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SILICA GEL: A KEYSTONE IN CHROMATOGRAPHIC TECHNIQUES

Pranali Bhaskar Bhore [*1] and Vineeta Vivek Khanvilkar [2]

Department of Quality Assurance [1], Department of Pharmaceutical Analysis [2], Bharati Vidyapeeth's College of Pharmacy, Navi Mumbai - 400614, Maharashtra, India.

Keywords:

Silica gel, Sol-gel synthesis, Monolithic silica columns, Mesoporous silica, Superficially porous silica columns, Applications of silica gel in chromatography

Correspondence to Author:
Ms. Pranali Bhaskar Bhore

Department of Quality Assurance, Bharati Vidyapeeth's College of Pharmacy, Navi Mumbai - 400614, Maharashtra, India.

E-mail:pranalibhore1994@gmail.com

ABSTRACT: Background: In research, the analyst gets samples for separation ranging from a simple mixture of two or more synthetic drugs to complex ones obtained from biological, environmental or industrial processes. Chromatography has extensively used in such separations where component separation based upon their affinity towards stationary phase and mobile phase. 90% of the stationary phases available use silica gel either in its original form or with advanced surface properties. **Objective:** To furnish the reader with detailed information on silica gel, the functional moiety in chromatography, from its synthesis, properties, types, modification of surface characteristics and applications. We aim to focus on novel silica materials evolved recently and getting attention due to their performance.

INTRODUCTION: Silica gel consists of granular, porous form of silicon dioxide synthetically made from sodium silicate and possesses significant characteristics of uniform pore and pore size due to which it becomes highly porous structure [1]. Historically silica gel was known to exist from the 1640s as scientific interest. In World War I silica gel was used in the adsorption of vapors and gases in gas mask canisters. In World War II, silica gel used for keeping penicillin dry, protecting military equipment from moisture damage. From the years 1914 to 1918 it was popularly used as a fluid cracking catalyst for production of high-octane gasoline and catalyst support for manufacturing of butadiene from ethanol. During this ranges, silica gel was also used as a feedstock for synthetic rubber program.

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Silica gel patented by chemistry professor Walter A. Patrick at Johns Hopkins University, Baltimore, Maryland in 1919. In 1959, silica gel was first supplied in "closed packages" for used as a buffering agent in museum applications to control relative humidity. Its use as a buffering agent rather than as a desiccant was unique during that mid-ranges [2]. Silica gel has characteristics of controlled structure, composition, morphology, and porosity. Thus, having hosting and recognition properties, as well as their wide-open structures containing many easily accessible active sites, make them particularly attractive for chromatographic separations. The purpose of this review article is to study what is silica gel, how does it get synthesize by various methods and how synthesis affects the quality of silica gel, various advancements in silica gel to enhance properties and its effectiveness in chromatographic separations [1-13].

2. Synthesis of Silica Gel:

2.1. Sol-gel Method for Synthesis of Silica Gel: The sol-gel process fabricates glass-like or ceramic materials through subsequent hydrolysis and

condensation of suitable alkoxides. Tetra-methoxy silane (TMOS) and tetraethoxysilane (TEOS) are one of the most popular alkoxides for the preparation of silicate materials. In a typical procedure, water and TMOS combined with a mutual solvent such as methanol followed by addition of a catalyst such as hydrochloric acid. During the sol-gel process, the viscosity of the solution gradually increases, and sol undergoes polycondensation reaction to form an interconnected rigid, porous network called as a gel.

2.2. Advantages of Sol-Gel Method:

1. Silica gel formed by the sol-gel method has found many advantages in chromatographic technique; particularly stationary phase development has been a prime area of interest.
2. By this method, silica gel in various forms like films, fibers, monoliths, powders can be readily made.
3. Sol-gel process readily forms thick, crack-free films due to improved flexibility of silica gel.
4. In the sol-gel process, functional groups can covalently be attached to the silicon dioxide network which inhibits loss of functional group in solution.
5. Reagents readily incorporated in the matrix by simply adding them to the sol before its gelation stage. Alternatively, reagents can be added by co-polymerizing tetramethoxysilane with organoalkoxysilanes. By this process, matrix stabilizes the entrapped reagent from photodegradation or caustic solution environments [3].

2.3. Synthesis of Silica Gel by Conventional Method: Using Sulphuric Acid and Sodium Silicate: Another conventional method for synthesis of silica gel other than sol-gel method was explained by Ali Hafiz *et al*. They synthesized silica gel by acidifying an aqueous solution of sodium silicate. The intermediate product formed at low pH called as a hydrosol which further undergoes agglomeration to form silica polymers. Governing subsequent washing of hydrosol at relatively low pH, the formation of silica gel occurs with high surface area (>700 m^2/g). When the viscosity of hydrosol increases it is termed as a hydrogel. Silica gel is a colloidal form possessing a continuous three-dimensional network of spherical particles. In the gel structure, both siloxane (-Si-o-Si-) and silanol (-Si-OH) bonds are present. In-gel structure, pores get filled with water from hydrolysis and condensation reaction.

The detailed stepwise process of the formation of silica gel and process to be performed at each step is tabulated in **Table 1**.

TABLE 1: STEPS INVOLVED INFORMATION OF SILICA GEL

S. no.	Name of the step	Process
1	Required materials	Slow addition of Liquid Sodium silicate having a specific gravity of 1.43 into the mixture of cobalt acetate and concentrated sulphuric acid having specific gravity 1.85 forms hydrosol
2	Washing of hydrosol	Washing of hydrosol with water is important to step for removing sodium sulphate which formed during the reaction and for the formation of silica gel with a high surface area
3	Drying	After washing of hydrosol, drying is an important step to remove adhered water from the gel
4	Screening	Finally, the product screened to get a uniform particle size

Following reaction shows the stepwise structures formed during the synthesis of silica gel.

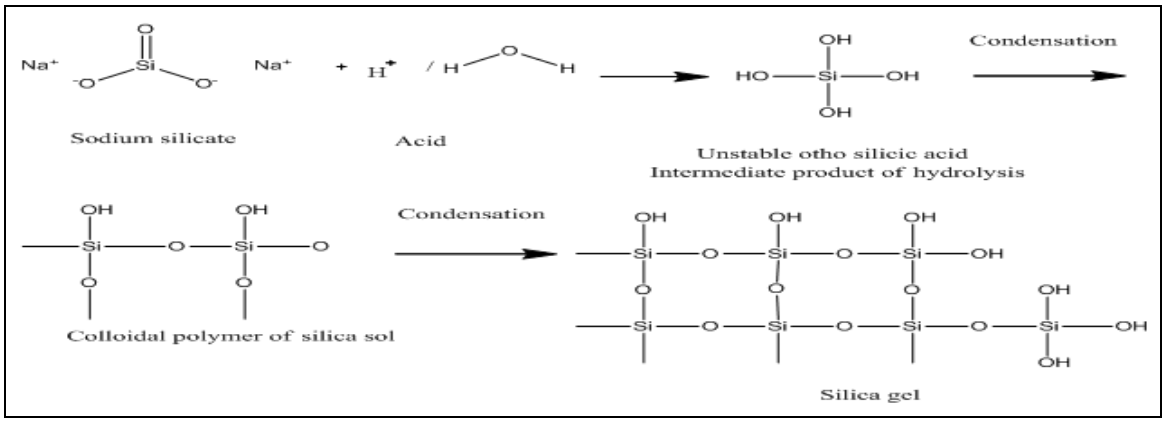

FIG. 1: REACTION SHOWING SYNTHESIS OF SILICA GEL BY CONVENTIONAL METHOD

2.4. Parameters Affecting Quality of Silica Gel during Synthesis by Conventional Method:

2.4.1. Effect of Temperature: Reaction of sulphuric acid and sodium silicate evolves great amount of heat, thus indicating that reaction is exothermic. Performing reaction at low temperature produces good quality silica gel.

2.4.2. Effect of pH: The pH is a major factor for the formation of granular and fine crystalline silica gel. Quality of synthesized product greatly depends upon the pH at which addition of sodium silicate will be done into sulphuric acid and hydrosol allowed to form a gel structure. At low pH, due to the growth of chain length, aggregation of particles occurs after the creation of three-dimensional gel networks. But very low pH 2.5 resists to the gel formation. The hydrosol formed at pH 2.5 does not get converted into hydrogel even after 24 h.

2.4.3. Effect of Concentration: Concentration of sodium silicate (Na_2SiO_3) solution has a profound effect on the characteristics of the product. Silica gel ready at pH 3 to 3.5 from the hydrosol, using a relatively concentrated solution of sodium silicate shows better characteristics for moisture absorptive capacity, bulk density, and iodine number compared to the less concentrated sodium silicate solution. The reverse of this observation seen at pH 6 where silica gel ready with a comparatively low concentrated sodium silicate solution shows better quality product.

2.4.4. Effect of Mixing Rate: By changing stirring rate, quality of the product and crystal size gets changed. By improper stirring, local coagulation resulted and after drying the product, silica gel has amorphous (powdered) structure [1].

2.4.5. Effect of Heat: Heat affects greatly on adsorption property of silica gel. Previous studies reported that the imbalance in the concentrations of vicinal and free silanol groups on the silica gel surface effects by decreasing adsorption of water by a silica gel. This imbalance occurs due to the thermal treatment or hydrothermal treatment of the samples. In both cases, the adsorption effectivity of water decreases. The high water adsorbing capacity can be obtained with a high surface area, a high concentration of silanol groups with a 1:1 ratio of vicinal and free silanol groups [4].

2.5. Characterizations: Prepared silica gel is characterized for its physical properties like free moisture contents, water-soluble matter, bulk density and iodine number using standard procedures and the values calculated with the help of equations 1 to 6.

$$\% \text{ Moisture} = \frac{\text{Loss in weight of silica gel}}{\text{Weight of silica gel}} \quad \text{Eq. 1}$$

% of water-soluble matter =

$$\frac{\text{Dry weight} - \text{Weight after extraction}}{\text{Dry weight}} \quad \text{Eq. 2}$$

$$\text{Bulk Density} = \frac{\text{Weight of silica gel}}{\text{Final volume of silica gel}} \quad \text{Eq. 3}$$

Iodine number expressed as the milliequivalent of iodine adsorbed per 100 g of silica gel.

Iodine Number = Millilitre titrating 25 ml original Iodine solution – Millilitre × Normality of thiosulfate Eq. 4

Water-soluble sulphates/chlorides calculated by using the equation 5.

% of water-soluble sulphates / chlorides =

$$\frac{\text{Weight of silica gel taken}}{\text{Weight of precipitates of sulphates or chlorides}} \quad \text{Eq. 5}$$

Heat resistance and moisture absorptive capacity of silica gel are calculated by equation 6.

Moisture absorptive capacity =

$$\frac{\text{Increase in weight due to moist air absorbed}}{\text{Weight of silica gel}} \quad \text{Eq. 6}$$

3. General classification of Silica Gel: By particle size, pore size, and shape, silica gel classified in three classes. The class and their characteristics tabulated in **Table 2**.

4. Properties of Silica Gel:

1. Silica gel is non-toxic, non-flammable and non-reactive, stable product.
2. It can react with various reagents such as with hydrogen fluoride, fluorine, oxygen difluoride, chlorine trifluoride, strong acids, strong bases, and oxidizers.
3. The dried form of silica gel called as silica xerogel which is tough and hard. It is naturally occurring mineral purified and processed into granular or beaded form.

4. Silica gel possesses a high specific surface area (around 800 m^2/g) allows it to absorb water readily. Silica gel removes moisture by adsorbing numerous pores and not absorbing it into the bulk of gel. Due to this mechanism silica gel is useful as a desiccant (drying agent) [1, 2, 3, 5].

TABLE 2: CLASSES OF SILICA GEL

Characteristics	Type A	Type B	Type C
Appearance	Clear pellets, drying and moisture proof properties. Also known as fine pore silica gel	Translucent white pellets	Translucent, micro-pored structure
Approximate pore diameter (nm)	2.5	4.5-7.0	-
Applications	Catalyst carriers, adsorbents, separators and variable- pressure adsorbent	Liquid adsorbents, drier, catalyst carriers	Additionally dried and screened form (macro-pored silica gel) used as drier, adsorbent and catalyst carrier

5. Advancements in Silica Gel:

5.1. Modified Silica Gel: Surface polarity can get dramatically modified by chemically bonding functional groups to silica gel, which produces chromatographic media with distinctive separation properties. Use of functionalized silica provides better resolution or separation of difficult compounds [3].

5.1.1. Bonded Phases: A previous report suggests that silica-based bonded phases made by depositing polymeric organic layer on the silica surface. There are three classes of bonded phase and they are "the brush phase", "the oligomeric phase" and "the bulk phase". There are different classes produced from the three different types of silane reagents, that is, the mono-substituted, di-substituted and tri-substituted silanes.

5.1.1.1. Bonded Phases for Reversed-Phase Chromatography:

Brush Phase: It is most widely used packing produced by silanol surface-reacted with monochloro organosilanes as shown in the following the figure.

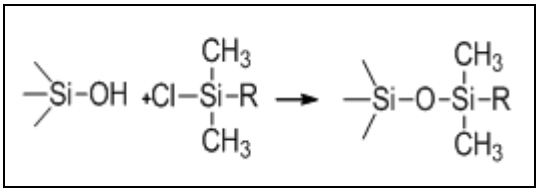

FIG. 2: REACTION OF SURFACE SILANOL WITH CHLORODIMETHYLSILANE

In the above reaction bonded phase packings are made by reaction of surface silanol with mono-functional reagents, such as chlorodimethylsilane. Various alkyl and substituted alkyl silicas are made by this reaction, such as, n-octadecylsilane (ODS or C_{18}) bonded phase materials.

Advantages of monofunctional silane reaction:

1. They are reproducible.
2. One silanol group reacts with one silane molecule, producing predictable structures.
3. Packing made by this route often shows the highest efficiency because of fast diffusion in and out of the thin stationary phase layer.

Oligomeric Phase: These types of bonded phases made by doing reaction of silanol surface with bifunctional silane.

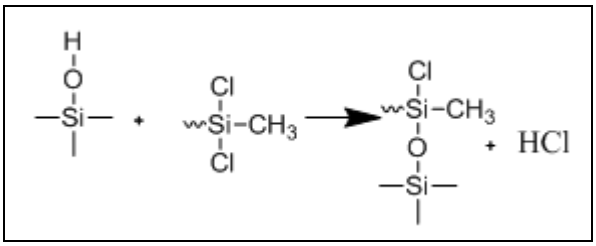

FIG. 3: FORMATION OF OLIGOMERIC BONDED PHASES

Bulk Phase: Some commercial packings made by doing reaction of surface layer resulting from the reaction of trifunctional silanes with the silica surface as shown in the following the figure.

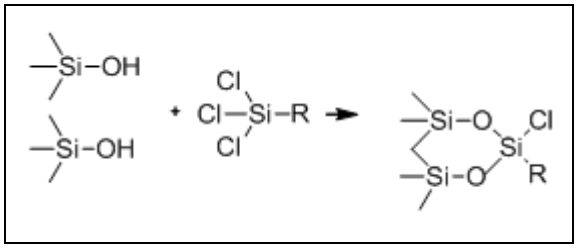

FIG. 4: REACTION OF SURFACE SILANOLS WITH TRIFUNCTIONALSILANE

Polymeric bonded phases shown in following reactions are more stable than monomeric phases at low pH.

However, packings made in this manner are more variable concerning retention and selectivity compared with monofunctional phases.

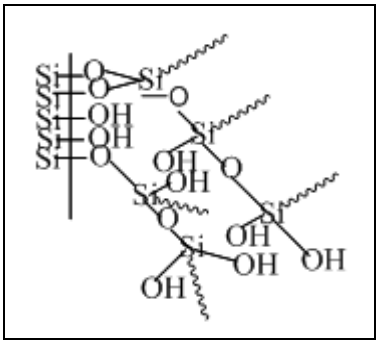

FIG. 5: LIGHTLY VERTICAL-POLYMERIZED PHASE

The following figure shows another type of bonded saline surface, called horizontal polymerization. These materials reported showing superior stability in low and high pH environments.

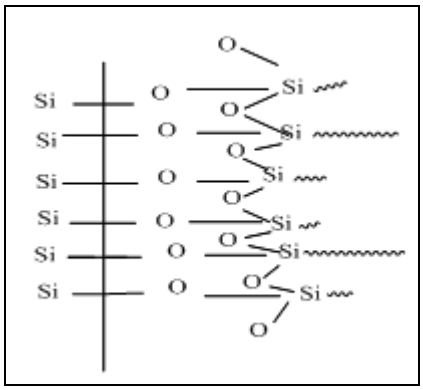

FIG. 6: HORIZONTAL POLYMERIZED BONDED PHASES

As the chain length or bulk of the silane increases, related silanol group percentage decreases. Even with the smallest silane (trimethyl or C_1), almost 50% of the silanol groups remain unreacted on the surface. These silanol groups are located under an umbrella of organic silane ligands but are still available for electrostatic interaction with appropriate solutes.

6. **Monolithic Silica Column:** Preparation and development of monolithic silica columns were first done in the 1990s. The primary feature of the monolithic column is they have high and variable external porosity.

Monoliths defined as the United continuous porous separation media without the interparticular voids and thus it is a promising tool for microscale separation. Their structure based on the continuous interconnected skeleton of mesoporous silica. These materials are assuring a bright future in separation science which is useful for pharmaceutical, biological, and food industries. They were able to give maximum efficiency with four times less back pressure in liquid chromatography as compared to columns packed with 5 µm particulate due to their greatest external porosity [6].

6.1. Synthesis of Monolithic Silica Column: For the preparation of monolithic columns by sol-gel method monomers are polymerized in a capillary column. The process typically involves polymer template inducing phase separation and poly-condensation of sol-gel transition due to the silica precursor, which leads to a co-continuous silica network of defined pore structure. Further, monolithic silica columns with a bimodal pore structure consisting of macropores and mesopores in the silica skeleton can be obtained with thermal treatment. In monolithic silica columns by keeping mesopores constant, smaller or larger macropores can be formed this feature makes the monolithic silica columns unique compared to their packed counterparts. The group of Tanaka prepared silica-based monolithic rods by hydrolytic polymerization of silanes, like tetramethoxysilane, the reaction is catalysed by aqueous acetic acid in the presence of polyethylene glycol which forms a sol. This sol is converted to monolithic silica having network structures attached to the tube wall in a fused silica capillary. Then ammonia is introduced for the formation of mesopores (in which analytes diffuse in and out), and thus creates a large specific surface area and a chromatographic efficiency corresponding to a 3-5 µm particulate column [7].

Following table shows a comparison between physical and chromatographic characterizations of monolithic and conventional packed columns.

TABLE 3: COMPARISON BETWEEN PHYSICAL AND CHROMATOGRAPHIC CHARACTERIZATIONS OF MONOLITHIC AND CONVENTIONAL PACKED COLUMNS

Characteristics	Monolithic column	Conventional particle-packed column
Structure	The monolithic column consists of a macroporous structure	The particle-packed column contains interparticular voids
Permeability	Column back pressure and permeability depends upon	Column back pressure depends upon interparticular

	the macroporous structure	voids
Performance and permeability	In monolithic silica columns, performance and permeability are independent of each other	In this type permeability is directly proportional to particle diameter and inversely proportional to plate number thus permeability and performance are dependent on each other
Separation capacity	These materials possess higher column efficiency at a high linear velocity	Separation efficiency is low as compared to the monolithic columns
Adsorption capacity	Adsorption capacity is 30 to 40% higher than that of particle packed column	Possess 30 to 40% lower adsorption capacity than monolithic columns

Characterization of porous form, flow characteristics, and column efficiency revealed the advantages of a monolithic silica column over a particle-packed column. Other important advantages of monolithic silica columns are listed below:

6.2. Advantages:

- Monolithic silica columns have the advantage of 5 to 10 time's faster operation leading to shorter analysis times.
- They own high linear velocity with high column efficiency per unit column length which offer a resolution at nearly 1/10 time compared with conventional packed columns.
- The high porosity leads to high permeability or low-pressure drop. Due to high permeability, monolithic silica columns are suitable for high throughput analysis.
- Monolithic silica columns provide fast separation especially in combination with Mass spectrometric (MS) detection.
- Monolithic columns effectively separate highly complex biological mixtures such as tryptic digest [6, 7].

7. Mesoporous Silica: Porous materials with regular geometries have been recently paid much attention following their great scientific importance and potentials in separation science. Depending upon the pore size, the porous materials are classified by IUPAC into three classes:

1. Microporous (Pore diameter in the size range of 0.2–2.0 nm).
2. Mesoporous (Pore diameter in the size range of 2.0–50.0 nm).
3. Macroporous (Pore sizes exceeding 50.0 nm).

Mesoporous silica possesses high surface areas as 1600 m^2/g and expected to provide superior separating ability which is essential for the chromatographic matrix. This silica's prepared by a sol-gel polymerization method which produces pore diameters adjustable between 20 and 300, with narrow pore-size distributions. Lack of pore size distribution makes microporous and macroporous materials incapable for selecting adsorbing broad spectrum of large organic molecules of technical interest.

The pore size of the mesoporous materials allows not only easy accessibility for molecules but also possible controllability in functions depending on pore geometries. Recently, the demand for mesoporous materials has triggered due to their high specific surface area, large specific pore volume, and pore diameter.

There are various methods for functionalization of mesoporous silica which is a necessary step for the applications of these materials in chromatographic separations

1. **Grafting (Post Synthetic Modification):** The grafting method used for modification of mesoporous silica, and the introduction of various groups like amino, thiol and alkyl groups.

2. Co-condensation: In co-condensation method, functionalized organosilicates, which have both organic functional group and condensable group, condensed together with silica sources such as tetra-methyl orthosilicate and tetra-ethyl orthosilicate. Therefore, mesoporous silica functionalized with organic group obtained directly in one-pot synthesis, and the functional groups spread homogeneously in the silica structures.

3. Periodic Mesoporous Organosilicate (PMO): One of the most different approaches in the preparation of mesoporous organic-inorganic hybrids is a synthesis of periodic mesoporous organosilicates. In this type of silicate materials created from organic molecules containing multiple alkoxysilane groups. These organic components play an important role as PMO pore wall.

Formation of crystalline pore wall in mesoporous silicate materials is one of the fantastic applications of PMO approach [8].

8. New Generation Silica-based HPLC Columns: Second generation silica-based monolithic columns, columns with sub 2 µm particles, superficially porous silica columns (core-shell packings) these are the new generation columns providing high throughput, high efficient separations.

First generation silica-based monolithic column (Chromolith, Merck Millipore) attracted a lot of attraction in 2000 because of its novelty. Silica-based monolithic columns consist of a single piece of porous silica having higher separation efficiency (plate count per meter = N/m up-to 200,000) and permeability. They provide lower back pressure, higher flow rate, faster analysis, and direct application of dirty samples without prior sample preparation during analysis. New generation silica-based monolithic HPLC columns (Chromolith High Resolution [HR]) provides more homogeneous porous silica network with well-designed silica skeleton in combination with bimodal pore structure than the first generation silica-based monolithic column. These columns have improved separation efficiency and peak symmetry for basic compounds.

8.1. Preparation of Second Generation Monolithic Columns using Sol-Gel Method: Tetramethoxysilane (TMOS) acting as silica precursor and polyethylene oxide (PEO) acting as a pore-forming agent both dissolved in slightly acidic conditions filled into gelation tube and heated at moderate temperature. After this stage sol forms silica gel and phase separation occurs separating silica-rich phase and solvent rich phase. The time required for phase separation of silica phase from solvent phase forms silica skeleton. Removal of solvent phase forms the macroporous structure of silica.

Further, polycondensation reaction causes shrinkage of the rod which detaches from the inner surface of gelation tube allowing its removal. A better control of this step in combination with smaller domain size and macroporous structure has led to the second generation of the monolith which shows improved separation on efficiency and peak symmetry. Monoliths of the second generation were prepared by systematically reducing the macropore size.

TABLE 4: COMPARISON BETWEEN SILICA-BASED MONOLITHIC HPLC COLUMNS: FIRST GENERATION *vs*. SECOND GENERATION

S. no.	First generation monolithic column (Chromolith Merck Millipore)	Second generation monolithic column (Chromolith High Resolution [HR])
1	Macropore size of chromolith column is around 2 µm	Macropore size of new generation Chromolith HR column is around 1.1 µm
2	Performance similar to 3.5-5 µm particle packed column with column back pressure similar to a 1.1 µm particle-packed column	Peak symmetry and separation efficiency achieved by symmetrically decreasing macropore size and corresponding domain size with homogeneous porous silica network
3	Possess separation efficiency of N/m>80,000	Possess much higher separation efficiency than first generation columns N/m>140,000
4	Separation efficiency increases due to higher column back pressure and due to decreased macropore size. 100 mm × 4.6 mm Chromolith column can be operated at 25 bar	Chromolith HR shows a column back pressure of around 65 bar. Typical length Chromolith HR columns can still be employed with low back pressure conventional HPLC instrumentation

8.2. Particle Packed Columns with Sub 2 µm Particles: Particle packed columns filled with sub 2 µm silica particles possess high separation efficiency about 200,000 N/m due to smaller porous particles. There are some disadvantages for working with 2 µm particle packed columns Require UHPLC system (instrument working with pressure above 1000 bar), frictional heat generation, blockage because of small porosity [9].

8.3. Superficially Porous Silica Columns: Nowadays, high-speed and efficiency in HPLC analyses are in the demands. Superficially porous silica columns with particle sizes of 2.5-3 µm possess higher separation efficiencies, column permeability, and lower column back pressure. The measure arenas for fast analyses are the pharmaceutical and food industries, agrochemical industries, biological and environmental sciences,

proteomic and genomic research. To meet these requirements, reduction in the column length, the linear velocity of the mobile phase and column particle size done respectively, but these are not economically feasible as they need costly UPLC machines. Due to a reduction in the particle, thermal deterioration of the column bed occurs which further resulted in efficiency losses.

Additionally, small particle columns need severe filtration of mobile phase to avoid blockage of 0.5µm frits of the column. These problems resulted in an advanced HPLC column technology, *i.e.*, the development of superficially porous silica particle columns (SPSPCs). This column contains particles of sub 3.0 µm and has a solid, nonporous core along with a shell of constant thickness made up of a porous silica gel. These SPSPCs are more efficient and reduce run time by 70%.

Generally, SPSPCs have twice the number of theoretical plates (>200 000 per meter) and resolving ability with moderate back pressure. The unretained solutes eluted quickly from these columns in comparison to monolithic columns. SPSPCs are suitable for separating small as well as large molecules with good sample loading capacities. The most commonly used mobile phases are water/acetonitrile, water/methanol, buffer/ acetonitrile, buffer/methanol, *etc*.

There are seven manufacturers in the market for supplying these columns, *i.e.* Advanced Material Technology, USA (Halo), Sigma-Aldrich, USA (Ascentis Express), Agilent Technologies, USA (Pro shell 120), Phenomenex, USA (Kinetex), Chromanik Tech., Japan (Sunshell), Macherey-Nagel, Germany (Nucleoshell), and Thermo Scientific, USA (Accucore). These new generation columns give super-fast separation by using conventional HPLC machine. Moreover, these columns need a low amount of solvents and handling time making them economical and less hazardous to the environment. These features are attracting industrialists, academicians, scientists, and regulatory authorities [10].

9. Applications of Silica Gel in Chromatography:

- Silica gel is most popular packing material which used as a stationary phase in chromatography. The stationary phase is a substance fixed in place for the chromatographic procedure.
- Silica-based packings are compatible with water and all organic solvents, and no dimensional variation (*e.g.*, swelling) in silica packings occurs with the change in solvents. This feature permits the formation of stabilization packed beds that are stable during use with various solvent types and during gradient elution.
- The hydroxy (OH) groups on the surface of silica can be functionalized to afford specialty silica gels that exhibit unique stationary phase parameters.
- It possesses high mechanical strength which permits the formation of efficiently packed beds that are stable under high operating pressures for long periods.
- The silica-based column provides the highest column efficiency of any of the materials used to produce columns that exhibit lower backpressures and longer lifetime.
- The stronger spherical particles are more easily and reproducibly packed into efficient columns.
- The surface of silica support gets chemically modified with a large variety of bonded phases having different functionalities.
- The hydrated silica gel surface has three kinds of silanol groups: free silanol, geminal, and associated silanols. Nonhydrogen-bonded free silanol is more acidic and can cause strong and deterious binding of basic solutes because of their highly acidic nature. Therefore, silica gel with a higher concentration of free and highly acidic silanols often shows increased retention and broad peak tailing for basic compounds.

  Fortunately, free silanols generally occur in a low concentration on the silica gel surface. The surface of silica gel with the highest concentration of geminal and associated silanols favored most for the chromatography of basic compounds because these silanols are less acidic [1-12].

Chromatographic types based on separation mechanism and uses of silica gel in all these types of chromatography tabulated in **Table 4**.

TABLE 5: APPLICATIONS OF THE SILICA GEL IN DIFFERENT TYPES OF CHROMATOGRAPHY

S. no.	Types of chromatography based on the separation mechanism	Uses of silica gel in chromatography
1	Partition Chromatography	In Partition Chromatography, silica gel used as a stationary phase
2	Adsorption Chromatography	Silica gel is used as an adsorbent and remains dominant stationary phase in Adsorption Chromatography
3	Ion exchange Chromatography	In ion Exchange Chromatography, silica gel used as a stationary phase which contains ionizable functional groups

1. Partition Chromatography: Partition chromatography classified as:

1.1. Liquid-Liquid Chromatography: Liquid-liquid chromatography is a part of partition chromatography and is further classified as normal phase chromatography and reversed phase chromatography.

1.1.1. Normal Phase Chromatography: In this type of chromatography, stationary phase (silica support) is a polar and mobile phase is non-polar. Due to silica gel's polarity, non-polar components tend to elute before more polar components, hence the name normal phase chromatography.

1.1.2. Reversed Phase Chromatography: In this type of chromatography, stationary phase (silica support) made non-polar by attaching C_{18} groups and polar solvent used as a mobile phase. When hydrophobic C_{18} groups attached to the silica gel, then polar components elute first, and the method referred to as reverse phase chromatography.

1.2. Gas-Liquid Chromatography: In this type of chromatography the substance to be separated is moved by an inert gas along with tube filled with the finely divided inert solid support of silica with a diatomaceous structure which further coated with non-volatile oil or waxy polymer.

2. Adsorption Chromatography or Liquid-Solid Chromatography: As an adsorbent silica gel has the following characteristics:

- Insoluble in the mobile phase.
- Inert to solute.
- Colorless especially when working with the color mixture.
- Suitable particle size is enough to give good separation and a reasonable flow rate.

In adsorption chromatography, various forms of silica gel can be used as an adsorbent such as: hydrated silica gel, silica gel G, silica gel H, silica gel S, silica gel N, silica gel GF_{254}, silica gel HF_{254}, silica gel PF_{254}.

Adsorption chromatography can further be classified as:

2.1. Column Chromatography: Column Chromatography consists of separation of each component from a mixture by loading the mixture into a column which packed with finely powdered adsorbent and then developing the column with a solvent, *i.e.* eluting the individual component in the mixture by passing a solvent through the column. The individual components collected as consecutive eluent fractions. Silica gel used as an adsorbent in column chromatography. In this type of chromatography, the stationary phase is most often composed of silica gel particles with 40-63 μm. For achieving the desired separation of certain molecular sizes, different particle sizes are used [10, 11].

2.1.1. Silica gel in High-Performance Liquid Chromatography (HPLC):

2.1.1.1. Column Packing Particles in HPLC: Particle type and size of the silica affects separation in the HPLC system. Several particle types available for HPLC application are as follows:

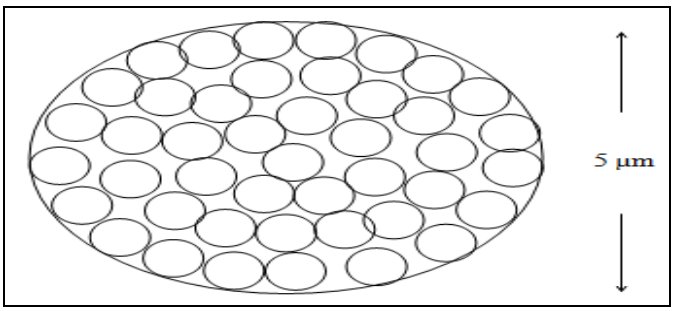

FIG. 7: POROUS MICROSPHERES

2.1.1.1.1. Porous Microspheres: Porous micro-spheres are most commonly used because of the favorable compromise of desired properties: efficiency, sample loading, a variety of diameter,

pore sizes, and surface areas so that all types of HPLC methods can be developed with these materials.

2.1.1.1.2. Micropellicular Particles: Micro-pellicular particles have a solid core with the thin outer skin of interactive stationary phase (silica gel). This silica particle, usually available in 1.5 to 2.5 µm sizes, displays outstanding efficiency for macromolecules because of fast mass transfer kinetics. Pellicular particles of sizes 1.5 µm are useful for extremely rapid separations of macromolecules such as proteins.

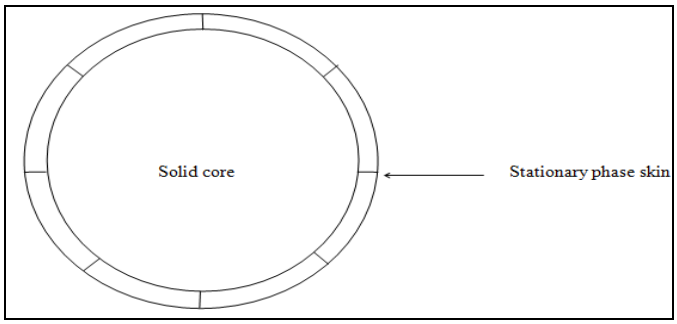

FIG. 8: MICROPELLICULAR PARTICLES

2.1.1.1.3. Perfusion Particles: Perfusion particles contain very large pores (*e.g.,* 4000 to 8000 Å) throughout the support and also include a network of smaller interconnecting pores solutes can enter (and leave) this pore structure through a combination of convective (flow) and diffusion. This effect minimizes band broadening so that large porous particles resemble smaller particles regarding column efficiency but with a fraction of the pressure drop.

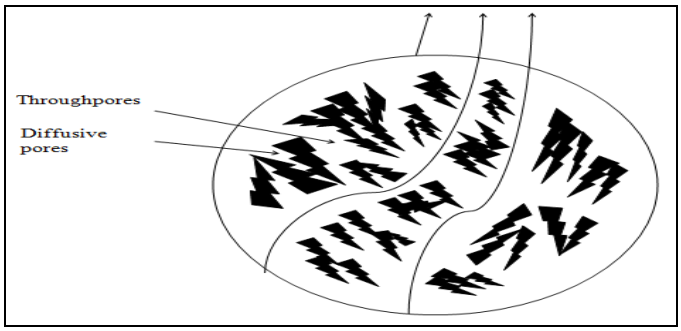

FIG. 9: PERFUSION PARTICLES

Particle size is very important in HPLC. A particle diameter of about 5 µm represents a good compromise for analytical columns regarding column efficiency, backpressure, and lifetime. Smaller porous particles (*e.g.,* 3 µm) are available for faster separations [5].

2.2. Thin Layer Chromatography (TLC): The silica gel used as an adsorbent for TLC plates has a mean particle size of approximately 12µm. The pore diameter is mainly 60 Å, and the surface area is approximately 500 m^2/g. The most frequently used thicknesses of silica-based thin layer for analytical procedures are approximately 200-250 µm. The thin layer for preparative purposes is available with thicknesses of up to 2 mm. The thin layer of the adsorbent can also contain a fluorescence indicator with a particle size less than the sorbent. The advantage of these indicators lies in the fact that all substances with a conjugated p-electron system (*e.g*., aromatic compounds) appear as dark spots on a bright emitting background when the chromatogram viewed under UV light.

An extension of TLC is High-Performance Thin Layer Chromatography (HPTLC). It is a robust, simplest, rapid, and efficient tool in the quantitative analysis of compounds. HPTLC is an analytical technique based on TLC, but with enhanced resolution of the compounds to be separated and to allow quantitative analysis of the compounds. The difference between TLC and HPTLC plate is particle size of coated material, which is 5 to 20 mm of TLC and 4 to 8 mm for HPTLC. Plates are produced from 4 to 5 mm silica gel with an inert binder to form a 200 mm layer. Plates of 20 × 20 cm and 5 × 7.5 cm used. Silica gel F_{254} having a pore size of 6 mm with a fluorescent indicator is a coating material [11, 12].

3. Ion-Exchange Chromatography: In this type of chromatography separation of molecules based on their respective charged groups and retention of analyte molecules based on ionic interactions. Mainly, molecules undergo electrostatic interactions with opposite charges bonded with stationary phase matrix. The stationary phase consists of an immobile matrix that bonded with charged ionizable functional groups. The silica-based stationary phase surface exhibit ionic functional groups (R-X) that interact with oppositely charged analyte ions. To achieve electroneutrality, these inert charges couple with counterions in the solution. Ionizable molecules that are to be purified compete with these counterions for binding to the immobilized charges on the stationary phase. These ionizable molecules are retained based on their charges [11, 13].

CONCLUSION: The review is a systematic compilation of data related to silica gel. In this review we have elaborated the sol-gel process which allows the association of mineral phases with organic or biological systems. Sol-gel process has been the cornerstone in the development of monolith columns and functionalized mesoporous silica particles providing wide open structures and gaining the popularity in chromatographic separations today. The article also describes the new generation columns packed with superficially porous silica particles providing stationary phases with moderate sample loading capacity and low back pressure useful in ultra-fast HPLC separations.

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CONFLICT OF INTEREST: Nil

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