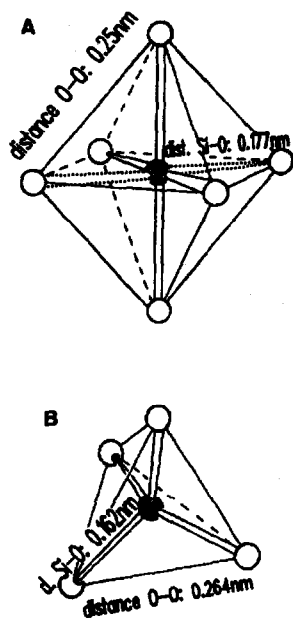


Fig. 1. Silica crystallography. (A) Octahedral organization; (B) tetrahedral organization.

the surface. As fully indicated elsewhere [62], the role of silanols in LC selectivity is essential. This is why it is very important to know the concentration of surface silanols for a given silica-based LC stationary phase.

2.1.3. Silanol assessment

There are several methods for the determination of surface hydroxyl groups, such as chemical, isotopic exchange and spectroscopic methods.

Chemical methods. All chemical methods use the acidic character of the hydrogen of the silanol hydroxyl group. The water hydrogens are more acidic than the silanol hydrogens, so all physisorbed water must be removed by heating the silica

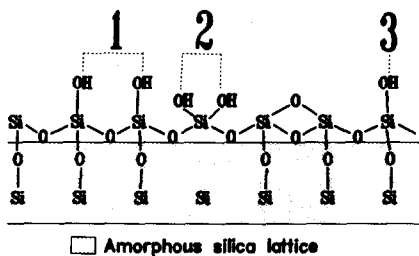


Fig. 2. Three different types of hydroxyl groups on the amorphous silica surface: (1) vicinal silanols; (2) geminal silanols; (3) isolated silanol.

sample at 150°C, under vacuum, for several hours. After the drying treatment under vacuum, the sample must not be exposed to the open air because water molecules of the atmosphere can be physisorbed again and rehydrate the sample. All chemicals must be added under a dry nitrogen atmosphere.

The most reliable estimate of surface silanol concentration is obtained by reaction of the hydroxyl groups with methyllithium [2,8]. Methyllithium is dissolved in a solvent [diisopentyl or diethyl ether, 5% (w/v)]. Provided that the sample was carefully dried, the solution reacts with hydroxyl groups as follows:

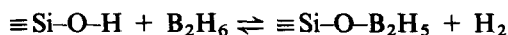


The amount of methane evolved is a direct measure of the silanol group surface content. If water molecules are present, methyllithium reacts with them first, producing lithium oxide (Li_2O) and methane; then it reacts with silanols. In such a situation, the measurement of methane is pointless. The methane is determined by gas chromatography [9,10]. The gas chromatograph is calibrated by injecting a known volume, C , of methane, which allows the determination of the volume, V , obtained when a mass, m , of dry silica with a specific surface area, S , is titrated by methyllithium. The surface silanol concentration, σ_{SiOH} , is then calculated with the equation

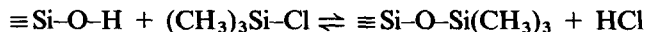
$$\sigma_{\text{SiOH}} = (V/22\,400) \cdot (P/101.5) \cdot (273/T) \cdot (10^6/mS) \quad (1)$$

where P and T are the measurement pressure (kPa) and temperature (K), respectively; V , m , S and σ_{SiOH} are measured in ml, g, m^2/g and $\mu\text{mol}/\text{m}^2$, respectively. The reaction is sterically hindered with silicas of an average pore diameter of $p_d < 6$ nm, owing to the formation of an etherate complex [11].

Diborane has also been used for silanol determination [12]. The reaction is

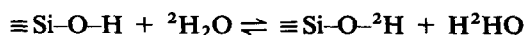


the hydrogen evolved being titrated. However, the result is very dependent on the stoichiometry of the reaction, which makes the method highly unreliable [8]. Trimethylchlorosilane has also been used:



Owing to the difference in the molecular cross-sectional area of a trimethylsilyl group relative to a hydroxyl group (factor of two), only half of the total concentration of the surface hydroxyl group content is measured on a fully hydroxylated surface.

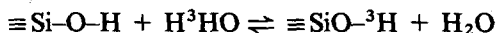
Isotopic exchange methods. Heavy water was used to determine the surface silanol concentration by isotopic exchange [2]:



σ_{SiOH} is determined by weighing the dry sample before and after the exchange and/or by using spectroscopic methods [13].

Tritium-labelled water, H^3HO , is preferably employed instead of heavy water,

because radioactive counting of the exchanged sample is easy [11,14]. The exchange reaction is



A detailed procedure is given in ref. 11.

A dynamic isotopic exchange method was recently developed [15]. An LC column is packed with the silica under investigation and dehydrated with a dry solvent that cannot exchange protons (*e.g.*, dichloromethane or benzene). Next, the eluent is switched to the same one but containing a 1% (v/v) concentration of methanol or another miscible proton-exchange solvent. The breakthrough volume is measured. The solvent delivery system is then switched to an isocomposition eluent, but the methanol is isotopically tagged with tritium. The breakthrough volume of the tagged eluent is measured. The difference between the two measured volumes give the amount of silanols in the column [15].

Spectroscopic methods. Infrared (IR) spectroscopy has been used to assess surface silanol concentration [16]. The dried silica sample is formed in pellets under high pressure in a steel die. The IR spectrometer must be able to work under vacuum or a dry nitrogen atmosphere [17]. Three bands, located at 3747, 3680 and 3535 cm^{-1} , correspond to isolated hydroxyl groups, internal hydroxyl groups and vicinal hydrogen-bonded hydroxyl groups, respectively. On heating at 600°C, the 3680 and 3535 cm^{-1} bands disappear, showing the sensitivity of surface silanols to annealing treatment. IR spectroscopy can distinguish isolated silanol groups from vicinal groups. However, this technique cannot discriminate geminal silanol groups (Fig. 2, region 2) [16].

Nuclear magnetic resonance (NMR) of ^{29}Si with cross-polarization and magic angle spinning (MAS) can assess free and geminal silanol groups and also silicon atoms with no silanol [17–20]. NMR bands are observed at 91, 100 and 109 ppm, corresponding to geminal silanol groups, isolated silanol groups and siloxane without hydroxyl, respectively. Proton MAS-NMR can also be used. Broad bands are observed at 1 and 2.2 ppm for isolated and geminal hydroxyl groups. The sharpness and intensity of the band can be greatly enhanced on isotopic exchange with fully deuterated methanol [20]. The combination of MAS with multiple-pulse line narrowing (^1H combined rotation and multiple-pulse spectroscopy, ^1H CRAMPS) is a powerful method for detecting hydrogen-bonded hydroxyl groups and physisorbed water [19].

Other methods, such as thermogravimetric analysis, diffuse reflectance Fourier transform infrared spectroscopy or pH measurements, can be used for silanol determination. Several methods were compared by Köhler *et al.* [20].

2.2. Origin of amorphous silica

Crystalline forms of silica are found in nature. The unique natural amorphous silica source has a biological origin: it is diatomaceous earth. All other amorphous silicas used in industry are made by synthetic means. There are two methods to obtain pure silica: the liquid-phase route and the gas-phase route.

2.2.1. Natural silica: diatomites

Diatoms are microscopic aquatic, single-cel algae, disk-like or elongated in shape. They consist of single cells surrounded by two half-cell walls or valves of transparent silica about $0.1 \mu\text{m}$ thick and made up of patterns of chambers and lace-like partitions of extreme beauty and complexity [4]. Fig. 3 shows an intact shell of a diatom. Most natural deposits of diatom skeletons are millions of years old and partially crystalline.

Diatomaceous earth or diatomite contains mainly silica. However, the exact composition of a given sample differs depending on the extraction point. The silica content is often around 90% with minor elements such as aluminium, calcium, iron, magnesium and sodium [21]. Diatomite is occasionally used as an LC packing but most often in gas chromatography (*e.g.*, Chromosorb stationary phases).

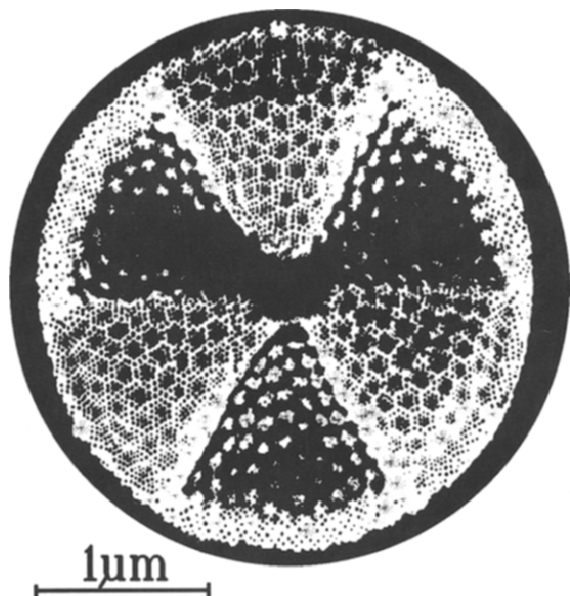


Fig. 3. An intact diatom.

2.2.2. Liquid-phase synthesis of silica: silicate hydrolysis

Most porous silicas used in LC packings originate from the liquid-phase synthesis route. Fig. 4 is a simplified diagram of the industrial scheme used to produce 99% pure silica gel or silica powder. Sand or a silica-containing ore is heated with sodium carbonate to form a vitreous sodium silicate, which is dissolved in water. The basic solution is filtered and then acidification with sulphuric acid induces silicic and polysilicic acid formation. The polysilicic acids are the nuclei for the formation of colloidal silica particles. Next these microparticles can form a three-dimensional aggregate of pure and porous silica gel, or a precipitate. The silica gel is washed and dried. The drying process produces a hard silica gel or xerogel. On drying the washed precipitate produces a polydisperse porous silica powder.

Drying the bulk wet silica gel produces shrinkage. The wet silica gel consists of

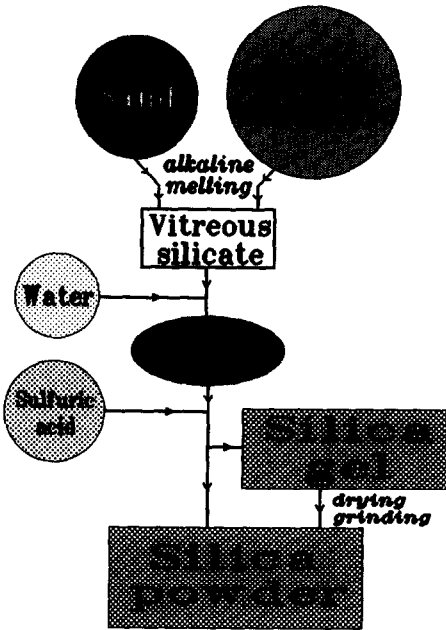


Fig. 4. Preparation of synthetic silica.

very fine grains separated by water capillary tubes, and arranged in a three-dimensional network. As the gel dries, shrinkage occurs owing to the surface tension of water in the tubes that become pores. A certain number of particle-to-particle bonds are created during the process, which makes it irreversible [4]. The solid pieces of hard silica gel are ground and sieved to give porous silica powders with angular particules.

If the neutralized silicic acid solution is allowed to age with gentle stirring, Ostwald ripening occurs, *i.e.*, the larger silica particles grow at the expense of the smaller particles. Ageing and ripening increase the size of silica particles, decreasing the specific surface area and increasing the pore volume.

To obtain spherical porous silica powder, it is necessary to dry it without shrinkage. The aqueous phase can be replaced with an organic phase, *e.g.*, an alcohol. Heating above the critical temperature so that there is no meniscus between the liquid and gas phases and venting off the vapours allows drying without shrinkage [4]. Perfectly spherical silica particles for LC packing are often prepared by subdividing the neutralized silicate solution (the colloidal silica sol) into fine droplets before gelling. Next, the droplets are suspended in air and dried. It is also possible to disperse the colloidal silica sol in an immiscible liquid (oil) to form emulsions in which the sol droplets solidify in a spherical form [22]. Also, coacervation of the colloidal silica sol can be induced by an organic agent to form droplets which are subsequently solidified. A heat treatment burns out the organic material and induces some sintering that strengthens the silica framework and produces spherical porous silica particles [4,22].

As shown, the drying process can dramatically change the characteristics of the porous silica obtained. The problem is that many parameters influence the final prop-

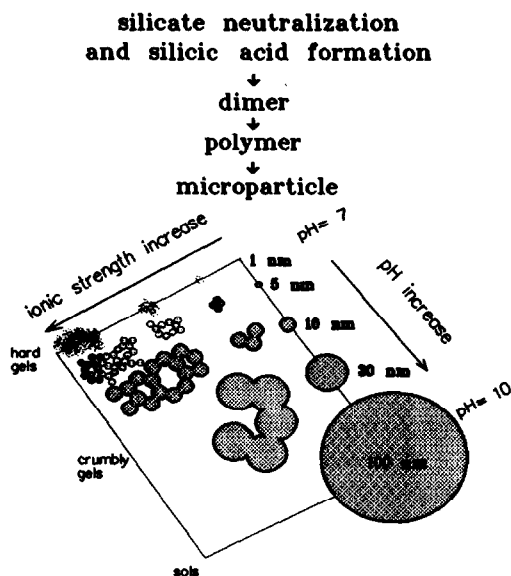


Fig. 5. Effect of ionic strength and pH gradient on the structure of the silica obtained.

erties of porous silica, namely the temperature, the concentration of the silicate solution and the neutralizing acid solution, the pH gradient, the ionic strength, the final pH, the delay time for gel ageing, the effect of additives (*e.g.*, ammonia acts as a coagulant) and the stirring energy or other external pressure or forces applied to the reactor.

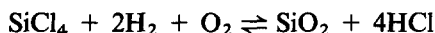
Fig. 5 illustrates the effect of two parameters, ionic strength and pH gradient. The pH value of sodium silicate is *ca.* 12. On addition of an acid, the pH can be rapidly lowered to 7 or slowly lowered to 10. A rapid decrease in pH induces very small microparticles, with diameters in the nanometre range. A gentle decrease in pH produces 100 times larger initial particles. A low ionic strength does not favour the three-dimensional organization and produces precipitates. A high ionic strength favours gel formation (Fig. 5) [4]. The crucial effect of these two parameters on the final porous silica obtained shows the importance of know-how in silica-based LC packing preparation. Each manufacturer has developed their own skills to prepare porous silica with the required particle size, specific surface area, pore volume, pore size and pore size distribution.

Trace amounts of sodium may be present in silicas obtained by silicate hydrolysis. Highly pure silicas, without sodium, withstand alkaline solutions: they are stable in aqueous solutions of pH 9. Such silicas are synthesized by hydrolysis of purified tetraethoxysilane $[\text{Si}(\text{OC}_2\text{H}_5)_4]$.

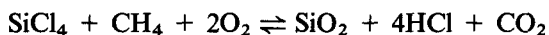
2.2.3. The gas-phase route for amorphous silica synthesis: pyrogenic silica

Pyrogenic silica are non-porous micro-powders with particle diameters in the few nanometres range. The industrial process uses the oxidation of silicon tetrachloride vapour. Silicon tetrachloride (SiCl_4) is easily prepared from lump silicon

which burns in chlorine gas. SiCl_4 is purified by distillation (boiling point 57.6°C). The oxidation reactions [23]



or



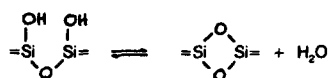
occur in a flame. Changing the combustion speed and the gas composition will affect the particle diameter and hydrophobicity. An excess of hydrogen or methane produces silanol-containing particles. An excess of oxygen gives hydrophobic particles without silanols. However, the gas-phase route is not well suited for the preparation of silica LC packings because poreless silica microspheres with too small diameters are obtained.

2.3. Chemical properties of synthetic silica

Most chemical properties of silica are due to surface silanols. Interactions of silica with water are silanol dependent. The hydroxyl acidity is responsible for the acid-base properties of silica. Silanols are used to graft various organic moieties on the silica surface and to obtain the wide variety of bonded silica essential for LC packings.

2.3.1. Water adsorption and desorption

As stated before, silica hydroxyl groups are associated with water molecules by hydrogen bonding. This adsorbed water is tightly bound to the silica surface, and is removed by heating at 150°C under vacuum for several hours. Overheating a silica sample produces dehydroxylation. Geminal silanols are reversibly dehydroxylated giving siloxane bridges:



Between 200 and 800°C , the temperature treatment under vacuum of a silica sample induces a monotonous decrease in the hydroxyl group concentration with siloxane bridge formation. If the calcination temperature does not exceed 600°C , the reactive siloxane group completely rehydroxylates when exposed to water [24].

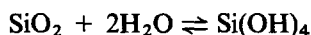
Complete dehydroxylation and sintering occur on heating above 1200°C . At this elevated temperature, the amorphous character of the silica can be slowly lost. The sample crystallizes in the stable cristobalite form. The hydrophilic surface is converted into a hydrophobic surface [24,25]. The conversion of amorphous silica into the crystalline form is very dependent on the purity of the silica, particularly on its sodium content. A highly pure silica remains amorphous even on exposure to a temperature of 1400°C for several weeks.

At room temperature, synthetic porous silica can adsorb water reversibly. Silica

powder is used to dry gases and is added to powders to reduce agglomeration due to humidity.

2.3.2. Water solubility

Water solubility is very dependent on the crystalline or amorphous form of the sample, and also on physico-chemical characteristics such as porosity, surface area and particle size. The water temperature, pressure and pH change the solubility of silica. The solubilization equation is



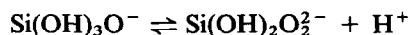
The solubility of crystalline and glass forms of silica is in the parts per million range (ppm = mg/l) [4]. The concentration of dissolved silica in rivers varies from 5 to 20 ppm. In sea water, the range is from 0.01 ppm in the Pacific Ocean to 10 ppm in the Mediterranean Sea. The dissolved silica concentration is very sensitive to the presence and concentration of other salts. All living cell fluids contain a few ppm of dissolved silica [4]. The solubility of silicic acid $[\text{Si(OH)}_4]$ is greatly dependent on the pH of the solution.

2.3.3. Acid-base properties

Silicic acid is a weak acid. The ionization constant of the reaction

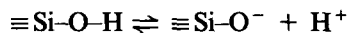


is $1.6 \cdot 10^{-10}$ at 20°C, which corresponds to a $\text{p}K_a$ value of 9.8. The $\text{p}K_a$ value of the second ionization:



is 11.7. At a pH of 11.7, all silanol groups ionize simultaneously. Most glasses contain silica and they can be attacked by a strongly alkaline solution.

The hydroxyl groups on the porous silica surface have also an acidic character. The $\text{p}K_a$ value of the reaction



is about 6.8 (± 0.5), which is 3 pH units lower than the free silicic acid first ionization [26]. There are several explanations for the higher mobility of the silanol proton compared with the silicic acid. The tendency for splitting off a proton from a particular silanol group is markedly promoted by its environment. For example, hydrogen bonding between adjacent surface silanols can facilitate the reaction with hydroxide ions [2,26].

The acidic character of surface silanols confers some ion-exchange properties on porous silica. In buffer or electrolyte solutions, the hydronium ions (protons) of the silanol groups are exchangeable by cations of the solution. The ion-exchange properties of silica are highly dependent on the pH of the solution, the surface area and the silanol concentration. At pH 7, silica bears negative charges in solution. The

isoelectric point of silica is close to pH 2.5 [2,4], but is at higher pH for some silica samples [27]. Owing to its low capacity, between 0.1 and a maximum of 2 mequiv./g, porous silica is not a suitable sorbent for ion-exchange chromatography. However, its mechanical and chemical properties make silica useful as an ion exchanger for the removal of cations in radioactive waste materials [28,29]. This ability to exchange ions must be known when porous silica is used as an LC column packing.

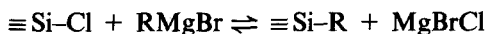
The other result of the mobility of proton silanols is well known by chromatographers namely the rapid dissolution of silica at mobile phase pH values higher than 10 and/or at temperatures higher than 40°C. A slow and insidious solubilization occurs from pH 8. This can produce small voids in the column packing, inducing changes in solute retention and especially efficiency losses. This is known as column ageing. It can be reduced by the use of a guard column or a precolumn installed between the pump and the injection valve to saturate the mobile phase in soluble silica. Important work has been done to improve the pH stability. As the solubility is a function of the purity of silica, it is economically worthwhile to make highly pure silica from alkoxysilanes (*e.g.*, Kromasil, Table 2). Such silicas are stable for months in aqueous solutions at pH 9.2 (ammonia buffer) [30]. Another way to reduce the dissolution problem is the "doping" of silica with metal oxides such as alumina or zirconia. Zirconia-treated silica were found to be stable at pH 9.5 for a long period [31].

The acid-base properties of porous synthetic silica are due to surface hydroxyl groups. There are several other chemical reactions involving surface silanols.

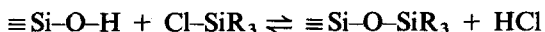
2.3.4. Chemical reactions involving surface silanols

As far as LC packing are concerned, the most important chemical reactions involving surface silanols are the surface changes of silica by organic bonding reactions. Such reactions are used to produce the wide assortment of bonded phases for LC use [24]. The goal of such reactions is to create Si-C bonds on the silica surface. The Si-C bond was found to be the most suitable for chromatographic use of the derivatized silica. The Si-C bond withstands hydrolysis when the derivatized silica is used as packing in reversed-phase LC with aqueous-organic mobile phases; Si-O-C, Si-N-C and Si-O-C=O bonds do not.

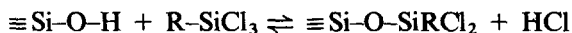
Bonding reactions. The full description of the wide variety of bonding reactions used to produce LC packings is beyond the scope of this section; such a description can be found elsewhere [62]. Only the mechanism of silica bonding reactions is given here. It is possible to create Si-C bonds directly by reaction of a Grignard organometallic compound with the chlorinated silica [32]:



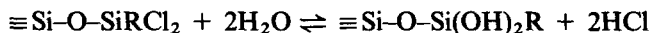
where R is an alkyl group. This route is less frequently used to prepare bonded silica. Most often, the Si-C bond pre-exists in the bonding reagent. Alkylchlorosilanes and alkylaminosilanes are used. They react directly with silanols after desorption of adsorbed water by heating the silica sample at 200°C under vacuum for about 12 h. With an alkylmonochlorosilane, the reaction in dry toluene is



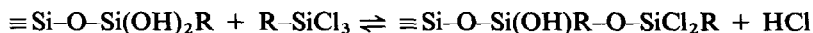
Usually the three R alkyl groups are not identical for steric hindrance reasons. For example, two of them may be methyl groups and the third a long-chain alkyl (butyl, octyl, decyl or octadecyl). Bonding with an alkyl monochlorosilane produces a monomeric or monofunctional "brush-type" bonded silica. Polymeric or polyfunctional bonded layers can be obtained using an alkyldi- or -trichlorosilane bonding reagent. With a trichlorosilane, the reaction is



Trace amounts of water may hydrolyse some Si-Cl bonds:

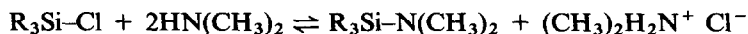


and another trichlorosilane molecule can react with newly created silanols:

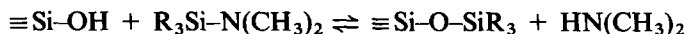


and so on. A polymer layer is generated. The structure of the bonded organosilane layer was elucidated by ^{13}C and ^{29}Si cross-polarization MAS-NMR spectroscopy [33].

Alkyldimethylaminosilanes can react with more silanol groups than chlorosilanes. They produce the highest bonding density [34]. Alkyldimethylaminosilanes are synthesized from the corresponding alkylnonochlorosilane:

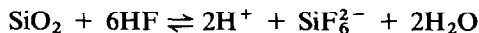


The alkylammonium chloride precipitates. The bonding reaction in dry toluene or dichloromethane is



Because there is one additional step, the aminosilane route is seldom used to produce derivatized silica in industry.

Reaction with HF. Silica is acid resistant, but hydrofluoric acid (HF) can convert it rapidly to fluorosilicic acid at room temperature:



Although HF, at any concentration, is a dangerous reagent, this reaction can be useful for silica characterization. HF destroys and dissolves the silica backbone of bonded silica used as LC packings without disrupting the Si-C bonds. The bonded moieties are released as volatile organo mono-, di- or trifluorosilanes, $\text{R}_3\text{-Si-F}$, $\text{R}_2\text{-SiF}_2$ or R-SiF_3 , respectively. The determination, by gas chromatography, of the volatile organosilanes released allows the characterization of the structure of the bonded layer of the silica attacked by HF [35-37].

3. PHYSICO-CHEMICAL PROPERTIES OF SILICA

The three most important physico-chemical parameters for silica are the particle size, the surface area and the porosity. Other important parameters are the particle shape, the carbon percentage, the ligand density and bonding structure and the surface silanol concentration.

3.1. Particle size

3.1.1. Definition

Silica packings are always obtained with a particle distribution, *i.e.*, a stated 5- μm particle diameter is an average diameter. The average particle diameter can be defined as the average diameter in number, d_n , the average particle diameter in surface, d_s , or the average particle diameter in mass, d_m , as follows:

$$d_n = \Sigma n_i d_i / N = \Sigma n_i d_i / \Sigma n_i \quad (2)$$

$$d_s = (1/S) \Sigma n_i d_i^3 \pi / 4 = \Sigma n_i d_i^3 / \Sigma n_i d_i^2 \quad (3)$$

$$d_m = (4\pi\rho/3M) (\Sigma n_i d_i^3) = \Sigma n_i d_i^3 / \Sigma n_i d_i^3 \quad (4)$$

where n_i is the number of particles with diameter d_i , N is the total number of particles in the studied sample, S is the surface area of the sample, M is the sample mass and ρ is the density of the particles.

Fig. 6 illustrates the case of an imaginary sample which contain 10% of 1- μm , 20% of 2- μm , 40% of 3- μm , 20% of 4- μm and 10% of 5- μm particles. The average diameters are 3, 3.71 and 3.95 μm in number, surface and mass, respectively. The values d_{90} and d_{10} are the diameters of which 90% and 10% of the particles are smaller than or equal to, respectively; they are 4 and 2 μm , respectively, for the sample in Fig. 6. The ratio d_{90}/d_{10} gives information on the width of the particle size distribution.

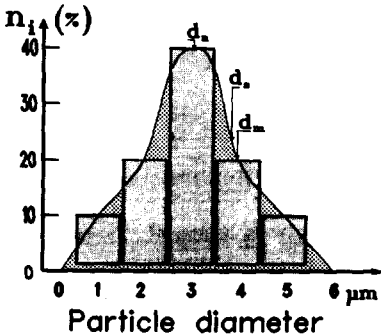


Fig. 6. Histogram of the hypothetical silica sample described in the text. d_n = Mean particle diameter in number; d_s = mean particle diameter in surface; d_m = mean particle diameter in mass.

For spherical packings, most manufacturers give the average particle diameter in number, seldom with the d_{90}/d_{10} ratio. For irregularly shaped particles, the average particle diameter given is often expressed as an equivalent mass diameter of a sphere.

3.1.2. Particle size determination

Silica-based LC packings have a particle size ranging from 1 to 100 μm . Microscopy is the simplest method to determine particle sizes. Electronic counting scanners can give a particle size distribution, with little or no assumptions on the particle shape. Sedimentation is another widely used method for particle size analysis. The particle sample is suspended in a liquid at a concentration below 1% (v/v). The suspension is homogenized in an ultrasonic bath and poured into the cell of the apparatus. Some types of apparatus use a centrifugal field with optical detection to analyse the particle size distribution. Others use a laser beam whose diffused light is analysed and deconvoluted to produce the results. Most often a particle size histogram is printed together with the average diameter in number and in mass. Brinkman, Coulter, Duke, Malvern and Perkin-Elmer are some companies making reliable particle size analysers [38]. However, these analysers, being based on different principles, yield different diameter values (number, surface or mass) for the same material. Monodisperse latex beads are used for calibration.

3.2. Surface area and porosity

3.2.1. Surface area

The specific surface area of a porous solid is equal to the sum of its internal and external surface areas [2,4]. The external surface area corresponds to the geometric surface of particles per gram of sample. For spherical particles of equal size, d_p , the external surface area is given by

$$A_s = 6/d_p\rho \quad (5)$$

where ρ is the particle density, defined as the ratio of particle mass to particle volume. For non-porous particles, ρ is the solid silica density, 2.2 g/cm^3 . The external surface area is inversely proportional to the particle diameter. A monodisperse non-porous silica, with a particle size of 3 μm , has a surface area of 0.91 m^2/g . Polydisperse non-porous silica with an average particle diameter of 3 μm (Fig. 6) has a surface area of 0.74 m^2/g , which corresponds to a monodisperse silica with a particle diameter of 3.71 μm , by definition.

Surface area is routinely measured using the BET method [39]. Gas adsorption isotherms are determined at different partial pressure and at a temperature at which the gas is in the liquid state (77 K for nitrogen). The surface area is directly proportional to X_m (mol/m^2), the amount of gas corresponding to a monolayer of adsorbed molecules:

$$A_s = X_m a_m N \quad (6)$$

where a_m is the area of one gas molecule (0.162 nm^2 per nitrogen molecule) and N is Avogadro's number ($6.02 \cdot 10^{23}$ molecules/mol). The BET equation is [40]

$$[P/X(P - P_0)] = (1/X_m C) + [(C - 1)/X_m C](P/P_0) \quad (7)$$

where P is the gas partial pressure, P_0 is the saturation vapour pressure, X is the amount of gas adsorbed, X_m is the amount of gas adsorbed when the monolayer is complete and C is a constant related to the energy of interaction between the adsorbate (gas) and the adsorbent (silica).

Using a mixture of 3% (v/v) nitrogen in helium, it is possible to determine the adsorbed amount, X , by gas chromatography [41]. Increasing stepwise the pressure of the gas mixture on the sample at 77 K increases the partial pressure, P , of nitrogen, giving several X values that allow one to extrapolate and to calculate X_m and C [41,42].

The complete theory of gas adsorption and specific surface area determination can be found in the book by Gregg and Sing [40]. The surface area of a silica sample is greatly dependent on the porosity which produces the internal surface area. The specific surface area of silicas used in high-performance liquid chromatography ranges from 10 to about $500 \text{ m}^2/\text{g}$, depending on the average pore diameter.

3.2.2. Porosity

A pore can be defined as a hole, a cavity or a channel communicating with the surface of the solid. The spaces or interstices between particles are voids rather than pores. A cavity that does not communicate with the surface is called a closed pore or an internal void and will not contribute to the porosity or specific surface area [40].

Pore systems and their characteristics are the topic of numerous works [43]. The pore shape may have a wide variety. The pore size covers a range of several orders of magnitude. A feature of special interest is the width, p_d , of the pores which is the diameter of an equivalent cylindrical pore. The classification of Dubinin [44], adopted by IUPAC [45], is micropores with $p_d < 2 \text{ nm}$, mesopores with $2 < p_d < 50 \text{ nm}$ and macropores with $p_d > 50 \text{ nm}$.

The porosity of a sample, ϵ_p , is defined as the total pore volume, V_p , divided by the volume of the sample, V_T :

$$V_T = V_p + V_s \quad (8)$$

where V_s is the volume of solid silica, assuming there are no closed pores. The pore volume and pore size distribution are estimated by gas adsorption-desorption isotherms or by mercury intrusion techniques [2,40].

The adsorption-desorption isotherms of gas onto solids can be arranged in six different types [40,43,45]. Type I, IV and V isotherms show hysteresis, *i.e.*, the desorption branch of the curve does not coincide with the adsorption branch. Both branches are used in pore shape and volume determination. Types I, IV and V correspond to microporous, mesoporous and polydisperse porosity, respectively. Types II and III correspond to non-porous samples with strong interaction and non- or macroporous samples with weak interaction, respectively. Type VI, the step-like isotherm, corresponds to non-porous material [45]. A mathematical treatment with as-

sumptions allows one to estimate the mean pore diameter with some information on the pore size distribution. Nitrogen, at the nitrogen boiling point (77 K), is the most commonly used gas for sorption studies. Experimentally, before any measurement, the sample must be carefully degassed under vacuum and heated at about 200°C.

The mercury intrusion method is used in automated apparatus to produce the pore size distribution and the mean pore diameter. Mercury does not wet a solid surface. At atmospheric pressure it does not penetrate pores whose diameters are smaller than 15 μm . To force mercury to penetrate a pore with diameter p_d , a pressure ΔP must be applied:

$$\Delta P = 4\gamma_{\text{Hg}} \cos \theta / p_d \quad (9)$$

where γ_{Hg} is the surface tension of mercury (0.48 N/m at 25°C) and θ the mercury contact angle (140° at 25°C). It can be calculated that a pressure of 100 MPa (1000 bar or 14 300 p.s.i.) is necessary to push mercury into a 15-nm pore [40]. The volume taken up by the porous sample, V_{Hg} , is measured as the applied ΔP pressure is gradually increased. At any ΔP value, V_{Hg} corresponds to the cumulative volume of all pores having a radius greater than or equal to the corresponding p_d (eqn. 9). The pore size distribution curve is the derivative of the V_{Hg} versus ΔP curve.

For a sample with a wide pore size range, the gas sorption and mercury intrusion methods must be used. The gas sorption method produces accurate results in the pore size range between 0.5 and 50 nm (micro- and small mesopores), whereas mercury porosimetry measures pores larger than 5 nm up to 15 μm (large macropores). The minimum pore size measurable is determined by the maximum pressure of mercury; 5 nm corresponds to 300 MPa (3000 bar or 43 000 p.s.i.). The two methods are complementary [40].

3.2.3. Relationship between surface area and porosity

Pore size, pore volume and specific surface area are related. Assuming there are no closed pores, the specific pore volume, V_p , in cm^3/g , is related to ρ_a , the apparent density of the particle defined as the ratio of the mass of the porous particle to its total volume (g/cm^3):

$$\rho_a = \rho / (1 + V_p \rho) = 2.2 / (1 + 2.2 V_p) \quad (10)$$

The density of solid silica is 2.2 g/cm^3 . Assuming a monodisperse distribution of perfectly cylindrical pores with diameter p_d , the internal surface area due to the pores is expressed by

$$A_p = 4V_p / p_d \quad (11)$$

The specific surface area is the sum of the external surface area (eqn. 5) with the corrected density (eqn. 10) and the internal surface area (eqn. 11):

$$A_s = 6(1 + V_p \rho) / d_p \rho + 4V_p / p_d \quad (12)$$

Table 1 shows the great importance of the pore diameter on the surface area and the

relative weight of the internal and external surface areas. The ranges of value for particle diameters, pore volumes and pore diameters are typical for silica used in LC packings. For most samples, more than 97% of the specific surface area is internal surface area due to pores. Hence, the particle diameter has little importance with regard to the surface area, which depends mainly on the pore size and volume. A silica sample with pore volume $0.5 \text{ cm}^3/\text{g}$ and mean pore size 10 nm has a specific surface area of $200 \text{ m}^2/\text{g}$, whatever the particle size is (Table 1).

4. APPLICATIONS TO LC COLUMN PACKINGS

Most of the examples given to illustrate the chemical or physico-chemical properties of silica were taken from silica samples typically used for LC packings. The chromatographic technique constrains the range of the particle size, the pore size and volume for reasons considered below.

4.1. Particle size, pressure and efficiency

Particle size is one of the most important parameters acting on chromatographic efficiency. The smaller the LC packing particle size, the higher is the efficiency [46]. The plate height, H , of a well packed column can be as low as twice the mean particle diameter ($100\,000 \text{ plates/m}$, $H = 10 \mu\text{m}$ with $d_p = 5 \mu\text{m}$).

TABLE I
EFFECT OF PORE VOLUME AND PORE SIZE ON SPECIFIC SURFACE AREA

Particle diameter (μm)	Pore volume (cm^3/g)	Pore diameter (nm)	Surface area ^a	
			Total (m^2/g)	Internal (% of total surface)
100	1.0	50	80.1	99.9
		10	400.1	99.9
		5	800.1	100
	0.5	50	40.1	99.8
		10	200.1	99.9
		5	400.1	100
	0.1	50	8.0	99.6
		10	40.0	99.9
		5	80.0	99.9
3	1.0	50	82.9	96.5
		10	402.9	99.3
		5	802.9	99.6
	0.5	50	41.9	95.4
		10	201.9	99.1
		5	401.9	99.5
	0.1	50	9.1	87.8
		10	41.1	97.3
		5	81.1	98.6

^a Surface area calculated with eqn. 12. Internal % due to pores in the ratio A_p (eqn. 11) to total surface area.

Unfortunately the particle size affects the column permeability, \bar{P} (cm²), in the following way:

$$\bar{P} = (d_p^2/Y)[\varepsilon_o^2/(1 - \varepsilon_o)^2] \quad (13)$$

where Y is a dimensionless shape factor ($Y = 180$ for spherical particles) and ε_o is the interstitial volume or external porosity (cm³). The permeability is a parameter of the column pressure drop, ΔP

$$\Delta P = u\eta L/\bar{P} \quad (14)$$

where u is the linear mobile phase velocity (cm/s), η the mobile phase viscosity (g/cm · s) and L the column length (cm). If the particle diameter is divided by a factor of two, the column driving pressure should be four times higher in order to obtain the same flow-rate.

The optimum particle size with regard to analysis time, plate number and pressure drop is *ca.* 2–4 μm [47,48]. Packings with average particle diameter 5 μm are the most commonly used for 10–25-cm analytical columns with I.D. 4–4.6 mm. Packings of 3 μm are used to obtain high efficiency with short columns; 10- μm , 20- μm or larger particle size packings are used when pressure is an important factor, such as in preparative chromatography.

Pyrogenic silicas cannot be used directly for LC packings as their mean diameter, in the few nanometres range, is too small. The column permeability (eqn. 13) would be very low and a tremendous driving pressure (eqn. 14) would be necessary to push any mobile phase, which is mechanically impossible. Pyrogenic non-porous silicas have been used to coat 30- μm glass beads to obtain a 1- μm superficially porous layer. Such packings were popular in the 1970s and commercialized under trade names such as Corasil, Pellosil, Perisorb, Vydac 101 and Zypax [2].

4.2. Polarity, surface, porosity and surface modification

4.2.1. Surface silanols and polarity

Silanol groups are responsible for the polarity of the silica surface. A silica completely dehydroxylated by prolonged heating at 1300°C is hydrophobic. The silanol concentration of a fully hydroxylated silica is about 9 $\mu\text{mol}/\text{m}^2$. The pretreatment temperature under vacuum can decrease the silanol concentration and adjust the hydrophilic character of a sample [49]. Fig. 7 shows the evolution of silanol concentration *versus* temperature for a precipitated silica. Above 200°C, the hydroxyl surface concentration decreases rapidly. At 500°C, only one quarter of the initial silanols remain (concentration between 2 and 3 $\mu\text{mol}/\text{m}^2$). However, as long as the temperature does not exceed 1200°C, the dehydroxylation is partially reversible.

It has been shown that the selectivity with a thermally treated silica used in normal-phase LC is greatly dependent on the apolar mobile phase water content [3,50]. Reproducible results with activated silicas and apolar solvents such as *n*-hexane are only obtained when the water content of the silica and the mobile phase is adjusted and controlled [51]. Water molecules, always present in trace amounts in an apolar solvent, adsorb on the most polar silanol sites, decreasing the retention of

TABLE 2
SOME SILICA-BASED LC STATIONARY PHASES

Name	Manufacturer ^a	Particle size (μm)	Particle shape ^b	Surface area (m^2/g)	Pore diameter (nm)	Pore volume (cm^3/g)	Bonding Type ^c	C (%)	Density ($\mu\text{mol}/\text{m}^2$)
Accusphere	J&W	3, 5, 7	O	210	12	0.63	C ₁	2.6	3.6
							C ₈	7.0	3.1
Adsorbosphere	Alt	3, 5, 7, 10	O	200	8	0.40	C ₁₈ m ec	10.5	2.4
Adsorbosphere HS	Alt	3, 5, 7, 10	O	350	6	0.53	C ₁₈ m ec	12	3.0
Apex	JoC	8, 15, 20	O	190	13	0.80	C ₈	20	3.2
							C ₁₈	7	3.4
Bakerbond	JTB	3, 5, 10	K	200	15	0.75	Bare	11	2.8
							C ₈ ec	—	—
							C ₁₈ ec	15	3.9
Bakerbond Wp	JTB	3, 15, 40	K	100	30	0.75	C ₄	—	—
							C ₈	—	—
							C ₁₈	—	—
Econosil	Alt	5, 10	K	450	6	0.68	C ₈ m	10	2.2
							C ₁₈ m	15	1.7
Econosphere	Alt	3, 5, 10	O	200	8	0.80	C ₈ m	5	2.2
							C ₁₈ m	10	2.4
Hypersil	Shd	3, 5, 10	O	170	12	0.60	C ₁ m ec	2.6	4.5
							C ₈ m ec	7	3.8
							C ₁₈ m ec	10	2.8
Inertsil	Int	5	K	320	15	1.20	Bare	—	—
							C ₈ m ec	10.5	3.2
							C ₁₈ m ec	18.5	3.2
Intersphere	Int	5, 7	O	500	8	1.0	Bare	—	—
							C ₁₈ m ec	18	2.0
							C ₁₈ p ec	22	—
Kromasil	Eka	5-20	O	340	10	0.90	Bare	—	—
							C ₈ m	12	3.5
							C ₁₈ m	19	3.1
LiChrosorb Si 100	Mer	5, 10	K	300	10	0.75	C ₈	8	2.5
							C ₁₈	13	2.2

LiChrosorb Si 60	Mer	5, 10	K	500	6	0.75	C ₈	9	1.7				
LiChrosorb 100	Mer	5, 10	O	340	10	1.0	C ₁₈ p	16	1.7				
Nucleosil	Nag	3, 5, 7, 10	O	500	5	0.80	C ₁₈ p ec	21	—				
				350	10	1.0	Bare	21.5	—				
				350	10	1.0	Bare	—	—				
				350	10	1.0	C ₈ m	8	2.1				
				200	10	0.7	C ₁₈ m	14	2.0				
				100	30	0.75	Bare	—	—				
				35	50	0.9	Bare	—	—				
				25	100	0.75	Bare	—	—				
				10	400	0.7	Bare	—	—				
				350	8.5	0.74	C ₈ m ec	9	2.5				
				Partisil	Wht	5, 10	K	—	—	—	C ₁₈ p	5	—
				Partisphere	Wht	5, 10	O	160	12	0.48	C ₁₈ p	15	—
Rexchrom	Reg	3, 5	O	200	10	0.5	C ₁₈ p ec	10.5	—				
				—	—	—	C ₈ m ec	6	3.4				
R-Sil	Mer	5, 10	K	400	8	0.8	C ₁₈ m ec	11	3.3				
RoSil	Alt Bro	5, 10	O	250	9	0.56	C ₁₈ m	18	3.9				
				—	8	—	C ₈ ec m	—	—				
Spherisorb	PhS	3, 5, 10	O	220	8	0.44	C ₈ p	—	—				
				—	—	—	C ₁₈ m ec	—	—				
				—	—	—	C ₁₈ p	—	—				
				—	—	—	C ₁ m	2	2.6				
				—	—	—	C ₈ m ec	6	2.5				
Spherisorb WP	PhS	5, 10	O	190	30	1.43	C ₁₈ m	6	1.2				
				—	—	—	C ₁₈ m ec	12	2.7				
				—	—	—	C ₁	2	3.0				
				—	—	—	C ₃	3	2.8				
—	—	—	C ₆	5	3.0								
—	—	—	C ₁₈	12	3.1								

(Continued on p. 22)

TABLE 2 (continued)

Name	Manufacturer ^a	Particle size (μm)	Particle shape ^b	Surface area (m^2/g)	Pore diameter (nm)	Pore volume (cm^3/g)	Bonding	
							Type ^c	C (%)
Supelcosil	Sup	3, 5	O	170	10	0.43	C_6m C_{18}m	— —
Ultrapase	SFC	5, 10	O	340	10	0.90	C_6m C_{18}m	12 19
Ultraporb	Phe	5, 7 5, 10	O O	550 370	6 9	0.83 0.82	C_{18}m C_{18}	31 22
Ultrapore	Bec	5	O	—	30	—	$\text{C}_3\text{m ec}$ $\text{C}_6\text{m ec}$	— —
Ultrasil	Bec	10	K	—	8	—	$\text{C}_6\text{m ec}$ $\text{C}_{18}\text{m ec}$	— —
Ultrasphere	Bec	5	O	—	8	—	$\text{C}_6\text{m ec}$ $\text{C}_{18}\text{m ec}$	— —
Versapak	Alt	10	K	200	8	0.4	$\text{C}_{18}\text{m ec}$ $\text{C}_{18}\text{m ec}$	10 13
Vydac HS	TSG	5, 10, 20	O	400	8	0.65	C_{18}m	2.8
Vydac TP	TSG	5, 10, 20	K	100	30	0.7	C_4m C_{18}m	9 4.2
Zorbax	DPN	5, 7, 10	O	300	8	0.6	Bare	—

^a Manufacturers' abbreviations and addresses, see Table 3.

^b Shape: O = spherical; K = irregular.

^c Type: m = monomer bonding; p = polymer bonding; ec = end-capping treatment. When the bonding type was not given, a monomer bonding was assumed in order to calculate the bonding density.

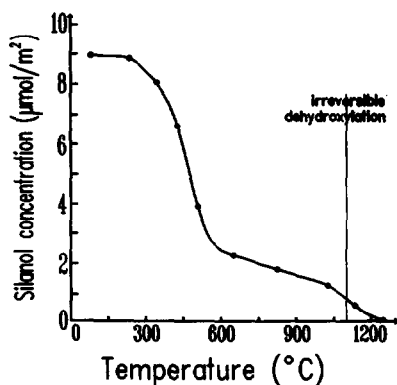


Fig. 7. Evolution of silanol concentration *versus* temperature. Dotted area: the dehydroxylation becomes irreversible. Data from ref. 2.

polar solutes and modifying the solute-stationary phase exchanges [52]. Peak shape and efficiency are also affected by the adsorption of water on silica. Methanol and other polar solvents can be added to the apolar mobile phase to adjust the selectivity [3].

As already described, surface silanols are used as anchor points to graft organic moieties. For steric reasons, it is not possible to fix an organic derivative on every hydroxyl group. Of the $9 \mu\text{mol}/\text{m}^2$ initial silanol concentration, only 50% can be derivatized. The maximum bonding density is about $4.5 \mu\text{mol}/\text{m}^2$ [53].

Acid silanols have a great affinity for basic groups. The low efficiencies and peak tailings obtained with the amino-containing solutes are well documented. They are due to $\equiv\text{Si}-\text{OH} \cdots \text{NH}-$ acid-base interactions. To reduce the residual silanol concentration of bonded silica, the end-capping treatment was used. The small trimethylchlorosilane molecule was used to react with silanols that were not accessible to the large octadecyl-containing reagent. An end-capped silica still bears unreacted silanols. The modern trend in manufacturing base-deactivated silica packings is to increase the ligand density (see section 4.2.3 and ref. 62).

4.2.2. Silica surface and porosity

The specific surface area of silica has an important impact on solute retention and selectivity. Obviously, the higher the specific surface area of a silica, the higher is the solute-stationary phase exchange area [3,54]. Solute retention is roughly proportional to the specific surface area, all other parameters being constant (same kind of stationary phase, same column length, same mobile phase, same flow-rate, etc.). For LC, Snyder [3] recommends the use of stationary phases possessing a specific surface area of at least $50 \text{ m}^2/\text{g}$. Silicas with A_s values higher than $500 \text{ m}^2/\text{g}$ have been used (Table 2).

Surface area and porosity are related (Table 1 and section 3.2.3). Eqn. 10 shows that the apparent particle density depends on the pore volume. With $V_p = 0.4 \text{ ml/g}$, the apparent particle density is $1.2 \text{ g}/\text{cm}^3$. The density drops to $0.5 \text{ g}/\text{cm}^3$ when the pore volume is 1.4 ml/g . By actually measuring the volume of 1 g of packing, it is

possible to define the actual packing density of a bonded phase. A 1-g amount of packing occupies between 1.2 and 5 cm³. The packing density varies between 0.2 and 0.8 g/cm³. It affects the specific surface area in m²/(cm³ of packing) in the column. A 10-cm column filled with a 150 m²/g packing of density 0.6 g/cm³ has twice the surface area of a 10-cm column filled with the same 150 m²/g packing but with density only 0.3 g/cm³.

4.2.3. Carbon content and bonding density

The bonding reactions described in paragraph 2.3.4 lead to partial coverage of the silica surface. The maximum concentration of 4.5 μmol/m² is difficult to achieve. It is important to characterize the chemically treated silica. The two important parameters for the characterization of bonded silica are the carbon content, in grams of C per 100 g of packing, and the surface concentration or bonding density, in micro-moles of bonding moiety per square metre of initial silica surface area. The assessment of these two parameters is treated in depth in ref. 62.

Although the bonding density is almost never given by manufacturers (see section 4.4), it is one of the most important parameters for a bonded silica-based stationary phase. A high bonding density means a low residual silanol concentration; such a phase has a better resistance to elevated pH mobile phases and a better ability to separate amino-containing compounds. A pragmatic test procedure for comparison and evaluation of reversed-phase silica-based LC packings was proposed recently [55].

4.3. Chemical and physical chromatographic requirements

Owing to dissolution of silica, basic and hydrofluoric acid aqueous solutions must not be used as mobile phases in LC with silica-based stationary phases. The safe working pH range for the mobile phase is 2–9. Below pH 2, Si–C bonds of derivatized LC packings can be split; above pH 9, silica is slowly solubilized into silicate. The working pH range increases when the bonding density and/or the silica purity is high. A small addition of sodium silicate to the mobile phase greatly reduces the dissolution of silica at elevated pH (pH 12) [56]. Zirconia-cladded silicas were introduced to enhance the resistance of silica to alkaline solutions [31].

The stationary phase must have some mechanical strength to withstand the column back-pressure, usually in the range 1–20 MPa (10–200 bar or 140–2900 p.s.i.). With a typical pore volume in the cm³/g range, the maximum pore size available for LC packings is 100 nm; in rare cases 400-nm pore size silicas can be used. Wide-pore silica particles have a low mechanical resistance and can break during the slurry packing procedure which operates at high pressures of 50 MPa or more (500 bar or 7200 p.s.i.). Particle breakage during the column packing procedure produces interstitial clogging; the column permeability becomes zero and the column and the packing are useless.

The silica pore size may be a critical parameter in the case of bulky solute analysis. For example, large-pore silicas are required for biopolymer separations, peptides or proteins [57]. In such analyses, 30- and 50-nm pore size packings are commonly used to enhance external silica–solute interactions and to avoid internal interactions such as restricted and slow diffusion of biopolymers [19]. The large mole-

TABLE 3
LIST OF SUPPLIERS

Name	Abbreviation	Address ^a
Alltech	Alt	Alltech Associates Inc., Applied Science Labs., 2051 Waukegan Road, Deerfield, IL 60015, USA
Asahi	Asa	Asahi Chemical Industry, Ltd., 1-3-2 Yakoo, Kawasaki-Ku, Kawasaki-Shi, 210, Japan
Beckman	Bec	Beckman Instruments Inc., 160 Hopper Avenue, Waldwick, NJ 07463, USA; Beckman Instruments International SA, 22 rue Juste-Oliver, CH-1260 Nyon, Switzerland
Brownlee	Bro	Brownlee Applied Biosystems, 850 Lincoln Center Dr., Foster City, CA 94404, USA
DuPont	DPN	E. I. DuPont de Nemours, 1007 Market St., Wilmington, DE 19898, USA
Eka Nobel Interchim	Eka Int	Eka Nobel AB, S-445 01 Surte, Sweden Interchim, 213 avenue J. F. Kennedy, B.P. 15, 03103 Montlucon, France
J&W Scientific	J&W	J&W Scientific, Inc., 91 Blue Ravine Rd., Folsom, CA 95630, USA
Jones Chromatography	JoC	Jones Chromatography, Ltd., New Road, Hengoed, Mid-Glamorgan CF8 8AU, UK
J. T. Baker	JTB	J. T. Baker, Inc., 222 Red School Lane, Phillipsburg, NJ 08865, USA
Macherey, Nagel & Co.	Nag	Macherey, Nagel GmbH & Co. KG, Neumann-Neander Str., P.O. Box 101 352, W-5160 Düren, Germany
Merck	Mer	E. Merck, Frankfurter Strasse 250, Postfach 4119, W-6100 Darmstadt, Germany
Phase Separations	PhS	Phase Separations Ltd., Deeside Industrial Park, Queensferry, Clwyd CH5 2NU, UK
Phenomenex	Phe	Phenomenex, 6100 Palos Verdes Drive S., Rancho Palos Verdes, CA 90274, USA
Regis	Reg	Regis Chemical Co., 8210 N Austin Avenue, Morton Grove, IL 60053, USA
Shandon	Shd	Shandon Southern Products Ltd., Chadwich Road, Astmoor, Runcorn, Cheshire WA7 1PR, UK
Separations Group	TSG	The Separations Group, Vydac, P.O. Box 867, 17 434 Mojave Street, Hesperia, CA 92345, USA
SFCC	SFC	Société Française Chromato Colonne, Z. I. des Renouillères, 9 rue Marcel Dassault, 93360 Neuilly-Plaisance, France
Supelco	Sup	Supelco, Inc., Supelco Park, Bellefonte, PA 16823-0048, USA
Whatman	Wht	Whatman Ltd., Springfield Mill, Maidstone, Kent ME14 2LE, UK

^a From *International Laboratory*, 1989 Buyer's Guide Edition, and Labguide, *Analytical Chemistry*, 61 (1989) 1G-294G.

cules can be trapped inside medium-size pores, inducing peak tails [24,58]. This is why a large pore size with a narrow pore size distribution should be used [59]. A narrow pore size distribution is also required in size-exclusion chromatography to obtain sufficient efficiency [60].

4.4. Examples

A non-exhaustive list of some commercially available silica-based LC packings is given in Table 2. Owing to the multiplicity of different bondings, only the linear alkyl bondings were considered together with the bare silica. Commercial catalogues give the characteristics of the products available in a form such as particle diameter, 100%; pore size, 94%, bonding moiety, 91%; particle shape, 90%; surface area, 53%; pore volume, 37%; bonding reaction (monomeric, polymeric and/or end-capping), 23%; carbon content, 21%; and bonding density, 4%. In Table 2, the pore volume and the bonding density were calculated when they were not given by the manufacturer. The bonding densities listed are only indicative because it is not possible to consider exactly the effect of the end-capping treatment on the carbon content and bonding density, and when the bonding reagent was not indicated a monomeric layer was assumed for the calculation. The abbreviations in the "Manufacturer" column refer to the company names and addresses listed in Table 3.

5. CONCLUSIONS

The qualities of silica, *i.e.*, high surface area and porosity, facile and versatile preparation, adjustable polarity and good mechanical strength, explain its widespread use in LC stationary phases. Its weak point is its low resistance to alkaline solutions. pH-resistant substitutes are commercially available today; they are mainly organic polymer-based materials. Styrene-divinylbenzene copolymer can be processed into spherical and macroporous particles with surface area 400 m²/g (Benson Polymeric, EM Science, Brownlee Applied Biosystems, Hamilton, Polymer Labs.). Vinyl alcohol copolymer is also used to mimic silica. The hydroxyl groups (alcohol groups) of this polymer can be chemically modified to form a bonded polymer that can withstand strongly alkaline solutions because it contains only C–C bonds (Asahi Chemical). Such packings and uses are discussed in ref. 62. Eventually, these polymers or others may supplant silica-based packings. Composite materials, such as polymer-coated silicas, are another promising route to design of LC packings of tomorrow [61]. Meanwhile, silica is likely to be used satisfactorily for many more years.

6. SYMBOLS

a_m	area of a gas molecule in the BET method (0.162 nm ² per N ₂ molecule)
A_s	surface area (m ² /g)
A_p	internal surface area due to pores (m ² /g)
C	BET constant
d_p	particle diameter (μm)
d_m	average particle diameter in mass (μm)
d_n	average particle diameter in number (μm)
d_s	average particle diameter in surface (μm)
L	column length (cm)
n_i	number of particles with a diameter d_i
N	Avogadro's number (6.02 · 10 ²³ molecules/mol)

P	gas partial pressure in BET measurements (Pa)
\bar{P}	column permeability (cm^2)
ΔP	back pressure (pascal, bar or p.s.i.)
P_0	gas saturation vapour pressure in BET measurements (Pa)
p_d	pore diameter (nm)
S	surface area of a sample (m^2/g)
u	linear mobile phase velocity (cm/s)
V_{Hg}	mercury volume pushed into pores at a pressure ΔP (cm^3)
V_p	pore volume (cm^3/g)
X	amount of adsorbed gas (mol/m^2)
X_m	amount of adsorbed gas corresponding to a monolayer (mol/m^2)
Y	dimensionless shape factor in column permeability equation

Greek letters

ε_0	external porosity or interstitial volume (cm^3)
ε_p	particle porosity (ratio of particle pore volume to total particle volume)
γ_{Hg}	surface tension of mercury (0.48 N/m at 25°C)
ρ	density of solid silica (2.2 g/cm^3)
ρ_a	apparent particle density in the case of a porous silica particle (g/cm^3)
η	mobile phase viscosity ($\text{g}/\text{cm} \cdot \text{s}$)
σ_{SiOH}	surface silanol concentration ($\mu\text{mol}/\text{m}^2$)
θ	mercury contact angle (140° at 25°C)

REFERENCES

- 1 C. R. Hammond, in R. C. Weast (Editor), *Handbook of Chemistry and Physics*, CRC Press, Boca Raton, FL, 67th ed., 1987, p. B34.
- 2 K. K. Unger, *Porous Silica (Journal of Chromatography, Library, Vol. 16)*, Elsevier, Amsterdam, 1979.
- 3 L. R. Snyder, *Principles of Adsorption Chromatography*, Marcel Dekker, New York, 1968.
- 4 R. K. Iler, *The Chemistry of Silica*, Wiley, New York, 1979.
- 5 R. B. Sosman, *Phases of Silica; Am. Ceram. Soc. Bull.*, 43 (1964) 213.
- 6 F. Liebau, *Structural Chemistry of Silicates, Structure, Bonding and Classification*, Springer, Berlin, 1985.
- 7 S. E. Frazier, J. A. Bedford, J. Hower and M. E. Kennedy, *Inorg. Chem.*, 6 (1967) 1693.
- 8 M. V. Tongelen, J. Uytterhoeven and J. J. Fripiat, *Bull. Soc. Chim. Fr.*, 31 (1965) 2318.
- 9 L. Nondek and V. Vyskocil, *J. Chromatogr.*, 206 (1981) 581.
- 10 S. C. Antakli and J. C. Serpinet, *Chromatographia*, 23 (1987) 767.
- 11 K. K. Unger and E. Gallei, *Kolloid Z. Z. Polym.*, 237 (1970) 358.
- 12 J. Shapiro and H. G. Weiss, *J. Phys. Chem.*, 57 (1953) 219.
- 13 L. T. Zhuralev, *Langmuir*, 3 (1987) 316.
- 14 P. Roumeliotis and K. K. Unger, *J. Chromatogr.*, 149 (1978) 211.
- 15 J. Goworek, F. Nooitgedacht, M. Rikhof and H. Poppe, *J. Chromatogr.*, 352 (1986) 399.
- 16 B. Camara, H. Dunken and P. Fink, *Z. Chem.*, 8 (1968) 155.
- 17 G. E. Maciel and D. W. Sindorf, *J. Am. Chem. Soc.*, 102 (1980) 7606.
- 18 B. A. Morrow and I. D. Gay, *J. Phys. Chem.*, 92 (1988) 5569.
- 19 C. E. Bronnimann, R. C. Zeigler and G. E. Maciel, *J. Am. Chem. Soc.*, 110 (1988) 2023.
- 20 J. J. Köhler, D. B. Chase, R. D. Farlee, A. J. Vega and J. J. Kirkland, *J. Chromatogr.*, 352 (1986) 275.
- 21 G. K. Barashkov, *Bot. Zh.*, 45 (1960) 1350.
- 22 K. K. Unger, *Angew. Chem., Int. Ed. Engl.*, 11 (1972) 267.
- 23 H. Kloepfner (Degussa), *Ger. Pat.*, 762 723 and 830 786 (1942).

- 24 K. K. Unger, R. Janzen, G. Jilge, K. D. Lork and B. Anspach, in Cs. Horvath (Editor), *High Performance Liquid Chromatography: Advances and Perspectives*, Vol. 5, Academic Press, London, 1988, pp. 1-93.
- 25 A. V. Kiselev and V. J. Lygin, *Infrared Spectra of Surface Compounds*, Wiley-Interscience, New York, 1975.
- 26 M. L. Hair, in E. R. Corey, J. Y. Corey and P. P. Gaspar (Editors), *Silicon Chemistry*, Ellis Horwood, Chichester, 1988, Ch. 44, pp. 481-489.
- 27 R. Demoyel, F. Rouquerol and J. Rouquerol, in A. Liapis (Editor), *Fundamentals of Adsorption, Proceedings of the Engineering Foundation Conference*, Engineering Foundation, New York, 1987, pp. 199-210.
- 28 I. L. Cunha and L. G. Audrade e Silva, *J. Radioanal. Nucl. Chem.*, 104 (1986) 293.
- 29 M. Montjoie, J. R. Costes and F. Josso, *Proc. Inst. Mech. Eng.*, 1988, 127.
- 30 B. Law and P. F. Chan, *J. Chromatogr.*, 467 (1989) 267.
- 31 R. W. Stout, S. I. Sivaroff, R. D. Ricker, H. C. Palmer, M. A. Jackson and T. J. Odiorne, *J. Chromatogr.*, 352 (1986) 381.
- 32 J. J. Pecsck and J. A. Graham, *Anal. Chem.*, 49 (1977) 133.
- 33 E. Bayer, K. Albert, J. Reiners, M. Nieder and D. Muller, *J. Chromatogr.*, 264 (1983) 197.
- 34 K. D. Lork, K. K. Unger and J. N. Kinkel, *J. Chromatogr.*, 352 (1986) 199.
- 35 J. F. Erard and E. sz. Kováts, *Anal. Chem.*, 54 (1982) 193.
- 36 S. D. Fazio, S. A. Tomellini, H. S. Hsien, J. B. Crowther, T. V. Raglione and T. R. Hartwick, *Anal. Chem.*, 57 (1985) 1559.
- 37 C. Gaget, D. Morel, M. Traore and J. Serpinet, *Anal. Chem.*, 12 (1984) 386.
- 38 Labguide 90, *Anal. Chem.*, 61 (1989) 150G.
- 39 S. Brunauer, P. H. Emmett and E. Teller, *J. Am. Chem. Soc.*, 60 (1938) 309.
- 40 S. J. Gregg and K. S. W. Sing, *Adsorption, Surface Area and Porosity*, Academic Press, New York, 2nd ed., 1982.
- 41 B. Pommier, F. Juillet and S. J. Teichner, *Bull. Soc. Chim. Fr.*, 38 (1972) 1268.
- 42 J. Serpinet, G. Untz, C. Gachet, L. Demourgues and M. Perrin, *J. Chim. Phys.*, 71 (1974) 949.
- 43 D. H. Everett, in K. K. Unger, J. Rouquerol, K. S. W. Sing and H. Kral (Editors), *Characterization of Porous Solids*, Elsevier, Amsterdam, 1988, pp. 1-21.
- 44 M. M. Dubinin, *Zh. Fiz. Khim.*, 34 (1960) 959.
- 45 K. S. W. Sing, *Pure Appl. Chem.*, 54 (1982) 2201.
- 46 J. C. Giddings, *Dynamics of Chromatography*, Marcel Dekker, New York, 1965.
- 47 J. N. Done and J. H. Knox, *J. Chromatogr. Sci.*, 13 (1972) 606.
- 48 P. A. Bristow and J. H. Knox, *Chromatographia*, 10 (1976) 279.
- 49 R. P. W. Scott and P. Kucera, *J. Chromatogr. Sci.*, 13 (1975) 837.
- 50 Z. El Rassi, C. Gonnet and J. L. Rocca, *J. Chromatogr.*, 125 (1976) 179.
- 51 H. Engelhardt and H. Elgass, in Cs. Horvath (Editor), *High Performance Liquid Chromatography - Advances and Perspectives*, Vol. 2, Academic Press, London, 1980, pp. 57-108.
- 52 R. P. W. Scott, *Faraday Symp. Chem. Soc.*, 15 (1980) 49.
- 53 F. Gobet and E. sz. Kováts, *Adsorpt. Sci. Technol.*, 9 (1984) 77.
- 54 H. Engelhardt, *High Performance Liquid Chromatography*, Springer, Berlin, 1979.
- 55 H. Engelhardt and M. Jungheim, *Chromatographia*, 29 (1990) 59.
- 56 N. T. Miller and J. M. Di Bussolo, *J. Chromatogr.*, 499 (1990) 317.
- 57 M. A. Stadalius, H. S. Gold and L. R. Snyder, *J. Chromatogr.*, 327 (1985) 27 and 93.
- 58 K. K. Unger and R. Jansen, *J. Chromatogr.*, 373 (1986) 227.
- 59 H. J. Richtie, P. Ross and D. R. Woodward, *Chromatogr. Anal.*, 13 (1990) 9.
- 60 F. E. Regnier, *Anal. Chem.*, 55 (1983) 1298A.
- 61 H. Figge, A. Deege, J. Köhler and G. Schomburg, *J. Chromatogr.*, 351 (1986) 393.
- 62 K. K. Unger, F. E. Regnier and R. E. Majors (Editors), *Liquid Chromatography Packings; J. Chromatogr.*, 544 (1991).